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A novel strategy of acrylamide mitigation in fried potatoes using asparaginase and high pressure technology

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Abstract

The potentiality of high pressure processing (HPP) to possibly enhance diffusion of asparaginase into raw potato sticks, and consequently on reduction of acrylamide levels in fried potatoes was evaluated. Raw potato sticks were immersed in asparaginase (10,000 ASNU/L) and immediately subjected to 0.1, 100, 200 and 400 MPa for 5 min, with total enzymatic reaction times of 5, 10 and 20 min and room temperature. Pressurized raw potato sticks became softer, more flexible, and required lower energy for cutting (up to 47% less); the roughness of potato surface and moisture content were slightly reduced; and the concentration of soluble solids in the exterior solutions increased, indicative of a leaching effect. Due to changes induced by asparaginase and/or HPP on raw potatoes, fried potatoes exhibited higher weight loss after frying, and higher hardness (crispness). The combined treatment with asparaginase and HPP showed to reduce acrylamide levels by 26-47%, while with asparaginase or HPP alone there was no significant reduction.

Keywords: high pressure processing, asparaginase, frying, acrylamide, raw potatoes, fried potatoes

1. Introduction

In early 2002, Swedish researchers presented preliminary findings of acrylamide in a range of heated foods, and moderate levels (5-50 μg/kg) were measured in protein-rich foods and higher contents (150-4000 μg/kg) in carbohydrate-rich foods, such as potato chips, French fries, beetroot and crispbread **(Tareke et al., 2002)**. These findings caused a worldwide concern because acrylamide is classified by the World Health Organization and the International Agency for Research on Cancer as a Group 2A carcinogen ("probably carcinogenic to humans") due to its carcinogenic implication in rats **(Food et al., 2002; IARC, 1994)**.

Acrylamide is primarily formed as a by-product of Maillard reaction, a complex series of non-enzymatic reactions, mainly between the amino acid asparagine and reducing sugars (fructose and glucose) found in foods when heated to high temperatures, typically at temperatures above 120 ºC **(Miranda & Aguilera, 2006; Pedreschi et al., 2008)**. Potato products are strongly susceptible to acrylamide formation due to their asparagine and reducing sugars contents, as well as the traditional applied cooking conditions (temperatures > 120 ºC), like frying and roasting. Thus, the potential strategies to prevent acrylamide formation may be covered in two major approaches: removing acrylamide precursors (glucose, fructose and asparagine) or interfering with the Maillard reaction **(Capuano & Fogliano, 2011; Genovese et al., 2019; Powers et al., 2013; Singh & Kaur, 2016)**.

Several strategies for acrylamide mitigation have been tested in fried potatoes **(FDE Food Drink Europe, 2019; The European Commission, 2017)**, including both time and temperature control during frying, and potato treatments prior to frying. These pre-treatments include blanching **(Mestdagh, De Wilde, Delporte, Van Peteghem, & De Meulenaer, 2008)**; use of additives, such as sodium acid pyrophosphate **(Lindsay**

& Jang, 2005; Pedreschi & Zuñiga, 2009b), organic acids **(Jung et al., 2003; Medeiros Vinci et al., 2011)**, divalent cations, and amino acids (not asparagine), such as glycine and glutamine **(Medeiros Vinci et al., 2011)**; lactic acid fermentation **(Baardseth et al., 2006)**; application of ultrasounds **(Antunes-Rohling et al., 2018)**; and addition of asparaginase **(Hendriksen et al., 2009; Kalum & Hendriksen, 2016; Pedreschi et al., 2008; Zuo et al., 2015).**

Asparaginase (L-asparagine amidohydrolase EC 3.5.1.1) is an enzyme that can reduce acrylamide formation in foods since it catalyses the hydrolysis of asparagine into ammonia and aspartic acid, which are not acrylamide precursors **(Capuano & Fogliano, 2011)**. The first results using a commercial asparaginase (Acrylaway®) for acrylamide mitigation in French fries were published by **Pedreschi et al. (2008)**. In the study, a reduction of about 60% in acrylamide levels was achieved in French fries whose potato strips were blanched at 75 ºC for 10 min and immersed in asparaginase (10,000 ASNU/L) at 40 ºC for 20 min. Another study carried out by **Hendriksen et al. (2009)** showed that a combined pretreatment with blanching and asparaginase (1,000 – 105,000 ASNU/L) resulted in 60 to 85% acrylamide reductions in French fries, while **Zuo et al. (2015)** has reached 80% reductions with an asparaginase solution (10 U/mL) at 80 ºC for 4 min. Asparaginase application on potato products is a complex process because these consist of solid cut pieces, and thereby a blanching step prior to enzyme application was used in all studies presented previously, as a strategy to change the microstructure of the potato strips, and, consequently, to increase the asparaginase-asparagine contact **(Pedreschi et al., 2008)**. However, as reviewed by **Dourado et al. (2019)**, some non-thermal processing technologies have proved to modify several physico-chemical properties of raw potato tubers, namely high pressure technology

(Oliveira et al., 2015; Saraiva & Rodrigues, 2011) and pulsed electric fields (PEF) **(Fauster et al., 2018; Ignat et al., 2015)**.

High pressure processing (HPP) has been mostly employed as a cold pasteurization technology, together with several other applications, such as for the modification of food biopolymers (including starch and proteins) and physiological processes (like potato sprouting) **(Alexandre et al., 2016; Balasubramaniam et al., 2015; Saraiva & Rodrigues, 2011)**, to accelerate infusion processes, namely in potato cubes subjected to 100 – 400 MPa **(Sopanangkul et al., 2002)**, and to change the tissue structure of several vegetables and tubers **(Oey et al., 2008; Oliveira et al., 2015).** However, few studies are reported in the literature regarding the effect of HPP on potato tubers, and no studies were performed regarding its use instead of blanching, as a strategy either to possibly enhance the infusion rate of asparaginase or to make the process energetically and economically less expensive. Thus, as the authors are aware, this is the first study assaying the potentiality of HPP on asparaginase infusion into raw potato sticks before frying, as a novel strategy to reduce acrylamide levels in fried potatoes.

2. Materials and methods

2.1. Materials

White potatoes (*Solanum tuberosum* L., Agria variety) were chosen due to their suitability for frying and availability in a local market (Aveiro, Portugal). Potatoes were stored up to 1 month in a room protected from light at 10 ºC, and were selected by shape uniformity and absence of injuries. The frying oil used (*Fula* brand, special for frying, Sovena) is a commercial combination of sunflower and rapeseed oil, widely

available in the Portuguese market (three different bottles were used in triplicate assays). Asparaginase enzyme (Acrylaway®) was kindly provided by Novozymes A/S, Basvaerd, Denmark. The reagents (analytical and chromatographic grade) were purchased in diversified suppliers.

2.2. Pre-frying processing

Potato tubers were randomly selected, washed in running water, manually peeled, and sliced by using an appropriate cutting tool (Actuel, Jumbo, Portugal). The potato sticks with 0.9 cm of width, 0.9 cm of thickness and between 4 and 4.5 cm of length were placed in polyamide/polyethylene bags (PA/PE, Plásticos Macar, Indústria de Plásticos Lda, Santo Tirso, Portugal). An asparaginase solution containing 10,000 ASNU/L (1 ASNU is defined as the amount of asparaginase that produces 1 µmol of ammonia per minute under the conditions of the assay (pH = 7 ± 0.005; 37.0 ± 0.5 ºC)) was prepared from commercially available Acrilaway®, using tap water as solvent. The asparaginase solution (in a proportion of 2:1 (solution: potato - g/g)) was added to the bags and these, in turn, were heat sealed, avoiding to leave air inside as much as possible. The potato sticks were immediately subjected to pressure treatments of 0.1 (control), 100, 200, and 400 MPa for 5 min, at room temperature, by using a pilot-scale HP equipment (Hiperbaric 55 L, Burgos, Spain) with a pressure vessel of 55 L. Thereafter, a first set was immediately removed from the asparaginase solution, a second set was removed 5 min after the end of pressurization treatment, and a third set was removed 15 min after the end of processing, obtaining samples with 5, 10 and 20 min of enzyme reaction, respectively. All of this process occurred at room temperature. Treatments were performed in triplicate for each pressure condition. From this point on,

samples will be designated by "pressure (MPa)/ reaction time (min)", such as 0.1/5, 0.1/10, 0.1/20, etc.

Simultaneously, control samples were prepared in a similar way, but using tap water instead of asparaginase solution, and potato sticks were subjected to 0.1, 200 and 400 MPa for 5 min. Pressure condition of 100 MPa was not tested since no significant changes were found in physico-chemical properties of raw potato tubers compared to unpressurized potatoes **(Dourado, 2019)**. Treatments were performed in triplicate for each pressure condition and samples will be designated by "pressure (MPa)/ enzymatic reaction time (min)", i.e. 0.1/0, 200/0, and 400/0.

After each treatment, the surrounding water was collected for further analysis of total soluble solids, and potato sticks were immediately characterized by measuring their texture, colour, and moisture.

2.3. Deep-frying

Pretreated potato sticks were deep-fried by using a domestic electric fryer with 4 L of capacity (JATA, FR700 model, Portugal). The electric fryer was pre-heated to 180 ºC, a portion of potato sticks (about 600 g) was fried in 3 L of frying oil during 7 min. The temperature was periodically controlled with a digital thermometer and each frying was performed in triplicate. Fried potatoes were drained over paper, and left to rest for 2 min.

Fried potatoes were immediately analysed for weight loss after frying, texture, colour and moisture. The remaining samples were stored at -40 ºC until further analyses (acrylamide content).

2.4. Characterization of potato samples (raw and fried potatoes)

All analyses were performed in triplicate (n=3), except texture analysis, in which 10 data points (n=10) were obtained from five potato sticks of each raw or fried potato sample; colour analysis, where 9 data points (n=9) were registered from five potato sticks of each raw or fried potato sample; and acrylamide quantification, that was performed in duplicate (n=2) for each fried potato sample.

2.4.1. Weight difference after frying and moisture determination

Potato sticks were weighed before and after frying, and their weight difference was expressed in %. Moisture was determined by drying at 105ºC for 24h, being expressed in g/100 of potatoes.

2.4.2. Texture analysis

After processing/frying, five potato sticks were analysed in a texture analyser equipment (model TA.Hdi, Stable Micro Systems) equipped with a load cell with 5 kg, and using a knife with 6 cm of width, 10 cm of height and 1.2 mm of thickness. The velocity parameters were fixed as 0.50 mm/s (pre-test), 1.00 mm/s (test), and 2.00 mm/s (post-test), and the cutting distance was 5.0 mm. The parameters obtained from Force *vs* Distance graphics were the maximum force, the initial slope and the total area for raw potato sticks, and the maximum force for fried potato sticks.

2.4.3. Colour determination

Five raw or fried potato sticks of each replicate sample were used for colour analyses. Potatoes colour was measured directly on the surface, in three different locations of three different sides of raw or fried potato stick (n=9). A Konica Minolta CM 2300d colorimeter (Minolta Konica, Osaka, Japan) and the SpectraMagicTM NX program (Konica Minolta, Osaka, Japan) were used for colour assays. The colour space

system used was CIE-$L^*a^*b^*$ to represent the following colour parameters: L^* value (0, dark; 100, light), a^* value (+, red; -, green), and b^* value (+, yellow; -, blue). The total colour change (ΔE) was calculated according to the following equation: $\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$, wherein ΔL^*, Δa^* and Δb^* correspond to the difference of L^*, a^* and b^* values, respectively, between each sample and the control.

2.4.4. Acrylamide determination

Acrylamide determination of fried potatoes was performed as described in detail in **Molina-Garcia et al. (2015)**, by GC-MS after derivatization with xanthydrol. LODs and LOQs of this method are 4 and 10 µg/kg, respectively, and results were expressed in µg/kg of fried potatoes.

2.5. Characterization of the exterior water samples

2.5.1. Total soluble solids (TSS)

Total soluble solids (TSS) present in the water samples were determined by drying water samples at 105 ºC for 24h. Through the quotient between TSS weight after and before drying, TSS in water were expressed in %.

2.6. Statistical analysis

All analyses were statically analysed using one-way Analyses of Variance (ANOVA), followed by Tukey's HSD test at 5% of significance. The results were expressed as mean ± standard deviation.

3. Results and Discussion

3.1. Characterization of pre-treated raw potato sticks

3.1.1. Texture properties of raw potato strips

The texture of raw potato sticks (**Table 1**) was represented by their firmness (N, the maximum force applied to cut the potato strip); energy for cutting (N.mm, calculated as the area bellow the force-displacement curve); and stiffness (N/mm, the slope of the linear proportion of the respective graphics). Although potato sticks treated at 100 MPa exhibited no significant changes ($p<0.05$) relatively to unpressurized samples, a slight tendency to texture parameters decrease was detected. Raw potato sticks treated at 200 and 400 MPa, either immersed in water or in asparaginase solution, showed significant reductions in all texture parameters, but no changes were detected between them for all the reaction times ($p<0.05$). Thus, in pressurized samples immersed in asparaginase solution, it was detected a decrease of 25-30% of firmness, 20-41% of stiffness, and 27-41% of energy for cutting, compared to the respective unpressurized samples. These results were similar to those obtained for raw potato sticks immersed in water, since at 200 and 400 MPa, reductions of 28-31% of firmness, 9-38% of stiffness, and 22-42% of energy for cutting were obtained. It means that the addition of asparaginase did not influence the texture of potato strips. Actually, the applied pressure level was the major factor leading to the decrease of maximum force required to cut potato tissue, the increase in elasticity and flexibility, and the reduction of energy for cutting potato strips, regardless the reaction time. These results were visually perceived, as evidenced in Error! Reference source not found.**S**. Furthermore, according to the results of atomic force microscopy (AFM) analyses of **Dourado (2019)**, raw potato sticks treated by HPP exhibited a smoother and slippery surface than unpressurized samples.

The obtained results are in accordance with data of studies which applied HPP in solid vegetables and fruits, wherein pressure levels equal to or higher than 100 MPa had led to textural changes in those foods, causing reductions in their firmness **(Al-Khuseibi et al., 2005; Eshtiaghi & Knorr, 1993; Oey et al., 2008; Oliveira et al., 2015)**. Moreover, **Ignat et al. (2015)** showed that a reduction of 35% in the energy for cutting and a decrease in the firmness were achieved in potato sticks treated by PEF (9,000 pulses at 0.75 kV/cm electric field and 810 pulses at 2.50 kV/cm electric field). Comparing with the obtained results, HPP showed to be able to cause higher reductions (up to 42%) when applying medium pressure levels (400 MPa). Indeed, both PEF and HPP seem to induce similar modifications in potato tubers, namely an increase of potato cell permeabilization, softness of potato sticks, and smoothness of potato surface, but simultaneously, HPP can reduce more considerably the energy required for the cutting process compared to PEF.

3.1.2. Colour properties of raw potato strips

Colour of raw potato sticks was expressed according to lightness (L^*), green to red (a^*) and blue to yellow (b^*) parameters, whose results are shown in **Table 1S**. All samples presented L^* values in the range of ~64.9 – 71.9, a^* values between -0.6 and 1.9, and b^* values between 23.8 and 34.9. In a first approach, the influence of asparaginase reaction time was analysed for each pressure condition, and in a second approach, the influence of pressure level was analysed for each asparaginase reaction time. In both cases, sporadic significant changes were observed in the several colour parameters, probably due to differences in the potato tubers/sticks themselves, since no consistent tendency was observed. ΔE of all samples was low and did not change with the applied pressure nor with the enzymatic reaction time, being possible to conclude

that the combination of asparaginase and pressure treatments did not considerably affect raw potatoes colour.

Also, we are aware that HPP treatment *per se* can lead to the browning of potato tissue since it induces the loss of membrane integrity, which consequently promotes the contact between polyphenol oxidase and phenolic compounds (substrate) **(Oey et al., 2008; Oliveira et al., 2015).** However, the presence of O_2 is also required. So, we used a strategy to avoid the contact with oxygen: packaging potato sticks in water, and thus browning of the tissue was prevented. And according to the colour results of raw potato sticks, no significant changes were observed comparing with control samples, which proves that there was no significant enzymatic browning.

3.1.3. Moisture content of raw potato sticks and total soluble solids in the exterior solutions

Table 2 shows the results of moisture content of raw potato sticks and percentage of TSS present in the respective exterior solutions. In samples immersed in asparaginase solution, the enzymatic reaction time (5, 10 and 20 min) showed not to affect moisture of raw potato sticks ($p<0.05$). However, samples subjected to 200 and 400 MPa exhibited lower moisture content than those treated at the same pressure level but immersed in water without enzyme (0 min of reaction time). It means that probably asparaginase addition caused a higher loss of water/intracellular liquids after HPP treatments. Overall, HPP-treated samples exhibited equal to or lower percentage of moisture than unpressurized samples, which is in accordance with other studies concerning the effect of HPP in other vegetables and tubers. **Oliveira et al. (2015)** reported a greater moisture reduction in Peruvian carrot, sweet potatoes and cocoyam samples subjected to HPP at 600 MPa for 5 and 30 min than in unprocessed samples.

Yucel et al. (2010) noted that HPP lead to 2-5% moisture loss for carrot, apple, and green bean subjected to 100, 200, 250 and 300 MPa for 5, 15, 30 and 45 min, and **Rastogi and Niranjan (1998)** showed that compression and decompression processes during HP treatments (at 100 - 700 MPa for 5 min) of pineapples led to a significant ($p<0.05$) moisture loss. Moreover, during catalysis promoted by asparaginase, one molecule of water is consumed for each asparagine molecule that is catalysed into aspartate. Thus, the moisture loss of potato tubers treated by HPP and asparaginase can be explained as a result of a combined effect of: (1) HPP treatment on potato tissue, mainly due to the damage of cell structure, cell permeabilization and softening of vegetable tissues caused by HPP ; (2) the consumption of water molecules during the reaction catalysed by asparaginase.

In relation to the results of TSS (**Table 2)** in the exterior solutions of raw potato sticks, as the pressure intensity increased, the presence of TSS in water samples increased proportionally, and this increase was not influenced by the asparaginase reaction time ($p<0.05$). Water samples of potato tubers pressurized at 100 MPa did not show significant differences ($p<0.05$) in TSS, but the application of pressures of 200 and 400 MPa caused a significant increase ($p<0.05$) of up to ~5-fold when compared to control samples. This increase of TSS may be due to the release of sugars and other intracellular soluble components. As the increase of pressure leads to increased cell damage **(Oliveira et al., 2015; Sopanangkul et al., 2002)**, the components inside are released to the outside solution proportionally to tissue damage.

3.2. Characterization of fried potato sticks

3.2.1. Weight and moisture differences after frying

Raw potato sticks were fried, and their weight was measured before and after frying. The weight loss of each sample is shown in **Figure 1**. Results evidenced that the reaction time of asparaginase did not change ($p<0.05$) the weight loss of potato sticks after frying, for any pressure tested. However, a slight tendency to increase the weight loss was observed for potato sticks pre-treated at 200 and 400 MPa immersed in asparaginase solution (for 5, 10 and 20 min), when compared to potato sticks immersed in water without asparaginase (0 min). Unpressurized samples (0.1 MPa) lost about 46-48%, while pressurized samples lost about between 48-55% after frying at 180 ºC for 7 min, leading to the conclusion that the pressure intensity increased ($p<0.05$) the percentage of weight loss for the majority of samples.

Oliveira et al. (2015) observed an increase in drying rate of HPP-treated tubers. As frying is mainly a drying process based on both heat and mass transfer **(Aguilera & Gloria-Hernandez, 2000)**, the higher the drying rate of potato sticks, the faster the loss of water during frying **(Ziaiifar et al., 2008)**. Therefore, the increase of weight loss in HPP-treated fried potatoes was possibly related with cellular and textural changes in raw potato sticks caused by HPP, which accelerated drying processes (including frying) and facilitates the release of water from the sticks core.

3.2.2. Moisture content of fried potato sticks

Moisture content of fried potato sticks was measured (**Table 3**) in order to assay if differences observed in the percentage of weight loss after frying caused distinguished changes in the amount of water of fried products. Only 200/5 samples showed significant differences ($p<0.05$) compared to the control. The remaining samples presented equal to or lower moisture percentage than non-pressurized samples, proving that samples that lost more weight after frying, contained a lower amount of water.

3.2.3. Effect of asparaginase and HPP on texture of fried potatoes

In a fried potato stick, there is a crispy and dehydrated layer called crust, and the increase of crust dehydration enhances the crispness of fried potatoes. Indeed, crispiness is a major textural property of fried products, and brittle materials exhibit a larger hardness, which results from the low moisture content induced by frying **(Vincent, 1998; Yee & Bussell, 2007)**.

Fried potato sticks were analysed in relation to their texture by an instrumental method. In **Table 3**, the results of hardness (maximum force required to cut a fried potato) are exhibited. The enzymatic reaction time in each pressure level did not affect ($p<0.05$) hardness values. Analysing the effect of pressure intensity, although no significant change ($p<0.05$) had been detected among the different conditions, it was observed a slight tendency to increase the hardness values of fried potatoes pre-treated at 200 and 400 MPa, which means that crispness of fried potatoes slightly increased as a consequence of pre-treatments on raw potatoes. **Mestdagh et al. (2008)** reported that the product crispiness was positively correlated with the taste and general appraisal, so fried potatoes pre-treated by the combined treatment of asparaginase and HPP, or by HPP alone could have a good appraisal by consumers, but a sensory evaluation is required to prove this hypothesis and the taste in general.

These results may be related with the slight reduction of moisture content in fried potatoes pre-treated by HPP, as well as with results obtained in the texture analysis of the respective raw potatoes (**Table 1**). That is, in raw potato sticks, significant modifications ($p<0.05$) were only achieved when pressure treatments of 200 and 400 MPa were applied. For this reason, pressure levels above 100 MPa are needed in order to induce enough textural changes in the potato tuber, which result in more severe

textural changes in fried potatoes. As the enzymatic reaction time in each pressure level did not affect ($p<0.05$) the hardness values, the pressure level applied in pre-treatments was possibly the major factor to induce these textural changes in fried products.

3.2.4. Effect of asparaginase and HPP on colour of fried potatoes

The colour of a fried potato is an important attribute that affects the perception of the product's quality by a consumer **(Krokida et al., 2001)**, and thereby, this quality property was also measured in fried potatoes (**Figure 2S**), represented by the colour coordinate parameters of CIE-$L^*a^*b^*$ colour space system. According to the literature, desirable fried potatoes show high L^* values (lighter colour), coordinate a^* values between -5 and 0, and coordinate b^* values higher than 10 **(Krokida et al., 2001)**.

Analysing the obtained results (**Table 4),** fried potatoes showed high L^* values (superior to 66), b^* parameter higher than ~33, and a^* values about 0-3. Therefore, all parameters are in accordance with the desirable tonality, except coordinate a^*, showing a more red tonality that is less desirable for consumers. The enzymatic reaction time did not change ($p<0.05$) colour parameters and no clear tendency was detected. In contrast, although pressure level did not induced changes ($p<0.05$) for the majority of colour parameters, a slight tendency to decrease was detected in L^* parameter and an increase in b^* parameter. Thus, fried potatoes pre-treated either by asparaginase and HPP or by HPP alone led to the formation of fried potatoes with a darker, more red and yellow colour than control samples. These differences are possibly related with changes observed in the texture, weight loss after frying, and moisture content, associated with a slightly higher degree of doneness despite using the same frying time. As fried potatoes pre-treated by HPP increased their hardness and lost more water (and weight) after frying than control samples, possibly it decreased the thickness of potato strips during

frying. Since the reduction of potato sticks thickness has a negative effect on *L**, *a** and *b** parameters of fried products **(Krokida et al., 2001)**, it could explain why *L** values were lower, and *b** values were higher.

3.2.5. Acrylamide content of fried potato sticks

Acrylamide content was determined (**Table 3**) in order to assay the efficacy of the combined treatment of asparaginase with HPP to mitigate acrylamide formation in fried potatoes. Firstly, samples treated only by HPP, immersed in water without asparaginase (0 min of reaction time), exhibited acrylamide levels in the range of 300.5 and 387.8 μg/kg. These results are similar to data provided by EFSA, in which acrylamide levels of French fries were between 356 and 338 μg/kg, from 2007 to 2010 **(EFSA, 2012)**. Since no significant differences ($p<0.05$) among pressurized and unpressurized samples were detected, it means that HPP treatment alone (without asparaginase) was not enough to affect significantly the precursors of acrylamide in raw potatoes, and consequently was not effective to decrease significantly acrylamide levels in fried potatoes. Secondly, fried potatoes pre-treated at 0.1 MPa (unpressurized) immersed in asparaginase from 5 to 20 minutes showed similar acrylamide levels (285.0 - 339.8 μg/kg). So, no significant changes ($p<0.05$) were detected among samples treated at 0.1 MPa, either immersed in asparaginase or in water. In contrast, when the combined treatment with asparaginase and HPP was applied, fried potatoes pre-pressurized exhibited lower acrylamide levels ($p<0.05$) than the respective control samples (181.1 - 246.8 μg/kg). This reduction was independent ($p<0.05$) of the enzymatic time and pressure level used in the pre-treatment of raw potato sticks.

As stated in the *Introduction* section, asparaginase application on potato products has shown to be a complex process because these consist of solid cut pieces,

and thereby the contact between enzyme and substrate is not favoured. For that reason, a blanching step is usually required since it changes the microstructure of potato strips and increases the asparaginase-asparagine contact **(Pedreschi et al., 2011)**. Due to the reduction of acrylamide in fried potatoes pressurized before frying, pressure treatments possibly induced infusion of asparaginase into the potato sticks, probably due to structural changes caused in potato tissues and the increase of cell permeability, and thereby it could be an alternative to blanching treatments. Beyond that, HPP is described as a process that changes enzyme functionality, inactivating enzymes at higher pressures (>400 MPa) and, in several cases, activating enzymes at lower pressures (<200 MPa) **(Eisenmenger & Reyes-De-Corcuera, 2009).** Thus, it is possible that HPP could also induce the activation of asparaginase, but no information in the literature was found regarding this enzyme. So, more tests evaluating the effect of HPP on the asparaginase activity are needed.

The results also show that (1) the increase in soluble solids leached during HPP treatment -where acrylamide precursors might be included - have a smaller contribution than the apparent infusion of asparaginase into the raw sticks; and (2) the inner effect might be more relevant than a surface one as it was apparently independent of the contact time, with a probable higher relevance of the contact surface (infusion of the enzymes into the inner portions). Therefore, although isolated HPP treatments did not reduce acrylamide levels in fried potatoes, HPP combined with asparaginase seemed to be efficient in the mitigation of this carcinogen compound in fries, with reductions from 26% (in samples 100/10) up to 47% (in samples 400/20). Thus, among all the tested conditions, pretreatment of raw potato sticks at 400 MPa for 5 min jointly with 15 min of reaction under atmospheric pressure (20 min of total enzymatic reaction) was efficient for acrylamide reduction.

In the literature, there are several studies that used asparaginase to tentatively reduce acrylamide concentration in fried potatoes. Although reductions higher than 60% of acrylamide were obtained, all of them used temperature (50-60 ºC) to increase the enzymatic activity, as well as higher treatment times, and some of them resorted to blanching in order to induce microstructure changes in potato tissue and increase the asparaginase-asparagine contact. In the present study, no thermal step was applied either during or after HPP treatment, and a short HPP processing time was applied (only 5 min), which can make this process energetically less costly. Even then, reductions of acrylamide levels of up to 47% were obtained, proving to be an interesting method needing to be further studied to be optimised.

4. Conclusion

Raw potato sticks pre-treated either by HPP or by the combination of asparaginase and HPP exhibited reductions in firmness (up to 35%), stiffness (up to 38%), and energy required to cut (up to 47%), showing the potentiality of this non-thermal technology as a pretreatment to improve the cutting step of the industrial production of fried potatoes. In addition, HPP tended to induce the reduction of moisture content of raw potato strips and to increase the release of soluble solids into the outside solutions. Fried potatoes, whose raw potato strips were pre-treated either by HPP or by the combination of asparaginase and HPP, showed higher weight loss after frying than control samples. It means that HPP may be able to increase the frying rate since this technology can increase the rate of drying. Consequently, moisture content of fried potatoes slightly decreased, and their hardness increased, which could be a good indicator of higher crispness. Fried potatoes exhibited a slightly darker, and more red and yellow colour than control samples. Finally, although isolated HPP treatments did

not reduce acrylamide levels in fried potatoes, HPP combined with asparaginase was efficient in the mitigation of this carcinogen in fries, with reductions from 26 to 47%.

To sum up, HPP proved to be a potential non-thermal technology to be applied on modification of potato tubers, not only to improve energetically some industrial steps (for instance, cutting step and frying time), but also to be applied as a pretreatment for the production of potato products with different properties. In addition, the application of asparaginase and HPP (with low time processing (only 5 min) and no thermal step) on raw potatoes proved to be a novel efficient strategy to reduce acrylamide levels in fries, by an energetically less costly treatment.

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Conflict of interest

The authors have declared no conflict of interest.

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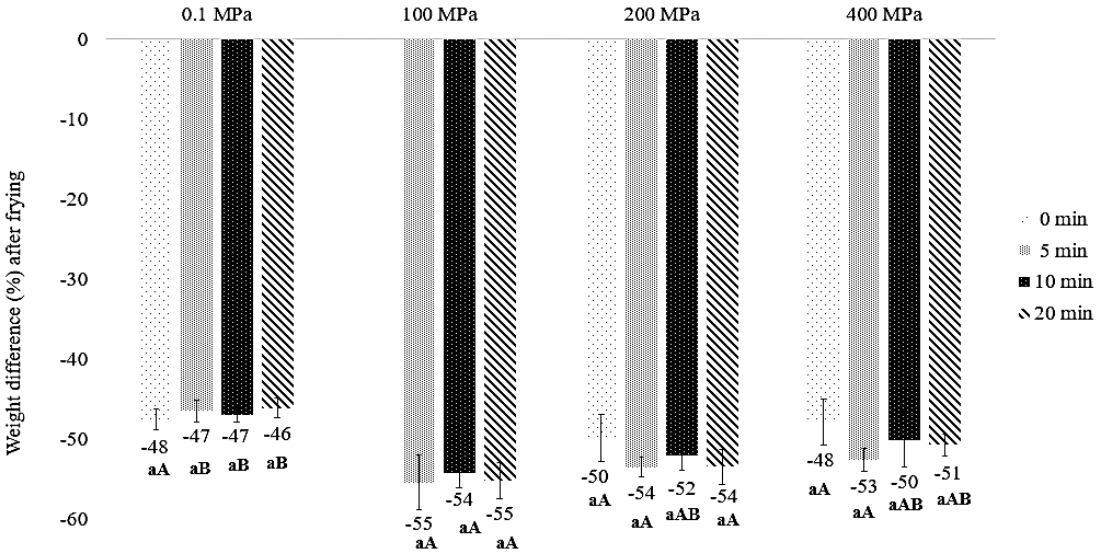

Figure 1 - Weight difference of potato sticks after frying, expressed in %. "0 min", "5 min", "10 min", and "20 min" indicate the enzymatic reaction time.
[a] small different letters mean significant differences ($p<0.05$) among reaction time of enzyme for the same pressure treatment; [A,B] capital different letters mean significant differences ($p<0.05$) among the pressure treatment for the same reaction time of enzyme.

Table 1 - Texture of raw potato sticks, expressing results of firmness, stiffness and energy required to cut raw potatoes.

Pressure (MPa)	Total asparaginase reaction time (min)	Firmness (N)	Stiffness (N/m)	Energy of cutting (N.mm)
0.1	**0**	10.38 ± 0.57 ^{aB}	10.39 ± 1.83 ^{aB}	38.20 ± 1.64 ^{aC}
	5	11.68 ± 0.67 ^{aB}	12.61 ± 1.83 ^{aB}	42.75 ± 2.51 ^{aB}
	10	11.30 ± 0.28 ^{aB}	13.03 ± 0.47 ^{aC}	42.01 ± 0.88 ^{aC}
	20	11.23 ± 0.62 ^{aB}	12.80 ± 0.82 ^{aB}	40.86 ± 2.30 ^{aB}
100	**5**	10.98 ± 0.42 ^{aB}	10.81 ± 0.52 ^{aB}	39.83 ± 0.69 ^{aB}
	10	10.28 ± 0.58 ^{aB}	10.58 ± 0.58 ^{aBC}	37.87 ± 1.01 ^{aC}
	20	9.48 ± 1.34 ^{aAB}	10.22 ± 0.77 ^{aAB}	39.08 ± 0.60 ^{aB}
200	**0**	7.52 ± 0.54 ^{aA}	9.43 ± 0.47 ^{aB}	29.92 ± 2.82 ^{aB}
	5	8.23 ± 0.48 ^{aA}	10.03 ± 0.92 ^{aAB}	29.91 ± 2.67 ^{aA}
	10	8.49 ± 0.52 ^{aA}	9.92 ± 1.61 ^{aA}	30.58 ± 2.91 ^{aB}
	20	8.04 ± 0.43 ^{aA}	9.79 ± 1.61 ^{aA}	26.91 ± 2.92 ^{aA}
400	**0**	7.15 ± 0.64 ^{aA}	6.44 ± 0.82 ^{aA}	22.22 ± 2.82 ^{aA}
	5	8.71 ± 0.30 ^{aA}	8.70 ± 0.52 ^{aA}	25.07 ± 1.62 ^{aA}
	10	8.28 ± 0.89 ^{aA}	7.74 ± 0.92 ^{aA}	24.41 ± 1.18 ^{aA}
	20	8.04 ± 0.23 ^{aA}	7.81 ± 0.38 ^{aA}	25.66 ± 1.45 ^{aA}

Results are expressed as the mean ± the standard deviation.
^{a} small different letters indicate significant differences ($p<0.05$) among reaction time of enzyme for the same pressure treatment; ^{A,B} capital different letters indicate significant differences ($p<0.05$) among the pressure treatment for the same reaction time of enzyme.

Table 2 - Moisture of raw potato sticks and total soluble solids present in the exterior solutions of potatoes, both expressed in %.

Pressure (MPa)	Total asparaginase reaction time (min)	Moisture (%)	Total soluble solids (%)
0.1	**0**	84.45 ± 0.67 ^{aA}	0.06 ± 0.03 ^{aA}
	5	84.66 ± 0.71 ^{aA}	0.09 ± 0.02 ^{aA}
	10	84.85 ± 2.45 ^{aB}	0.12 ± 0.02 ^{aA}
	20	83.99 ± 0.89 ^{aA}	0.09 ± 0.02 ^{aA}
100	**5**	83.06 ± 1.33 ^{aA}	0.12 ± 0.02 ^{aAB}
	10	83.21 ± 0.97 ^{aAB}	0.13 ± 0.04 ^{aA}
	20	83.32 ± 2.84 ^{aA}	0.17 ± 0.07 ^{aAB}
200	**0**	83.73 ± 0.71 ^{bA}	0.15 ± 0.02 ^{bB}
	5	81.82 ± 2.31 ^{aA}	0.21 ± 0.04 ^{bB}
	10	84.88 ± 1.26 ^{aB}	0.27 ± 0.03 ^{bB}
	20	83.49 ± 2.22 ^{aA}	0.32 ± 0.09 ^{bBC}
400	**0**	83.85 ± 1.87 ^{bA}	0.33 ± 0.02 ^{aC}
	5	80.56 ± 2.60 ^{aA}	0.36 ± 0.04 ^{aC}
	10	80.60 ± 1.11 ^{aA}	0.40 ± 0.05 ^{aC}
	20	81.83 ± 1.55 ^{aA}	0.44 ± 0.01 ^{aC}

Results are expressed as the mean ± the standard deviation.
^{a} small different letters indicate significant differences ($p<0.05$) among reaction time of enzyme for the same pressure treatment; ^{A,B} capital different letters indicate significant differences ($p<0.05$) among the pressure treatment for the same reaction time of enzyme.

Table 3 – Moisture (expressed in %), hardness (expressed in N), and acrylamide(expressed in μg/kg)of fried potato sticks.

Pressure (MPa)	Total asparaginase reaction time (min)	Moisture (%)	Hardness (N)	Acrylamide (μg/kg)
0.1	0	62.04 ± 1.70 ^{aA}	2.48 ± 0.55 ^{aA}	309.1 ± 51.2 ^{aA}
	5	59.98 ± 2.26 ^{aB}	2.82 ± 0.16 ^{aAB}	285.0 ± 46.5 ^{aB}
	10	61.02 ± 3.06 ^{aA}	2.76 ± 0.43 ^{aAB}	332.8 ± 21.3 ^{aB}
	20	60.90 ± 1.23 ^{aA}	2.58 ± 0.09 ^{aA}	339.8 ± 10.2 ^{aB}
100	5	56.48 ± 1.36 ^{aAB}	2.67 ± 0.55 ^{aA}	207.9 ± 19.3 ^{aA}
	10	59.14 ± 1.41 ^{aA}	2.30 ± 0.46 ^{aA}	246.8 ± 32.2 ^{aA}
	20	60.15 ± 4.05 ^{aA}	2.59 ± 0.46 ^{aA}	198.2 ± 37.3 ^{aA}
200	0	59.66 ± 1.62 ^{aA}	2.82 ± 0.32 ^{aA}	387.8 ± 29.9 ^{bA}
	5	54.64 ± 0.72 ^{aA}	3.81 ± 0.39 ^{aB}	*
	10	56.52 ± 3.35 ^{aA}	3.45 ± 0.13 ^{aB}	192.8 ± 42.1 ^{aA}
	20	56.37 ± 3.80 ^{aA}	3.39 ± 0.32 ^{aA}	*
400	0	59.75 ± 1.71 ^{aA}	2.84 ± 0.43 ^{aA}	300.5 ± 60.9 ^{bA}
	5	59.92 ± 1.11 ^{aB}	3.37 ± 0.35 ^{aAB}	187.8 ± 18.8 ^{aA}
	10	57.18 ± 3.07 ^{aA}	3.46 ± 0.47 ^{aB}	203.3 ± 50.3 ^{aA}
	20	60.44 ± 2.25 ^{aA}	3.02 ± 0.45 ^{aA}	181.1 ± 25.2 ^{aA}

Results are expressed as the mean ± the standard deviation.
^{a} small different letters indicate significant differences ($p<0.05$) among reaction time of enzyme for the same pressure treatment; ^{A,B} capital different letters indicate significant differences ($p<0.05$) among the same pressure treatment for the reaction time of enzyme.
*200/5 and 200/20 samples had a problem that prevented the determination of acrylamide content.

Table 4 - Colour of fried potato sticks, expressed according to *L**, *a**, *b**, and ΔE parameters.

Pressure (MPa)	Total asparaginase reaction time (min)	*L**	*a**	*b**	ΔE
0.1	**0**	70.0 ± 2.3 ^{aA}	0.4 ± 0.3 ^{aAB}	35.8 ± 1.1 ^{aB}	
	5	73.5 ± 2.7 ^{aA}	1.2 ± 1.3 ^{aA}	34.5 ± 1.5 ^{aA}	
	10	72.6 ± 1.1 ^{aA}	0.3 ± 0.7 ^{aA}	35.8 ± 1.8 ^{aA}	
	20	72.3 ± 1.7 ^{aA}	0.5 ± 1.3 ^{aA}	34.5 ± 0.9 ^{aAB}	
100	**5**	68.8 ± 1.0 ^{aA}	2.2 ± 0.8 ^{aA}	37.4 ± 0.9 ^{aA}	5.7 ± 1.2 ^{aA}
	10	66.3 ± 3.7 ^{aA}	3.2 ± 1.3 ^{aA}	38.2 ± 1.0 ^{aA}	7.6 ± 3.2 ^{aA}
	20	68.4 ± 1.5 ^{aA}	2.3 ± 0.7 ^{aA}	38.0 ± 1.2 ^{aC}	5.6 ± 1.7 ^{aA}
200	**0**	69.1 ± 1.3 ^{aA}	1.1 ± 1.3 ^{aB}	37.6 ± 1.2 ^{aB}	2.7 ± 0.7 ^{aAB}
	5	70.5 ± 3.4 ^{aA}	0.8 ± 0.6 ^{aA}	35.7 ± 3.1 ^{aA}	4.9 ± 1.6 ^{aA}
	10	66.4 ± 3.8 ^{aA}	3.0 ± 1.7 ^{aA}	36.9 ± 1.6 ^{aA}	7.0 ± 4.2 ^{aA}
	20	68.8 ± 0.9 ^{aA}	1.5 ± 1.5 ^{aA}	37.1 ± 1.3 ^{aBC}	4.6 ± 1.7 ^{aA}
400	**0**	70.2 ± 1.1 ^{aA}	0.3 ± 1.48 ^{aAB}	35.9 ± 0.9 ^{aB}	1.7 ± 0.3 ^{aA}
	5	69.8 ± 1.5 ^{aA}	0.9 ± 0.2 ^{aA}	36.0 ± 1.5 ^{aA}	4.1± 1.7 ^{aA}
	10	71.3 ± 1.2 ^{aA}	0.7 ± 0.5 ^{aA}	35.3 ± 1.3 ^{aA}	1.9 ± 1.1 ^{aA}
	20	71.6 ± 1.9 ^{aA}	0.1 ± 0.5 ^{aA}	33.4 ± 1.1 ^{aA}	2.1 ± 1.1 ^{aA}

Results are expressed as the mean ± the standard deviation.
^{a} small different letters indicate significant differences ($p<0.05$) among reaction time of enzyme for the same pressure treatment; ^{A,B} capital different letters indicate significant differences ($p<0.05$) among the pressure treatment for the same reaction time of enzyme.

Highlights

- HPP induces changes on potato texture, increasing softness
- The potato cutting process/energy needed can be improved/reduced by high pressure
- Asparaginase/HPP pre-processing effectively reduced acrylamide content in fried potatoes
- Acrylamide reduction by asparaginase/HPP is faster than by other common or emergent methodologies

Industrial relevance

Industrial relevance text: HPP is a non-thermal technology that may be used as a pre-treatment for the production of fried potatoes with different/better textural and nutritional properties, as well as to reduce energetic costs of some industrial steps of the production of fried potatoes (for instance, the cutting process and frying time). Also, a combined pre-treatment with HPP and asparaginase may be used as a strategy of acrylamide mitigation in fried potatoes.