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ESTUDO DO EFEITO DO PROCESSAMENTO POR ALTA PRESSÃO NO PROCESSO DE FRITURA DA BATATA

STUDY OF THE EFFECT OF HIGH PRESSURE PROCESSING ON THE POTATO FRYING PROCESS

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biotecnologia, ramo Biotecnologia Alimentar, realizada sob a orientação científica do Doutor Jorge Manuel Alexandra Saraiva, Investigador Auxiliar do Departamento de Química da Universidade de Aveiro

Dedico este trabalho aos meus pais, irmãos e Ivan pelo apoio incondicional ao longo de todos estes anos

o júri

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palavras-chave

Batata, processamento por alta pressão, asparaginase, fritura, acrilamida, tubérculo de batata, batata frita

resumo

A fritura é a técnica mais usada para o processamento de batatas antes do seu consumo, e apesar de algumas das alterações texturais, nutricionais e sensoriais resultantes serem desejáveis, outras são prejudiciais à saúde, nomeadamente os elevados níveis de acrilamida (composto carcinogénico) e de gordura. Assim, o maior desafio na produção de batatas fritas consiste na redução da formação das características não desejáveis, não comprometendo as qualidades sensoriais. A tecnologia de processamento por alta pressão (do inglês *High Pressure Processing*, HPP) tem sido usada para acelerar processos de infusão, bem como alterar o amido, enzimas, textura e processos fisiológicos das batatas. Portanto, pretendeu-se com este trabalho, avaliar a potencialidade do HPP na modificação das propriedades físico-químicas dos tubérculos de batata e das propriedades sensoriais, nutricionais e físico-químicas das respetivas batatas fritas, bem como na infusão de asparaginase em palitos de batata, e consequente redução dos níveis de acrilamida em batatas fritas.

Os tubérculos de batata inteiros (descascados e não descascados, embalados em água ou em vácuo) processados a 100-500 MPa por 2.5 min exibiram um decréscimo até 42% na sua firmeza, e um aumento de até ~12 vezes da libertação de líquido para o exterior, devido às alterações texturais e na permeabilidade celular induzidas pelo HPP. Palitos de batata processados por HPP (200-600 MPa, 2.5 e 10 min) ou infundidos com asparaginase por HPP (100-400 MPa, 5 min) apresentaram reduções de até 35% na firmeza, 38% na rigidez, e 47% na energia necessária para o corte; a rugosidade à superfície e o conteúdo em água também diminuíram, e a concentração de açúcares/sólidos solúveis na água envolvente aumentou. Além disso, o HPP induziu a gelatinização do amido, seguida de retrogradação a 600 MPa.

Devido às alterações induzidas pelo HPP nas batatas cruas, as respetivas batatas fritas apresentaram uma maior perda de peso após a fritura, uma ligeira diminuição no conteúdo em água, um aumento na sua rigidez à superfície (crocância), e uma cor mais clara. O teor em lípidos, perfil lipídico, e a concentração de acrilamida não foram afetados pelo HPP. Porém, quando a asparaginase foi adicionada, os níveis de acrilamida nas batatas pré-tratadas a 100-400 MPa reduziram-se em 26-47%, enquanto só com asparaginase (sem HPP) não se verificou redução. Assim, o tratamento combinado entre o HPP e a asparaginase poderia ser uma nova estratégia a ser aplicada na redução dos níveis de acrilamida em batatas fritas.

Em suma, o HPP afetou a qualidade dos tubérculos de batata ao nível textural, físico e químico, e foi eficiente na infusão de asparaginase nos palitos de batata, e por isso, poderia ser usado não só como pré-tratamento para a produção de batatas fritas com diferentes/melhoradas propriedades sensoriais, texturais e nutricionais, mas também para melhorar energeticamente alguns processos industriais (por exemplo, o corte e o tempo de fritura).

keywords

Potato, high pressure processing, asparaginase, frying, acrylamide, potato tuber, fried potatoes

abstract

The frying process is the most used technique in potato processing before consumption. Some of the textural, nutritional, and sensory changes induced by frying are desirable, but others are harmful to human health. Thus, the main challenge in potato frying is to reduce the formation of the undesirable characteristics, without compromising the sensorial attributes. High Pressure Processing (HPP) technology has been used to accelerate infusion processes, as well as to modify the potato starch, enzymes, texture and physiological processes, and thereby this work aimed to evaluate the potentiality of HPP on modification of physico-chemical properties of raw potato tubers, and of sensory, nutritional and physico-chemical properties of the respective fried potatoes, as well as on asparaginase infusion into raw potato sticks, and consequently on reduction of acrylamide levels in fried potatoes.

Whole potato tubers (peeled and unpeeled, packaged in water or under vacuum) subjected to 100-500 MPa for 2.5 min exhibited a decrease of up to 42% in their firmness, and an increase of up to ~12-fold in water exudation of peeled potatoes, due to the changes on texture and cell permeability caused by HPP. Raw potato sticks treated by HPP (200-600 MPa, 2.5 and 10 min) or infused with asparaginase by HPP (100-400 MPa, 5 min) exhibited reductions in firmness (up to 35%), stiffness (up to 38%), and energy for cutting (up to 47%); the roughness of potato surface was also reduced and moisture content slightly reduced; and the concentration of soluble solids/sugars in the exterior water increased. Also, HPP induced starch gelatinization, followed by starch retrogradation at 600 MPa. Due to changes induced by HPP on raw potatoes, HPP pre-treated fried potatoes exhibited higher weight loss after frying, slight lower moisture content, higher hardness (crispness), and a lighter colour. Lipid content, lipid profile and acrylamide levels were quality parameters that were not affected by HPP. When asparaginase was added, acrylamide levels of fried potatoes pre-treated at 100-400 MPa reduced of 26-47%, while with asparaginase alone (without HPP) there was no reduction. Thus, this combined asparaginase and HPP treatment could be a novel strategy for acrylamide mitigation in fried potatoes.

In sum, HPP showed to affect the quality of potato tubers at the textural, physical and chemical level, and was efficient on the asparaginase infusion into raw potato sticks. Therefore, it may be used as a pre-treatment for the production of fried potatoes with different/better sensorial, textural and nutritional properties, as well as to improve energetically some industrial processes (for instance, the cutting process and frying time).

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List of abbreviations

Abbreviation	Designation
APCI	Atmospheric pressure chemical ionization
Asn	Asparagine
a_w	Water activity
CE	Capillary electrophoresis
Da	Dalton
DNS	3,5 – dinitrosalicylic acid
DSC	Differential Scanning Calorimetry
DW	Dry weight
EFSA	European Food Safety Agency
ELISA	Enzyme-Linked Immunosorbent Assay
EPA	Environmental Protection Agency
ESI	Electrospray ionization
FW	Fresh weight
FDA	Food and Drug Administration
FA	Fatty Acids
GRAS	Generally recognized as safe
GC	Gas chromatography
HMF	Hydroxymethylfurfural
HPLC	High performance liquid chromatography
HPP	High pressure processing
HP	High pressure
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC	Liquid chromatography
MS	Mass spectrometry
MSPD	Matrix solid-phase dispersion
MUFA	Monounsaturated Fatty Acids
NIR	Near infrared
NEODA	National Edible Oil Distributors Association
PA/PE	Polyamide/ Polyethylene
PEF	Pulsed electric field
PPO	Polyphenol oxidase
POD	Peroxidase
PUFA	Polyunsaturated Fatty Acids
SAPP	Sodium Acid Pyrophosphate
SEM	Scanning electron microscopy
SIM	Selected ion monitoring
SPE	Solid phase extraction
SPME	Solid phase microextraction
U.S.	United States
USDA	United States Department of Agriculture
w.b.	Weight basis

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Contextualization and thesis structure

This thesis is composed of five chapters, wherein the first one comprises a literature review regarding the subject of potato tubers and potato cooking, with focus on frying process and the problems associated with fried products, as well as the effect of non-thermal technologies, emphasizing high pressure processing (HPP), on potato tuber and fried potato quality, followed by the work objectives. Then, the second chapter describes in detail the materials and methods used, and the third chapter comprises the presentation and discussion of the results obtained. The main conclusions of this thesis are provided on the fourth chapter, followed by the proposed future work on the fifth chapter, and the list of the consulted literature in the aim of the present work. Finally, an appendix section is also included, concerning data that, due to its extension, could not be presented on the corresponding chapters.

Chapter I – Literature Review

This section comprises an extensive, briefly compiled literature review regarding the subjects of high pressure processing as a technology affecting potato tubers, frying process, and problems underlying fried products

(this chapter of the thesis was partially adapted from a paper already published, **Dourado et al. (2019)**)

1. Potatoes

The potato is a tuberous crop produced by a perennial herb (potato plant), which belongs to the family *Solanaceae*, species *Solanum tuberosum* L. **(Singh and Kaur, 2016)**. The potato is one of the most important crops for human consumption and it is original from the Andean regions of South America **(O'Connor et al., 2001).** Nowadays, there are more than four thousand and five hundred (4500) varieties of potatoes growing worldwide (differing in texture, flavour, shape and colour) being cultivated in more than one hundred and sixty (160) countries. The global total production of potato exceeds three hundred and seventy four (374) million tons per year and this tuber is consumed by more than a billion people worldwide **(Camire et al., 2009; Cipotato, 2018; Singh and Kaur, 2016)**.

2. Potato tuber morphology and composition

The potato tubers are essentially underground stem. Morphologically, they have several differences in size, shape and flesh/skin colour, depending on the genetics of the cultivar. Nevertheless, the potato tubers are usually oval to round in shape, with pale brown skin and white flesh **(Singh and Kaur, 2016)**. Their outer skin is formed by dead cells that do not contain starch or protein grains, and underlying the skin is the cortex composed by cells with multiple oval-shaped starch granules stored as a reserve material. The middle lamella, the outer layer of the cell wall, is composed by pectic material that cements cells together and is dissolved during heating **(Miranda and Aguilera, 2006)**. In the central zone of the potato tuber is the pith (also called medulla or water core), composed of smaller cells with lower starch content **(Troncoso et al., 2009)**. Due to these microstructural features and the histological variability, the potato tuber is an anisotropic material, being an important factor when determining the textural properties of finished products **(Miranda and Aguilera, 2006)**.

Potatoes are considered an essential and safe source of energy and dietary fibre, whose nutritional characteristics (such as starch digestibility and glycaemic index) are important in human health **(Camire et al., 2009).** Their nutritional composition vary with potato varieties, soil type, crop practices, location, weather conditions and postharvest storage conditions **(Miranda and Aguilera, 2006)**. **Table 1** shows the composition variability of raw potatoes expressed on fresh weight (FW) basis.

Table 1 - Nutritional composition of raw potato tubers and French fries produced by McDonald's company, per 100g of edible portion. Values taken from **USDA and ARS (2018)** and **USDA (2018)**, respectively.

Nutrient	Raw potato tuber (value per 100g)	French fries (value per 100g)
Water (g)	79.25	36.63
Carbohydrates (g)	17.46	42.58
Sugars, total (g)	0.82	3.41
Fibre, total dietary (g)	2.1	3.9
Protein (g)	2.05	3.41
Lipid (g)	0.09	15.47
Sugars, total (g)	0.82	0.21
Minerals		
Potassium (mg)	425	596
Phosphorus (mg)	57	127
Magnesium (mg)	23	37
Calcium (mg)	12	19
Iron (mg)	0.81	0.80
Sodium (mg)	6	189
Zinc (mg)	0.30	0.51
Vitamins		
Vitamin C, total ascorbic acid (mg)	19.7	5.6
Thiamin (mg)	0.081	0.180
Riboflavin (mg)	0.032	0.037
Niacin (mg)	1.061	3.220
Vitamin B-6 (mg)	0.298	0.380
Folate (µg)	15	-
Vitamin A (IU)	2	0
Vitamin E (α-tocopherol)	0.01	1.38
Vitamin K (µg)	2.0	16.0
Lipids		
Fatty acids, total saturated (g)	0.025	2.271
Fatty acids, total monounsaturated (g)	0.002	7.379
Fatty acids, total polyunsaturated (g)	0.042	4.727
Fatty acids, total trans (g)	0.000	0.064

Generally in a potato tuber about 1-2% is dietary fibre, being supplied especially by the thickened cell walls of the peel, 20% is dry matter and the remaining fraction is water **(Singh and Kaur, 2016)**. The water is contained in different compartments inside the potato cells, being 84% in the vacuoles, the nucleus and the cytoplasm, 13% inside starch granules and 3% in the cell wall **(Rutledge et al., 1994)**. Starch is the major component of the dry matter, accounting for approximately 70% of the total solids, and consists of amylose and amylopectin **(Singh and Kaur, 2016)**. Thus, potatoes are regarded as an excellent source of carbohydrates. In addition, they have very low fat content and supply protein of high relative biological value (90-100). Regarding the free amino acids, asparagine (Asn) is an important amino acid for plant growth, playing a central role in nitrogen storage and transport **(Lea et al., 2006)**, and is the most abundant

free amino acid in potatoes, usually accounting for 0.2-4% for dry matter and 20-60% of total free amino acids **(Food Drink Europe, 2013)**. Lastly, potatoes are also composed by several micronutrients, namely essential minerals like potassium, phosphorus, calcium and magnesium, and bioactive compounds, such as vitamins C (the major vitamin in raw potato), E and B (folic acid, niacin, pyridoxine, riboflavin and thiamine), carotenoids and phenolic compounds (phenolic acids, flavonoids, anthocyanins) **(Camire et al., 2009; Singh and Kaur, 2016)**.

3. Potato cooking

Before consumption, potatoes need to be processed mainly due to the indigestibility of their ungelatinized starch, which has very low digestibility in the raw state since potato starch granules have a β-crystalline structure that is resistant to amylase digestion. Indeed, potato cooking greatly improves the digestibility of potato starch due to the conversion of the natural resistant starch into highly digestible starch (**Figure 1**) **(García-Alonso and Goñi, 2000)**.

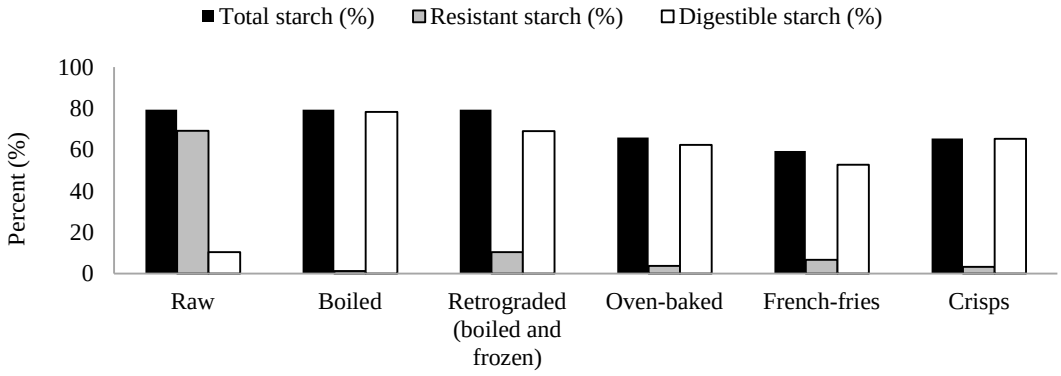

Figure 1 - Impact of processing on total, resistant and digestible starch in potatoes. Values taken from **García-Alonso and Goñi (2000)**.

The most popular cooking methods include boiling, baking, toasting, roasting, frying and microwaving **(Decker and Ferruzzi, 2013; García-Alonso and Goñi, 2000)**. Considering all the products resulted from the potato cooking (**Figure 2)**, the most important one refers to French fries, with an annual consumption of approximately 7 million metric ton worldwide, and the second one refers to potato chips **(Miranda and Aguilera, 2006; Mordor Intelligence, 2016; National Potato Council, 2018)**. The manufacture and characteristics of these potato products will be discussed later.

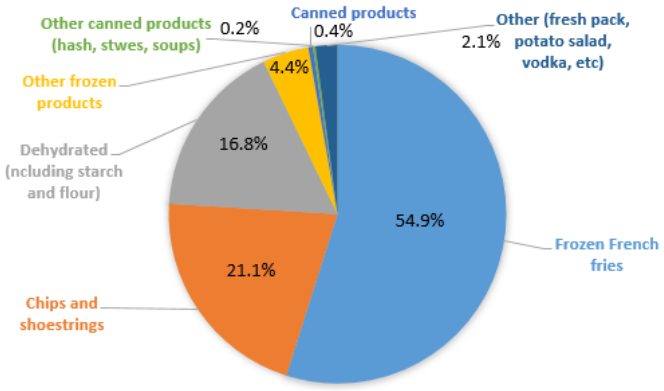

Figure 2 - Different products and applications of processed potatoes. Values taken from **National Potato Council (2018)**.

3.1. Frying process

Frying is a process used worldwide, both industrially and at home, characterized by an operational simplicity and speed. It also creates unique sensorial qualities in foods, namely unique flavour and textures **(Gertz, 2014)**, and for these reasons, frying is the most common technique used in potato processing **(Miranda and Aguilera, 2006)**. Deep-fat or immersion frying is extensively used in food processing, being defined as a cooking food operation that involves the immersion of food pieces in edible oil or fat at a temperature above the boiling point of water. Fried potato products are one of the largest applications of deep-fat frying **(Hubbard and Farkas, 2000)**.

Frying is mainly a drying procedure based on heat transfer by convection from the surrounding oil of the surface and afterwards, by conduction within the potato core (interior). In addition, mass transfer also takes place resulting in water removal and oil uptake by the potato strips **(Aguilera and Gloria-Hernandez, 2000)**. As a result, several physical, structural, chemical and nutritional changes are induced by the potato frying process **(Miranda and Aguilera, 2006)**, which are represented schematically in **Figure 3.**

3.1.1 Physical and structural changes induced by frying

During frying, the crust and the core of fried products suffer different changes at microstructural level. When the potato pieces are placed into the hot oil (160-180 ºC), the temperature of the surface layers rises rapidly, the surface water boils and evaporates,

starch granules undergo gelatinization and the tissues quickly dehydrate. Consequently, the surface porosity increases, as well as the shrinkage and roughness **(Aguilera et al., 2001; Arslan et al., 2018; Bouchon and Aguilera, 2001; Gertz, 2014)**. As frying proceeds, the dehydrated crust develops and increases, a temperature gradient is formed in the interior (not exceeding 100 ºC), as a result of heat transfer by conduction from the crust into the core, and the core is cooked. More specifically, starch granules inside cells undergo hydration by the water surrounding them, causing the starch swelling **(Aguilera et al., 2001)**, and the middle lamellae between cells becomes softened and disintegrates, resulting in the so-called mealy texture **(Bouchon and Aguilera, 2001)**.

3.1.2 Chemical and nutritional changes induced by frying

During frying, food products suffer changes in their surface colour as well as in their aromatization. At higher temperatures, the Maillard reaction between amino acids (or free amino groups of proteins and peptides) and reducing sugars occurs, which is responsible either for the colouring of fried products, changing their colour to golden yellow and later to brown, or for their aromatization due to the formation of volatile compounds as secondary products **(Miranda and Aguilera, 2006; Moreira et al., 1999)**. In addition, some food components are lost by evaporation, leaching or degradation, such as ascorbic acid and total flavonoids, while new compounds are formed, both highly pleasant ones (as the typical fried volatile flavours), and unhealthy ones (as degraded lipids and toxic compounds formed during Maillard reaction, namely acrylamide, hydroxymethylfurfural (HMF), furan, heterocyclic amines, and polycyclic aromatic hydrocarbons) **(Anese et al., 2013; Balagiannis et al., 2019; Camire et al., 2009; Pedreschi et al., 2008; Qi et al., 2018)**. The loss of healthy compounds and the formation of unhealthy ones, which can be absorbed by the product, lead to a reduction of the nutritional value of fried foods **(Ziaiifar et al., 2008)**. Furthermore, prolonged use of oil at high temperature and in the presence of air leads to several reactions such as hydrolysis, oxidation and polymerization, resulting in the formation of volatile and non-volatile compounds within the oil, and some of which have been reported to pose risks to health **(Miranda and Aguilera, 2006)**. The undesirable volatile products formed include peroxides, hydroperoxides, aldehydes, ketones and carboxylic acids. The undesirable non-volatile compounds include free fatty acids, di- and monoglycerides formed by the hydrolysis of oil **(Yee and Bussell, 2007)**. Moreover, when starch is gelatinized, it can

interact with polar and non-polar compounds, like fatty acids. The hydrocarbon portion of the lipid locates within the helical cavity of amylose, forming helical inclusion complexes **(De Pilli et al., 2008)**, which modify some properties of starch, decreasing its solubility in water, retarding retrogradation and reducing the viscosity of gelatinized starch **(Meng et al., 2014)**.

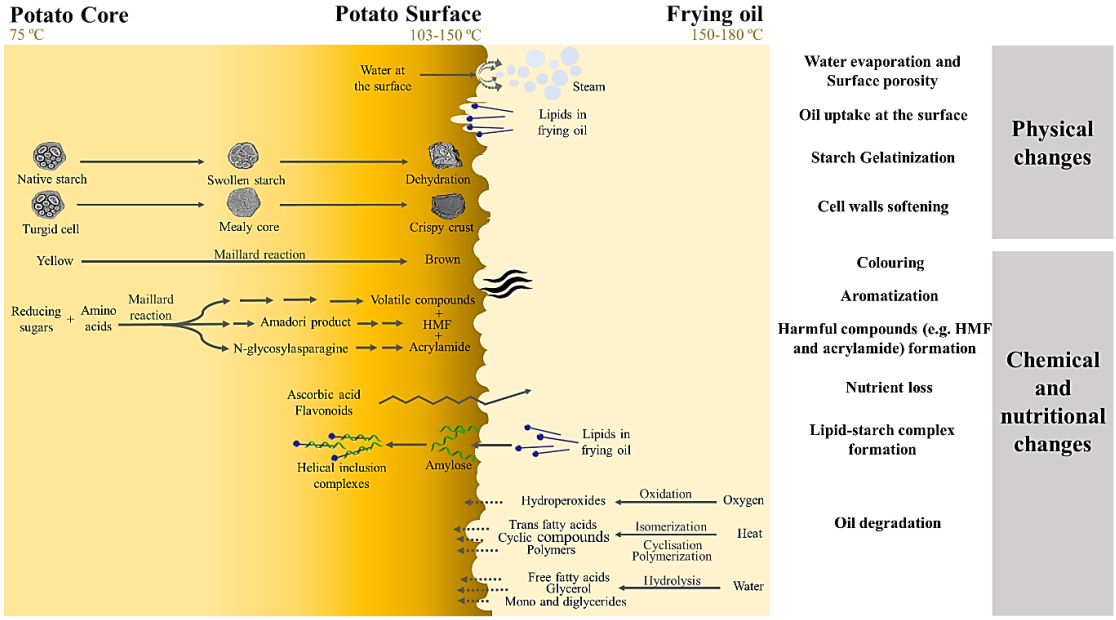

Figure 3 - Physical, chemical and nutritional changes induced by potato frying. Produced and published in **Dourado et al. (2019)**.

3.1.3 Oil uptake during frying process

Aguilera and Gloria (1997) have reported that oil absorption is principally a surface phenomenon and French fries have almost six times as much oil in the crust that in the inner part. In addition, **Bouchon et al. (2001)** have studied the oil distribution in fried potato cylinders of 1 cm diameter and reported that the maximum penetration of oil in the crust was about 300 μm. This result reflected that the structure developed during deep-fat frying had an anisotropic nature.

During deep-fat frying, the water present in fried potatoes is converted into steam. The generated steam escapes from deformities, channels and open capillaries of the cellular structure, a positive water vapour pressure is created and consequently, oil adheres to fried potatoes and flows toward the large cavities **(Rice and Gamble, 1989)**. However, this mechanism does not fully explain the oil uptake process **(Arslan et al.,**

2018). Several studies have noted that a small amount of oil is absorbed during frying, and oil absorption phenomenon mainly occurs at the end of frying and on cooling, involving an equilibrium between adhesion and drainage of oil after the removal of the fried products from the fryer **(Gamble et al., 1987; Ufheil and Escher, 1996; Ziaiifar et al., 2008)**. When the chips or fries are removed from the frying medium, their temperature immediately starts to decrease. Below 100 ºC, water vapour condenses and the internal pressure drops on the surface, resulting in the creation of a positive pressure vacuum, which favours the oil present on the surface to be absorbed **(Miranda and Aguilera, 2006)**. The decrease of the oil absorption during the cooling phase could be achieved by using superheated steam or hot air to keep the high temperature and to avoid instant cooling, and vacuum or absorbent paper to remove the surface oil **(Arslan et al., 2018)**. However, due to the complexity of oil uptake phenomenon, it is affected by many other factors, as shown in **Table 2**.

3.1.4 Post-Frying and loss of texture quality

Miranda and Aguilera (2006) reviewed the several post-frying changes that occur in the texture of French fries and the authors have reported that the highest quality of fried products is achieved rapidly after removal from the fryer and drainage of the excess oil (a maximum of 8-10 minutes after frying). During the post-frying period, the development of limpness (also known as "sagging" or "drooping") is a phenomenon that reduce textural quality of French fries **(Miranda and Aguilera, 2006)**, and it is correlated with moisture uptake and in some cases with starch retrogradation during cooling after removal from the oil, a process in which amylose and amylopectin molecules of gelatinized starch reassociate, increasing the hardness of fried potatoes **(Goñi et al., 1997)**.

In French fries, although the crust is below the equilibrium moisture content with the relative humidity of the surrounding air, the main source of moisture transfer to the dry crust is the moist core. Thus, French fries get soggy within minutes after preparation and thereby they are supposed to remain hot for immediate food service **(Weaver and Huxsoll, 1970).**

Table 2 - Factors affecting oil uptake before, during and after frying.

Factors that affect oil uptake		Effects in oil uptake	Cause/ explanation	Reference
Product composition	Higher initial water content	Increasing	-	**(Ufheil and Escher, 1996)**
	Higher potato density	Reduction	-	
Surface area	Surface roughness	Increasing	Surface roughness increases overall surface area and the surface porosity, increasing the amount of oil absorbed.	**(Paul et al., 1997)**
	Food dimensions	Increasing	Reduced product thickness and increased product surface result in increasing of oil uptake.	
Pre-processing	Blanching	Reduction	Migration of water-soluble components from the product to the blanching water, decreasing dry-mass content; surface starch gelatinization, forming a protective thin layer.	**(Pedreschi and Moyano, 2005)**
	Drying	Reduction	Creation of a firm and dried layer around the product, decreasing the water content; shrinkage during drying reduces total surface area, reducing mass transfer.	
	Osmotic dehydration	Reduction	Osmotic dehydration reduces initial water content, decreasing oil uptake.	
Frying temperature and time	Higher temperature and shorter frying time	Reduction	Formation of a better developed crust, which would act as a barrier for oil absorption; reduction of porosity of the crust.	**(Mehta and Swinburn, 2001)**
Dehydration	Higher amounts of water removed from the surface	Increasing	Dehydration occurs at above 100 ºC, water is lost, increasing surface porosity and thus the oil uptake.	**(Ziaiifar et al., 2008)**
Post-frying conditions	Shaking the frying basket	Reduction	Draining the surface oil; oil penetration into the pores is limited.	**(Thanatuksorn et al., 2005; Topin and Tadrist, 1997)**
	Post-frying drying	Reduction	Reducing the contact time between the oil and the product.	

4. Acrylamide formation

In early 2002, Swedish researchers presented preliminary findings of acrylamide in a range of foods heated during production or preparation. Moderate levels of acrylamide (5-50 μg/Kg) were measured in heated 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: (i) acrylamide is present in high concentrations in several highly popular and of high consumption foods; (ii) neurotoxicity of acrylamide as result of high dietary exposure and cumulative effects **(LoPachin, 2004)** (iii) 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 implication in cancer in rats **(Food et al., 2002; IARC, 1994)**; (iv) high doses of this compound also have effects on neurological and reproductive systems **(Friedman, 2003).** For these reasons, it is important to reduce the contaminant levels in products that contain high level of acrylamide **(Pedreschi et al., 2008)**.

4.1. Formation mechanism in foods

It has been stated that acrylamide is primarily formed as a by-product of Maillard reaction, a complex series of non-enzymatic reactions which are responsible for the brown colour and tasty flavour of baked, fried, toasted and roasted starchy products **(Novozymes, 2017; Pedreschi et al., 2008)**. More specifically, acrylamide results mainly from the reaction 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 and Aguilera, 2006; Novozymes, 2017; Pedreschi et al., 2008)**. In French fries and chips, acrylamide formation occurs predominantly at the surface, where the (oil) temperature is high and the moisture content low **(Parker et al., 2012)**.

Zyzak et al. (2003) presented a possible mechanism for the formation of acrylamide from the reaction of the amino acid asparagine and a carbonyl-containing compound (preferably an α-hydroxycarbonyl) at typical cooking temperatures (**Figure 4**). The mechanism involves formation of the N-glycosylasparagine and a de-carboxylated Schiff base (after dehydration under high temperatures). The de-carboxylated Schiff base may lead directly to acrylamide and an imine or be followed by

hydrolysis to 3-aminopropamide (3-APA) and carbonyl compounds. Subsequent elimination of ammonia from 3-APA under heat can yield acrylamide. The confirmation of this mechanism was accomplished through selective removal of asparagine using asparaginase that results in a reduced level of acrylamide in a selected heated food.

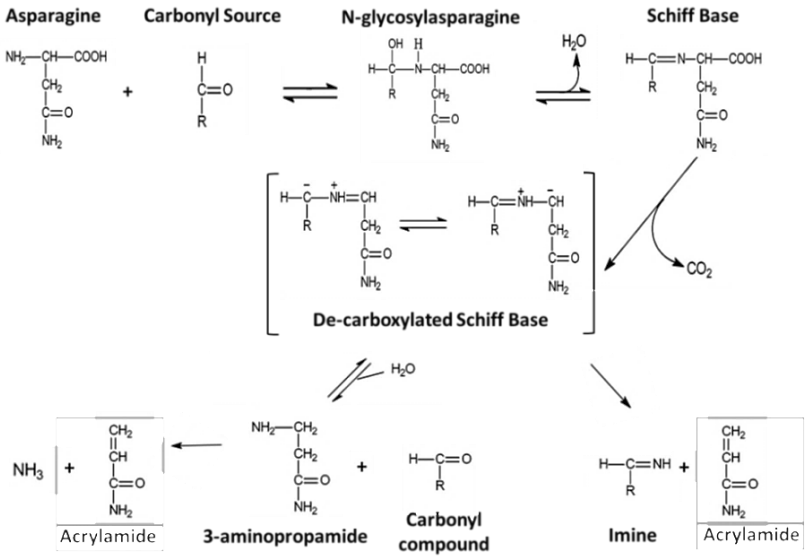

Figure 4 - Proposed mechanism for acrylamide formation in heated foods. Adapted from **Zyzak et al. (2003)**.

4.2. Occurrence in foods and Dietary Exposure

Powers et al. (2013) have performed a statistical analysis of a large dataset of manufacturers' measurements of acrylamide levels and they reported a decrease of about 53% (from 763 ng.g^{-1} in 2002 to 358 ng.g^{-1} in 2011) in potato crisp. In addition, the proportion of samples containing acrylamide levels above 1000 ng.g^{-1} reduced from 23.8% in 2002 to 3.2% in 2011. Despite this, a wide range of food (prepared industrially, in catering or at home) still contains high levels of acrylamide. Fried potato products (French fries and potato crisps), bread and bakery products, coffee and breakfast cereals are the food commodities that contribute the most (about 90%) to dietary acrylamide exposure. Nevertheless, acrylamide has not been detected in unheated or boiled foods (< 5-50 μg/Kg) and therefore it was considered to be formed during heating at high temperatures **(Capuano and Fogliano, 2011; Powers et al., 2013)**.

Boon et al. (2005) have reported a great variability in acrylamide levels between different products of each food category, as well as between different brands of the same

product. The main reasons are the difference in the concentration of asparagine and reducing sugars (acrylamide precursors) in raw materials, and the difference in food composition and in process conditions applied. In addition, a great variability of dietary acrylamide intake between populations has been found according to population's eating habits and the way the foods are processed and prepared. In 2011, the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) estimated the mean dietary acrylamide intake for the general population (including children) to be between 1 and 4 μg/Kg of body weight/day, while according to FDA, acrylamide intake should be around 0.4 μg/Kg of body weight/day **(JEFCA et al., 2011)**. Furthermore, it was noted that children have dietary acrylamide exposures at least twice as high as adult consumers, probably because of their higher caloric intake relative to the body weight and their higher consumption of certain acrylamide-rich foods, such as French fries and potato crisps **(Capuano and Fogliano, 2011)**.

4.3. Health risks and risk assessment

Acrylamide contain an electrophilic double bond, which can react with nucleophilic groups. Covalently interactions with cellular nucleophiles, such as sulfydryl groups in reduced glutathione and in proteins, occur *in vivo*. It has been postulated that acrylamide is carcinogenic through a genotoxic pathway, since it leads to gene mutations and changes in chromosomes**.** Although the reactivity of the double bond of acrylamide, the epoxide group of glycidamide is generally more reactive. Glycidamide is formed by the cytochrome P450-catalyzed epoxidation of acrylamide (**Figure 5**), is 100-1000 times more reactive with DNA than acrylamide and leads to gene mutations and changes in chromosomes. For these reasons, this compound is considered the major carcinogenic factor related to the excessive ingestion of acrylamide **(Friedman, 2003; Granath et al., 2001; Klaunig, 2008)**.

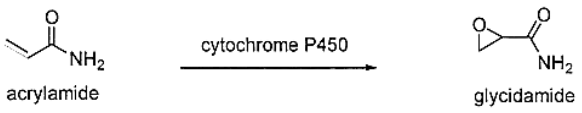

Figure 5 - Formation of the reactive epoxide glycidamide, through the reaction of acrylamide and cytochrome P450. Adapted from **Friedman (2003)**.

4.4. Factors affecting acrylamide formation in fried potato products and possible mitigation strategies

Potato products are strongly susceptible to acrylamide formation due to asparagine and reducing sugars content, as well as the traditional applied baking conditions (temperatures > 120 ºC), like frying and roasting, which favour the Maillard reaction and consequently the acrylamide formation (**Figure 4**). Thus, the potential strategies to prevent acrylamide formation may be covered in two major approaches: removing the acrylamide precursors (glucose, fructose and asparagine) or interfering with the Maillard reaction **(Singh and Kaur, 2016)**.

Biedermann-Brem et al. (2003) have reported that potatoes, which may be used for roasting and frying, should contain between 0.2 and 1.0 g/Kg fresh weight of reducing sugars, because below 0.2 g/Kg there is production of insufficient browning and flavour, and above 1.0 g/Kg roasted and fried products contain more than 500 μg/Kg acrylamide. However, the Maillard reaction is essential for the desired and characteristic flavour and colour formation of potato products and thereby, the major challenge is reducing acrylamide formation while not affecting the qualities that are demanded by consumers. For these reasons, an understanding of the kinetics of the reactions which lead to acrylamide formation has considerable importance in the development of such strategies. **Parker et al. (2012)** have presented a mathematical model that, on basis of the composition of the par-fried potato strips, can predict accurately the acrylamide concentration in French fries after frying for a certain time at a particular temperature. Implicit in the model is that the fructose/glucose ratio is one parameter that has a strong effect on acrylamide formation, as well as the asparagine/total free amino acids ratio, and thus is important to change the ratio either by enzymatic modification or crop selection.

4.4.1 Factors affecting acrylamide formation during pre-frying process

Beyond the concerns about the farming and storage conditions, aspects related with the pre-frying process are also important, namely the following over.

4.4.1.1 Potato storage

Although asparagine contents appear not be susceptible to various storage temperatures and long storage time **(De Wilde et al., 2005)**, certain storage conditions can lead to starch-to-sugar conversion and cause sugar accumulation mainly due to senescence sweetening and cold temperatures **(Singh and Kaur, 2016)**. On one hand, senescence sweetening results from an enzymatic process that occurs more rapidly at higher storage temperatures (> 8 ºC) and is related to the start of sprout growth **(Amrein et al., 2003)**. On the other hand, cold temperatures (< 8 ºC) lead to a quick accumulation of reducing sugars in stored potato tubers (cold sweetening). Therefore, ideally potato tubers should be stored at 8-10 ºC, since reducing sugar content is not significantly influenced and although sprouting would occur at this temperature, it can be controlled using sprout suppressants such as chloropham **(De Wilde et al., 2005, 2006; Halford et al., 2012)**. Furthermore, the storage place should be as dark as possible and the relative humidity should be 60% **(Yee and Bussell, 2007)**.

4.4.1.2 Size and cut shape of the raw materials

Acrylamide is formed in the surface layer of the potato product, so thinner and smaller cut sizes result in increased acrylamide formation upon final frying **(Matthäus et al., 2004)**.

4.4.1.3 Treatments prior to frying and use of additives or processing aids

Blanching is an important operation in the industrial process of French fry production since during this step enzymes are inactivated, a layer of gelatinized starch is formed, limiting oil absorption and improving texture, and reducing sugars are leached out, resulting in lower acrylamide contents in the final product **(Moreira et al., 1999; Pedreschi et al., 2004, 2009; Pedreschi and Zuñiga, 2009)**. However, **Mestdagh et al. (2008)** have noted that blanching conditions (time and temperature) should be manipulated to achieve an optimized reducing sugars extraction, to avoid textural and nutrient losses in extreme blanching conditions.

In addition, several papers in the literature describe some food additives to influence acrylamide formation in potato products (**Table 3**), namely:

I. Sodium acid pyrophosphate (SAPP, E450) is added (pH level ≈ 4.7) to reduce the darkening of the blanched potato strips (caused by air exposure or by the ferridichlorogenic acid complex formation during cooking) **(Singh and Kaur, 2016)**;

II. Dextrose (glucose) contributes to a uniform and standardized colour of the final product, according to customer demands. Although in North America, the use of colour additives such as caramel and annatto instead of dextrose is permitted, in Europe this is still restricted for potato processing **(Singh and Kaur, 2016)**;

III. Organic acids are known for their mitigating effect due to the protonation of asparagine amino groups at low pH, blocking the nucleophilic addition of asparagine with a carbonyl compound. Thus, the formation of the corresponding Schiff base is prevented, as well as the Maillard reaction and consequently the formation of acrylamide **(Jung et al., 2003; Mestdagh et al., 2008d; Pedreschi et al., 2004; Pedreschi and Zuñiga, 2009b)**. Unfortunately, this type of treatments may also have an impact on the sensorial product quality resulting in sour product tastes, since low pH also suppresses the Maillard reaction, responsible for the generation of desirable flavours and colours. According to **Kita et al. (2004)** this effect however depends upon the type and concentration of the acid used, suggesting that acetic acid would be a better acidulant compared to citric acid, due to the less appearing sourness;

IV. Mono- and divalent cations (such as Na^+ and Ca^{2+}) can interact with asparagine, preventing the formation of the Schiff base **(Gökmen and Şenyuva, 2007a; Mestdagh et al., 2008a; Pedreschi and Zuñiga, 2009b)**;

V. NaCl has also been proposed to accelerate acrylamide elimination via polymerization in a model food matrix **(Kukurová et al., 2009)**;

VI. The presence of free amino acids (not asparagine), such as glycine, cysteine and lysine, have been suggested to decrease acrylamide formation, either by promoting competitive reactions or by covalently binding the forming acrylamide, resulting in adduct formation **(Friedman, 2003; Mestdagh et al., 2008a)**;

VII. Antioxidants have been reported to influence the Maillard reaction and may have various effects. Although their mechanism of action is not yet fully

understood, it is known that some antioxidants (e.g., chlorogenic acid) may facilitate sucrose degradation, increasing the reducing sugar contents and consequently the formation of acrylamide; others (e.g., epicatechin) may trap Maillard intermediates; and others may react with acrylamide too **(Jin et al., 2013)**;

VIII. Lactic acid fermentation with *Lactobacillus plantarum* before deep-frying has reduced acrylamide formation in fried potato products due to the rapid decay of the levels of glucose, fructose and some amino acids involved in Maillard reactions (specially alanine, arginine, phenylalanine and serine) during the fermentation and due to an acidifying effect caused by lactic acid production **(Baardseth et al., 2006)**;

IX. 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) by hydrolysing the amine group in the side chain of asparagine **(Capuano and Fogliano, 2011)**. The majority of asparaginases are quite specific for asparagine, but some enzymes also have a low activity towards glutamine **(Krasotkina et al., 2004)**. Commercially there are two asparaginase products currently available for acrylamide mitigation in the food industry. These are PreventASe^{TM} from DSM (Heerlen, The Netherlands) and Acrylaway® from Novozymes A/S (Bagsvaerd, Denmark), and both have shown high asparagine specificity and minimum activity towards glutamine and other amino acids **(Novozymes, 2017; Xu et al., 2016)**. These enzymes are produced by specific fungal strains of *Aspergillus niger* and *Aspergillus oryzae*, respectively, fungi that have been widely used in commercial products and have been proved to be safe by Joint FAO/WHO Expert Committee on Food Additives **(JECFA, 2010)**. Moreover, asparaginase has received "generally recognized as safe" (GRAS) status from the U.S. FDA and the JECFA **(JECFA, 2009)** and during heating process the enzyme is deactivated, ensuring its safe application in food products **(Xu et al., 2016)**.

The first results using a commercial asparaginase (Acrylaway®) for acrylamide mitigation in potato tubers were published by **Pedreschi et al. (2008)**. In the study, a reduction of 67% in acrylamide was achieved in French fries, at 60ºC and pH 7.0, and the

researchers highlighted the importance of blanching and temperature control of asparaginase treatment. In fact, asparaginase application on potato products is more complex because these consist of solid cut pieces and thereby the contact between enzyme and substrate is not ideal. For that reason, a blanching step to enzyme application is usually required since it changes the microstructure of the potato strips and increases the asparaginase-asparagine contact. Therefore, another study by the same group focused on the combination of asparaginase (Acrylaway®) treatment and conventional blanching to treat potato tuber samples **(Pedreschi et al., 2011)**. The authors found that by combining the two methods (treatment with asparaginase solution (10000 ASNU[1]/L) at 50ºC for 20 min and blanching in hot water at 85 ºC for 3.5 min), almost 90% of acrylamide was mitigated. Although acrylamide in these scientific studies was significantly reduced, no sensory analysis of the product was performed.

Furthermore, other studies using asparaginase for the treatment of potato tubers for French fries' production are shown in **Table 3**. However, the majority of mitigation measures proposed so far were only tested at laboratory scale, thus it is not clearly known if the results reached at laboratory scale could ever be achievable in food processed at an industrial scale. Moreover, although asparaginase is being already used for some products at industrial scale, the high cost of the enzyme represents a serious limitation on its application on a large scale **(Capuano and Fogliano, 2011)**. However, **Xu et al. (2016)** have highlighted that if the application of asparaginase became commercially attractive, its use alongside raw materials low in asparagine might provide the solution to the acrylamide formation.

4.4.2 Factors affecting acrylamide formation during frying process

Finally, processing conditions also affect acrylamide levels of the fried potato products, since acrylamide is actually formed during this last step. **Williams (2005)** has investigated the influence of the inclusion of a water soak prior to frying and some frying conditions (temperature, time and oil type) on acrylamide levels of fried potato crisps, which is shown in **Figure 6** as a graph. The author concluded that cooking time and

[1] ASNU is defined as the amount of asparaginase that produces 1 μmol of ammonia per min under the conditions assay (pH = 7 ± 0.005; 37 ± 0.5 ºC) using Acrylaway®.

temperature had the greatest influence on acrylamide formation, while cooking oil type and soaking had an insignificant effect.

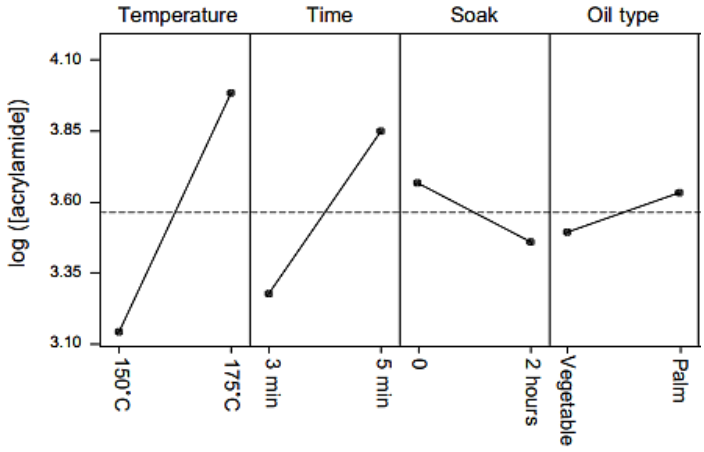

Figure 6 - Processing conditions (water soak prior to frying, cooking temperature, cooking time and cooking oil type) that influence the acrylamide levels of fried potato products. Adapted from **Williams, (2005)**.

During frying, the Maillard reaction is equally responsible either for the acrylamide formation or the browning, texture and flavour development. For that reason, acrylamide formation is correlated with colour development and, moreover, the applied frying conditions (time and temperature) affect both in similar manners. Thus, intense frying conditions (time and temperature) lead to darker fries and higher acrylamide contents. On the other hand, frying at lower temperatures (below 140 ºC) results in increased frying time which in turn leads to an increase of oil uptake and higher acrylamide contents. Therefore, frying time and oil temperatures should be controlled, meaning that temperature should not exceed 170-175ºC, in order to avoid high acrylamide levels **(Gökmen et al., 2007b; Pedreschi et al., 2004; Pedreschi and Moyano, 2005)**.

The selection of the frying oil considers the degradation it suffers while it is used and the absence of saturated and *trans* fats **(Moreira et al., 1999)**. Moreover, the type of oil used for frying was also investigated but there is some controversy regarding the influence of the type of oil on the acrylamide levels in the final products. **Becalski et al. (2003)** and **Gertz and Klostermann (2002)** have reported that palm oil and olive oil generated higher acrylamide contents in comparison to rapeseed, sunflower and corn oils. However, there are other authors reporting that oil type did not influence acrylamide levels **(Matthäus et al., 2004; Mestdagh et al., 2008a)**.

Table 3 - Summary of mitigation strategies of acrylamide formation, specifically in the raw material production, the recipe production and the potato pre-processing, tested in French fries.

Mitigation strategy	Ways to achieve the mitigation strategy	Results of acrylamide (AA) reduction in French fries	Sensorial aspects	Reference
Addition of other minor ingredients **(Development)**	Addition of amino acids in lab trials, such as Glycine (Gly) and Glutamine (Gln)	Gly and Gln: ↓ 55% in French fries	-	**(Bråthen et al., 2005)**
		Gly: ↓ 7% in frozen par-fried French fries	Acceptable	**(Medeiros Vinci et al., 2011)**
	Addition of antioxidants of bamboo leaves in lab	↓ 74-76% in French fries	No significant changes	**(Zhang et al., 2007)**
Decreasing of pH **(Development)**	Addition of acids: acetic, citric, lactic, succinic and ascorbic acids	Citric acid: ↓ 75% in French fries, lab scale	Sour taste and harder texture with 2% acid; no changes with 1%	**(Jung et al., 2003; Pedreschi et al., 2004)**
		Acetic, citric, lactic, L-ascorbic and succinic acids: ↓ 45%, 64%, 30%, 25% and 7% respectively in frozen par fried French-fries, lab scale	Acceptable	**(Medeiros Vinci et al., 2011)**
		Citric and acetic acid: ↓ 39% in frozen par-fried French fries, industrial scale	Negative impact on sensorial properties	
Pre-treatments **(SAPP - Commercial application; Others - in Development)**	Blanching of potato strips prior to processing	Blanching at 70 ºC, 10-15 min: ↓ 65% in French fries	-	**(Mestdagh et al., 2008b)**
	Addition of sodium acid pyrophosphate (SAPP) after blanching in lab scale	↓ 95% in French fries when samples were blanched at 70 ºC for 30 min	**-**	**(Lindsay and Jang, 2005)**
		↓ 17% in French fries	-	**(Pedreschi and Zuñiga, 2009b)**
	Addition of sodium chloride (NaCl) in lab scale	↓ 49% in French fries ↓ 62-97% in frozen par-fried French fries	**-**	**(Gökmen et al., 2007b)**

Table 3 - Summary of mitigation strategies of acrylamide formation, specifically in the raw material production, the recipe production and the potato pre-processing, tested in

Mitigation strategy	Ways to achieve the mitigation strategy	Results in acrylamide (AA) reduction	Sensorial aspects	Reference
Pre-treatments **(Development)**	Addition of divalent cations, such as calcium lactate (Ca lactate), calcium chloride ($CaCl_2$), magnesium lactate (Mg lactate) and magnesium chloride ($MgCl_2$);	$CaCl_2$: ↓ 93% in French fries, lab scale	No changes	**(Gökmen et al., 2007b)**
		$CaCl_2$, Ca lactate, $MgCl_2$ and Mg lactate: ↓ 44%, 12%, 32% and 18% in frozen par-fried French fries, lab scale	Acceptable	**(Medeiros Vinci et al., 2011)**
		Ca lactate: ↓ 36% in frozen par-fried French fries, industrial scale	Negative impact on sensorial properties	
	Lactic acid fermentation in French fries, lab scale	↓ 79% and 94% in French fries, with blanching + 45 and 120 min fermentation, respectively	No changes	**(Baardseth et al., 2006)**
Application of enzymes **(Development)**	Addition of Asparaginase	↓ 62% in French fries, lab scale	-	**(Pedreschi et al., 2008)**
		↓ 60-85% in French fries, lab scale;	-	**(Hendriksen et al., 2009)**
		↓ 80 % in French fries (asparaginase 0-20 U)	-	**(Zuo et al., 2015)**
		↓ 65% in frozen par-fried French fries, lab scale	-	**(Medeiros Vinci et al., 2011)**
		↓ 66% in frozen par-fried French fries, lab scale (SAPP treatment, pH 4.7, asparaginase 5000-20,000 ASNU[a]/L)	-	
		↓ ~100% in chilled (not par-fried) French fries, industrial scale (SAPP treatment, pH 4.7, asparaginase 625-2500 ASNU[a]/L)	No effect detected	

[a] ASNU is defined as the amount of asparaginase that produces 1 μmol of ammonia per min under the conditions assay (pH = 7 ± 0.005; 37 ± 0.5 ºC) using Acrylaway®.

5. Quality and safety parameters of fried products

The overall quality of foods is a combination of the sensorial perception of appearance, texture, taste and consumer acceptability **(Miranda and Aguilera, 2006; Yee and Bussell, 2007).** In the specific case of fried products, their quality is a product of the quality of the tubers used in the manufacture and the manufacturing process applied. The fried products manufacturing process produces hazardous substances of chemical and physical origin, such as acrylamide and trans-fatty acid are considered harmful to consumers if present in levels exceeding tolerance limits. For this reason, the levels of these undesirable compounds are monitored. Moreover, physic-chemical properties of fried products which affect consumer perception and are related to their sensory properties are also evaluated. It includes texture/mechanical, colour and nutritional properties **(Yee and Bussell, 2007).**

5.1. Oil content

Although the frying oil contributes to the improvement of the organoleptic properties (such as, flavour and palatability) of potato chips and French fries, the amount of oil absorbed during frying process greatly influences the quality of these food products **(Arslan et al., 2018)**. The fried potatoes with high-oil content are associated with the higher incidence of obesity, cholesterol level and high blood pressure, and thereby fat uptake is a major health concern for the potato processing industry **(Arslan et al., 2018; Yee and Bussell, 2007)**. In addition, as mentioned in section *3.1.2 Chemical and nutritional changes induced by frying*, some of the volatile and non-volatile compounds generated within the oil are undesirable products, which are absorbed into the fried products and eventually eaten by consumers. Thus, the main challenge is to improve the frying process by controlling and lowering the final fat content of fried products **(Yee and Bussell, 2007)**. In par-fried strips, the oil content varies from 5-7% weight basis (w.b.) while in finished French fries, the oil content is between 10 and 15% w.b. **(Moreira et al., 1999)**.

Oil content may be measured by several methods, such as, Soxhlet extraction with petroleum ether **(Egan et al., 1981)**, using Near Infrared Spectroscopy FT-NIR **(Fauster et al., 2018)** and differential scanning calorimetry (DSC) **(Aguilera and Gloria, 1997)**.

5.2. Acrylamide analysis

The U.S. Environmental Protection Agency (EPA) requires the limit for acrylamide content in water to be less than 0.5 ppb (corresponding to 0.5 μg/Kg) **(CSPI, 2003)**. However, starch rich products, like those produced from potato tubers, have a much higher content of acrylamide (170-3700 ppb) than the level identified as safe by EPA **(Becalski et al., 2003)**. Advanced methods of acrylamide quantification are necessary to accurately assess the human exposure to this harmful compound. Acrylamide quantification in food is difficult because of three main reasons: (i) food products consist in complex matrices, rich in interfering compounds; (ii) amounts of acrylamide are usually minimal, being crucial to concentrate acrylamide and to remove interfering compounds from sample matrices; (iii) acrylamide is a compound with low molecular mass (71.08 Da), high polarity, very good water solubility (215.5 g/100mL), high reactivity and low volatility **(Friedman, 2003; Oracz et al., 2011; Zhou et al., 2007)**.

Currently, acrylamide has been quantified in various food products by using chromatography techniques, like gas chromatography (GC), liquid chromatography (LC) or high performance liquid chromatography (HPLC), and selective and specific detectors **(Oracz et al., 2011)**. GC methods have generally involved the derivatization of acrylamide before the analysis, in order to improve selectivity and precision of GC assays **(Kepekci Tekkeli et al., 2012)**. The derivatization step may be performed using potassium bromate or potassium bromide **(Fernandes and Soares, 2007)**, xanthydrol **(Molina-Garcia et al., 2015)** or by silylation followed by solid phase microextraction (SPME) **(Ridgway et al., 2007)**. LC is commonly used in separation and quantification of compounds which are well soluble in water and nonvolatile, being HPLC coupled with mass spectrometry (MS) the most often used for the determination of the acrylamide concentration **(Oracz et al., 2011)**.

The choice of acrylamide analysis technique has a significant influence on the results **(Wenzl et al. 2004)**, as well as the acrylamide extraction step(s). **Oracz et al. (2011)** have reviewed the conventional methods applied currently in acrylamide quantification in foods and the most promising novel approaches which can replace the first ones. The authors reported that although conventional analytical methods have high reliability, efficiency and sensitivity, they also require high time-consuming preparation and expensive equipment. Thus, in order to overcome these problems, novel analytical techniques have been developed, such as capillary electrophoresis (CE),

immunoenzymatic tests (ELISA) and electrochemical biosensors, since they are sufficiently sensitive and selective, have high resolution power and short time of analysis, and allow for fast screening of numerous samples without the usage of sophisticated apparatuses **(Oracz et al., 2011)**. Additionally, **Sasi et al. (2015) and Veselá and Šucman (2013)** have reported that acrylamide levels may be also determined by using Ultraviolet Spectroscopy and Adsorption Stripping Voltammetry, respectively.

5.3. Texture/mechanical properties

Bourne (2002) defined textural properties as a group of physical characteristics that: i) arise from the structural elements of the food; ii) are sensed by the feeling of touch; iii) are related to the deformation, disintegration and flow under a force, and; iv) are measured objectively by functions of mass, time and distance. Textural properties of foods can be determined by instrumental analysis or sensory evaluation **(Yee and Bussell, 2007)**.

Sensory methods are the primary tool for determination of texture **(Miranda and Aguilera, 2006)**. **Mestdagh et al. (2008a)** have evaluated the impact of several additives in the sensorial quality of potato crisps, by means of a sensory panel. The authors have concluded that the product crispness, snap and fried potato taste were positively correlated with the taste and general appraisal, while sourness showed a negative correlation either with the product appreciation parameters or with the fried potato taste. Bitterness and popcorn-like flavours were also found, which induced the suppression of the regular taste of fried potatoes, leading to an unacceptable final product quality.

Although sensory methods are the primary means of determining the textural characteristics that are relevant to consumers, their complexity has led to the development of instrumental methods **(Miranda and Aguilera, 2006)**. Instrumentation techniques providing force-deformation (or force-distance) curves (**Figure 9**), which are widely used due to the simplicity of their implementation, low cost, easier interpretation of the results and avoidance of dealing with human responses (sensory methods). Destructive methods are preferred since they are usually better related to sensorial responses. The most common destructive tests used in assessing the texture of fried products include puncture, compression, shearing and bending tests **(Miranda and Aguilera, 2006)**. **Figure 7** shows a typical force-deformation curve obtained by puncture testing of a brittle material (exhibit a relatively small strain up to the point of rupture and a low work to fracture).

According to **Vincent (1998)**, parameters that are commonly derived from these curves in the case of fried products are: i) maximum force (also called hardness, fracturability, peak force or rupture force), detected as a peak of the force with a pronounced change in curvature; ii) springiness (also called stiffness, deformability modulus, modulus of deformation or firmness), defined as the slop of the initial "linear region" of the force-distance curves and represents the bending resistance of the material before rupture; iii) work to fracture (known in engineering as toughness), defined as the area under the force-displacement curve to the initial fracture point; iv) core force, the resistance opposed by the core of fried foods after the probe has passed through the crust. However, experimental texture data of fried products have exhibited variability, namely due to the anatomical and chemical heterogeneity of the potato tuber, as well as to the irregular distribution of gelatinized starch, oil, cell sizes and other compounds of the tissue structure after frying **(Miranda and Aguilera, 2006)**.

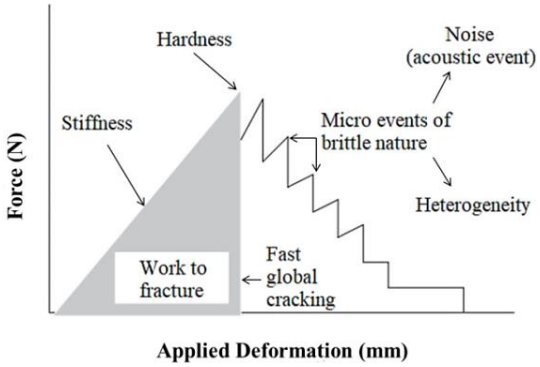

Figure 7 - Typical force-deformation curve for the first bite on a brittle material. Adapted from **Vincent (1998)**.

A brittle object will exhibit a large hardness, low work to fracture and a sudden drop in force. In products that are heterogeneous at the microstructural level, as deformation increases the phenomena repeat as a few smaller events of rise, and sudden drop of the force giving a jagged appearance to the diagram (**Figure 7**). This mechanical behaviour is known in textural terms as crispness **(Miranda and Aguilera, 2006)**. Therefore, crispness is a quality of brittle materials that rapidly fracture under stress at small strains and is a major textural property of fried foods, resulting from the low moisture content induced by frying. For that reason, brittle behaviour is lost as moisture content increases, being detected either by instrumental methods, through a change in the

force-deformation curve pattern and an increase in the hardness and mechanical work values, or by sensory methods **(Vincent, 1998; Yee and Bussell, 2007)**.

5.4. Colour properties

The colour of a fried potato is an important attribute that affects the perception of the product's quality by a consumer. Potato fries with a light golden colour are considered desirable by consumers whereas those with a darker colour are considered undesirable and associated with burnt potato fries **(Maga, 1973)**. Moreover, colorimetric studies have shown that acrylamide levels of fried potatoes are strongly correlated with their colour **(Halford et al., 2012; Kita et al., 2004)**. For this reason, monitoring the colour of potato fries and only passing onto consumers those with a light golden colour (*i.e.* removing the darkest ones) is a mean of satisfying consumer sensory expectations and preventing fries with high levels of acrylamide being consumed **(Yee and Bussell, 2007)**. Laboratory measurements of potato fries are usually performed using a colorimeter because of its high level of accuracy. In turn, colour determination in the processing factory is performed by optical colour sorter/scanner, which uses either a line scanning camera or a digital imaging camera **(Yee and Bussell, 2007)**.

6. Effect of emergent methods on potato quality

The increased consumers' interest in high quality foods with fresh-like sensory and additive free attributes led to the development of novel food processing technologies as alternative to conventionally heat treatments. As described above, although thermic processes provide desirable food characteristics they also lead to unwanted effects **(Jaeger et al., 2010)**. Accordingly, much of the recent scientific research has focused on non-thermal processing methods, for preservation (cold pasteurization) of foods as well as for structural modification, allowing to reduce heat induced changes (nutritional and sensorial properties) in product quality **(Jaeger et al., 2010)**. Irradiation, cold plasma, ultrasound, pulsed electric field (PEF) and high pressure processing (HPP) are non-thermal technologies **(Fauster et al., 2018; Huang et al., 2017)** which have already been applied in the treatment of potatoes. The effects of these non-thermal emergent methods on the quality of potato tubers and fried potatoes were already reviewed on the study of

Dourado et al. (2019). However, this thesis is focused on HPP technology and thereby, a more extensive review of the literature will be in this scope.

6.1. High Pressure Processing (HPP)

HPP, also known as high hydrostatic pressure or high isostatic pressure, is an emerging technology in food processing and preservation, which uses elevated hydrostatic pressure (up to 600 MPa) to induce pasteurization effect. This technology is based on two essential principles: (i) Le Chatelier's Principle, which claims that a change in a system under equilibrium and accompanied by a decrease in volume is compensated by an increase in pressure, and vice-versa; (ii) the isostatic principle, which states that pressure is equally, uniformly and instantaneously distributed by the entire samples, regardless of its shape or size **(Elamin et al., 2015)**.

HPP is currently being employed as a cold pasteurization technology, providing the possibility to produce microbiologically safe foods, eliminating vegetative microorganisms (both pathogens and spoilers) at and below room temperature, which extends the shelf life of foods. Furthermore, this technology allows to maintain the organoleptic properties and nutritional value of foods, which are usually lost during the conventional thermal pasteurization processes **(Ramirez et al., 2009; Rastogi et al., 2007)**. The maintenance of nutritional and sensory characteristics of foods after HPP process is based on: (i) HPP is not able to break covalently bonded molecules, resulting in nutritional (e.g., vitamins) and sensorial (coloured compounds and aromas) preservation, which is crucial for thermolabile foods, namely fruit and vegetable products **(Oey et al., 2008);** (ii) HPP process follows the isostatic principle, *i.e.* no pressure gradient is observed and thereby all regions are equally and instantaneously pressurized **(Buzrul and Alpas, 2012)**.

In 2004, the U.S. FDA has officially approved HPP as a cold pasteurization technology that can replace traditional pasteurization in the food industry **(FDA, 2004; Huang et al., 2017)**. HPP has been increasingly investigated and used at the industrial level, being one of the most promising novel processing technology due to the high number of equipment operating worldwide, as shown in **Figure 19** of **Annex I (Bermúdez-Aguirre and Barbosa-Cánovas, 2011; Buzrul and Alpas, 2012; Oey et al., 2008)**.

Besides its use in food preservation technology, has also been studied for several other applications, such as for the modification of physiological processes, like potato sprouting **(Alexandre et al., 2016; Saraiva and Rodrigues, 2011)** , to accelerate infusion processes **(Sopanangkul et al., 2002)** and to the modification of food biopolymers, such as starch and proteins **(Balasubramaniam et al., 2015)**. Pressure induces changes in the structure and function of proteins, leading to protein denaturation, aggregation or gelation **(Angioloni and Collar, 2013; Galazka et al., 2000)**. Starch is also affected by pressure treatments, being gelatinization and gelling the main influenced phenomena **(Kim et al., 2012)**. The next topics will focus on the influence of HPP treatments on potato quality, beginning with the effects on potato starch, potato enzymes and, finally, on fresh vegetables and potato tubers.

6.1.1 Effect of HPP on potato starch

A study performed using several starches had concluded that the pressure range required to cause their gelatinization varies with the starch origin, being the potato starch more baroresistant than other starches. Indeed, in order to achieve a complete pressure gelatinization of the potato starch, a pressure about 800-900 MPa is required. In addition, B-type starches (**Figure 8 (b)**) in water suspensions showed to be more resistance to the pressure than C- and A-type starches (**Figure 8(a)**) **(Stute et al., 1996)**. Moreover, **Katopo et al. (2002)** have found that HPP can convert starches that display the A-type pattern to the B-type pattern.

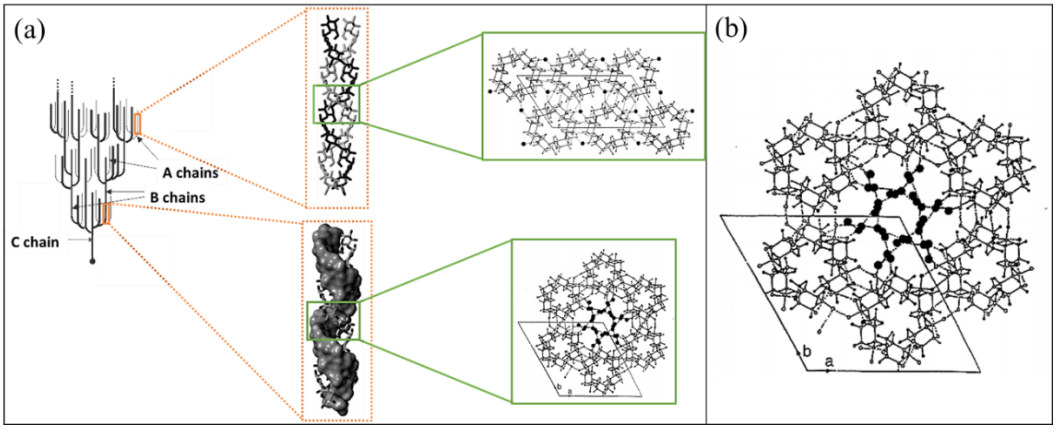

Figure 8 - Molecular drawing and crystalline packing of double helix in A and B starches (a), showing in more detail the structure of the B-type starch (wild-type potato) (b). For each unit cell, four water molecules are located between the helices in A-type starch, and a complex network of water molecules are in the center of the crystal structure of B-type starch. Projection of the structures onto de (a, b) plane, water molecules are indicated as black points and hydrogen bonds as broken lines. Adapted from **Buléon et al. (1998); Pérez et al. (2009)**.

Błaszczak et al. (2005) have studied the effect of high pressure (HP) processing on the structure of potato starch. For this purpose, a potato starch-water suspension (10%) was subjected to HP treatment at 600 MPa for 2 and 3 min, 20 ºC. The researchers observed a decrease in gelatinization temperatures as well as a substantial reduction in the total enthalpy of pressurized starches along with the time of HP treatment. Moreover, the scanning electron microscopy (SEM) analysis confirmed that HP altered the starch granule structure (**Figure 9**), showing that, like in native starch, the majority of starch granules treated with HP retained a granular shape. However, the inner part of the granule was almost completely filled with gel-like network since some fibrillary structures were clearly visible. This gel-like structure formed inside the granule might result from hydration of the amorphous phase and/or melting of the crystalline structures. In turn, the outer part of the granule seemed to be more resistant to HP treatment, showing a very compact condensed layer.

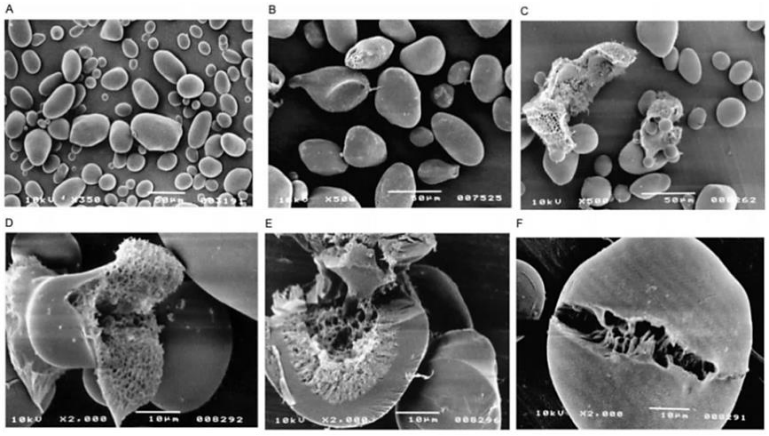

Figure 9 - SEM microstructure of native potato starch (A), potato starch treated with HP at 600 MPa for 2 min (B) and 3 min (C). Images D, E and F show details of starch structure treated for 3 min. Adapted from **Błaszczak et al. (2005)**.

6.1.2 Effect of HPP on potato enzymes

HPP is described as a process that changes enzyme functionality, inactivating enzymes at high pressures (>400 MPa) and, in several cases, activating enzymes at lower pressures (<200 MPa) **(Eisenmenger and Reyes-De-Corcuera, 2009; Mújica-Paz et al., 2011)**. Thus, the pressure needed strongly depends on the enzyme **(Hendrickx et al., 1998)**.

Enzyme activation may occurs due to two main reasons: (i) interactions between food constituents and enzymes and substrates released from vegetables membranes under pressure; (ii) activation of latent isoenzymes due to changes in the enzyme conformation that possibly exposes active sites, leading to an increase in enzyme activation **(Huang et al., 2013; Rastogi et al., 2007)**. Nevertheless, the oxidative enzymes peroxidase (POD) and polyphenoloxidase (PPO) are reported as being baroresistent, remaining active at pressures up to 600 MPa in various vegetable products **(Bayindirli et al., 2006; Cano et al., 1997; Huang et al., 2013)**. However, some authors have found significant inactivation of these enzymes in other vegetables sources, since in each tuber the amount and conformation of the enzymes are different, as well as the quantity and tissue distribution of the substrates **(Cao et al., 2011; Eshtiaghi and Knorr, 1993; Soysal et al., 2004; Yamaguchi et al., 2010)**. In addition, baroresistance is also dependent of the food matrix (such as tuber cubes, puree and extract) since the substrates and/or other tuber constituents can modify the effect of HPP on the enzymes **(Tribst et al., 2016)**.

6.1.3 Effect of HPP on fresh vegetables and fruits

The effect of the HPP on fresh vegetables and fruits structure has been investigated. **Oey et al. (2008)** have reported a through overview related to the effect of HPP on colour, flavour and texture of fruit and vegetable-based food products.

Concerning colour changes, the authors stated that HPP (at low and moderate temperatures) has a limited effect on pigments (such as chlorophyll, carotenoids and anthocyanins) responsible for the colour of fruits and vegetables. However, during storage of HP processed fruits and vegetables, colour changes can occur due to incomplete inactivation of enzymes and microorganisms, which can result in undesired either enzymatic or non-enzymatic reactions in the food matrix **(Oey et al., 2008)**.

Regarding to flavour changes, it is generally assumed that the fresh flavour of fruits and vegetables is not influenced by HPP, since the structure of small molecular flavour compounds is not directly affected by HP. However, HPP can enhance and retard enzymatic and chemical reactions and, thereby, it could indirectly alter the content of some flavour compounds. Due to interactions between individual flavour compounds, even a small change in the concentration of one compound may have major effects on the overall flavour **(Oey et al., 2008)**.

When it comes to texture changes, it can be related to changes in cell wall polymers of fruits and vegetables due to enzymatic and non-enzymatic reactions. During HPP, fruits and vegetables are susceptible to the softening of the tissues. This effect is dependent on pressure and cell structure of each vegetable and has been attributed to changes in cell wall structure and architecture **(Oey et al., 2008)**. Some authors have reported that HPP favoured physical disruption of the cell wall structure of vegetables during pressurisation, allowing the contact between substrate and hydrolytic enzymes (such as pectin methyl esterase, pectinesterase, polygalacturonase and pectate lyase), which accelerated the enzymatic lysis of the structural wall of vegetable tissues **(Basak and Ramaswamy, 1998; Oey et al., 2008; Rastogi et al., 2007)**. In addition, the cell permeability increases, as well as the movement of water and metabolites (substrates, ions and enzymes) that are located in different compartments **(Oey et al., 2008; Rastogi et al., 2007).** This effect highlights HPP as an interesting tool for nutrients diffusion in foods **(Rastogi et al., 2007; Sopanangkul et al., 2002)** and as a pre-drying treatment **(Al-Khuseibi et al., 2005).**

Concerning HPP effects on texture of (solid) fruits and vegetables, hardness or firmness is mostly used as a parameter. **Basak and Ramaswamy (1998)** have studied the effect of HPP (100-400 MPa, 5-60 min, room temperature) on the firmness of different fruits and vegetables. The authors observed a rapid firmness loss during compression. However, upon HPP, an increased hardness is usually observed, mainly due to pectinmethylesterase activity **(Oey et al., 2008)**. Actually, when the enzyme is liberated and contacts with the substrate (the highly methylated pectin), pectin demethylation occurs. Consequently, the de-esterified pectin is capable of forming a gel-network with divalent cations (for example, generating calcium bridges), which results in increased hardness **(Al-Khuseibi et al., 2005; Oey et al., 2008)**.

6.1.4 Effect of HPP on potato and other fresh tubers

The main research work on HPP using fresh vegetables has been performed in fruits, whose structure and composition are fundamentally different from tubers **(Oliveira et al., 2015)**. The effects of HPP on fresh tubers were only evaluated in a few studies. **Oliveira et al. (2015)** have investigated the effect of HPP on the physical characteristics of cocoyam, Peruvian carrot and sweet potato. The HP treatment at 600 MPa applied for 5 and 30 min caused physical damage in the structure of the cellular structure of the

tubers, evidenced by the presence of starch outside the cells, lack of cellular definition, agglomeration of the starch granules and increase of the granule volume, possibly due to hydration of starch. The researchers highlighted that HPP lead to distinct physical changes in tubers when compared to thermal processes, since degradation of pectin substances by depolymerization or demethoxylation are induced by heating processes and are not observed after HPP. In addition, water exudation and an increase in syneresis (up to 12%) were also observed, as well as the increasing of the drying rate (~30%) and the reduction of the firmness (up to 60%). Additionally, the researchers found that 5 min process may have affected the starch structure, while more drastic conditions (30 min process) induced starch gelatinization in tubers.

Eshtiaghi and Knorr (1993) have studied the effect of HPP on potato cubes and found that the process at 400 MPa and 20 ºC for 15 min provided loss of firmness similar to blanching in boiling water. Furthermore, **Sopanangkul et al. (2002)** have studied the effect of HPP on the diffusion coefficient of sucrose in potato cylinders, at various pressures and temperatures. The authors reported that the pressure opened up the tissue structure, increased the cell permeability and facilitated the diffusion to a certain extent. Actually, higher pressures (above 400 MPa) also induced starch gelatinization and hindered diffusion. The maximum value of diffusion coefficient was 8-fold higher than the atmospheric pressure value, and the authors highlighted that application of appropriate levels of pressure (100 to 400 MPa) can be used to accelerate mass transfer during ingredient infusion into foods and to reduce processing times.

The texture of potato tubers may be also altered by physiological processes, namely by the sprouting. Therefore, the control of sprouting is essential for potato tuber storage since it causes softening, shrinkage, formation of toxic alkaloids and consequently it reduces the weight, the nutritional and processing quality of tubers, being responsible for important economic losses **(Sorce et al., 2005)**. The storage at low temperatures, the use of chemical sprouting inhibitors and the irradiation are the primary methods used to control potato sprouting of stored tubers. However, potatoes for processing cannot be stored al low temperatures for a long time because these storage conditions promote the conversion of starch to sugars, increasing tubers' sweetness, with the consequent change in taste and undesirable browning because of Maillard reactions, when tubers are processed at high temperature **(Alexandre et al., 2016; Saraiva and Rodrigues, 2011).**

Saraiva and Rodrigues (2011) have shown that HPP could be used as a nonthermal and chemical-free alternative to control sprouting of potato tubers. The researchers

studied the effect of HP treatments (100 MPa applied for 5 and 10 min) on inhibition of sprouting of potato tubers that were stored for 3 months before the pressure treatments and they concluded that this processing inhibited potato tuber sprouting up to 6 weeks at 18 ºC. In addition, the effect of the pressure treatment on textural properties (firmness and stiffness) of the potato tubers was also evaluated, being observed a decrease in firmness and stiffness at higher pressure levels, but a significant decrease was verified only for the tubers treated at 100 MPa.

Furthermore, **Alexandre et al. (2016)** have studied the effect of short thermal treatments (60 and 65 ºC for 1 min) and low intensity HP treatments (15 and 30 MPa for 10 min) on the sprouting of potato tubers. The most pronounced inhibitory effect on potato tuber sprouting was achieved when treatments were sequentially combined. Thus, the researchers have highlighted the potential of the HPP for industrial application on the sprouting control and, consequently, on the tuber texture control.

Objectives

Fried products are widely consumed, and the extent of toxic compounds formation and oil absorption during frying are important with regard to public health. On the other hand, several studies have proved that some non-thermal processing technologies, namely HP processing, lead to modification in several quality aspects of potato tubers. However, the effect of pressurized potatoes on the characteristics of potato fried had never been studied before. Also, no study was performed regarding infusion of asparaginase into raw potatoes using HPP as a technology to enhance the infusion rate, and subsequently to mitigate acrylamide levels in fried potatoes.

Thus, this research work had two main objectives: (1) assay the potentiality of HPP pretreatment on modification of physico-chemical properties of raw potato tubers and sticks, and subsequently of sensory, nutritional and physico-chemical properties of the respective fried potatoes; (2) evaluate the potentiality of HPP on asparaginase infusion into raw potato sticks, as a novel strategy to reduce acrylamide levels in fried potatoes.

Chapter II – Materials and Methods

This section comprises all the methodologies employed on this work

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 the light at 10 ºC, and were selected by shape uniformity and absence of injuries. The frying oil used (*Fula* brand) is a commercial combination of sunflower and rapeseed oil, whose nutritional composition is shown in **Table 31** (**Annex II**), 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. Samples preparation

A preliminary test was performed in order to evaluate the effect of pressure treatments in whole potato tubers with and without peel, involved in water or under vacuum. For this purpose, potato tubers were randomly selected, washed in running water and four sets of potato tuber samples were prepared:

(i) "*Peeled potatoes + Water*": washed potato samples were manually peeled, cut in half and placed in polyethylene bags. Tap water (in a proportion of 2:1 (water: potato - g/g)) was added, and the bags were heat sealed;

(ii) "*Peeled potatoes + Vacuum*": washed potato samples were manually peeled, cut in half and placed in polyethylene bags. The air was removed from the bags using a vacuum packaging machine at 95% of vacuum (Vacupack 2; Krups, Offenbach am Main, Germany), and the bags were heat sealed;

(iii) "*Unpeeled potatoes + Water*": whole and unpeeled potato samples were placed in polyethylene bags, tap water (in the same proportion as set **(i)**) was added, and the bags were heat sealed;

(iv) "*Unpeeled potatoes + Vacuum*": whole and unpeeled potato samples were placed in polyethylene bags, vacuum packaged as described for set **(ii)**, and the bags were heat sealed.

The potato tubers were subjected to HP treatments of 0.1 (control), 100, 200, 300, 400 and 500 MPa for 2.5 min, at room temperature, by using a pilot-scale HP equipment (Hiperbaric 55 L, Burgos, Spain) with a pressure vessel of 55 L. It is noted that in samples

(i) "Peeled potato + Water" and *(ii) "Peeled potato + Vacuum"*, each half of processed potato was compared with the other unprocessed half. In samples *(iii) "Unpeeled potato + Water"* and *(iv) "Unpeeled potato + Vacuum"*, processed potatoes were compared with unprocessed potatoes which had similar shape and dimensions. After pressure treatment, the exterior water samples, which were involving the potato tubers, were collected and total soluble solids content was measured. Potato tubers were characterized by measuring their weight difference after HPP, moisture content, syneresis, texture properties, and optical microscopy observations.

3. Pre-frying processing

After this preliminary test, two sets of conditions were prepared:

(i) "*Potato sticks + Pressure*": 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). Tap water (in a proportion of 2:1 (water: potato - g/g)) was added, and the bags were manually heat sealed. The potato sticks were subjected to pressure treatments of 0.1 (control), 200, 300, 400, 500 and 600 MPa for 2.5 min, and 600 MPa for 10 min. Pressure treatments were performed in triplicate.

(ii) "*Potato sticks + Asparaginase + Pressure*": Potato sticks (with equal dimensions) were prepared as described above and placed in PA/PE. 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 since it is the most used in food industry. 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. The potato sticks were subjected to pressure treatments of 0.1 (control), 100, 200, and 400 for 5 min. Thereafter, a first set of potato sticks 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. Treatments were performed in triplicate for each pressure condition.

After treatments, the involving water was collected for further analysis (sugars content, and total soluble solids in water), and potato sticks were immediately characterized by measuring their weight difference after HPP, texture, colour, and moisture. The remaining potato sticks were stored at -20 ºC and used for scanning electronic microscopic (SEM) observations, differential scanning calorimetry (DSC), and reducing sugars analyses.

4. Deep-frying

After characterization of potato sticks, these 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 immediately analysed for weight loss after frying, texture, colour and moisture. The remaining samples were stored at -40 ºC until further analyses (oil content, fatty acids composition, and acrylamide content).

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

5.1. Weight differences after HPP

The percentage of weight difference, between initial and final weight of potato samples, was calculated in triplicate for initial pre-test samples (peeled and unpeeled potato tubers packaged in water), potato sticks treated by pressure, and potato sticks treated by the combination of pressure and an asparaginase solution. **Equation 1** was used for the calculation of this parameter.

$$Weight\ difference\ (\%) = \frac{potato\ weight\ after\ HPP\ (g) - potato\ weight\ before\ HPP\ (g)}{potato\ weight\ before\ HPP\ (g)} * 100 \quad \textbf{(Equation 1)}$$

5.2. Weight difference after frying

The percentage of weight difference after frying, between initial and final weight of fried potato sticks, was calculated in triplicate for fried potatoes pre-treated by HP and those pre-treated by the combination of pressure and an asparaginase solution. **Equation 2** was used for the calculation of this parameter.

$$Weight\ difference\ (\%) = \frac{potato\ sticks\ weight\ after\ frying\ (g) - potato\ sticks\ weight\ before\ frying\ (g)}{potato\ sticks\ weight\ before\ frying\ (g)} * 100$$ **(Equation 2)**

5.3. Syneresis

Syneresis was calculated for initial pre-test samples (peeled and unpeeled potato tubers) packaged under vacuum in order to assess the effect of HPP treatments on water exudation from each tuber. This parameter was calculated according to **Equation 3** and the results were expressed in %.

$$Syneresis\ (\%) = \frac{tuber\ weight\ before\ HPP\ (g) - tuber\ weight\ after\ HPP\ (g)}{tuber\ weight\ before\ HPP\ (g)} * 100$$

(Equation 3)

5.4. Moisture

Five raw or fried potato sticks of each replicate sample were used for moisture determination (**Equation 4**). Potato sticks were weighted before and after drying at 105 ºC for 24h, and moisture was calculated by applying the following expression:

$$Moisture\ (\%) = \frac{sticks\ weight\ before\ drying\ (g) - sticks\ weight\ after\ drying\ (g)}{sticks\ weight\ before\ drying\ (g)} * 100$$

(Equation 4)

5.5. Optical microscopy observation

Potato tubers pressurized under 0.1 (control), 100, 300 and 500 MPa for 2.5 min were cut by a scalpel, obtaining 3 slices with 2.5 cm of length, 1.5 cm of width, and 1 mm of thickness, for each replicate of sample. Five drops of iodine solution were applied under each slice of potato. After 10 min of reaction, observations in an optical microscopy (Carls Zeiss, serie number 3108010130, Thornwoodby, United States) using a 10x objective were carried out. For each slice of potato, 3 images were captured with a mobile camera.

5.6. Scanning electronic microscopy (SEM)

Scanning electronic microscopy (SEM) observations were carried out to raw potato sticks pre-treated by HPP, at 100-600 MPa for 2.5 min. Pre-treated potato pieces with 1 cm width, 2 cm length, and 0.5 cm thickness were freeze at -80 ºC, then lyophilized for 7 days, and stored in a desiccator to prevent hydration. Lyophilized samples were cut in thinner pieces with 0.5 cm width, 1 cm length, and 0.5 cm thickness, and were fixed (with adhesive tape on the base) to a support, with the transverse plane facing upwards. The support containing samples was placed in the tabletop microscope (Hitachi, TM4000Plus), and analyses were performed under vacuum, with an electron acceleration of 10 kV, and several magnification levels (80, 100, 200, 250, 400 and x600).

5.7. Differential scanning calorimetry (DSC)

Differential Scanning Calorimetry (DSC) analyses were performed in the samples (potato sticks) pressurized at 0.1, 200, 400 and 600 MPa for 2.5 min to evaluate the effect of pressure on gelatinization temperature of potato tubers. For this purpose, a Shimadzu DSC-50 instrument under nitrogen (N_2) atmosphere was used. Samples (about 8-13 mg) were sealed in hermetic aluminium pans, rested for 5 min at 25.00 ºC and heated from 25.00 to 95.00 ºC at a heating rate of 10.00 ºC/min. Onset temperature (T_o), maximum peak temperature (T_p), and enthalpy of gelatinization (ΔH expressed as J/g) were determined **(Karlsson and Eliasson, 2003)**.

5.8. Texture analysis

Two methods were applied for textural characterization:

5.8.1. Texture analysis by compression

A texture profile analysis (TPA) by compression were used in the preliminary tests to evaluate the effect of pressure treatments on whole potato tubers. After processing, three cylinders with 1 cm of diameter and 2 cm of length were obtained from each sample, and each cylinder was placed in a vertical position on a fixed and flat surface. TPA analyses were performed in a texture analyser equipment (model TA.Hdi, Stable Micro Systems) equipped with a load cell with 5 Kg, and using a platen with 6 cm of diameter (**Figure 20 – A, Annex III**). The velocity parameters were fixed as 0.50 mm/s (pre-test, test and post-test), and the compression distance was 3.0 mm (15% of deformation). The maximum force of the first peak was obtained from TPA (Force vs Distance) graphics.

5.8.2. Texture analysis by cutting with a knife

A texture analysis by cutting were performed either on raw potatoes or fried potato sticks. After processing/frying, 5 potato sticks were analysed and 2 assays were performed in each potato stick, obtaining a total of 10 experimental points for each replicate sample. The analyses were performed by using the same texture analyser equipment as described above and a knife with 6 cm of width, 10 cm of height and 1.2 mm of thickness (**Figure 20 – B1-B2, Annex III**). 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.

5.9. Colour

Five raw or fried potato sticks of each replicate sample were used for colour analyses. Potatoes colour was measured directly in the surface on three different locations of three different sides of raw or fried potato stick, obtaining a total of 9 experimental points for each replicate sample. 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 difference (ΔE*) was calculated according to **Equation 5**:

$$\Delta E = \sqrt{\Delta L *^2 + \Delta a *^2 + \Delta b *^2} \qquad \textbf{(Equation 5)}$$

where Δ*L**, Δ*a** and Δ*b** correspond to the difference of *L**, *a** and *b** values, respectively, between each sample and the control.

5.10. Reducing sugars content

Reducing sugars content is an important parameter to be measured due to its participation in the acrylamide formation. The analysis of reducing sugars concentration present in raw potatoes ("*Potato sticks + Pressure*") was performed by applying the 3,5-dinitrosalicylic acid (DNS) method **(Miller, 1959)**.

For DNS reagent preparation, 10 g of DNS were weighted and dissolved in 200 mL of a 2 N NaOH solution, under intense heating and stirring. Simultaneously, a solution with 300 g of potassium tartrate and 500 mL of distilled water was prepared, under intense heating and stirring. Finally, both solutions were mixed with stirring and distilled water was added up to a total volume of 1 L.

For determination of reducing sugars concentration, 5 raw potato sticks of two replicates of each sample were weighted (~10 g) and 30 g of distilled water were added. An Ultra-Turrax homogenizer (MICCRA D-9, ART Prozess & Labortechnik GmbH & Co. KG, Germany) as used to homogenize the samples, at maximum speed for 3 min, samples were centrifuged, and supernatants were filtered. The filtrate was used for reducing sugars analysis and each filtrate was analysed in triplicate. Briefly, 1.0 mL of DNS reagent was added to 1.0 mL of sample filtrate, stirred in a vortex and placed in a boiling water bath (at 100 ºC) for 5 min. Then, the mixture was placed on ice (in order to stop the reaction) diluted with 10 mL of distilled water, and finally, the absorbance was read in duplicate using a microplate spectrometer (Thermo Scientific, Thermo Fisher Scientific Inc., USA) at 540 nm. Reducing sugars concentration was calculated by using a calibration curve (**Figure 26** of **Annex IX**) that was previously prepared from glucose

solutions with 0.00 – 1.00 g/L (**Equation 6**), and results were expressed as g of reducing sugars/ 100g of raw potato.

Abs (540 nm) = 0.353 [Glucose] + 0.0366 R^2=0.9953

(Equation 6)

5.11. Lipid extraction

Extraction of oil from fried potato sticks for the determination of lipid content in fried potatoes pre-treated by HPP was performed using a mixture of organic solvents, according to the method described by **Tabee et al. (2009)**, with slight modifications. Ten fried potato sticks (about 20 g) was placed in a plastic bottle and 60 mL of a mixture of petroleum ether: diethyl ether (90:10; v:v) were added. An Ultra-Turrax homogenizer (MICCRA D-9, ART Prozess & Labortechnik GmbH & Co. KG, Germany) was used to homogenize the samples, at maximum speed for 4 min. 100 mL of the mixture of petroleum ether: diethyl ether (90:10; v:v) were then used to wash the homogenizer, and lipids were extracted by stirring vigorously, using a magnetic stirrer, for 10 min. The upper solvent (where lipids were dissolved) was filtrated four times using anhydrous sodium sulphate, in order to remove water molecules that could be dissolved. The solvent was evaporated under vacuum in a rotary evaporator, at 30 ºC for 15 min. Fat content (%) was determined by the quotient between the final lipid weight and initial samples weight.

5.12. Lipid profile

Lipid profile of fried potato sticks was determined according to **Santos et al. (2017)** with some modifications. Firstly, an internal standard was prepared containing two non-natural lipids, C11:0 and C13:0, with concentration of 10.74 mg/mL and 17.24 mg/mL, respectively. C11:0 (non-methylated fatty acid) served as a sample control and C13:0 (methylated fatty acid) served as a control of methylation process. Secondly, 1 g of homogenized sample were mixed with 50 µL of the internal standard, 1.58 mL of isopropanol (for protein precipitation), and 2.04 mL of cyclohexane. After a reduced period of manual vortexing, a binary mixture was obtained, and samples were preserved overnight under refrigeration. 2.250 mL of aqueous sodium chloride (1%) were added in order to remove the non-lipid compounds, obtaining a ternary mixture. After

centrifugation (3000 rpm, 5 min), the upper phase (cyclohexane + lipid compounds) was transferred to dark glass vials and evaporated under nitrogen stream (70 ºC). Anhydrous sodium sulphate and 2 mL of hexane were added, as well as 200 µL of 2 M KOH in MeOH for methylation of fatty acids (FA). After 1 min of manual vortexing, anhydrous sodium hydrogen sulphate was added to stop methylation reaction and the mixture was centrifuged at 3000 rpm for 5 min. 1 mL of supernatant was transferred to dark glass vials and FA composition was evaluated by gas chromatography (GC) technique, using a FAME CP-Select CB column (50 m x 0.25 mm) on a Chrompack CP 9001 gas chromatograph (Chrompack, Middelburg, the Netherlands). A certified standard mixture of FA methyl esters (TraceCert – Sulpelco 37 component FAME mix, USA) was used to support FA identification and FID calibration. The three replicates of each sample were analysed in duplicate, so for each sample, six chromatograms were acquired. In turn, for each chromatogram, the areas of the identified FA and the total areas (*raw areas*) of chromatograms were obtained.

For the calculation of the FA composition in potatoes (g/100 g of potatoes), a correction factor for each identified FA was determined through the analysis of the standard mixture (Sulpelco 37). Raw areas of each FA were multiplied by the correction factor, obtaining the respective real (corrected) areas. Finally, the concentration of each FA (g/100 g potatoes) was calculated through **Equation 7**:

$$FA\ (\text{g}/100\ \text{g}) = \frac{Real\ area\ of\ FA}{\sum Real\ area\ of\ total\ identified\ FA} * 100$$

(Equation 7)

Additionally, the relative percentages of saturated FA, monounsaturated FA (MUFA), polyunsaturated FA (PUFA), trans FA, and w-3 and w-6 PUFA were calculated by summing the concentration of each type of FA mentioned.

5.13. Acrylamide content

The quantification of acrylamide was performed as described by **Molina-Garcia et al. (2015)**, with minor modifications. Briefly, 2 g of homogenized sample were placed in a centrifuge tube, 200 µL of $AA^{13}C_3$ (internal standard, 10 ppm) were added, and left in contact for a few minutes. 20 mL of water and 5 mL of 1,2-dichloroetane were added in

order to solubilize acrylamide and remove fat, respectively. After 15 min of shake by using an homogenizer, the mixture were centrifuged (3000 rpm, 5 min), and the lower phase (1,2-dichloroetane + fat) was removed. A second addition of water (5 mL) and 1,2-dichloroetane (5 mL) took place, the mixture was shaken and centrifuged again, and the upper phase (water + acrylamide) was placed in a round bottom flask. Subsequently, 6 mL of diethyl glycol solution (10% in MeOH) were added and the extract was concentrated in a rotary evaporator (40 ºC of bath temperature) until a final volume of 5 mL. The derivatization reaction occurred by adding 1.65 mL of xanthydrol solution (5% in MeOH), the derivatization agent, and 1 mL of HCL (1.5 M), since the acidic medium is the most conducive to the occurrence of this reaction. The derivatization reaction was conducted in a water bath at 40 ºC, for 50 min. Afterwards, 700 µL of KOH solution (2.5 M) was added in order to alkalinize the solution to a pH at least of 9.0 and, then, it was buffered with 30.8 mg of $NaHCO_3$ and 169.2 mg of K_2CO_3. 2 mL of water saturated with NaCl (1 g/mL) were added and the acrylamide derivative was extracted from the saturated aqueous phase with two additions of 1 mL of ethyl acetate, being vigorously shaken for 1 min and centrifuged at 3000 rpm for 5 min. Finally, the upper layer was transferred to a vial and anhydrous sodium sulphate to remove water molecules that could be dissolved. Two replicates of each sample were used for extraction of acrylamide and injections of extracted acrylamide were performed in duplicate; therefore, four results for each sample were obtained.

For the quantification of acrylamide in samples, a calibration curve was constructed by using raw potato tubers (2 g) as matrix due to the absence of acrylamide. Several standard solutions of acrylamide (0, 100, 250, 500, 1000, and 1500 ppm) were added and all remaining steps of the procedure were performed as previously described.

Extracted acrylamide was analysed by gas chromatography – mass spectrometry (GC-MS) by using a DB-XLB column (Agilent, 0.25 mm I.D., 30 m length, 0.10 µm film thickness), and a gas chromatograph Agilent (model GC-6890 N) equipped with a split-splitless injector and coupled to a mass selective detector Agilent (model MSD-5975 N, Palo Alto, CA, USA).

6. Characterization of involving water of potatoes

6.1. Weight difference after HPP

The percentage of weight difference, between initial and final weight of exterior water samples after HPP, was calculated in triplicate for water samples of initial pre-test samples (peeled and unpeeled potato tubers packaged in water), potato sticks treated by pressure, and potato sticks treated by the combination of pressure and an asparaginase solution. **Equation 8** was used for the calculation of this parameter.

$$Weight\ difference\ (\%) = \frac{water\ weight\ after\ HPP\ (g) - water\ weight\ before\ HPP\ (g)}{water\ weight\ before\ HPP\ (g)} * 100 \quad \textbf{(Equation 8)}$$

6.2. Total soluble solids (TSS)

Total soluble solids (TSS) present in the water samples that were involving potato tubers/sticks were determined by two different ways:

(i) Measuring ºBrix through a refractometer (ATAGO Refractometer, ATC-1E), at room temperature;

(ii) Drying water samples at 105 ºC for 24h and applying the following expression (**Equation 9**):

$$TSS\ (\%) = \frac{TSS\ weight\ after\ drying\ (g)}{water\ weight\ before\ drying\ (g)} * 100 \quad \textbf{(Equation 9)}$$

7. Statistical analysis

All analyses, except SEM, optical microscopic and DSC 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.

Chapter III – Results and Discussion

This section comprises all the obtained results and the respective discussion for both raw potatoes and fried potatoes

1. Effect of HPP on raw potato tubers

The objective of this research work was to evaluate the effect of HPP on the quality of fried potatoes. However, a pre-test was necessary in order to better understand the effect of pressure on raw potato tubers and, for this purpose, four conditions were assayed: the presence and absence of potato peel, and the packaging in water and under vacuum. The conditions of packaging in water and under vacuum were also selected considering that HPP, generally, increases the activity of oxidative enzymes in potatoes **(Tribst et al., 2016)**. Therefore, packaging the potato tubers in water and under vacuum were two strategies to avoid the contact of polyphenol oxidase and/or peroxidase with oxygen and, consequently, to avoid potato tubers oxidation. A few experimental analyses were performed, namely weight difference of potato tubers and water samples after HPP, as well as syneresis of potato samples that were packaged under vacuum, moisture measurements, optical microscopy, texture analyses through a compression test, and the total soluble solids present in exterior water of potato tubers. The obtained results and the respective discussion are present bellow.

1.1. Weight difference after HPP of raw potato tubers

Weight difference after HPP was calculated for peeled and unpeeled potato, and for the respective exterior water samples, whose results are present in **Table 4** and **Figure 21** (**Annex IV**). In relation to weight difference of potato samples, it is notorious that peeled potato tubers showed a different behaviour compared to unpeeled ones. In the first case, although potato tubers weight increased significantly ($p<0.05$) when subjected at 100 MPa (12.4 ± 1.5%), from this pressure level, a reduction in weight gain was observed as the pressure intensity increased, and negative levels of weight difference were obtained at 500 MPa (-3.8 ± 0.5%). However, in the second case, weight difference of unpeeled potato tubers was not significantly different ($p<0.05$) over the pressure, keeping their levels of about 0.1-0.3%.

The water samples showed negative weight differences which decreased (became more positive) over the pressure. The exterior water samples of potato sticks processed at 100 MPa presented the highest weight loss (-7.2 ± 0.4%), while those of potato sticks subjected to 500 MPa for 2.5 min showed a weight gain of 0.3 ± 0.4%. In contrast, the

exterior water samples of unpeeled potato tubers exhibited no changes ($p<0.05$) in their weight after HPP, showing losses between -1.3 and -0.9%.

According to the presented results, it is possible to state that, as the intensity of pressure treatment increased, peeled potato tubers probably lost components that, in turn, solubilized in water and led to an increase in water weight. In contrast, unpeeled potato tubers did not suffer variations, either in potato tubers or in exterior water, since potato peel is a physical barrier that blocks the output and/or input of compounds from/into potato tubers.

Table 4 - Weight difference, expressed in %, of peeled and unpeeled potato tubers, and the exterior water samples, along the pressure (at 0.1 - 500 MPa, for 2.5 min).

Processing conditions (MPa/min)	Potato weight difference (%)		Exterior water weight difference (%)	
	Peeled potato	Unpeeled potato	Peeled potato	Unpeeled potato
0.1/2.5	3.8 ± 0.9 ^{c}	0.3 ± 0.3 ^{a}	-3.9 ± 0.4 ^{bc}	-1.3 ± 0.3 ^{a}
100/2.5	12.4 ± 1.5 ^{d}	0.2 ± 0.1 ^{a}	-7.2 ± 0.4 ^{a}	-1.4 ± 0.2 ^{a}
200/2.5	5.6 ± 1.0 ^{c}	0.3 ± 0.1 ^{a}	-5.4 ± 1.3 ^{ab}	-1.1 ± 0.1 ^{ab}
300/2.5	2.7 ± 0.4 ^{bc}	0.1 ± 0.2 ^{a}	-3.0 ± 0.6 ^{c}	-1.0 ± 0.3 ^{ab}
400/2.5	0.4 ± 0.9 ^{b}	0.1 ± 0.1 ^{a}	-2.5 ± 0.8 ^{c}	-0.9 ± 0.1 ^{ab}
500/2.5	-3.8 ± 0.5 ^{a}	0.3 ± 0.3 ^{a}	0.3 ± 0.4 ^{d}	-1.3 ± 0.3 ^{a}

Results are expressed as the mean ± the standard deviation.
^{a,b,c,d} Different letters indicate significant differences ($p<0.05$) among the pressure treatment for the same evaluated parameter and sample.

1.2. Syneresis of raw potato tubers packaged under vacuum

After the evaluation of the effect of HPP on potato tubers packaged in water, the effect of HPP on potato tubers packaged under vacuum was also evaluated. For this purpose, syneresis was measured for both peeled and unpeeled potato tubers (**Figure 10**), in order to assay the effect of HP treatments on the release of liquid from potato tuber. Comparing the global results presented in **Figure 10**, it was evident that the presence or absence of potato peel had influence on the release of exudates from potato tubers. For this reason, in peeled potatoes, a gradual increase of syneresis was observed as the intensity of pressure increased, with a release of exudates up to ~12-fold higher than control samples. Only the samples subjected to HPP at 400 and 500 MPa presented a greater percentage of syneresis ($p<0.05$) when compared to the control samples (0.1 MPa). Actually, these results corroborate the study of **Oliveira et al. (2015)**, who found that HP induced a greater release of exudate in peeled tubers (packaged under vacuum)

subjected to HPP at 600 MPa, specifically in Peruvian carrot (~10-fold increase), sweet potato (~5-fold increase), and cocoyam (~3.7-fold increase) tubers. The differences between the percentage of exudation of this study and the study of **Oliveira et al. (2015)** are probably related with the utilization of tubers other than potato tubers, and the application of different pressure conditions.

In contrast, in unpeeled potatoes, few changes ($p<0.05$) were observed in the syneresis values along the pressure treatments, and a release of exudates only up to ~3-fold higher than unpressurized samples was noted. No study in the literature have studied the effect of HPP on potato tubers in the presence of potato peel, and for this reason, it was not possible to compare the obtained results. However, it is known that potato peel consists of a layer with approximately ten cells deep, and these cells have thick cell walls rich in pectin polysaccharides **(Miranda and Aguilera, 2006; Scharf et al., 2018)**. On the one hand, some studies have reported that HPP lead to changes in the structure and architecture of the cell walls of vegetable tissues, and thereby HP may also induce changes in the cell walls of potato peel. Nevertheless, once the cell walls of potato outer skin are thicker than those of parenchyma cells **(Miranda and Aguilera, 2006)**, they are probably more resistance to the pressure. For this reason, higher pressure treatments are needed to cause changes in the cell walls of potato peel and, consequently, lead to the release of liquids from potato tubers. This probably explains that a higher water exudation ($p<0.05$) was observed only in unpeeled potato tubers subjected at 500 MPa for 2.5 min.

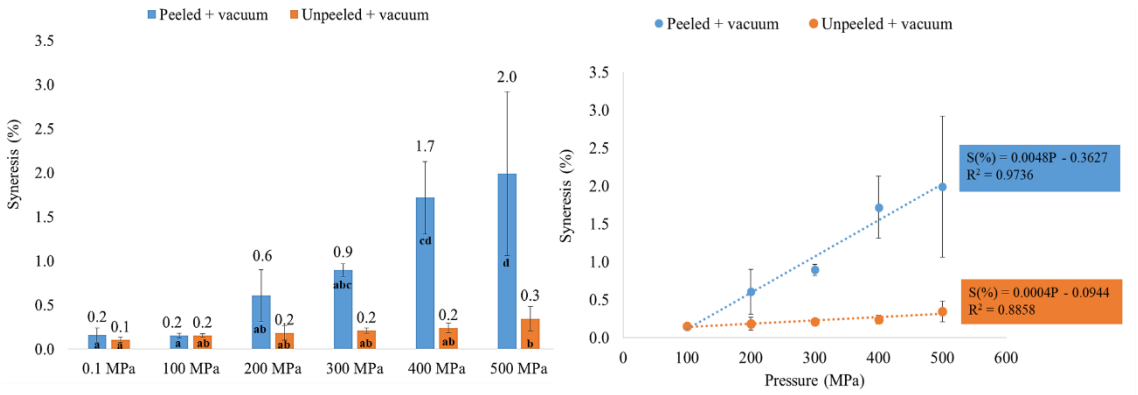

Figure 10 – Graphical representation on the left shows syneresis values for peeled and unpeeled potato tubers packaged under vacuum, over the pressure (at 0.1 - 500 MPa, for 2.5min). Graphical representation on the right shows the respective linearizations. "S(%)" and "P" mean Syneresis (%) and Pressure (MPa), respectively.
[a,b,c] Different letters indicate significant differences ($p<0.05$) among the pressure treatment for the analysed sample.

1.3. Optical microscopy observation of raw potato tubers

The purpose of this analysis was to evaluate if pressure treatments would induce the release of intracellular starch to the extracellular environment. Therefore, thin pieces of potato tubers treated at 0.1 (control), 100, 300 and 500 MPa for 2.5 min were dyed with an iodine solution, in order to promote the formation of starch (amylose)-iodine complexes, and consequently, to enable its observation under the optical microscope (**Figure 11**). Although potato tuber tissue had shown a huge variability, in general, potato tubers treat at 100 MPa showed a similar appearance to control samples, but a gradual increase of extracellular starch granules was observed as the pressure intensity increased. A possible justification for this happening was related with textural changes caused by pressure, which led to an increase in the release of intracellular cytoplasm, and consequently, intracellular components could fulfill the extracellular environment of the potato tissue.

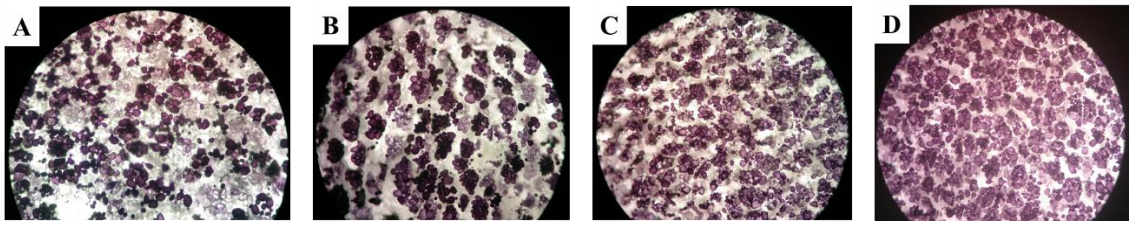

Figure 11 - Images of optical microscopy of potato tubers pressurized under (A) 0.1, control; (B) 100; (C) 300, and (D) 500 MPa for 2.5 min, using iodine solution as dyed agent.

1.4. Moisture of peeled potato tubers packaged in water and total solids of the respective exterior water samples

Moisture of unpeeled potato tubers packaged in water was measured and results are present in **Table 5**. Although significant differences ($p<0.05$) were not observed among all samples, it was noted that moisture of tubers subjected to pressure tended to be lower (~1-7%) than control tubers. In fact, this result is in accordance with other studies which studied 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 moisture loss. The obtained results (for potato tubers) and the results recovered from the literature (for other vegetables) are mainly due to the damage of cell structure, cell permeabilization and softening of vegetable tissues caused by HPP, which cause the release of water from vegetables.

Two possible explanations may justify the lack of significant differences in the moisture of potato tubers. Firstly, cell permeabilization and the release of cell liquid could not be enough to cause significant changes ($p<0.05$). Secondly, as potato tubers were packaged in water, some of the exterior water could diffuse into the potato tuber simultaneously with the release of water from its interior, counterbalancing the water loss.

Table 5 - Moisture of peeled potato tubers packaged in water, expressed in %, and total soluble solids present in the respective water samples, along the pressure.

Processing conditions (MPa/min)	Moisture (%)	Total soluble solids in exterior water (%)
0.1/2.5	83.0 ± 2.8 ^{a}	0.02 ± 0.01 ^{ab}
100/2.5	81.2 ± 3.9 ^{a}	0.01 ± 0.01 ^{a}
200/2.5	82.0 ± 1.6 ^{a}	0.08 ± 0.02 ^{bc}
300/2.5	77.6 ± 3.3 ^{a}	0.16 ± 0.04 ^{cd}
400/2.5	78.2 ± 2.5 ^{a}	0.21 ± 0.02 ^{d}
500/2.5	79.3 ± 2.6 ^{a}	0.24 ± 0.03 ^{d}

Results are expresses as the mean ± the standard deviation.
^{a,b,c,d} Different letters indicate significant differences ($p<0.05$) among the pressure treatment for the same evaluated parameter.

The results of total soluble solids (TSS) in the exterior water of peeled potato tubers are shown in **Table 5**. As the pressure intensity increased, the presence of TSS in water samples increased proportionally. Water samples of potato tubers pressurized at 100 MPa did not showed significant differences ($p<0.05$), but the application of pressures above of 200 MPa caused a significant increase ($p<0.05$) of up to 12-fold when compared to control samples. This increase of TSS may be due to the release of sugars and other soluble components which were in potato cells. As the increase of pressure lead to an increase of cell damage **(Oliveira et al., 2015; Sopanangkul et al., 2002)**, the components present in the interior of cells are released to the exterior water, which is greater as the greater the tissue damage.

1.5. Texture analysis of raw potato tubers

The texture analysis of unpeeled and peeled potato tubers packaged in water and under vacuum was performed immediately after pressure treatments. An example of a set of Texture Profile Analysis (TPA) graphic is present in **Figure 22** of **Annex V** and refers to peeled potato tubers packaged in water. By analysing TPA graphic of **Figure 22** and the results of **Table 6**, it is remarkable a decrease in the maximum force (firmness) of processed samples as the processing intensity increases, for all the tested conditions. Comparing the results for peeled pressurized potatoes, either for those packaged in water and vacuum, a significant reduction ($p<0.05$) was observed in samples subjected to pressures above 200 MPa. Moreover, there were no significant differences ($p<0.05$) between the packaging in water or under vacuum. In unpeeled potato tubers, a reduction in firmness ($p<0.05$) was observed from 100 MPa, and no changes ($p<0.05$) were observed between samples packaged in water or under vacuum.

These results are in accordance with some studies present in the literature. Several works evaluated the effect of pressure treatments in the texture of various solid vegetables and fruits, and reported that firmness was unaffected by HPP up to 100 MPa. At higher pressures (≥100 MPa), pressure treatments affect the organization of the parenchyma cells, leading to cell permeability, and even cell disruption, whose degree of cell disruption is dependent either of the applied pressure intensity or the type of plant cell **(Oey et al., 2008)**. The effect of HPP in whole potato tubers was studied in only one research work in the literature. **Saraiva and Rodrigues (2011)** subjected unpeeled potato tubers to 30, 50, and 100 MPa for 5 and 10 min, and the authors observed a significant decreased ($p<0.05$) in firmness and stiffness only for the tubers treated at 100 MPa, which corroborates the results obtained for unpeeled potato tubers treated at this pressure level. In relation to the remaining pressure tested, no data in the literature is available, but the decrease in firmness along the pressure was possibly due to the increase of cell permeability and damage of cell structure which, in turn, increased the softness of potato tissue.

In quantitative terms, peeled potato tubers packaged in water suffered a reduction of up to ~31% in firmness comparing to the control samples; peeled potatoes packaged under vacuum had a decrease of up to ~37%; unpeeled potato tubers packaged in water showed a diminution of up to ~36%; and a reduction of up to ~32% was observed in

unpeeled potato tubers packaged under vacuum. In all the conditions, the maximum reduction in the firmness was achieved at 400 MPa.

Table 6 - Maximum force of raw potato tubers (peeled/unpeeled potatoes packaged in water/ under vacuum) treated by different conditions of pressure (0.1-500 MPa), for 2.5 min.

Processing conditions (MPa/min)	Peeled + H2O	Peeled + vacuum	Unpeeled + H2O	Unpeeled + vacuum
0.1/2.5	50.63 ± 1.68 ^{cA}	53.36 ± 3.20 ^{dA}	49.97 ± 3.08 ^{cA}	49.44 ± 1.76 ^{cA}
100/2.5	50.12 ± 2.23 ^{cC}	52.87 ± 4.83 ^{dBC}	40.84 ± 3.70 ^{bA}	38.77 ± 9.31 ^{bAB}
200/2.5	42.38 ± 0.27 ^{bBC}	44.87 ± 3.03 ^{cdC}	37.02 ± 1.61 ^{abA}	37.86 ± 2.06 ^{abAB}
300/2.5	38.72 ± 1.31 ^{abA}	42.74 ± 3.34 ^{bcB}	36.51 ± 1.87 ^{abA}	35.32 ± 1.06 ^{aA}
400/2.5	35.03 ± 2.63 ^{aA}	33.40 ± 0.31 ^{aA}	32.03 ± 2.33 ^{aA}	33.87 ± 1.23 ^{aA}
500/2.5	36.15 ± 1.42 ^{aA}	34.44 ± 6.16 ^{abA}	33.49 ± 2.32 ^{aA}	34.12 ± 3.07 ^{aA}

Results are expresses as the mean ± the standard deviation.
^{a,b,c} Small different letters indicate significant differences ($p<0.05$) among the pressure treatment for the same sample condition; ^{A,B,C} Capital different letters indicate significant differences ($p<0.05$) among peeled samples for the same pressure treatment and among unpeeled samples for the same pressure treatment.

Through graphical representations of the percentage of maximum force (of pressurized potato tubers relatively to unpressurized ones) as a function of pressure, four linear representations were obtained and are shown in **Figure 12**. For peeled potato tubers, slopes of -0.07 and -0.09 were obtained, which means that an increase of 100 MPa caused a reduction of the maximum force in 7-9%. For unpeeled potato samples, slopes of -0.06 were obtained and it means that an increase of 100 MPa led to a decrease in the maximum force in only 6%. A possible justification for this result is that when potato peel is present, there is a higher resistance to the pressure and thereby the reduction of maximum force is lower than in peeled potato tubers. These results could be interesting for industrial application because, once the reduction of maximum force is directly proportional to the pressure intensity, it could be possible to select exactly the pressure condition which would provide a potato tuber with a desired softness for a specific application.

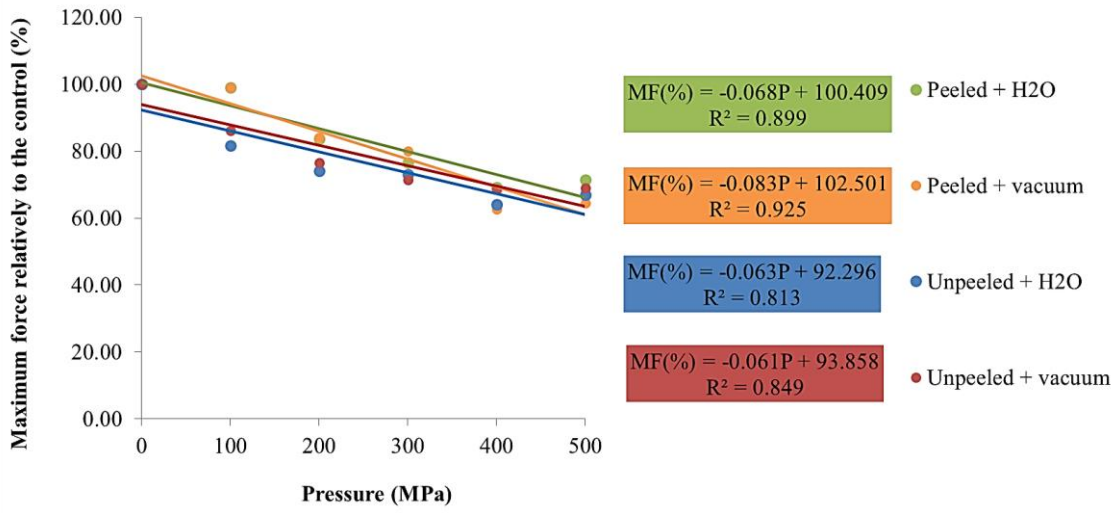

Figure 12 - Graphical representation of percentage of maximum force, relatively to the control, as a function of pressure, for peeled/unpeeled potato tubers packaged in water and under vacuum. "MF(%)" and "P" mean Maximum Force relatively to control (%) and Pressure (MPa), respectively.

2. Effect of pressure treatment on raw potato sticks

After characterization of whole potato tubers, raw potato sticks were subjected to pressure and then deep-fried. The results obtained in the pre-test with whole potato tubers were important to define the treatment conditions of potato sticks. Unpeeled potato tubers showed higher resistance to the pressure due to the presence of thicker cell walls in the potato peel, and thereby this condition was not selected for further tests. In relation to peeled potato tubers, the packaging in tap water is a more common and cheaper operation at industrial level than the packaging under vacuum. Considering the results obtained for this condition, potato tubers treated at 100 MPa showed lower changes ($p<0.05$) compared to control samples than those treated at ≥ 200MPa. For this reason, pressure levels above 200 MPa were selected for the treatment of potato sticks before deep-frying. Pressure of 600 MPa were also applied, for 2.5 and 10 min, to find out if either an elevated pressure level or a higher processing time led to more severe modifications, namely in starch gelatinization. Peeled raw potato sticks packaged in tap water and the respective exterior water were characterized as presented above.

2.1. Weight differences of potato sticks and exterior water samples after HPP

Weight difference after HPP was calculated for raw potato sticks and for the respective exterior water samples, whose results are present in **Table 7** and **Figure 23** (**Annex VI**). Comparing the results of the pressurized potato sticks with the unpressurized ones, it was detected a significant weight increase ($p<0.05$) only at 200 MPa. From this level of pressure, a reduction in weight gain was observed as the pressure intensity increased. Samples subjected to 300 and 400 MPa for 2.5 min showed a lower weight gain than those pressurized at 200 MPa for 2.5 min, but no differences were obtained compared to the control sticks. At higher pressure levels (500 and 600 MPa), potato sticks presented the lowest weight difference after HPP.

In relation to weight difference of the exterior water samples of peeled potato tubers, a contrary behavior was observed compared to that of potato samples. In other words, the water samples showed negative weight differences which decreased (became more positive) over the pressure. The exterior water samples of potato sticks processed at 200 MPa presented the highest weight loss (-6.8 ± 0.8%), while those of potato sticks subjected to 600 MPa for 2.5 min showed the lowest weight loss (-2.6 ± 1.7%).

Table 7 - Weight difference, expressed in %, of potato sticks and the respective exterior water samples, relatively to initial weight, along the pressure.

Processing conditions (MPa/min)	Potato sticks weight difference (%)	Exterior water weight difference (%)
0.1/2.5	5.2 ± 0.8 [b]	-4.7 ± 0.7 [ab]
200/2.5	11.5 ± 1.3 [c]	-6.8 ± 0.8 [b]
300/2.5	6.4 ± 1.8 [b]	-5.1 ± 0.3 [ab]
400/2.5	6.1 ± 0.9 [b]	-4.8 ± 0.6 [ab]
500/2.5	0.9 ± 1.4 [a]	-3.4 ± 1.3 [ab]
600/2.5	0.4 ± 1.9 [a]	-2.6 ± 1.7 [a]
600/10	-1.0 ± 1.9 [a]	-2.8 ± 2.0 [a]

Results are expressed as the mean ± the standard deviation.
[a,b,c] Different letters indicate significant differences ($p<0.05$) among the pressure treatment for the same evaluated parameter.

The results obtained for raw potato sticks were similar to those obtained for whole potato tubers and thereby justifications are identical. That is, as the intensity of pressure treatment increased, potato sticks lost components that, in turn, solubilized in water and led to an increase in water weight.

2.2. Scanning Electronic Microscopy (SEM) observations

SEM images of potato tubers treated by pressure at 0.1, 200, 400 and 600 MPa for 2.5 min were captured, and were compiled in **Figure 13**. The main goal of this analysis was to assay visual changes in potato tissue caused by HPP treatments, and thus, samples processed at lower, medium and higher pressures were analysed.

Images on the left were captured with a magnification of x100, allowing a global perception of potato tissue, and images on the right were observed with x400 of magnification, allowing a more detailed observation of starch granules. All samples exhibited the presence of fosses (large empty cavities) and starch granules (spherical structures). Possibly, fosses represented spaces that were occupied by water, and after lyophilization of potato sticks, these spaces became empty. Unpressurized samples (**Figure 13 – A1-A2**) showed a more structured tissue, with higher amount of fosses and lower amount of spread starch granules. In addition, starch granules of these samples seemed smaller than starch granules of the pressurized samples. As the pressure intensity increased, the relative amount of fosses seemed to reduce, and the size and amount of starch granules seemed to increase, probably because of the textural and cellular changes induced by HPP, which caused the release of intracellular material to the extracellular

environment, filling the empty spaces. The largest changes occurred at 600 MPa (**Figure 13 – D1-D2)**, since these samples exhibited a lot of material spread through the potato tissue, low amount of fosses, and large and burst starch granules. These results are in accordance with the study of **Błaszczak et al. (2005)**, in which SEM images showed that a pressure treatment of 600 MPa for 2 and 3 min led to changes in the granule structure of potato starch.

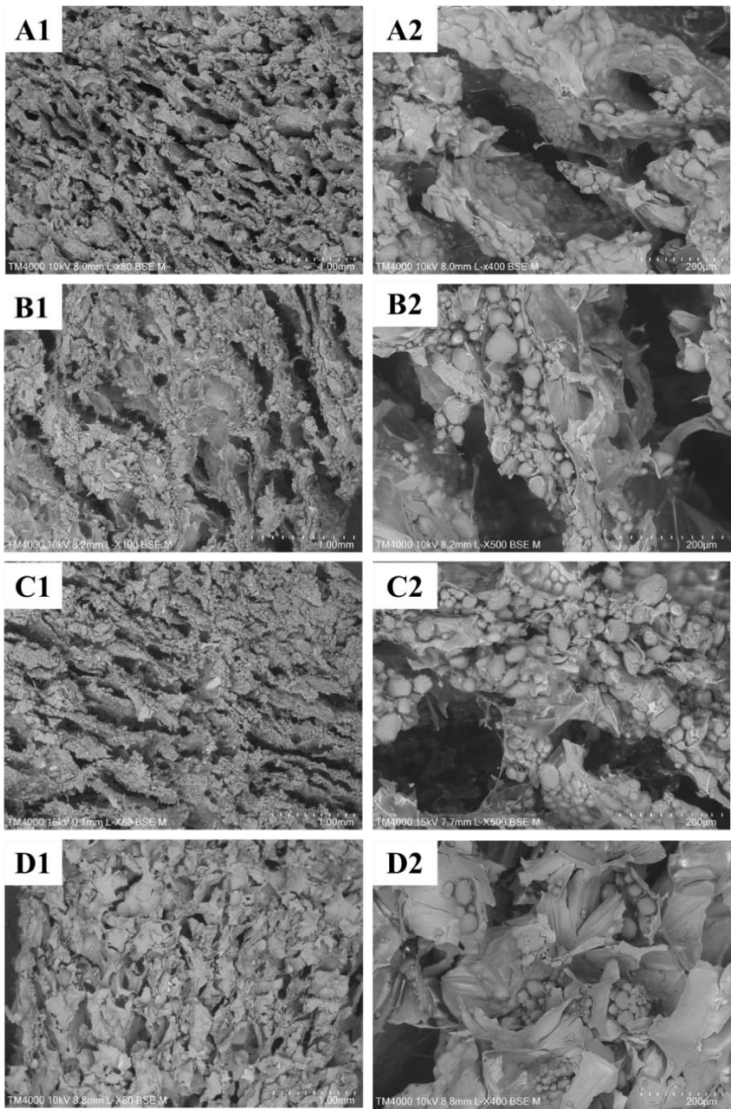

Figure 13 - SEM images of potato sticks pre-treated at (A) 0.1 MPa; (B) 200 MPa; (C) 400 MPa; and (D) 600 MPa. Images on the left (numbered with 1) correspond to images of potato tissue observed with a magnification of x100, and images on the right (numbered with 2) correspond to images captured at x400 of magnification.

2.3. Effect of HPP on gelatinization properties of raw potato sticks

DSC is an important analysis to assay thermal properties of starchy foods, once provides information about the level of starch gelation, as well as the composition and organization of the crystalline structure in the starch granules. In the literature, there are few studies evaluating the effects of HPP on fresh tubers, since most are focused on pure or isolated starch **(Karlsson and Eliasson, 2003; Kawai et al., 2012; Pei-Ling, 2012)**.

One replicate of potato sticks treated at 0.1, 200, 400, and 600 MPa for 2.5 min were analyzed by DSC in order to assess if the pressure level and time of processing caused changes in gelatinization properties of potato tubers. **Figure 24** of **Annex VII** shows the DSC curves obtained for each sample, and the respective gelatinization (onset and peak) temperatures and enthalpy of gelatinization (ΔH) are exposed in **Table 8**. As the pressure intensity increased, onset and peak temperatures showed a tendency to decrease. As opposed to samples treated up to 400 MPa, those subjected to 600 MPa exhibit a considerable increase in ΔH of about 1.7-fold compared to control samples.

Pei-Ling, (2012) reported a decrease in gelatinization temperatures and ΔH in starch (waxy corn and tapioca starch) suspensions treated at 450-600 MPa, suggesting that pressure had effect on physicochemical properties of native starch by modifying the microstructure of starch granules, facilitating granule hydration (swelling), and thus inducing gelatinization. **Błaszczak et al. (2005)** also found out similar results in potato starch-water suspensions subjected to 600 MPa for 2 and 3 min, observing either a decrease in gelatinization temperatures or ΔH of pressurized samples. Although the majority of HPP-treated starch granules had retained their granular shape, the inner part of the granule was almost completely filled with a gel-like structure which might result from hydration of the amorphous phase and/or melting of the crystalline structures. **Karlsson and Eliasson (2003)** compared the thermal (gelatinization and retrogradation) properties of distinct potato tissue zones and starch isolated from different potato tuber zones. Peak temperature values were in a similar range to those obtained (~70 ºC), but ΔH values were higher (~8-14 J/g). The various parts of potato tissue showed higher differences in gelatinization temperatures than the isolated starch, and thereby the authors suggested that, although starch is the main solid constituent, other components like cell walls, proteins and pectin may have impact in thermal properties of potato tuber. The potato tuber size did not affect the gelatinization properties. **Oliveira et al. (2015)** observed that different tubers exhibited distinct thermal properties after HPP (600 MPa

for 5 and 30 min), probably due to the differences among tubers starches. The authors found out that the increase in process time from 5 to 30 min increased the percentage of starch gelatinization, which was represented by a reduction in ΔH values. Tubers with type-B starches presented smaller variations of gelatinization temperatures, between the control and pressurized samples, than tubers containing type-A or C starches. **Kawai et al. (2012)** studied not only the HPP-induced gelatinization but also retrogradation in potato starch-water mixtures. Pressure treatments can gelatinize starch even at low temperatures, but retrogradation may also be induced by HPP at low temperatures, and immediately after the pressure treatment. Although there are few studies in the literature about this, amylose gelation had been suggested as an explanation for the occurrence of HPP-induced retrogradation. The authors concluded that HPP at temperatures below 50 ºC can produce HPP-gelatinized and/or HPP-retrograded starch.

Comparing the obtained results with the studies reported in the literature and, taking into account that the higher differences observed were only in ΔH value of potato sticks treated at 600 MPa, it evidenced that this is the required pressure to induce changes in starch structure of potato tubers. At pressures below 600 MPa, there were no distinguished modifications since potato tuber starch belongs to the type-B starch, which is more baroresistant than other starches **(Stute et al., 1996)**. As described previously, HPP-induced gelatinization is represented by a decrease in gelatinization temperatures and ΔH. However, ΔH of potato sticks treated at 600 MPa for 2.5 min increased in relation to control samples, which means that this HPP conditions induced starch gelatinization , which was immediately followed by starch retrogradation, being in agreement with what has been reported in the study of **(Pei-Ling, 2012)**. Although retrogradation is frequently associated with the loss of quality of gelatinized starchy foods, it can have potential commercial applications in the use of retrograded starch as resistant starch.

Table 8 - Gelatinization properties (onset and peak temperature, and enthalpy of gelatinization) of raw potato sticks treated by pressure, measured by DSC.

Processing conditions (MPa/min)	Onset temperature (ºC)	Peak temperature (ºC)	ΔH (J/g)
0.1/2.5	70.17	72.46	2.94
200/2.5	69.66	72.13	2.98
400/2.5	68.56	71.47	3.10
600/2.5	67.26	69.68	5.19

2.4. Effect of HPP on texture of raw potato sticks

The effect of HPP on the texture of raw potato sticks was also assayed, but the applied method was different from that used for whole potato tubers. Thus, the values obtained for each parameter cannot be compared with those obtained for whole potato tubers. Firmness, energy for cutting (N.mm), and stiffness (N/mm) were the parameters determined from the force-displacement curves (**Figure 25** of **Annex VIII**), and results are shown in **Table 9**. Firmness (N) was determined as the maximum force applied to cut the potato stick; energy for cutting (N.mm) was calculated as the area below the force-displacement curve; and stiffness (N/mm) as the slope of the linear portion of the respective graphic.

Table 9 - Results from texture analysis (by using a cutting knife) of raw potato sticks treated by HPP.

Processing conditions (MPa/min)	Firmness (N)	Energy for cutting (N.mm)	Stiffness (N/mm)
0.1/2.5	10.38 ± 0.57 ^{b}	38.20 ± 1.64 ^{c}	10.39 ± 0.46 ^{b}
200/2.5	7.52 ± 0.54 ^{a}	29.92 ± 2.82 ^{b}	9.43 ± 0.24 ^{b}
300/2.5	6.74 ± 0.37 ^{a}	22.33 ± 0.51 ^{a}	7.44 ± 0.88 ^{a}
400/2.5	7.15 ± 0.64 ^{a}	22.22 ± 2.82 ^{a}	6.44 ± 0.58 ^{a}
500/2.5	7.48 ± 0.06 ^{a}	21.25 ± 0.70 ^{a}	6.95 ± 0.35 ^{a}
600/2.5	7.75 ± 0.93 ^{a}	20.29 ± 1.52 ^{a}	6.65 ± 0.91 ^{a}
600/10	7.66 ± 0.59 ^{a}	20.92 ± 2.48 ^{a}	6.73 ± 0.95 ^{a}

Results are expressed as the mean ± the standard deviation.
^{a,b,c} Different letters indicate significant differences ($p<0.05$) among the pressure treatment for the same evaluated parameter.

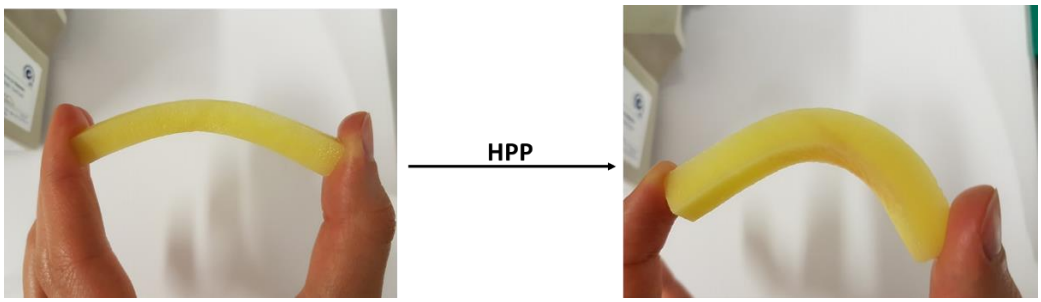

Figure 14 - Visual differences between the appearance and flexibility of a potato stick before HPP (image on the left) and after HPP (image on the right).

By analysing the profile of the force-displacement curves for each pressure condition (**Figure 25** of **Annex VII**), it was possible to note clear differences between the behaviour of control (unpressurized) and pressurized samples. The first ones showed

higher force values along the texture assay, as well as a higher area below the force-distance curve than pressurized samples. In opposition to control samples, the treated ones presented a distinct force peak, and its value was lower (about 25-35%) than maximum forces obtained for unpressurized potato sticks ($p<0.05$). Moreover, the maximum force of treated samples did not change as the pressure severity increased ($p<0.05$), which means that the perforation of the potato tissue required a similar force, independently of the pressure applied. As soon as the plant tissue was perforated, the average force required to continue cutting the interior of potato sticks decreased as the pressure increased, and consequently, the corresponding area below the curve decreased as well. In practical terms, the energy needed for cutting potato sticks was reduced over the pressure, as evidenced by the values of **Table 9**, up to ~47% (achieved by applying 600 MPa for 2.5 min). In addition, the slope of the elastic part of the force-distance curves (a measure of the stiffness) suffered some changes as the pressure severity increased. At pressures equal to or greater than 300 MPa, the stiffness of potato sticks decreased about 28-38% ($p<0.05$), which means that their rigidity was reduced, and their elasticity and flexibility was increased. This result was visually perceived, as evidenced in **Figure 14**. Furthermore, treated potato sticks showed a smoother and slippery surface than non-treated samples.

Another interesting observation consisted in the slight increase of firmness and stiffness in potato sticks treated at 500 and 600 MPa for 2.5 min, compared to the other pressure levels. In addition, increasing the time of process from 2.5 to 10 min at 600 MPa also led to a higher firmness and stiffness. Correlating this textural result with results obtained in DSC analysis, it is probable that this increase is due to HPP-induced retrogradation.

These results are in accordance with some studies present in the literature. In the previous section, it was reported that pressure levels equal to or higher than 100 MPa had led to textural changes in several solid vegetables and fruits, causing reductions in their firmness **(Oey et al., 2008; Oliveira et al., 2015)**. A few studies in the literature have evaluated the effect of HPP on the texture of potato sticks/cubes. **Eshtiaghi and Knorr (1993)** subjected potato cubes (2x2x2 cm) to 400 MPa for 15 min, and observed a decrease in firmness, which was similar to the loss of firmness achieved by blanching in boiling water. **Al-Khuseibi et al. (2005)** immersed potato cubes (1.5x1.5x1.5 cm) were immersed in 1% citric acid solution and processed at 400 MPa for 15 min. The authors

found no changes in the texture of potato cubes compared to fresh potatoes, which were possibly due to the calcium bridges formed with pectin after demethylation.

Moreover, the effect of another non-thermal technologies on the texture of raw potatoes were reviewed by **Dourado et al. (2019)**, and it was found that pulsed electric fields technology is one of the non-thermal technology with more studies in this area. Actually, **Ignat et al. (2015)** showed that a reduction of 35% in the energy for cutting and a decrease in the firmness were achieved in PEF-treated potato sticks. Comparing with the obtained result, HPP-treated potato sticks (≥ 300 MPa) showed reductions of 42-47% in the energy for cutting, a higher value than that obtained by PEF. Besides, **Fauster et al. (2018)** observed that the PEF pre-treatment of potato sticks led to an increase in softness of potato sticks, as well as the smoothness of the potato surface. The reasons given for these results were similar to those described in the studies with HPP, that is, PEF induced an increase of potato cell permeabilization and a reduction in the cell turgor pressure. Therefore, HPP is an alternative technology to PEF, causing similar modifications in potato tubers, and simultaneously a lower required energy to cut them.

2.5. Effect of HPP on colour of raw potato sticks

Colour of potato sticks treated by HPP was determined and it was expressed in lightness (L^*), green to red (a^*) and blue to yellow (b^*) parameters, whose results are shown in **Table 10**. Although no significant changes ($p<0.05$) were observed in all parameters, among the different samples, a tendency of reduction was detected, that is, HPP-treated potato sticks shown lower values of L^*, a^* and b^* than non-treated samples. This means that pressure treatments induced a general discolouration. The colour of foods is an important physical attribute which has influence in consumer decision or acceptance as well as provides information about the product quality, such as degree of ripeness, and product alteration **(Terefe et al., 2014)**. According to **Oey et al. (2008)**, HPP (at low and moderate temperatures) had shown a limited effect on pigments (such as carotenoids, anthocyanins, and phenolic compounds) that are responsible for the colour of fruits and vegetables. However, colour changes can be related to changes in textural properties of HPP-treated vegetables and fruits, and may occur due to incomplete inactivation of enzymes (such as the oxidative enzymes peroxidase, POD, and polyphenol oxidase, PPO) and microorganisms, and consequently result in undesired enzymatic or non-enzymatic reactions in thee food matrix. As already stated, HPP induces alterations in plant tissues,

which lead to the disruption of cellular compartments, and consequently increase the contact between oxidative enzymes and substrates. Moreover, potato PPO has been shown to be resistant to pressure, with no significant inactivation after 13 min of pressure treatments at 100 – 500 MPa, with a temperature of -26 – 20 ºC, either in cell-fresh extracts or in potato pieces **(Van Buggenhout et al., 2006)**.

For all these reasons, the discolouration observed in HPP-treated potato sticks was probably due to the potato cells damaged caused by HPP, which induced the contact between PPO and its substrates (O_2 and phenols). As potato PPO was not inactivated by pressure, enzymatic and non-enzymatic reactions resulted in the formation of darker polymers (melanins), and consequently in a slight discolouration of potato tissue. Anyway, this modification appeared moderated and did not affecting the fresh-like appearance of potato sticks, as clearly observed in images **Figure 14**.

Table 10 - Colour of raw potato sticks treated by pressure, showing the results obtained for *L**, *a**, *b**, and ΔE parameters.

Processing conditions (MPa/min)	*L**	*a**	*b**	ΔE
0.1/2.5	71.2 ± 3.8 ^{a}	1.2 ± 0.2 ^{abc}	34.9 ± 2.7 ^{a}	
200/2.5	64.9 ± 0.8 ^{a}	1.7 ± 0.4 ^{c}	32.5 ± 0.7 ^{a}	6.8 ± 0.8 ^{a}
300/2.5	67.3 ± 2.7 ^{a}	1.6 ± 0.2 ^{bc}	33.1 ± 2.2 ^{a}	4.5 ± 3.1 ^{a}
400/2.5	68.9 ± 1.5 ^{a}	1.4 ± 0.5 ^{abc}	32.3 ± 0.4 ^{a}	3.6 ± 1.1 ^{a}
500/2.5	67.6 ± 2.8 ^{a}	1.4 ± 0.2 ^{abc}	31.5 ± 2.6 ^{a}	5.1 ± 3.4 ^{a}
600/2.5	69.5 ± 1.3 ^{a}	0.6 ± 0.5 ^{a}	29.5 ± 0.9 ^{a}	5.8 ± 0.7 ^{a}
600/10	70.7 ± 2.6 ^{a}	0.7 ± 0.2 ^{ab}	29.6 ± 3.6 ^{a}	5.6 ± 3.8 ^{a}

Results are expresses as the mean ± the standard deviation.
^{a,b,c} Different letters indicate significant differences ($p < 0.05$) among the pressure treatment for the same evaluated parameter.

2.6. Effect of HPP on moisture of raw potato sticks

In a first approach, moisture of raw potato sticks subjected to 0.1, 200, 300, 400, 500 and 600 MPa for 2.5 min, and 600 MPa for 10 min (initial defined conditions) were measured, and results are shown in **Table 11** designated by "Initial" test. Simultaneously, ºBrix of the exterior water were also determined and results are presented in the same table. It was possible to conclude that no changes in the moisture content were induced by pressure treatments ($p<0.05$). However, one objective of this study was to try to achieve fried potatoes with lower oil content by treating raw potato sticks with HPP.

Oliveira et al. (2015) observed an increase in drying rate of HPP-treated tubers. As frying is mainly a drying process, the higher the drying rate of potato sticks, the faster the loss of water, and consequently, the more the oil is absorbed **(Ziaiifar et al., 2008)**. For this reason, a strategy to reduce the final oil content in fried potato sticks could be by increasing the initial moisture of raw potato sticks. Indeed, **Ignat et al. (2015)** treated raw potato sticks with pulsed electric fields (PEF) and observed that PEF-treated sticks exhibited higher initial moisture content lost lower water during frying, and consequently fried potato sticks presented lower oil content. For this reason, some extra tests were performed to evaluate if pressure or/and time of processing, time of potato sticks in water after HPP, proportion of potato stick: water (g/g), and saline concentration in exterior water had influence on moisture of potato sticks. The following conditions were tested:

(A) 0.1, 50 and 100 MPa for 2.5 min. Potato sticks were removed from water after 0 and 25 min of the end of processing;

(B) 0.1, 200 MPa for 2.5 min and 400 MPa for 2.5, 10 and 20 min. Potato sticks were removed from water after 0 min of the end of processing;

(C) 0.1 and 400 MPa for 2.5 min. Potato sticks were removed from water after 0, 5, 10, 15, 20, 30, 45, 60, 80, 100, 120, 150 and 180 min of the end of processing;

(D) 0.1 and 400 MPa for 2.5 min with a proportion of potato:water of 1:5 (g/g). Potato sticks were removed from water after 0 and 25 min of the end of processing;

(E) 0.1, 200 and 400 MPa for 2.5 min, with a saline concentration in exterior water of 1g/100 mL and 5g/100 mL. Potato sticks were removed from water after 45, 90 and 120 of the end of processing.

Moisture of potato sticks was measured for tests A, B, C, D and E, and ºBrix of exterior water samples were measured for tests A, B, C and D. Results are present in **Table 11**.

As previously mentioned, experimental conditions tested initially did not changed the amount of water in raw potato sticks ($p<0.05$). However, the increase in pressure intensity led to an increase release of soluble solids for the involving water.

When lower pressures were tested (test A), it was observed that neither moisture content of potato sticks nor ºbrix of exterior water were modified ($p<0.05$), comparatively to control samples, probably because there were no significant changes in the texture of potato sticks subjected to pressures below 100 MPa. Thus, treatments at lower pressures did not prove to be a great strategy to retain water in potatoes.

Regarding test B, moisture of potato samples did not change significantly ($p<0.05$), but it was observed a slight tendency to decrease as the processing time (at 400 MPa) increased. On the other hand, the amount of soluble sugars in water increased both with the intensity of pressure and with the processing time. Therefore, the increase of processing time was also not a good solution.

When test C was performed, potato sticks subjected at 400 MPa exhibited a percentage of moisture equal to or lower than unpressurized potato sticks, over the three hours after the treatment. In contrast to untreated samples, the amount of soluble solids increased continuously in the exterior water of HPP-treated potatoes over time after the end of processing. So, the time of potato sticks in water after processing proved to be a good strategy to increase the starch leaching, but not a good strategy for retaining water in potatoes.

In test D, although no statistical difference had been detected ($p<0.05$), potato sticks presented a slight decrease in moisture content compared to control samples, either after 0 or 25 min of the end of HPP. Relatively to the amount of soluble sugars in water, it was observed that ºBrix of the exterior water of HPP-treated potato sticks had a higher value than the exterior water of control samples. Thus, packaging potato sticks with a higher proportion of potato:water (g/g) did not showed to be a good solution.

Finally, tests (E and F) with addition of sodium chloride (1 and 5g/100mL) showed that moisture content of potato sticks did not changed significantly ($p<0.05$) when the pressure intensity was increased, but there was a decrease ($p<0.05$) over the time of potato sticks in water after HPP. These results were observed either for a saline concentration of 1g/100mL or for 5g/100mL. In addition, comparing the results for both tests, it was observed that as the saline concentration increased, the amount of water in potato sticks decreased. Probably, as the concentration of sodium and chloride ions increased, more water molecules were bound and captured for the exterior solution, decreasing the moisture content of potato sticks. For this reason, the salt addition to the exterior water was not a good strategy to maintained water in potatoes.

Table 11 - Moisture of raw potato sticks and ºBrix of exterior water of initial, A, B, C, D and E tests.

Test	Processing conditions (MPa/min)	Time in water after HPP (min)	Moisture (%)	º Brix (%)
Initial	0.1/2.5	25	84.45 ± 0.67 [a]	0.28 ± 0.03 [a]
	200/2.5		83.75 ± 0.71 [a]	0.40 ± 0.00 [b]
	300/2.5		84.73 ± 0.23 [a]	0.53 ± 0.05 [c]
	400/2.5		83.85 ± 1.87 [a]	0.58 ± 0.04 [c]
	500/2.5		84.03 ± 2.30 [a]	0.63 ± 0.05 [cd]
	600/2.5		84.53 ± 1.78 [a]	0.72 ± 0.04 [de]
	600/10		84.53 ± 1.58 [a]	0.75 ± 0.05 [e]
A	0.1/2.5	0	85.17 ± 0.93 [a]	0.30 ± 0.00 [a]
	50/2.5		84.73 ± 1.42 [a]	0.30 ± 0.00 [a]
	100/2.5		84.74 ± 1.23 [a]	0.30 ± 0.00 [a]
	0.1/2.5	25	83.91 ± 1.00 [a]	0.30 ± 0.00 [ab]
	50/2.5		85.23 ± 0.64 [a]	0.27 ± 0.06 [a]
	100/2.5		84.50 ± 1.19 [a]	0.30 ± 0.00 [ab]
B	200/2.5	0	82.89 ± 1.26 [a]	0.37 ± 0.03 [b]
	400/2.5		84.61 ± 1.15 [a]	0.43 ± 0.03 [c]
	400/10		82.73 ± 1.40 [a]	0.53 ± 0.03 [d]
	400/20		82.50 ± 0.85 [a]	0.58 ± 0.03 [d]
C	0.1/2.5	0	83.90	0.2
		5	85.49	0.3
		10	84.59	0.3
		15	84.89	0.3
		20	86.60	0.3
		30	87.39	0.3
		45	85.76	0.2
		60	85.32	0.3
		80	84.38	0.2
		100	84.29	0.2
		120	82.95	0.2
		150	84.68	0.2
		180	84.76	0.2
	400/2.5	0	83.20	0.4
		5	82.92	0.4
		10	84.15	0.5
		15	83.66	0.6
		20	82.74	0.6
		30	85.11	0.6
		45	81.50	0.7
		60	83.91	0.7
		80	82.62	0.8
		100	84.74	0.9
		120	81.04	0.9
		150	84.24	1.0
		180	83.55	1.0

Results are expressed as the mean ± the standard deviation, except for test C (only 1 replicate was analysed). [a,b,c,d] Different letters mean significant differences ($p<0.05$) among the pressure treatment for the same time of potatoes in water after HPP.

Table 11 (Cont.) - Moisture of raw potato sticks and ºBrix of exterior water of initial, A, B, C, D and E tests.

Test	Experimental conditions	Time in water after HPP (min)	Moisture (%)	º Brix (%)
D	0.1/2.5	0	83.95 ± 0.56 ^{a}	0.30 ± 0.00 ^{a}
	400/2.5		82.80 ± 1.55 ^{a}	0.38 ± 0.03 ^{bc}
	0.1/2.5	25	84.08 ± 0.76 ^{a}	0.28 ± 0.03 ^{a}
	400/2.5		83.47 ± 3.43 ^{a}	0.40 ± 0.00 ^{b}
E [salt]= 1g/100mL	0.1/2.5	45	83.90 ± 0.63 ^{aC}	
	200/2.5		82.64 ± 2.50 ^{aBC}	
	400/2.5		80.80 ± 1.14 ^{aABC}	
	0.1/2.5	90	83.85 ± 1.20 ^{aB}	
	200/2.5		82.81 ± 1.60 ^{aB}	
	400/2.5		81.76 ± 1.36 ^{aB}	
	0.1/2.5	120	81.11 ± 2.19 ^{aA}	
	200/2.5		82.93 ± 2.23 ^{aA}	
	400/2.5		81.92 ± 1.37 ^{aA}	
E [salt]= 5g/100mL	0.1/2.5	45	78.11 ± 1.10 ^{aAB}	
	200/2.5		78.57 ± 2.72 ^{aAB}	
	400/2.5		76.58 ± 0.43 ^{aA}	
	0.1/2.5	90	77.37 ± 0.67 ^{aA}	
	200/2.5		75.54 ± 1.00 ^{aA}	
	400/2.5		78.02 ± 1.78 ^{aA}	
	0.1/2.5	120	77.59 ± 2.42 ^{aA}	
	200/2.5		76.54 ± 3.19 ^{aA}	
	400/2.5		77.78 ± 3.01 ^{aA}	

Results are expressed as the mean ± the standard deviation. In test D, ^{a,b,c} different letters mean significant differences ($p<0.05$) among the pressure treatment for the same time of potatoes in water. In test E, ^{a,b,c} small different letters mean significant differences ($p<0.05$) among the pressure treatment for the same salt concentration; ^{A,B,C} capital different letters mean significant differences ($p<0.05$) among the pressure treatment and saline concentration for the same time of potatoes in water after the end of HPP. Empty spaces mean that no analysis was performed on the exterior water of the respective samples.

2.7. Total soluble solids in exterior water of potatoes

The exterior water samples of potato sticks were analysed in relation to their total soluble solids. Previously, the results of soluble sugars in water, represented as ºBrix, were presented (**Figure 15**) and it was observed an increase in their values both as the pressure severity and time processing increased, achieving a maximum of 0.75% in samples treated at 600 MPa for 10 min. Simultaneously, the percentage of total solids solubilized in water samples was also calculated, but applying another method. For the measurement of ºBrix, a refractometer was used, and for the measurement of total soluble solids, water samples were dried, and calculation was performed by mass differences. Probably because of this reason, different values were obtained comparing both methods.

Total soluble solids also exhibited an increase as the pressure intensity increased, but a maximum of 0.41% was achieved for samples subjected to 600 MPa for 10 min. Interestingly, this increase has a linear behaviour, that is the percentage of total solids dissolved in water increases proportionally with the pressure intensity, as shown in **Figure 15** (graphic on the right).

In the literature, no data were found for potato samples treated by HPP. However, studies with another non-thermal technology (PEF) showed similar results. That is, **Ignat et al. (2015)** pre-treated potatoes with PEF and visually observed an increase in turbidity of the aqueous phase after treatment. The authors suggested that PEF induced the release of intracellular cytoplasm solution to the extracellular environment due to the cell membrane disintegration and the increase of cell membrane permeabilization. Probably, mechanisms underlying HPP treatment are also related to the increase of cell wall damage and cell permeability, which enhances intracellular liquid release, and consequently the percentage of soluble solids/sugars in the exterior water of potato sticks. This effect is incremented when higher cellular damages are achieved, *i.e.*, when more severe pressure treatments are applied.

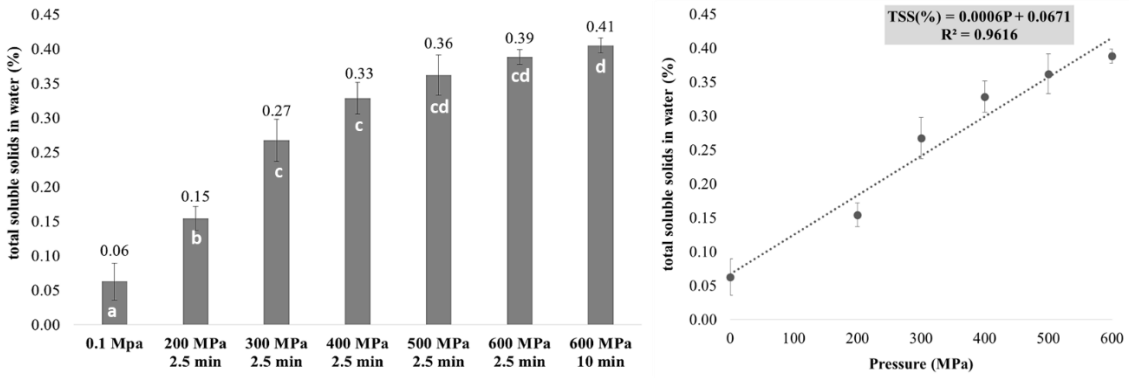

Figure 15 - Graphical representation on the left shows total soluble solids, expressed in %, of exterior water of potato sticks processing before frying, over the pressure. Graphical representation on the right shows the respective linearization. "TSS(%)" and "P" mean Total Soluble Solids in the exterior water, expressed in %, and Pressure (MPa), respectively.
[a,b,c,d] Different letters indicate significant differences ($p<0.05$) among the pressure treatment.

2.8. Effect of HPP on reducing sugars content of raw potato sticks

Reducing sugars are considered a precursor for the formation of acrylamide during frying of potatoes. As it was observed, the increase of pressure intensity led to an increase in the release and solubilization of sugars for the exterior water of potato sticks. Thus, a

question appeared: Did this starch leaching affect the final content of reducing sugars in potatoes? For this reason, reducing sugars content of HPP-treated potato sticks was measured and results are shown in **Table 12**.

First, reducing sugars content of all samples (pressurized and unpressurized) were about 0.6-0.8 g/Kg and no significant change ($p<0.05$) was observed. This results are in accordance with those of the study of **Biedermann-Brem et al. (2003)**, which obtained a value of 0.7 g/Kg of reducing sugars in potatoes of Agria variety. Besides, the same authors suggested that potatoes with 0.2-1.0 g/Kg of reducing sugars were the most suitable for roasting and frying. Therefore, samples used in this study belonged to an appropriate potato variety to produce fried potato products with low levels of acrylamide (<500 µg/Kg), without compromising browning and flavor.

Comparing this result with another study where PEF was used for the treatment of potato sticks, it was possible to observe that **Janositz et al. (2011)** also verified an enhance in the release of reducing sugars after PEF processing, but simultaneously they detected a reduction of one-third and almost half of the fructose and glucose content, respectively. Thus, although a release of reducing sugars had been observed either in HPP or PEF treatments, it was not enough to cause a significant reduction in reducing sugars content of HPP-treated potato sticks ($p<0.05$). A hypothetical strategy to reduce the reducing sugars concentration of potatoes could be through the infusion of an enzyme capable of reacting with these acrylamide's precursors, such as an oxireductase.

Table 12 - Reducing sugars content of raw potato sticks treated by pressure, expressed in g/100g of raw potato.

Processing conditions (MPa/min)	Reducing sugars (g/100g)
0.1/2.5	0.68 ± 0.10^{abc}
200/2.5	0.65 ± 0.09^{ab}
300/2.5	0.81 ± 0.09^{bc}
400/2.5	0.58 ± 0.06^{bc}
500/2.5	0.67 ± 0.18^{abc}
600/2.5	0.60 ± 0.08^{a}
600/10	0.85 ± 0.12^{c}

Results are expresses as the mean ± the standard deviation.
[a,b,c] Different letters mean significant differences ($p<0.05$) among the pressure treatment.

3. Effect of pressure pretreatment on quality of fried potato sticks

3.1. Weight difference after frying of potato sticks

Potato sticks were weight before and after frying, and their weight loss were calculated. The obtained results are shown in **Figure 16**, and it was verified that a loss of about 50% was obtained for all samples after frying at 180 ºC for 7 min. Although no changes ($p<0.05$) were observed between fried potatoes whose raw potato sticks were treated by HPP and those that were not treated, a slight increase of weight loss was detected in fried potato sticks pretreated by HPP.

As stated previously, frying is mainly a drying procedure based on both heat and mass transfer, resulting in a series of physico-chemical, structural and nutritional changes, including water removal and oil uptake of potato strips **(Aguilera and Gloria-Hernandez, 2000)**. For this reason, the amount of water loss is a factor that affect the amount of oil absorbed, that is the more the water removal from the potato surface, the more the oil uptake. Indeed, evaporation of the surface water leads to the formation of cavities and the frying oil occupies those voids spaces left by the escaping water **(Gamble et al., 1987; Mehta and Swinburn, 2001; Rice and Gamble, 1989)**. Thus, the slight increase of weight loss by fried potato sticks pretreated by HPP means that HP treatments induced the removal of a little more water, comparatively to control samples. Possibly, it is related with textural changes in raw potato sticks caused by HPP, which accelerated drying processes (including frying) and facilitates the release of water.

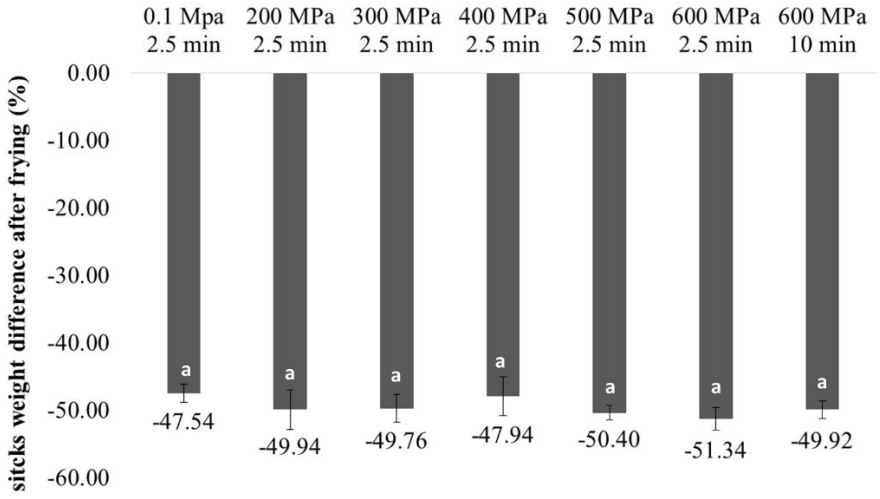

Figure 16 - Weight difference of potato sticks after frying, whose raw potato sticks were pre-treated with HPP.
[a] Different letters indicate significant differences ($p<0.05$) among the pressure treatment.

3.2. Effect of HPP pretreatment on moisture of fried potatoes

The percentage of moisture of fried potato sticks was measured in order to assay if differences observed previously caused distinguished changes in the amount of water of fried potatoes. Results are shown in **Table 13**, and although no significant differences ($p<0.05$) were observed between pre-pressurized and control samples, it is possible to verify that pre-treated potato sticks exhibited lower percentage of moisture than non-treated potato sticks. Actually, it proved that changes caused by HPP on raw potatoes had influence on water content of fried potatoes.

Table 13 - Moisture, expressed in %, of fried potatoes, whose raw potato sticks were treated by pressure before frying.

Processing conditions (MPa/min)	Moisture (%)
0.1/2.5	62.04 ± 1.70 [a]
200/2.5	59.66 ± 1.62 [a]
300/2.5	58.95 ± 5.93 [a]
400/2.5	59.75 ± 1.71 [a]
500/2.5	60.80 ± 2.01 [a]
600/2.5	59.32 ± 0.74 [a]
600/10	60.00 ± 2.41 [a]

Results are expresses as the mean ± the standard deviation.
[a] Different letters indicate significant differences ($p<0.05$) among the pressure treatment.

3.3. Effect of HPP pretreatment on texture properties of fried potatoes

Fried potato sticks were analysed in relation to their texture, and this property could be determined by instrumental methods or sensory evaluation. Once instrumentations techniques have an easier interpretation of the results, lower costs and complexity, and do not need to lead with human responses **(Miranda and Aguilera, 2006)**, the texture evaluation of fried potato sticks were performed through an instrumental method. Besides, destructive methods are preferred since they are usually better related to sensorial responses **(Miranda and Aguilera, 2006)**, and thereby texture analysis were carried out by using a knife. In **Table 14**, the results of hardness (maximum force required to cut a fried potato) are exhibited.

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 large hardness,

which result from the low moisture content induced by frying **(Vincent, 1998; Yee and Bussell, 2007)**. Analysing the obtained results, no significant change ($p<0.05$) was detected in hardness values among the different samples. However, it was possible to observe a slight increase of hardness in fried potatoes pre-treated by HPP, compared to control samples, which means that crispness of fried potatoes slightly increased as a consequence of HP treatments. Probably it is related with the slight reduction of moisture content in fried potatoes pre-treated by HPP. In addition, **Mestdagh et al., (2008a)** reported that the product crispiness was positively correlated with the taste and general appraisal, so fried potatoes pre-treated by HPP could have a good appraisal by consumers, but a sensory evaluation is required to prove this hypothesis and the taste in general.

Table 14 - Results of texture analysis (by using a cutting knife) of fried potatoes, whose raw potato sticks were treated by pressure before frying.

Processing conditions (MPa/min)	Hardness (N)
0.1/2.5	2.48 ± 0.55 [a]
200/2.5	2.82 ± 0.32 [a]
300/2.5	3.22 ± 0.43 [a]
400/2.5	2.84 ± 0.43 [a]
500/2.5	2.54 ± 0.25 [a]
600/2.5	2.57 ± 0.37 [a]
600/10	2.75 ± 0.25 [a]

Results are expresses as the mean ± the standard deviation.
[a] Different letters indicate significant differences ($p < 0.05$) among the pressure treatment.

3.4. Effect of HPP pretreatment 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, and several variables may affect it, namely oil type and temperature, frying time, and sample dimensions **(Krokida et al., 2001)**. Therefore, this quality property was also measured in fried potatoes pre-treated by HPP, which was 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 15**), it appears that the used frying conditions led to the production of fried potatoes with a desirable tonality, since L^* values are high (superior to 69), the majority of a^* values are between -1.63 and 1.14, and b^* values are always higher than ~28.

Considering coordinate *L** values, HP treatments did not significantly affect ($p<0.05$) this parameter, compared to control samples. However, a slight increase was detected in fried potatoes pre-treated at least at 300 MPa. Relatively to *a** parameter, although no changes ($p<0.05$) were also detected, a slight reduction was found out from samples pre-treated at 300 MPa. Finally, for *b** parameter, a significant decrease ($p<0.05$) was observed in fried potatoes pre-treated at 500 and 600 MPa, either for 2.5 and 10 min, while samples pre-treated at 200, 300, and 400 MPa exhibited higher values than non-treated samples. According to **Krokida et al. (2001)**, higher *L** and *b** parameters, and lower *a** parameter values are desirable for fried products. Considering the presented results, fried potatoes pre-treated at 300 and 400 MPa for 2.5 min seem to be the most desirable, once they exhibited simultaneously higher *L** and *b** values, and lower *a** values than control samples.

These results are similar to colour results obtained for the respective raw potatoes, which means that probably the effects caused by HPP caused on the colour of raw potato sticks had a direct effect on the colour of fried potatoes (became lighter, showing a more uniform and brighter colour). Thus, HPP pre-treatment could be a good alternative to the traditional pre-treatments, such as blanching, used to control the formation of brown compounds (melanoidins) in fried products.

Table 15 – Colour analysis of fried potato sticks of fried potatoes, whose raw potato sticks were treated by pressure before frying, showing the results obtained for *L**, *a**, *b**, and ΔE parameters.

Processing conditions (MPa/min)	*L**	*a**	*b**	ΔE
0.1/2.5	69.99 ± 2.29 ^{a}	0.40 ± 0.33 ^{ab}	35.82 ± 1.14 ^{b}	
200/2.5	69.09 ± 1.32 ^{a}	1.14 ± 1.27 ^{b}	37.57 ± 1.22 ^{b}	2.70 ± 0.72 ^{ab}
300/2.5	70.58 ± 2.16 ^{a}	-0.35 ± 1.09 ^{ab}	37.20 ± 3.17 ^{b}	3.62 ± 0.74 ^{abc}
400/2.5	70.19 ± 1.11 ^{a}	0.27 ± 1.48 ^{ab}	35.95 ± 0.91 ^{b}	1.69 ± 0.33 ^{a}
500/2.5	71.43 ± 1.74 ^{a}	-1.44 ± 0.58 ^{ab}	30.50 ± 0.86 ^{a}	5.94 ± 1.35 ^{cd}
600/2.5	71.44 ± 0.86 ^{a}	-0.98 ± 1.07 ^{ab}	30.89 ± 0.93 ^{a}	5.43 ± 0.91 ^{bc}
600/10	72.97 ± 1.08 ^{a}	-1.63 ± 0.10 ^{a}	27.96 ± 1.92 ^{a}	8.70 ± 1.86 ^{d}

Results are expresses as the mean ± the standard deviation.
^{a,b,c,d} Different letters indicate significant differences ($p<0.05$) among the pressure treatment for the same evaluated parameter.

3.5. Effect of HPP pretreatment on lipid content of fried potatoes

Lipid extraction was carried out using a mixture of organic solvents (petroleum ether and diethyl ether in the proportion 90:10 (v:v)), due to their suitability to solubilize lipids, lower costs than other organic solvents, low flammability and boiling point, and low time consuming than other methods of lipid extraction **(AAFCO, 2014; Thiex et al., 2003)**. The obtained results (**Table 16**) showed that pre-treatments with HPP did not change significantly ($p<0.05$) the lipid percentage of the respective fried potatoes, and all of them were inferior to the literature values of 10-15% **(Moreira et al., 1999)**. Although no distinguished tendency was detected over the pressure, all the samples showed a percentage approximately equal to or higher than control samples, *i.e.*, in the range of approximately 8-9%. Probably it is related with differences of moisture content of fried potatoes and weight loss after frying. Indeed, the more water is removed from the surface, the more the oil is absorbed **(Ziaiifar et al., 2008)**. For this reason, as potato sticks pre-treated by HPP lost a little more water than those unpressurized, it could explain why their lipid content did not decreased, what would be desirable. Another significant parameter conditioning oil absorption in potatoes frying is temperature **(Ziaiifar et al., 2008)**, but it kept constant during all the assays.

Moreover, it is known that when starch is gelatinized during frying, it can interact with polar and non-polar compounds (namely lipids), and the formation of lipid-amylose complexes modifies some properties of starch, such as the retardation of starch retrogradation **(De Pilli et al., 2008; Meng et al., 2014)**. Thus, fried potatoes pre-treated at 600 MPa for 10 min exhibited a slightly higher lipid content (9.16%) probably due to the starch modification induced by HPP in raw potato sticks, which were detected in DSC analysis. It could explain the observed differences between fried potatoes pretreated at 600 MPa for 2.5 and 10 min, since higher time processing possibly increases starch modification, which enhance the bound between potato starch and lipid absorbed from oil frying. Although the increase of lipid uptake is not desirable, this different bound between lipids and starch could increase the quality time of fried potatoes because starch retrogradation would be more delayed, and consequently, sensory and quality properties of fried potatoes would be changed. Anyway, once the fat percentage did not increase significantly ($p<0.05$), it is possible to state that HPP did not induce the production of less healthy fried potatoes.

Table 16 – Lipid content of fried potatoes whose raw potato sticks were treated by pressure before frying.

Processing conditions (MPa/min)	Lipid content (%)
0.1/2.5	8.05 ± 1.10 [a]
200/2.5	8.45 ± 0.78 [a]
300/2.5	8.40 ± 0.45 [a]
400/2.5	8.33 ± 0.42 [a]
500/2.5	8.00 ± 0.84 [a]
600/2.5	8.24 ± 0.72 [a]
600/10	9.16 ± 0.66 [a]

Results are expresses as the mean ± the standard deviation.
[a] Different letters indicate significant differences ($p<0.05$) among the pressure treatment.

3.6. Effect of HPP pretreatment on lipid profile of fried potatoes

Raw potato tubers contain reduced amounts of lipids (0.09% - **Table 1**), and thereby the composition of fatty acids (FA) in fried potatoes reflects directly the frying oil composition. Fula oil was the frying oil used, and consists of a mixture of colza and sunflower oil, whose nutritional composition is shown in **Table 29** of **Annex II**. Unfortunately, no data in the literature presents the fatty acid composition of this specific oil, but there is some data for sunflower and colza oil individually. **Rosa et al. (2009)** verified that sunflower oil is composed mainly of 5 - 7.6% of palmitic acid (C16:0), 2.7 - 6.5% of stearic acid (C18:0), 14.0 - 39.4% of oleic acid (C18:1), and 48.3 - 74.0% of linoleic acid (C18:2). Moreover, **Farahmandfar et al. (2015)** showed that the main fatty acids in colza oil are palmitic (4.29%), stearic (2.59%), oleic (65.39%), linoleic (16.32%) and α-linolenic acid (7.54%).

The main fatty acids of oil extracted from fried potatoes pre-treated by HPP (**Table 17**) are consistent with those identified in colza and sunflower oil, consisting of 5.34 - 5.85% of palmitic acid (C16:0), 2.60 - 2.68% of stearic acid (C18:0), 43.17 - 43.35% of oleic acid (C18:1c), 40.18 - 40.38% of linoleic acid (C18:2c), and 5.29 - 5.46% of α-linolenic acid (C18:3n3). Differences between the obtained percentages and those obtained by **Rosa et al. (2009)** and **Farahmandfar et al. (2015)** are due to the fact that *Fula* oil is a mixture of sunflower and colza oil.

According to the nutritional composition **Table 29** of **Annex II**, *Fula* oil has 8.8 g of saturated FA, 50 g of monounsaturated FA (MUFA), and 41 g of polyunsaturated FA (PUFA) in 100 mL. **Table 18** shows the relative percentage of different types of FA in oil extracted from fried potatoes, and results are in accordance with nutritional

composition of *Fula* oil because a percentage of ~9% of saturated FA, ~44% of MUFA and ~46% of PUFA were determined, which is relatively similar to the oil composition. Low percentage of *trans* FA was obtained (~0.4%), evidencing a low oil thermal degradation, and ~40% and ~5% of PUFA were omega-6 and omega-3 FA. Through the statistical analysis, no significant changes ($p<0.05$) were obtained for the majority of FA among the different samples, which means that HPP did not induce modifications on the bound tendency for certain types of FA.

Table 17 - Fatty acid composition of oil extracted from fried potatoes, expressed in g/100 g of fat, whose raw potato sticks were treated by HPP.

Processing conditions (MPa/min)	C16:0	C18:0	C18:1c	C18:2c	C18:3n3
0.1/2.5	5.34 ± 0.02 ^{a}	2.68 ± 0.01 ^{ab}	43.35 ± 0.03 ^{abc}	40.18 ± 0.02 ^{ab}	5.38 ± 0.03 ^{cdef}
200/2.5	5.38 ± 0.08 ^{ab}	2.67 ± 0.02 ^{ab}	43.22 ± 0.15 ^{ab}	40.28 ± 0.07 ^{ab}	5.46 ± 0.06 ^{f}
300/2.5	5.38 ± 0.10 ^{ab}	2.68 ± 0.06 ^{b}	43.18 ± 0.41 ^{abc}	40.29 ± 0.51 ^{ab}	5.46 ± 0.09 ^{def}
400/2.5	5.53 ± 0.26 ^{ab}	2.63 ± 0.06 ^{ab}	43.17 ± 0.07 ^{a}	40.31 ± 0.08 ^{ab}	5.42 ± 0.07 ^{ef}
500/2.5	5.55 ± 0.22 ^{ab}	2.63 ± 0.03 ^{ab}	43.33 ± 0.10 ^{abc}	40.38 ± 0.11 ^{b}	5.31 ± 0.05 ^{abcdef}
600/2.5	5.67 ± 0.17 ^{ab}	2.61 ± 0.03 ^{a}	43.33 ± 0.14 ^{abc}	40.29 ± 0.13 ^{ab}	5.33 ± 0.05 ^{bcdef}
600/10	5.85 ± 0.29 ^{b}	2.60 ± 0.03 ^{a}	43.30 ± 0.14 ^{abc}	40.18 ± 0.11 ^{ab}	5.29 ± 0.04 ^{abcde}

Results are expresses as the mean ± the standard deviation.
^{a,b,c,d,e,f} Different letters indicate significant differences ($p<0.05$) among the pressure treatment for the same evaluated parameter (fatty acid).

Table 18 - Relative percentage of saturated fatty acids (FA), monounsaturated FA (MUFA), polyunsaturated FA (PUFA), *trans* FA, omega-3 and omega-6 polyunsaturated FA (∑n-3 and ∑n-6 PUFA), and ratio between n-3 and n-6 PUFA (n3:n6 ratio) of oil extracted from fried potatoes pre-treated by HPP, expressed in g/100 g of fat.

Processing conditions (MPa/min)	Saturated FA	MUFA	PUFA	*Trans* FA	∑n-3 PUFA	∑n-6 PUFA	w3:w6 ratio
0.1/2.5	9.30 ± 0.01 ^{a}	44.31 ± 0.03 ^{ab}	45.91 ± 0.11 ^{a}	0.45 ± 0.02 ^{ab}	5.42 ± 0.03 ^{cdef}	40.25 ± 0.02 ^{ab}	0.13 ± 0.00 ^{bcde}
200/2.5	9.31 ± 0.05 ^{a}	44.17 ± 0.14 ^{ab}	46.07 ± 0.17 ^{bc}	0.43 ± 0.01 ^{ab}	5.48 ± 0.08 ^{f}	40.35 ± 0.09 ^{ab}	0.14 ± 0.00 ^{de}
300/2.5	9.31 ± 0.17 ^{a}	44.13 ± 0.43 ^{ab}	46.11 ± 0.61 ^{abc}	0.43 ± 0.01 ^{ab}	5.50 ± 0.10 ^{def}	40.36 ± 0.51 ^{ab}	0.14 ± 0.00 ^{e}
400/2.5	9.35 ± 0.11 ^{a}	44.10 ± 0.08 ^{a}	46.08 ± 0.07 ^{c}	0.44 ± 0.02 ^{ab}	5.46 ± 0.07 ^{ef}	40.38 ± 0.07 ^{ab}	0.14 ± 0.00 ^{acde}
500/2.5	9.36 ± 0.12 ^{a}	44.19 ± 0.18 ^{ab}	45.99 ± 0.13 ^{abc}	0.43 ± 0.03 ^{ab}	5.33 ± 0.03 ^{abcdef}	40.43 ± 0.11 ^{b}	0.13 ± 0.00 ^{abc}
600/2.5	9.38 ± 0.09 ^{a}	44.23 ± 0.11 ^{ab}	45.95 ± 0.16 ^{abc}	0.42 ± 0.03 ^{a}	5.37 ± 0.05 ^{bcdef}	40.35 ± 0.13 ^{ab}	0.13 ± 0.00 ^{abcde}
600/10	9.55 ± 0.17 ^{a}	44.18 ± 0.18 ^{ab}	45.80 ± 0.11 ^{abc}	0.45 ± 0.05 ^{ab}	5.33 ± 0.04 ^{abcdef}	40.23 ± 0.12 ^{ab}	0.13 ± 0.00 ^{abcd}

Results are expresses as the mean ± the standard deviation.
^{a,b,c,d,e,f} Different letters indicate significant differences ($p<0.05$) among the pressure treatment for the same evaluated parameter (type of lipids).

3.7. Effect of HPP pretreatment on acrylamide content of fried potatoes

Acrylamide is considered a group 2A carcinogen ("probably carcinogenic to humans"), and thereby it has received much attention both from the scientific community and consumers in general. Given the large consumption of fried potatoes worldwide and their high acrylamide levels **(Capuano and Fogliano, 2011; Powers et al., 2013)**, acrylamide content in fried potatoes pre-treated by HPP was measured. However, only samples pre-treated at 0.1, 200, 400 and 600 MPa for 2.5 min were assayed in order to evaluate if a low, medium, or HP level led to changes in the formation of acrylamide, and the results are presented in **Table 19**. According to data provided by EFSA, acrylamide levels of French fries were between 356 and 338 μg/Kg, from 2007 to 2010 **(EFSA, 2012)**, and acrylamide levels of fried potatoes pre-treated by HPP were similar to that range (between 300.5 and 387.8 μg/Kg). In addition, no significant differences ($p<0.05$) were detected among the different changes. Indeed, a major approach to mitigate the acrylamide formation consist of removing acrylamide precursors (reducing sugars and asparagine) in raw potatoes **(Singh and Kaur, 2016)**. Once acrylamide levels of fried potatoes pre-treated by HPP were not different ($p<0.05$) from those of control samples, it means that HP treatment alone was not enough to affect significantly precursors' acrylamide in raw potato sticks.

Table 19 - Acrylamide content in fried potatoes, expressed in μg/Kg, whose raw potato sticks were treated by HPP.

Processing conditions (MPa/min)	Acrylamide (μg/Kg)	Average reduction of acrylamide in relation to control (%)
0.1/2.5	309.1 ± 51.2 [a]	
200/2.5	387.8 ± 29.9 [a]	-25.5
400/2.5	300.5 ± 60.9 [a]	2.2
600/2.5	304.6 ± 29.0 [a]	1.5

Results are expresses as the mean ± the standard deviation.
[a] Different letters indicate significant differences ($p<0.05$) among the pressure treatment.

4. Effect of pressure treatment and asparaginase infusion on raw potato sticks

According to the results obtained previously, HP treatment alone did not seem to be an effective strategy to mitigate acrylamide formation in fried potatoes. However, some studies in the literature have proven that HPP can increase the rate of diffusion processes, which is naturally low. Indeed, **Sopanangkul et al. (2002)** has shown that HPP has ability to increase and accelerate mass transfer processes into potato cylinders when 100-400 MPa were applied, due to the enhancement of cell permeability. Thus, the infusion of asparaginase (an enzyme with ability to hydrolyse asparagine, an acrylamide precursor, into ammonia and aspartic acid) was a hypothesis that arise to be tested.

Both thermal and non-thermal methodologies were already tested for infusion of asparaginase into potato sticks before frying, as a strategy of acrylamide mitigation in fried potatoes, as mentioned in the introduction chapter. However, no study in the literature had tested the combination of asparaginase treatment and HPP. For this reason, an asparaginase solution (10 000 ASNU[2]/L) was prepared using tap water as solvent (because it is cheaper and more common industrially than distilled water), and exhibited a pH of 7.50, that belongs to the range of good asparaginase activity (5-9). Potato sticks were soaked in that asparaginase solution, then subjected to 0.1 (control), 100, 200 and 400 MPa, and three enzymatic reaction times were assayed (5, 10 and 20 min). 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. Raw potato tubers treated by Acrylaway® and pressure were characterized as to their weight difference after treatment, texture, colour, and moisture, and the respective exterior water were analysed as to their total soluble solids and sugars.

4.1. Weight difference of potato sticks and exterior water samples after HPP

Potato sticks were weighted before and after treatment (asparaginase and HPP), whose results of weight difference are present in **Table 20**, and the respective linearizations in **Figure 27** (**Annex X**).

Comparing the results of the pressurized potato sticks with the unpressurized ones, it was detected a significant weight increase ($p<0.05$) only at 100 MPa. From this level

[2] ASNU is defined as the amount of asparaginase that produces 1 μmol of ammonia per min under the conditions assay (pH = 7 ± 0.005; 37.0 ± 0.5 ºC) using Acrylaway®.

of pressure, a reduction in weight gain was observed as the pressure intensity increased, and samples treated at 200 MPa had a weight gain similar to control samples ($p<0.05$), while samples treated at 400 MPa showed a negative weight difference, and thus significantly inferior ($p<0.05$) to other samples. Comparing these results with those obtained for raw potato sticks treated only by HPP (**Table 7**), it was observed a faster and higher weight loss compared to samples treated to the respective pressure level. Thus, probably the addition of asparaginase induced not only a lower potato weight gain but also a faster weight loss over the pressure. In addition, similarly to what was observed in raw potato sticks pre-treated by HPP, the exterior water samples showed negative weight differences, which decreased (became more positive) over the pressure.

Weight differences of potato sticks and exterior water samples exhibited a linear behaviour as a function of the pressure level, as shown in **Figure 27** (**Annex X**). Beyond the results of the statistical analysis, through the analysis of the graphic of **Figure 27**, it was evident that the enzymatic reaction time did not have a significant influence in weight loss or gain, and pressure level was the main factor that induced these differences ($p<0.05$). As stated previously, possibly HP treatments induce the release of potato components to the involving water, leading to the decrease of potato sticks weight, and consequently to the increase of water weight.

Table 20 - Weight difference of raw potato sticks, expressed in %, treated by pressure and an asparaginase solution, and the respective exterior water samples.

Pressure (MPa)	Total asparaginase reaction time (min)	Raw potato sticks weight difference (%)	Exterior water weight difference (%)
0.1	5	2.8 ± 0.2^{aB}	-3.6 ± 1.0^{aB}
	10	3.1 ± 0.8^{aB}	-3.8 ± 0.6^{aB}
	20	3.4 ± 0.6^{aB}	-4.3 ± 0.3^{aB}
100	5	8.4 ± 2.7^{aC}	-7.1 ± 0.8^{aA}
	10	8.2 ± 2.4^{aC}	-6.1 ± 0.7^{aA}
	20	8.4 ± 1.6^{aC}	-6.6 ± 0.9^{aA}
200	5	4.7 ± 1.0^{aBC}	-5.0 ± 0.4^{aB}
	10	4.4 ± 0.4^{aBC}	-5.1 ± 0.4^{aAB}
	20	4.5 ± 0.7^{aB}	-4.7 ± 0.3^{aB}
400	5	-3.0 ± 2.0^{aA}	-3.3 ± 1.0^{aB}
	10	-2.2 ± 2.2^{aA}	-2.0 ± 0.9^{aC}
	20	-2.2 ± 1.6^{aA}	-1.5 ± 0.7^{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,C] capital different letters indicate significant differences ($p<0.05$) among the pressure treatment for the same reaction time of enzyme.

4.2. Effect of asparaginase and HPP on texture of raw potato sticks

The texture of potato sticks treated by the combination of asparaginase and pressure treatments were represented as their firmness, stiffness and energy for cutting. The obtained results are shown in **Table 21** and a decrease in all parameters was observed over the pressure. Although potato sticks treated at 100 MPa exhibited no significant changes ($p<0.05$) relatively to the control samples, a slight decrease was detected. Raw potato sticks treated at 200 and 400 MPa showed significant reductions in all texture parameters, but no changes were detected between them for all the reaction times ($p<0.05$). Thus, in this samples, it was detected a decrease of 25-30% of firmness, 20-41% of stiffness, and 27-41% of energy for cutting, compared to control samples. These results were similar to those obtained for raw potato sticks pre-treated with HPP (**Table 9**), once at 200 and 400 MPa, reductions of 28-31% of firmness, 9-38% of stiffness, and 22-42% of energy for cutting were detected, as well as a decrease in the roughness of potato surface. It means that the addition of asparaginase did not influence the texture of potato strips. Actually, pressure level was the major factor which led 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.

Table 21 - Texture of raw potato sticks treated by pressure (0.1, 100, 200 and 400 MPa for 5 min) and an asparaginase solution, with different times of enzyme reaction (5, 10 and 20 min).

Pressure (MPa)	Total asparaginase reaction time (min)	Firmness (N)	Stiffness (N/m)	Energy of cutting (N.mm)
0.1	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	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	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.

Once results were similar to those obtained in section *2.4 Effect of HPP on texture of raw potato sticks*, discussion and justifications are similar, that is, the obtained results are in accordance with data of studies which applied HPP in fruits and vegetables **(Al-Khuseibi et al., 2005; Eshtiaghi and Knorr, 1993; Oey et al., 2008; Oliveira et al., 2015)**, and moreover it showed that HPP induces similar changes in the texture of potatoes compared to PEF, but has an extra advantage: HPP-treated potatoes exhibit a lower energy for cutting than PEF-treated potatoes.

4.3. Effect of asparaginase and HPP on colour of raw potato sticks

Colour of raw potato sticks treated only by pressure were evaluated previously and it was already observed that, although no differences ($p<0.05$) were noted in all parameters for all samples, a slight discolouration was detected due to the reduction tendency of L^*, a^* and b^* parameters. Thus, colour of raw potato sticks treated by an asparaginase solution and pressure were determined in order to achieve if this combined treatment caused significant changes in this quality parameter. Results are shown in **Table 22**.

In a first approach, the influence of asparaginase reaction time was analysed for each pressure condition and results were compared with control samples. Colour parameters (L^*, a^* and b^*) did not changed significantly ($p<0.05$), except at 0.1/20 (higher L^* value); at 100/10 (higher b^* value); and at 200/20 (lower b^* value). In a second approach, the influence of pressure level was analysed for each asparaginase reaction time and results were compared with control samples. a^* and b^* parameters did not change significantly ($p<0.05$), except at 200/5 (higher b^* value); at 100/10, 200/10 and 400/10 (increasing a^* values); and at 400/20 (higher a^* value). L^* parameter values changed ($p<0.05$) in samples with a reaction time of 10 and 20 min. At 100 and 200 MPa, samples showed lower values of L^* parameter than control samples. However, at 400 MPa, the opposite behaviour was observed.

Sporadic changes observed in the several colour parameters were probably due to differences in the potato tubers themselves, since no consistent tendency was observed. Moreover, there were some differences between the colour of potato sticks treated by HPP and those treated by Acrylaway and HPP, which are also possibly related to differences in the potato tubers composition. ΔE of all samples were low and did not changed among the pressure or enzymatic reaction time, and for this reason it is possible

to concluded that the combination of asparaginase and pressure treatments did not greatly affect colour of raw potatoes.

Table 22 – Colour of raw potato sticks treated by pressure (0.1, 100, 200 and 400 MPa for 5 min) and an asparaginase solution, with different times of enzyme reaction (5, 10 and 20 min).

Pressure (MPa)	Total asparaginase reaction time (min)	*L**	*a**	*b**	ΔE
0.1	5	70.4 ± 0.5 ^{aAB}	-0.6 ± 0.5 ^{aA}	24.3 ± 0.8 ^{aA}	
	10	69.8 ± 0.5 ^{aB}	-0.4 ± 0.2 ^{aA}	25.2 ± 2.2 ^{aA}	
	20	71.9 ± 0.4 ^{bC}	-0.5 ± 0.3 ^{aA}	25.5 ± 0.3 ^{aA}	
100	5	66.7 ± 1.0 ^{aA}	0.0 ± 0.2 ^{aA}	24.4 ± 0.4 ^{aA}	3.7 ± 1.1 ^{aA}
	10	66.5 ± 1.0 ^{aA}	0.2 ± 0.2 ^{aB}	26.4 ± 0.4 ^{bA}	3.6 ± 0.7 ^{aA}
	20	66.4 ± 1.9 ^{aA}	0.1 ± 0.2 ^{aA}	24.5 ± 0.4 ^{aA}	5.7 ± 1.9 ^{aA}
200	5	68.3 ± 2.1 ^{aAB}	-0.1 ± 0.3 ^{aA}	26.3 ± 0.3 ^{bB}	3.1 ± 1.7 ^{aA}
	10	65.8 ± 0.5 ^{aA}	0.2 ± 0.2 ^{aB}	25.8 ± 0.7 ^{bA}	4.1 ± 0.3 ^{aA}
	20	67.7 ± 1.9 ^{aAB}	-0.1 ± 0.4 ^{aA}	23.8 ± 1.0 ^{aA}	4.8 ± 1.2 ^{aA}
400	5	71.7 ± 2.6 ^{aB}	0.5 ± 0.9 ^{aA}	26.0 ± 1.0 ^{aAB}	3.4 ± 0.6 ^{aA}
	10	71.4 ± 0.5 ^{aC}	1.9 ± 0.1 ^{aC}	25.5 ± 2.3 ^{aA}	3.4 ± 0.4 ^{aA}
	20	71.3 ± 1.7 ^{aBC}	1.4 ± 0.4 ^{aB}	23.7 ± 0.8 ^{aA}	3.1 ± 0.8 ^{aA}

Results are expressed as the mean ± the standard deviation.
^{a,b} 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.

4.4. Effect of asparaginase and HPP on moisture of raw potato sticks

Moisture of potato sticks treated by asparaginase and pressure was determined and results are present in **Table 23**. Enzymatic reaction time, in each pressure condition, showed not affect moisture of potato sticks ($p<0.05$), and the applied pressure level led to a slight change just in samples 400/20. Generally, HPP-treated samples exhibited equal to or lower percentage of moisture than unpressurized samples, which is in accordance with results obtained for samples subjected just to pressure treatments, and thereby, justifications are similar.

Table 23 - Moisture of raw potato sticks treated by pressure (0.1, 100, 200 and 400 MPa for 5 min) and an asparaginase solution, with different times of enzyme reaction (5, 10 and 20 min).

Pressure (MPa)	Total asparaginase reaction time (min)	Moisture (%)
0.1	5	84.66 ± 0.71 ^{aA}
	10	84.85 ± 2.45 ^{aB}
	20	83.99 ± 0.89 ^{aA}
100	5	83.06 ± 1.33 ^{aA}
	10	83.21 ± 0.97 ^{aAB}
	20	83.32 ± 2.84 ^{aA}
200	5	81.82 ± 2.31 ^{aA}
	10	84.88 ± 1.26 ^{aB}
	20	83.49 ± 2.22 ^{aA}
400	5	80.56 ± 2.60 ^{aA}
	10	80.60 ± 1.11 ^{aA}
	20	81.83 ± 1.55 ^{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.

4.5. Effect of asparaginase and HPP on reducing sugars and total soluble solids of the exterior water samples

The exterior water samples of potato sticks were analysed in relation to their soluble sugars, represented as ºBrix, and total soluble solids (**Table 24**). These analyses were performed applying two different methods, as described previously for raw potato sticks pre-treated by HPP, and probably because of this reason, different values were obtained for each method of analysis. However, the percentage of either soluble sugars or total soluble solids exhibited an increase as the pressure intensity increased, and a maximum of 0.65% (ºBrix) and 0.44% (total soluble solids) were achieved for samples 400/20. These values were similar to those obtained for raw potato sticks treated at the respective pressure level (**Table 11** and **Figure 15**), which means that the release of soluble sugars/solids is mainly due to the effect of HPP, and the addition of asparaginase did not influence.

Table 24 - Reducing sugars and total soluble solids in exterior water samples of raw potato sticks treated by pressure (0.1, 100, 200 and 400 MPa for 5 min) and an asparaginase solution, with different times of enzyme reaction (5, 10 and 20 min).

Pressure (MPa)	Total asparaginase reaction time (min)	Total soluble solids (%)	º Brix (%)
0.1	5	0.09 ± 0.02 ^{aA}	0.30 ± 0.00 ^{aA}
	10	0.12 ± 0.02 ^{aA}	0.37 ± 0.06 ^{aA}
	20	0.09 ± 0.02 ^{aA}	0.35 ± 0.05 ^{aA}
100	5	0.12 ± 0.02 ^{aAB}	0.33 ± 0.03 ^{aA}
	10	0.13 ± 0.04 ^{aA}	0.37 ± 0.06 ^{aA}
	20	0.17 ± 0.07 ^{aAB}	0.37 ± 0.06 ^{aA}
200	5	0.21 ± 0.04 ^{aB}	0.48 ± 0.03 ^{aB}
	10	0.27 ± 0.03 ^{aB}	0.52 ± 0.03 ^{aB}
	20	0.32 ± 0.09 ^{aBC}	0.57 ± 0.06 ^{aB}
400	5	0.36 ± 0.04 ^{aC}	0.57 ± 0.03 ^{aC}
	10	0.40 ± 0.05 ^{aC}	0.60 ± 0.05 ^{aB}
	20	0.44 ± 0.01 ^{aC}	0.65 ± 0.05 ^{aB}

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,C} capital different letters indicate significant differences ($p<0.05$) among the pressure treatment for the same reaction time of enzyme.

5. Effect of pressure treatment and asparaginase on fried potato sticks

5.1. Weight difference after frying

Raw potato sticks, which were pre-treated by a combined treatment of asparaginase and HPP, were fried and their weight before and after frying was measured. The weight loss of each sample is shown in **Figure 17**, and results evidenced that the reaction time of asparaginase did not changed the weight loss of potato sticks after frying, for any pressure tested. In contrast, the weight difference of control samples was between -47.00 and -46.14%, while the weight difference of pressurized samples presented a range of -55.49 and -50.16%, concluding that the pressure intensity increased ($p<0.05$) the percentage of weight loss for the majority of samples. As stated previously, the increase of weight loss by pre-treated fried potatoes was possibly related with the increase of water release, as a consequence of either the textural changes or the enhancement of drying processes (including frying) induced by HPP.

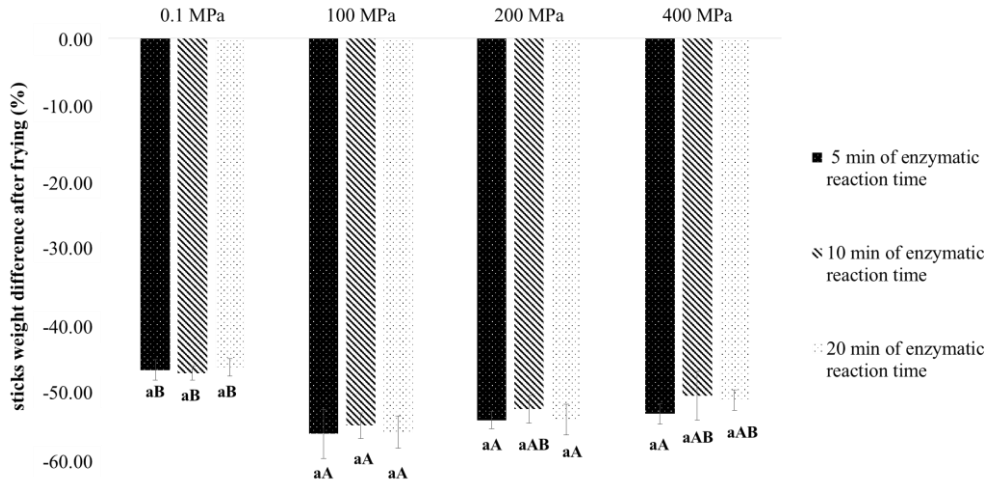

Figure 17 - Weight difference of potato sticks after frying, expressed in %, whose raw potato sticks were subjected to a combination of asparaginase and pressure treatment.
[a] small different letters mean significant differences ($p<0.05$) among reaction time of enzyme for the same pressure treatment; [A,B] capital different letters mean significant differences ($p<0.05$) among the pressure treatment for the same reaction time of enzyme.

Comparing these results with those obtained for fried potatoes pre-treated only by HPP, it was observed some differences. First, control samples pre-treated both by HPP and by asparaginase and HPP showed a weight loss after frying of ~47%, and thereby, the presence of asparaginase in the exterior solution of potato strips did not affect the amount of water release during frying. In contrast, while potato sticks pre-treated with

asparaginase and HP lost ~50-55% of their weight after frying, potato sticks pre-treated only with HP lost ~50%. This difference can be related with the application of 5 min of HPP in the first case instead of 2.5 min in the second case, resulting in more severe textural changes, and consequently in a higher water release during frying.

5.2. Effect of asparaginase and HPP on moisture of fried potatoes

As described in section *3. Effect of pressure pretreatment on quality of fried potato sticks*, moisture content of fried potato sticks was measured (**Table 25**) 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. In addition, comparing these results with results obtained for fried potatoes pre-treated by HPP (**Table 13**), it is possible to observe that the first ones had lower moisture content than the second ones, and thereby it could also explain the differences in the results of weight loss after frying, *i.e.*, why fried potato sticks pre-treated by asparaginase and HPP exhibited higher weight loss than potato sticks only pre-treated by HPP. Actually, it proved that changes caused by the combined treatment of asparaginase and HP had, not only influence on water content of fried potatoes, but also a greater effect than pressure treatment alone.

Table 25 - Moisture of fried potato sticks treated by pressure (0.1, 100, 200 and 400 MPa for 5 min) and an asparaginase solution, with different times of enzyme reaction (5, 10 and 20 min).

Pressure (MPa)	Total asparaginase reaction time (min)	Moisture (%)
0.1	5	59.98 ± 2.26^{aB}
	10	61.02 ± 3.06^{aA}
	20	60.90 ± 1.23^{aA}
100	5	56.48 ± 1.36^{aAB}
	10	59.14 ± 1.41^{aA}
	20	60.15 ± 4.05^{aA}
200	5	54.64 ± 0.72^{aA}
	10	56.52 ± 3.35^{aA}
	20	56.37 ± 3.80^{aA}
400	5	59.92 ± 1.11^{aB}
	10	57.18 ± 3.07^{aA}
	20	60.44 ± 2.25^{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.

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

The texture of fried potato sticks pre-treated with a combination of asparaginase infusion and HP was also evaluated by the same method applied in section ***Erro! A origem da referência não foi encontrada.*** *Effect of HPP pretreatment on texture properties of fried potatoes*. The obtained results (**Table 26**) showed that the enzymatic reaction time in each pressure level did not affect ($p<0.05$) hardness value. Analyzing the effect of pressure intensity, although no significant change ($p<0.05$) had been detected among the different conditions, it was observed a slight increase of hardness values of fried potatoes pre-treated at 200 and 400 MPa. Probably it is related with results obtained in the texture analysis of the respective raw potatoes (**Table 21**). 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. In a similar way, fried potato sticks pre-treated just by HPP (≥200 MPa) also exhibited a slightly higher hardness than control samples, so the pressure level applied in pre-treatments is probably the major factor to induce these textural changes in fried products.

As discussed above, the increase of hardness represents an increase of crispness, an important textural quality parameter, so fried potatoes pre-treated by the combined treatment of asparaginase and HPP could have a good appraisal by consumers, but a sensory evaluation is required to prove this hypothesis and the taste in general.

Table 26 - Texture of fried potato sticks treated by pressure (0.1, 100, 200 and 400 MPa for 5 min) and an asparaginase solution, with different times of enzyme reaction (5, 10 and 20 min).

Pressure (MPa)	Total asparaginase reaction time (min)	Hardness (N)
0.1	5	2.82 ± 0.16^{aAB}
	10	2.76 ± 0.43^{aAB}
	20	2.58 ± 0.09^{aA}
100	5	2.67 ± 0.55^{aA}
	10	2.30 ± 0.46^{aA}
	20	2.59 ± 0.46^{aA}
200	5	3.81 ± 0.39^{aB}
	10	3.45 ± 0.13^{aB}
	20	3.39 ± 0.32^{aA}
400	5	3.37 ± 0.35^{aAB}
	10	3.46 ± 0.47^{aB}
	20	3.02 ± 0.45^{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.

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

Colour of fried potatoes pre-treated with a combination of asparaginase and HP (**Figure 18**) was measured by the same reasons and method presented in section *3.4 Effect of HPP pretreatment on colour of fried potatoes*. As stated previously, 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 27**), fried potatoes showed with 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. Comparing these results with those obtained for fried potatoes pre-treated only by HPP ($L^* > 69$; a^* between -1.63 and 1.14; $b^* > 28$), it appears that the combined treatment of asparaginase and HP led to the formation of fried potatoes with a darker, more red and yellow colour than the treatment with HPP alone. Probably these differences are related with changes observed in weight loss after frying and moisture content. As fried potatoes pre-treated by asparaginase and HPP lost more water and weight after frying than those pre-treated by HPP alone, possibly it decreased the thickness of potato strips during frying. Once 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 a^* and b^* values were higher than the respective colour parameters of potato sticks pre-treated by HPP.

Considering coordinate L^* values, a slight decrease was detected for fried potatoes pre-treated with an asparaginase solution and HPP, although there were no statistical differences ($p<0.05$). Relatively to a^* parameter, neither statistical changes ($p<0.05$) were detected, nor a clear tendency. For b^* parameter, no changes ($p<0.05$) were observed, except for 100/20 samples, in which the pre-treatment at 100 MPa and for 20 min of enzymatic reaction showed to increase b^* parameter of fried potatoes. However, in general higher b^* values were observed in pre-pressurized fried potatoes, which is considered desirable for consumers. One more time, these results are similar to colour results obtained for the respective raw potatoes, which means that probably the effects caused by Acrylaway and HPP treatment on colour of raw potato sticks had a direct effect

on colour of fried potatoes (became slightly darker, and with a more red and yellow colour).

Table 27 - Colour of fried potato sticks treated by pressure (0.1, 100, 200 and 400 MPa for 5 min) and an asparaginase solution, with different times of enzyme reaction (5, 10 and 20 min).

Pressure (MPa)	Total asparaginase reaction time (min)	*L**	*a**	*b**	ΔE
0.1	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	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	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.

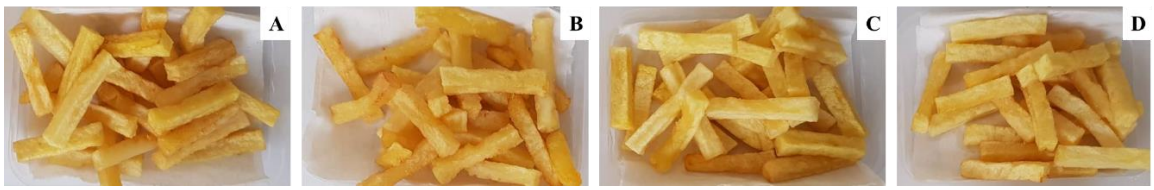

Figure 18 - Images of fried potatoes whose raw potato sticks were treated at (A) 0.1, (B) 100, (C) 200, and (D) 400 MPa.

5.5. Effect of asparaginase and HPP on lipid profile of fried potatoes

Potato sticks pre-treated by a combination of asparaginase and pressure treatments were also deep-fried using *Fula* oil as frying oil. Fried potato sticks pre-treated with asparaginase and pressure exhibited a qualitative and quantitative composition in fatty acids similar to those pre-treated by HPP. The main fatty acids identified (**Table 28**) were palmitic acid (C16:0, 5.46 - 5.82%), stearic acid (C18:0, 2.63 - 2.69%), oleic acid (C18:1c, 43.33 - 43.66%), linoleic acid (C18:2c, 39.86 - 40.11%), and α-linolenic acid (C18:3n3, 5.17 - 5.29%), and no significant changes ($p<0.05$) were detected among the

different samples, either over the pressure or the enzymatic reaction time. These FA were also mostly identified in sunflower and colza oil **(Farahmandfar et al., 2015; Rosa et al., 2009)**, but relative percentages were different because *Fula* oil is a mixture of sunflower and colza oil.

Table 29 shows the relative percentage of different types of FA in oil extracted from fried potatoes, and results are in accordance with nutritional composition of *Fula* oil (**Table 31** of **Annex II**) because a percentage of ~9% of saturated FA, ~44% of MUFA and ~45% of PUFA were determined, which is relatively similar to the oil composition. Low percentage of *trans* FA was obtained (~0.5%), and ~40% and ~5% of PUFA were omega-6 and omega-3 FA. No significant changes ($p<0.05$) were obtained among the different samples, either over the pressure or the enzymatic reaction time. These results were similar to those obtained for fried potatoes pre-treated by HPP (**Table 18**), which means that neither pressure treatment alone nor the combination of asparaginase and HP treatments led to changes in fatty composition of fried potatoes, or induced modifications on the bound tendency for certain types of FA.

Table 28 - Lipid profile of oil extracted from fried potato sticks treated by pressure (0.1, 100, 200 and 400 MPa for 5 min) and an asparaginase solution, with different times of enzyme reaction (5, 10 and 20 min).

Pressure (MPa)	Reaction time (min)	C16:0	C18:0	C18:1c	C18:2c	C18:3n3
0.1	5	5.67 ± 0.13 ^{abA}	2.64 ± 0.03 ^{abA}	43.53 ± 0.18 ^{abcA}	40.03 ± 0.17 ^{abA}	5.26 ± 0.04 ^{abcdBC}
	10	5.49 ± 0.11 ^{abA}	2.68 ± 0.01 ^{abA}	43.43 ± 0.06 ^{abcA}	40.05 ± 0.07 ^{abA}	5.29 ± 0.01 ^{abcdeB}
	20	5.82 ± 0.38 ^{bA}	2.63 ± 0.05 ^{abA}	43.33 ± 0.07 ^{abcA}	40.11 ± 0.12 ^{abC}	5.29 ± 0.06 ^{abcdeA}
100	5	5.61 ± 0.15 ^{abA}	2.68 ± 0.03 ^{abA}	43.57 ± 0.10 ^{abcA}	39.93 ± 0.12 ^{abA}	5.17 ± 0.03 ^{aA}
	10	5.55 ± 0.08 ^{abA}	2.68 ± 0.02 ^{abA}	43.63 ± 0.12 ^{bcA}	40.04 ± 0.18 ^{abA}	5.22 ± 0.01 ^{abA}
	20	5.53 ± 0.08 ^{abA}	2.68 ± 0.01 ^{abA}	43.63 ± 0.06 ^{bcC}	39.95 ± 0.03 ^{abAB}	5.18 ± 0.04 ^{aA}
200	5	5.46 ± 0.11 ^{abA}	2.67 ± 0.02 ^{abA}	43.50 ± 0.05 ^{abcA}	40.08 ± 0.05 ^{abA}	5.29 ± 0.02 ^{abC}
	10	5.52 ± 0.12 ^{abA}	2.67 ± 0.02 ^{abA}	43.50 ± 0.09 ^{abcA}	40.07 ± 0.09 ^{abA}	5.27 ± 0.02 ^{abcdAB}
	20	5.53 ± 0.11 ^{abA}	2.67 ± 0.01 ^{abA}	43.44 ± 0.04 ^{abcAB}	40.06 ± 0.06 ^{abBC}	5.29 ± 0.06 ^{abcdeA}
400	5	5.53 ± 0.09 ^{abA}	2.69 ± 0.02 ^{abA}	43.60 ± 0.06 ^{abcA}	39.93 ± 0.11 ^{abA}	5.19 ± 0.04 ^{abAB}
	10	5.56 ± 0.09 ^{abA}	2.69 ± 0.02 ^{abA}	43.66 ± 0.08 ^{cA}	39.86 ± 0.06 ^{aA}	5.22 ± 0.04 ^{abA}
	20	5.55 ± 0.14 ^{abA}	2.69 ± 0.02 ^{abA}	43.59 ± 0.09 ^{abcBC}	39.88 ± 0.08 ^{aA}	5.23 ± 0.02 ^{abAC}

Results are expressed as the mean ± the standard deviation.

^{a,b,c,d,e} small different letters indicate significant differences ($p<0.05$) among reaction time of enzyme for the same pressure treatment; ^{A,B,C} capital different letters indicate significant differences ($p<0.05$) among the pressure treatment for the same reaction time of enzyme.

Table 29 - Relative percentage of saturated fatty acids (FA), monounsaturated FA (MUFA), polyunsaturated FA (PUFA), *trans* FA, omega-3 and omega-6 polyunsaturated FA (∑n-3 and ∑n-6 PUFA), and ratio between n-3 and n-6 PUFA (n3:n6 ratio) of oil extracted from fried potatoes pre-treated by HPP, expressed in g/100 g of fat.

Pressure (MPa)	Reaction time (min)	Saturated FA	MUFA	PUFA	*Trans* FA	∑w-3 PUFA	∑w-6 PUFA	w3:w6 ratio
0.1	5	9.46 ± 0.05 ^{aAB}	44.43 ± 0.20 ^{abA}	45.63 ± 0.21 ^{abcA}	0.45 ± 0.02 ^{abA}	5.30 ± 0.04 ^{abcdBC}	40.10 ± 0.18 ^{abA}	0.13 ± 0.00 ^{abcB}
	10	9.43 ± 0.07 ^{aA}	44.37 ± 0.05 ^{abA}	45.71 ± 0.07 ^{abcB}	0.46 ± 0.01 ^{abA}	5.33 ± 0.01 ^{abcdefA}	40.14 ± 0.07 ^{abA}	0.13 ± 0.00 ^{abcdeA}
	20	9.57 ± 0.20 ^{aA}	44.24 ± 0.09 ^{abA}	45.73 ± 0.14 ^{abcC}	0.44 ± 0.02 ^{abA}	5.33 ± 0.07 ^{abcdefA}	40.17 ± 0.12 ^{abB}	0.13 ± 0.00 ^{abcdeA}
100	5	9.52 ± 0.07 ^{aB}	44.52 ± 0.14 ^{abA}	45.46 ± 0.13 ^{aA}	0.49 ± 0.03 ^{bA}	5.21 ± 0.02 ^{aA}	40.01 ± 0.11 ^{abA}	0.13 ± 0.00 ^{aA}
	10	9.44 ± 0.07 ^{aA}	44.46 ± 0.15 ^{abA}	45.61 ± 0.16 ^{abcAB}	0.47 ± 0.02 ^{abA}	5.26 ± 0.02 ^{abA}	40.12 ± 0.17 ^{abA}	0.13 ± 0.00 ^{aA}
	20	9.44 ± 0.07 ^{aA}	44.57 ± 0.06 ^{bB}	45.50 ± 0.03 ^{abAB}	0.46 ± 0.02 ^{abAB}	5.23 ± 0.04 ^{abA}	40.04 ± 0.02 ^{abAB}	0.13 ± 0.00 ^{aA}
200	5	9.35 ± 0.07 ^{aA}	44.44 ± 0.06 ^{abA}	45.73 ± 0.03 ^{abcA}	0.45 ± 0.02 ^{abA}	5.33 ± 0.02 ^{abcdefC}	40.16 ± 0.05 ^{abA}	0.13 ± 0.00 ^{abcdeB}
	10	9.40 ± 0.06 ^{aA}	44.44 ± 0.12 ^{abA}	45.69 ± 0.09 ^{abcB}	0.44 ± 0.03 ^{abA}	5.31 ± 0.02 ^{abcdeA}	40.14 ± 0.09 ^{abA}	0.13 ± 0.00 ^{abcA}
	20	9.42 ± 0.11 ^{aA}	44.39 ± 0.05 ^{abAB}	45.71 ± 0.13 ^{abcBC}	0.46 ± 0.02 ^{abAB}	5.33 ± 0.07 ^{abcdefA}	40.14 ± 0.07 ^{abB}	0.13 ± 0.00 ^{abcdeA}
400	5	9.45 ± 0.05 ^{aAB}	44.57 ± 0.07 ^{bA}	45.48 ± 0.12 ^{aA}	0.47 ± 0.03 ^{abA}	5.24 ± 0.04 ^{abAB}	40.01 ± 0.10 ^{abA}	0.13 ± 0.00 ^{aAB}
	10	9.48 ± 0.05 ^{aA}	44.60 ± 0.08 ^{bA}	45.42 ± 0.05 ^{aA}	0.47 ± 0.02 ^{abA}	5.25 ± 0.06 ^{abA}	39.93 ± 0.05 ^{aA}	0.13 ± 0.00 ^{abA}
	20	9.47 ± 0.08 ^{aA}	44.53 ± 0.12 ^{abB}	45.47 ± 0.07 ^{aA}	0.49 ± 0.02 ^{bB}	5.27 ± 0.02 ^{abcA}	39.97 ± 0.08 ^{abA}	0.13 ± 0.00 ^{abcA}

Results are expressed as the mean ± the standard deviation.

^{a,b,c,d,e,f} small different letters indicate significant differences ($p<0.05$) among reaction time of enzyme for the same pressure treatment; ^{A,B,C} capital different letters indicate significant differences ($p<0.05$) among the pressure treatment for the same reaction time of enzyme.

5.6. Effect of asparaginase and HPP on acrylamide content of fried potatoes

Acrylamide content was determined in order to assay the efficacy of the combined treatment of asparaginase with HPP to mitigate the acrylamide formation in fried potatoes. Comparing these results (**Table 30**) with those obtained for fried potatoes pre-treated only by HPP (300.5 - 387.8 μg/Kg), it is verified that only unpressurized samples showed similar acrylamide levels (285.0 - 339.8 μg/Kg), for any enzymatic reaction time. In contrast, fried potatoes pre-pressurized at 100, 200 and 400 MPa exhibited lower acrylamide levels ($p<0.05$) than 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.

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 ideal. For that reason, a blanching step had been required since it changes the microstructure of potato strips and increases the asparaginase-asparagine contact **(Pedreschi et al., 2011)**. However, due to the reduction of acrylamide in fried potatoes pressurized before frying, pressure treatments showed ability to induce the infusion of asparaginase into 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. 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 was the efficient for acrylamide reduction.

In the literature, there are several studies that used asparaginase and applied thermal and non-thermal technologies to try to reduce acrylamide concentration in fried potatoes. In **Table 3** are summarized the results of several studies that used asparaginase as an acrylamide mitigation strategy. Although reductions superior to 60% of acrylamide were obtained, all of them used temperature (50-60 ºC) to increase the enzymatic activity 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 HP treatment, and lower time processing were applied (only 5 min), which became the entire treatment energetically less costly. Even

then, reductions of acrylamide levels of up to 47% were obtained, proving to be a good result.

Table 30 - Acrylamide content in fried potato sticks treated by pressure (0.1, 100, 200 and 400 MPa for 5 min) and an asparaginase solution, with different times of enzyme reaction (5, 10 and 20 min).

Pressure (MPa)	Total asparaginase reaction time (min)	Acrylamide (µg/Kg)	Average of reduction of acrylamide in relation to control (%)
0.1	5	285.0 ± 46.5 ^{aB}	
	10	332.8 ± 21.3 ^{aB}	
	20	339.8 ± 10.2 ^{aB}	
100	5	207.9 ± 19.3 ^{aA}	27.3
	10	246.8 ± 32.2 ^{aA}	25.8
	20	198.2 ± 37.3 ^{aA}	41.7
200	10	192.8 ± 42.1 ^{aA}	42.1
400	5	187.8 ± 18.8 ^{aA}	34.3
	10	203.3 ± 50.3 ^{aA}	38.9
	20	181.1 ± 25.2 ^{aA}	46.7

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.

Chapter IV – Conclusions

This section comprises the main conclusions on the scope of this thesis

This research work had two main objectives: (1) assay the potentiality of HPP pretreatment on modification of physico-chemical properties of raw potato tubers and sticks, and subsequently of sensory, nutritional and physico-chemical properties of the respective fried potatoes; (2) evaluate the potentiality of HPP on asparaginase infusion into raw potato sticks, as a novel strategy to reduce acrylamide levels in fried potatoes.

Preliminary tests with peeled and unpeeled potato tubers packaged in water and under vacuum showed that HPP (>100 MPa) induced textural and cellular changes, and consequently, intracellular material was released into the extracellular medium and the potato tissue became softer. Once the reduction of firmness was directly proportional to the pressure intensity, it could be interesting for industrial application because it would be possible to select exactly the pressure condition that would result in potato tubers with specific desired softness for specific applications. Additionally, potato peel proved to be a physical barrier that blocked the release of intracellular liquid from unpeeled potatoes. In opposition, an increase of water exudation (up to ~12-fold, compared to untreated potatoes) was observed in peeled potatoes, as the pressure intensity increased.

Raw potato sticks pretreated 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. Moreover, the roughness of potato surface reduced in pressurized potatoes, and samples subjected to 600 MPa showed that starch gelatinization was promoted, followed by retrogradation. Despite the activation of the oxidative enzymes due to HPP, colour of raw potatoes was similar to control samples. In addition, HPP tended to induce the reduction of moisture content of raw potato strips, and this effect was more severe as the salt concentration in the exterior water increased. Although the concentration of soluble solids and sugars in the exterior water had increased over the pressure level and holding time after HPP, reducing sugars content in raw potato sticks did not change.

Fried potatoes whose raw potato strips were pretreated either by HPP or by the combination of asparaginase and HPP showed higher weight loss 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 pretreated by HPP exhibited a lighter colour, while those pretreated by HPP and asparaginase exhibited a darker, and more red and yellow colour

than control samples. Lipid content of fried potatoes pretreated by HPP did not changed, which proved that, although this problem has not been solved, the production of less healthy fried potatoes was not promoted. Lipid composition in oil extracted from fried potatoes remained the same regardless of the applied treatment. 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% (in samples 100/10) up to 47% (in samples 400/20). Furthermore, all of the studies in the literature, that used asparaginase as a strategy to reduce acrylamide levels in fries, have resorted to temperature (50-60 ºC) in order to increase the enzymatic activity and the asparaginase-asparagine contact. In opposition, in the present study, no thermal step was applied either during or after HP treatment, and lower time processing were used (only 5 min), which became the entire treatment energetically less costly.

In sum, this study showed that HPP is 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, given the ability of HPP to facilitate the diffusion of ingredients into potato sticks, it would be possible to promote infusion of other interesting enzymes, compounds with biological importance (such as, antioxidants, vitamins, etc), and others, as a strategy to improve sensory and nutritional properties of potato products.

Chapter V – Future Work

This section proposes the essential future work regarding the application of HPP on modification of the quality of fried potatoes

Further experiences regarding the evaluation of nutritional properties of fried potatoes should be carried out. It includes the measurement of asparagine content in fried potatoes pretreated by HPP and asparaginase (whose method required optimization and because of lack of time, it was not possible to conclude this analysis) in order to prove that acrylamide levels reduced due to the reduction of asparagine concentration; determination of oil content in fried potatoes pre-treated by HPP and asparaginase to evaluate if this treatment had influence on oil uptake; quantification of resistant starch in raw potato sticks to assay if HPP had ability to reduce resistant starch and consequently accelerate potato cooking processes in general; sensorial analysis of fried potatoes whose raw potato sticks were pretreatment by HPP and by the combination of asparaginase and HPP. Furthermore, it would be interesting to test double frying to simulate the industrial process of French fries' production.

In addition, there were no data in the literature about the effect of HPP on infusion of asparaginase into food products, and even on its enzymatic activity. Thus, a larger number of conditions should be optimized, namely the asparaginase concentration, pressure level, thickness of potato strips, and time processing, in order to improve asparaginase activity under pressure and the contact between asparaginase-asparagine. Lastly, it would be interesting to evaluate infusion of another enzymes and compounds as novel strategies to reduce the concentration of acrylamide precursors in raw potatoes, and consequently the acrylamide levels in fried or even roast potatoes.

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Annexes – Complementary information

This section comprises all the complementary information mentioned along the various thesis chapters

Annex I – Total number of HP equipment operating worldwide

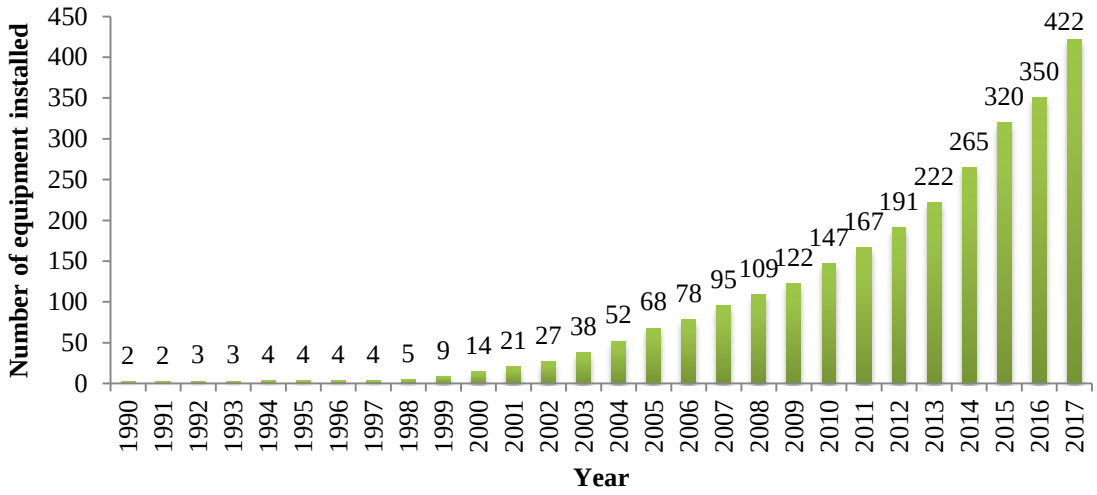

Figure 19 - Total number of HP equipment operating worldwide. Courtesy of Hiperbaric (Burgos, Spain).

Annex II – Nutritional composition of oil frying (*Fula* brand)

Table 31 - Nutritional composition of oil frying (*Fula* brand) used in frying assays, whose nutrient concentrations were expressed as value/100 mL of oil **(Sovena S.A., 2019)**.

Nutrient		Value per 100 mL of frying oil
Lipids		92 g
of which	saturated	8.1 g
	monounsaturated	46 g
	polyunsaturated	38 g
Carbohydrates		0 g
of which	sugars	0 g
Proteins		0 g
Salt		0 g
Vitamin E		60 mg

Annex III – Instrumental accessories (plate and knife) used in texture assays

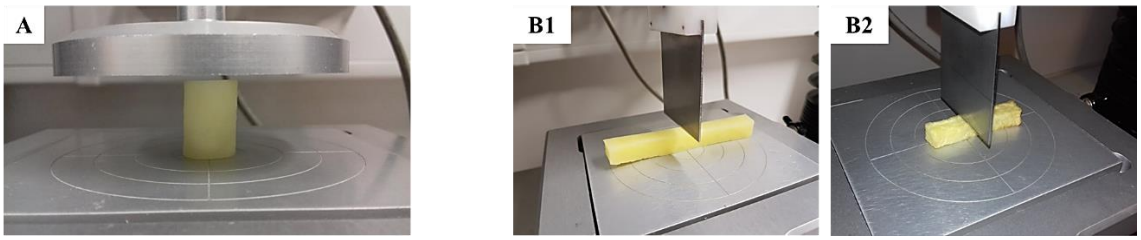

Figure 20 – Image A shows the platen (with 6 cm of diameter) and the potato cylinders (with 1 cm of diameter and 2 cm of length) used in texture assays by compression of whole potato tubers pre-treated by HPP. Images B show the knife (with 6 cm of width, 10 cm of height and 1.2 mm of thickness) used in texture assays by cutting of raw potato sticks (B1) and fried potato sticks (B2).

Annex IV – Weight different of potato tubers after HPP

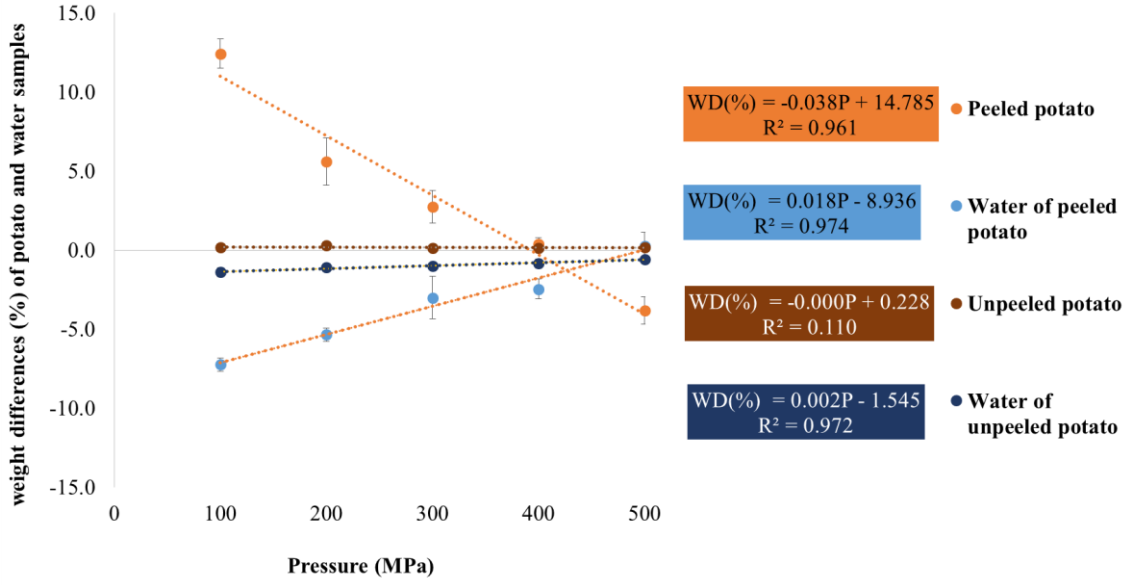

Figure 21 - Graphical representation of weight difference, expressed in %, of peeled and unpeeled potato tubers and the respective exterior water samples, along the pressure. In the linearization equations, "WD(%)" and "P" mean Weight Difference (%) and Pressure (MPa), respectively.

Annex V – Graphic of texture analysis, by compression, of raw potato tubers

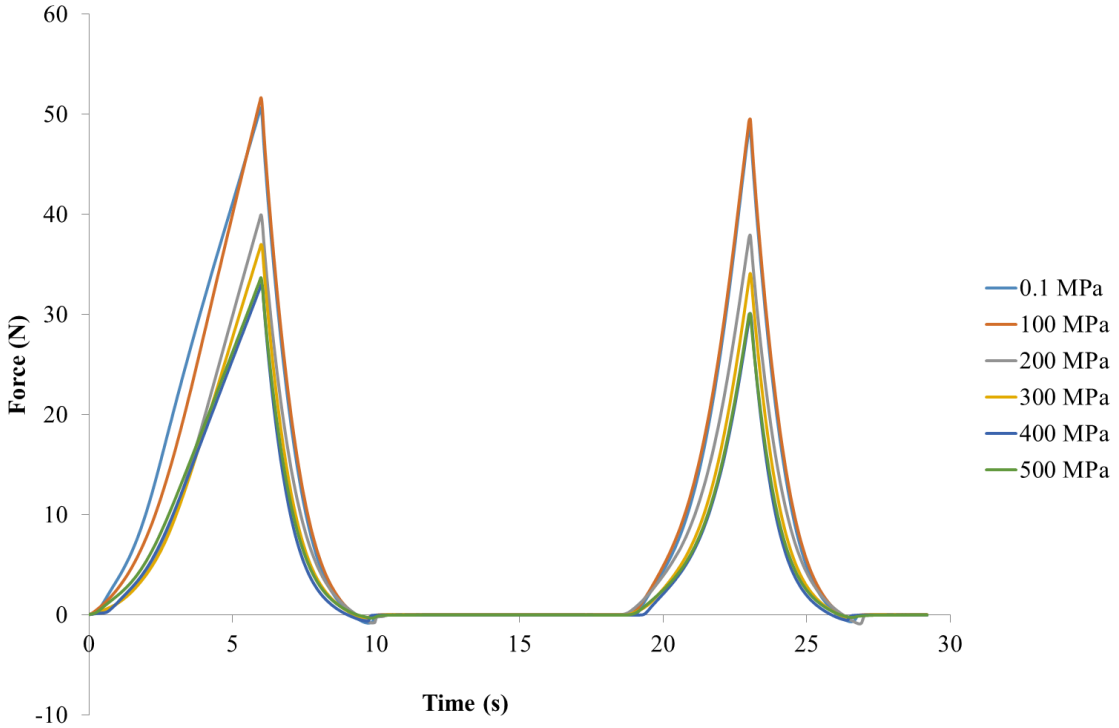

Figure 22 - Example of a set of Texture Profile Analysis (TPA) graphic obtained from the analysis of peeled potato tubers packaged in water.

Annex VI – Weight difference of raw potato sticks after HPP

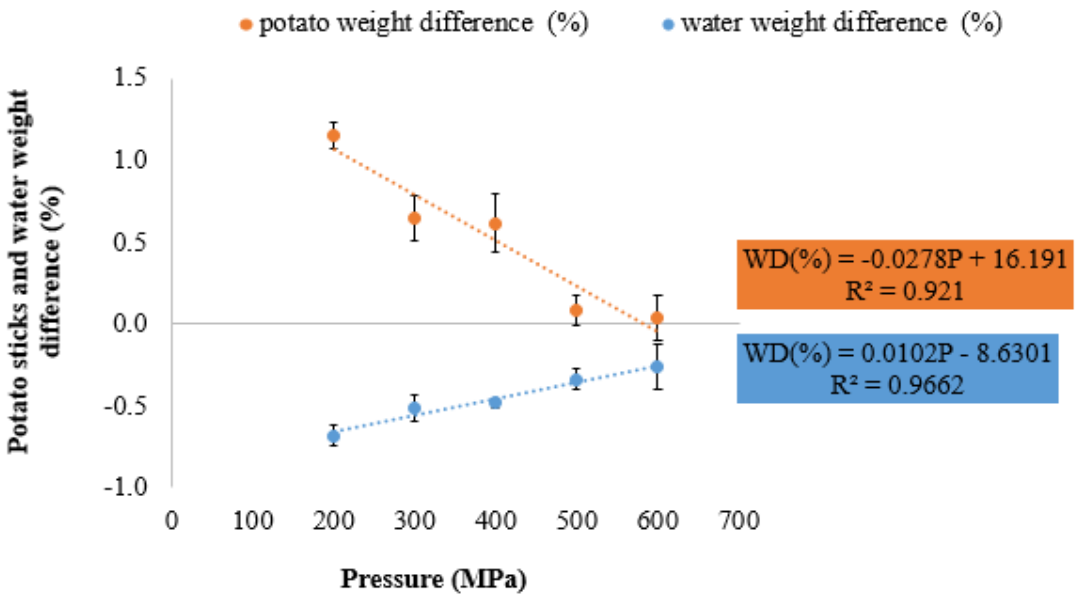

Figure 23 - Graphical representation of weight difference, expressed in %, of potato sticks subjected to 200, 300, 400, 500 and 600 MPa for 2.5 min, and the respective exterior water samples, along the pressure. In the linearization equations, "WD(%)" and "P" mean Weight Difference (%) and Pressure (MPa), respectively.

Annex VII – Graphical representations obtained from DSC analysis of raw potato sticks

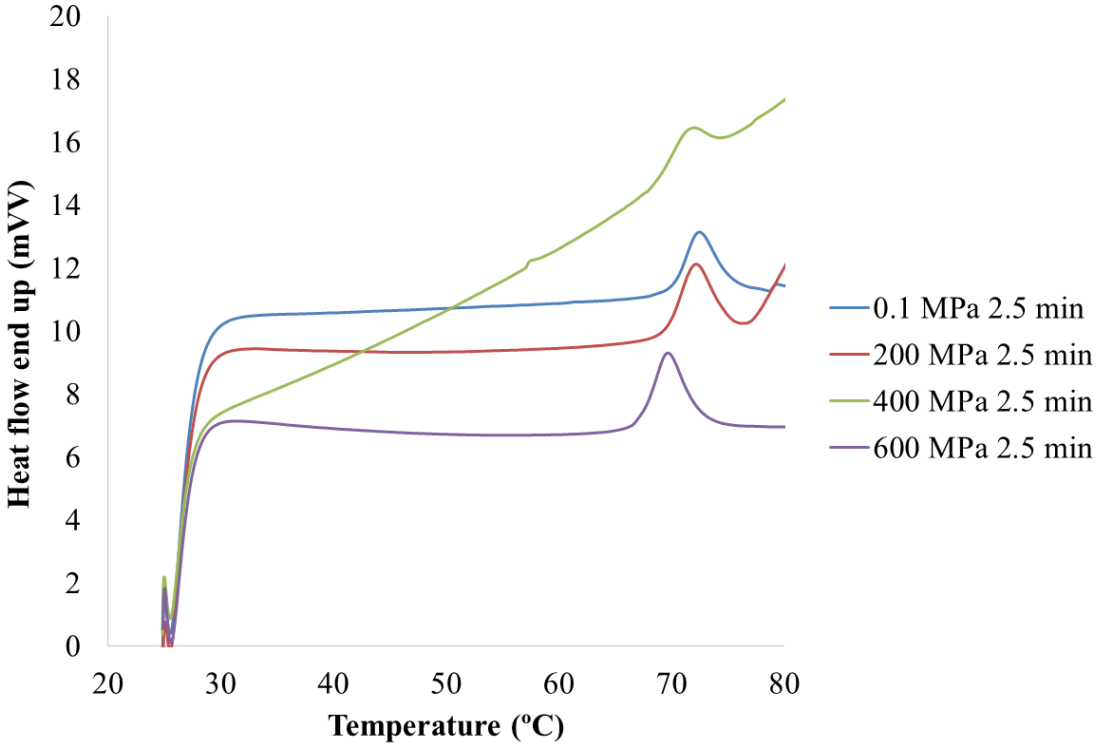

Figure 24 - Graphical representation of heat flow end up in function of temperature, during DSC assay, for raw potato sticks treated at 0.1, 200, 400 and 600 MPa for 2.5 min.

Annex VIII - Texture analysis, by a cutting test, of raw potato sticks treated by HPP

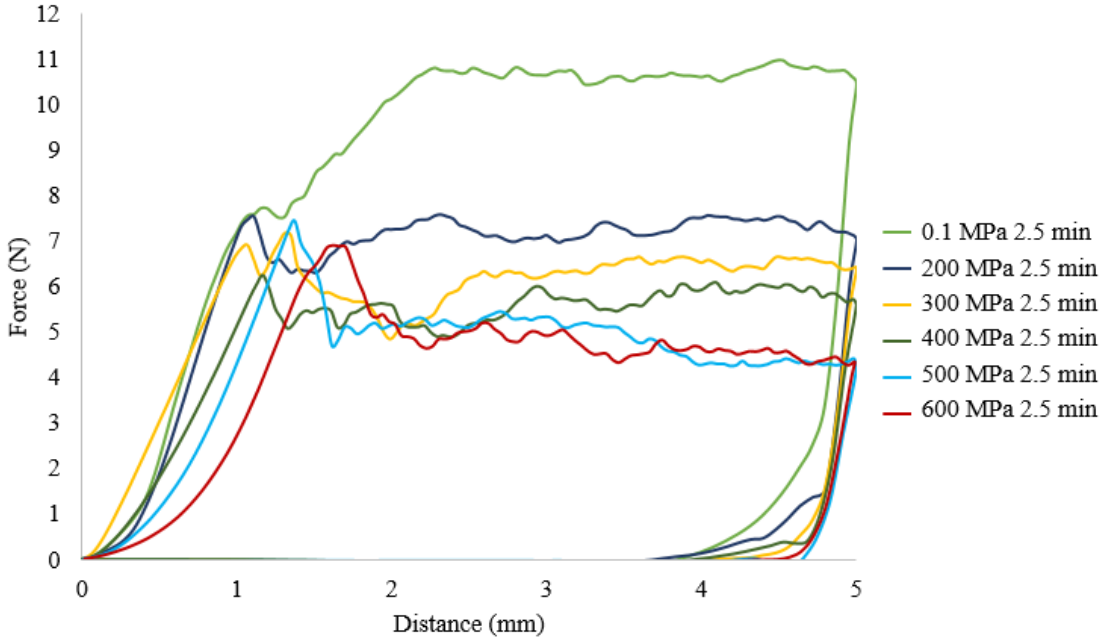

Figure 25 - Exemplary force-displacement curves obtained through the texture analysis of pressurized potato sticks, by a cutting test using a suitable knife.

Annex IX – Calibration curve of reducing sugars analysis

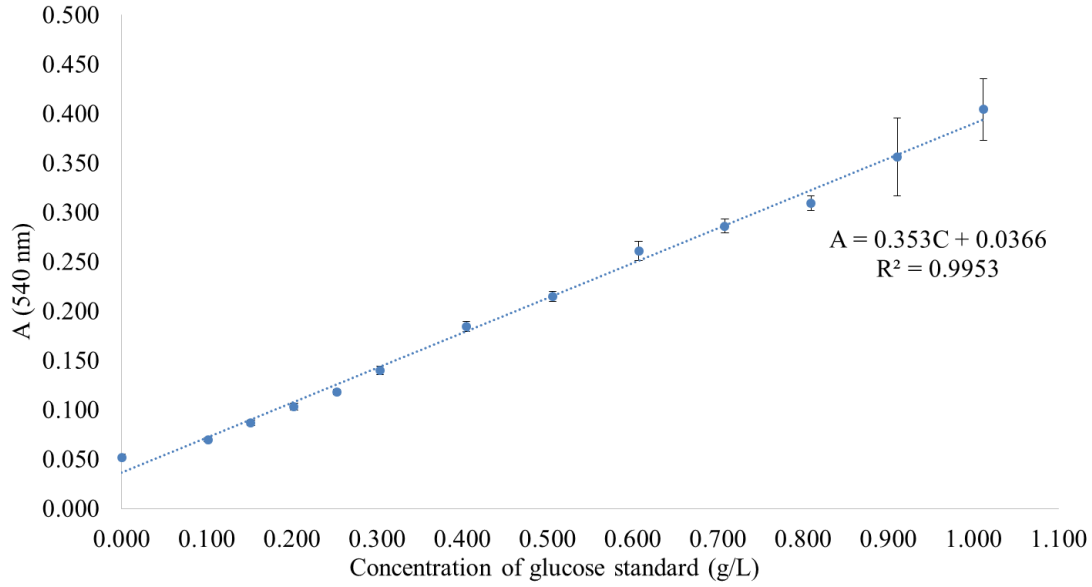

Figure 26 - Calibration curve prepared for reducing sugars analysis with several glucose standards. "A" and "C" mean Absorbance (540 nm) and Concentration of glucose standard (g/L).

Annex X – Weight difference of raw potato sticks after asparaginase and HPP treatment

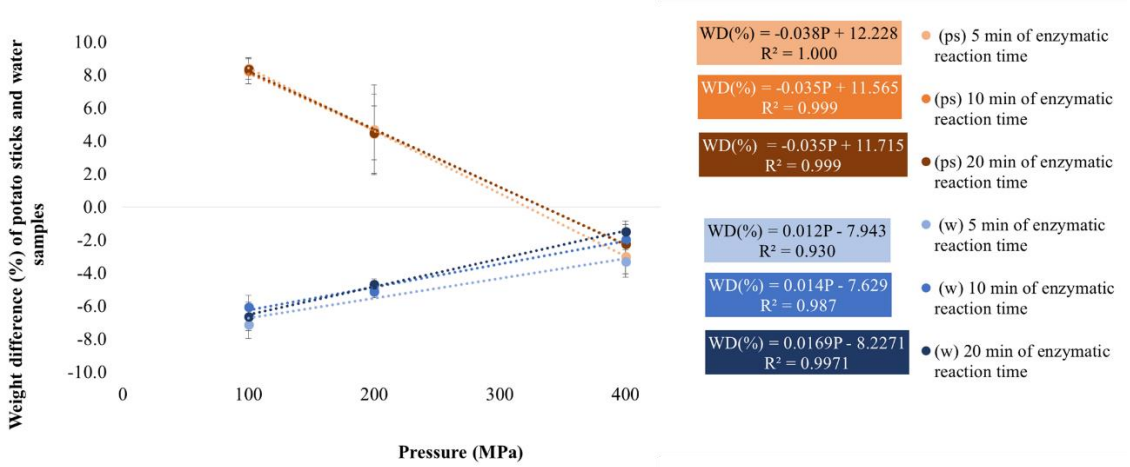

Figure 27 - Graphical representation of weight difference, expressed in %, of raw potato sticks treated by HPP and an asparaginase solution, and the respective exterior water samples, along the pressure. In the linearization equations, "WD(%)" and "P" mean Weight Difference (%) and Pressure (MPa), respectively. In the legend, "ps" and "w" mean potato sticks and water, respectively.