University of Aveiro Department of Chemistry

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RITA FERREIRA SARDÃO

Effect of high pressure processing to extend the shelf life of a functional acorn beverage

Efeito do processamento por alta pressão no prolongamento da vida útil de uma bebida funcional de bolota

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biotecnologia, Ramo de Biotecnologia Alimentar, realizada sob a orientação científica da Professora Doutora Maria Manuela Estevez Pintado, Professora Associada da Escola Superior de Biotecnologia da Universidade Católica Portuguesa, do Doutor Jorge Manuel Alexandre Saraiva, Investigador Auxiliar do Departamento de Química da Universidade de Aveiro, e da Doutora Elisabete Maria da Cruz Alexandre, Investigadora do Departamento de Química da Universidade de Aveiro.

Dedico este trabalho aos meus pais e irmã, pelo exemplo de vida que são, por todo o amor que me dão e por tornarem isto possível.

o júri

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Palavras-chave Bolota, processamento por alta pressão, microbiologia, cor, taninos hidrolisáveis, compostos fenólicos, atividade antioxidante, aminoácidos, ácidos gordos

Resumo O consumo de bebidas vegetais pode ser uma excelente forma de aumentar a ingestão de compostos bioativos que promovem a saúde. No entanto, as bebidas vegetais não processadas têm um tempo de prateleira curto e geralmente são necessários tratamentos térmicos, afetando a sua qualidade. O processamento por alta pressão (HPP) pode ser uma solução, pois tem sido reconhecido por manter a qualidade nutricional e sensorial de alimentos frescos. A bolota é muito abundante em Portugal, mas ainda é subvalorizada, a ponto de permanecer no campo sem utilidade, apesar de seu elevado valor nutricional, compostos fitoquímicos, propriedades antioxidantes, anticarcinogénicas, e cardioprotetoras. Assim, este trabalho teve como objetivo desenvolver uma bebida funcional de bolota, isenta de glúten e lactose. O impacto do HPP (450 e 600 MPa por 5, 12.5, e 20 min) e do processamento térmico convencional (85 ºC por 30 min) na segurança microbiológica (mesófilos, psicrófilos, bolores e leveduras, *Enterobacteriaceae*, *Staphylococcus,* e bactérias lácticas) e características físico-químicas (pH, cor, atividade antioxidante, e compostos antioxidantes) foi avaliado. A lixiviação inicial da bolota reduziu em 42.5 % o teor de taninos hidrolisáveis, responsáveis pela adstringência indesejável. As amostras pressurizadas mantiveram a estabilidade microbiológica ao longo de dez semanas de análise, o que não se verificou para amostras não tratadas. Além disso, o HPP a 450 MPa por 5 min foi suficiente para inativar *B. cereus*, *E. coli,* e *P. aeruginosa*. O HPP preservou melhor a cor (ΔE^* menor), mas o pH aumentou após ambos os tratamentos (de 5.1 para 5.9 e 6.2, em amostras processadas termicamente e pressurizadas, respetivamente). O grau brix foi muito baixo em todas as amostras (0,1 – 1,6 %), mas com valores mais altos nas processadas termicamente. Os fenólicos totais e a atividade antioxidante (ABTS, DPPH, e ORAC) foram semelhantes entre os diferentes tratamentos, sendo o ácido gálico o principal composto fenólico detetado. O conteúdo total em hidratos de carbono, lípidos, e proteínas foi semelhante, bem como a fração lipídica, que mostrou elevados valores de MUFA e PUFA, ácidos gordos nutricionalmente relevantes, como oleico, linoleico e linolénico. A fração lipídica revelou também baixos índices de trombogenicidade e aterogenicidade. Esta bebida é fonte de aminoácidos, uma vez que contém todos os aminoácidos essenciais determinados. Em relação ao conteúdo em minerais, o potássio foi o principal composto quantificado, mas outros foram encontrados em menores quantidades. A ausência de 5-hidroximetilfurfural foi confirmada em ambos os tratamentos. Na análise sensorial realizada, os consumidores preferiram a amostra pressurizada. Com este estudo foram realizados alguns ensaios a fim de prosseguir com o desenvolvimento de uma bebida funcional com este fruto que é um excedente em Portugal e no mundo.

Keywords Acorn, high pressure processing, microbiology, colour, hydrolysable tannins, phenolic compounds, antioxidant activity, amino acids, fatty acids

Abstract The consumption of plant-based beverages can be an excellent way to increase the intake of bioactive compounds promoting consumers' health. However, unprocessed plant-based beverages have a short shelf life and thermal treatments are usually required, affecting the overall quality of the beverages. High pressure processing (HPP) may be a solution since it has been recognized for retaining nutritional and sensorial quality of fresh foods. The acorn is very abundant in Portugal but still is sub-valorised to the point of staying in the field without any use, despite its high nutritional value, phytochemical compounds, antioxidant, anticarcinogenic, and cardioprotective properties. This work aimed to develop a functional acorn beverage, free of gluten and lactose. Thereafter, the impact of HPP (450 and 600 MPa for 5, 12.5, and 20 min) and conventional thermal processing (85 ºC for 30 min) on the microbiological safety (*Enterobacteriaceae*, mesophiles, psychrophiles, molds and yeasts, *Staphylococcus* and lactic bacteria) and physical-chemical characteristics (pH, colour, antioxidant activity, and food compounds) was assessed. The initial acorn leaching reduces in 42.5 % the content of hydrolysable tannins, which are responsible for the undesirable astringency. The pressurized samples maintained microbiological stability over ten weeks of analysis, which was not verified for untreated samples. Moreover, HPP at 450 MPa/5 min was enough to inactivate *B. cereus, E. coli* and *P. aeruginosa*. The HPP better preserved the colour (lower ΔE^*), but the pH increased after both treatments (from 5.1 to 5.9 and 6.2, for thermal processed and pressurized samples). The degree brix is very low in all samples (0.1 – 1.6 %), with higher values in the thermal processed samples. Total phenolics as well as antioxidant activity (ABTS, DPPH, and ORAC) were similar among the different treatments being gallic acid the main phenolic compound found in this beverage. The total carbohydrates, lipids, and protein contents were similar between the different treatments, as well as the lipid fraction, that shows elevated values of both MUFA and PUFA, namely nutritionally relevant fatty acids like oleic, linoleic, and linolenic. The lipid fraction also reveals low thrombogenicity and atherogenicity indices. This beverage is source of amino acids once it contains all the essential amino acids determinate. Regarding mineral content, potassium was the principal compound quantified but other minerals were found in minor amounts. The absence of 5-hydroxymethylfurfural was confirmed in both treatments. In the sensory analysis performed, the consumers preferred the pressurized treated sample. With this study some tests were performed in order to proceed with the development of a functional beverage with this fruit that is a surplus in Portugal and around the world.

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Abbreviations and Symbols

5-HMF	5-hydroxymethylfurfural
5-SMF	5-sulfoxymethylfurfural
AAPH	2,2′-azobis(2-methylpropionamidine) dihydrochloride
ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
AI	Index of atherogenicity
ALA	Alpha-linolenic acid
ANOVA	One-way analysis of variance
ATCC	American type culture collection
a_w	Water activity
a*	Red-greenness
BBS	Borate buffer
BGL	β-glucosidase
b*	Blue-yellowness
CFU	Colony forming units
CHD	Coronary heart disease
CIE	International Commission on Illumination
CINATE	Laboratório de análises e ensaios a alimentos e embalagens
CHTMAD	Centro hospitalar de Trás-os-Montes e Alto Douro
CoQ10	Coenzyme Q10
DF	Dilution factor
DHA	Docosahexaenoic acid
DMF	N,N-dimethylformamide
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC	European Commission
EPA	Eicosapentaenoic acid
FA	Fatty acids
FAME	Fatty acid methyl esters
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GLA	Gamma-linolenic acid
GLC-FID	Gas chromatography-flame ionization detector
HDL	High-density lipoprotein
HHP	High hydrostatic pressure
HIV	Human immunodeficiency virus
HPH	High pressure homogenization
HPL	Hydroperoxide lyase
HPLC	High-performance liquid chromatography
HPLC-DAD	High-performance liquid chromatography with a diode-array detector
HPP	High pressure processing
HTC	Hydrolysable tannins content
HTST	High-temperature short-time
ICP-OES	Inductively coupled plasma optical emission spectroscopy
ISO	International Organization for Standardization
K	Equilibrium constant
LDL	Low-density lipoprotein
LOD	Limit of detection
LOX	Lipoxygenase

L*	Lightness
MAP	Modified atmosphere packaging
MF	Membrane filtration
MRS	Man Rogosa and Sharpe
MUFA	Monounsaturated fatty acid
OMF	Oscillating magnetic fields
OPA	Phthaldialdehyde
ORAC	Oxygen radical absorbance capacity
P	Pressure
PATS	Pressure assisted thermal sterilization
PBS	Phosphate buffered saline
PCA	Plate count agar
PEF	Pulsed electric fields
PETS	Pressure enhanced thermal sterilization
PG	Polygalacturonase
PME	Pectinmethylesterase
POD	Peroxidase
POTS	Pressure ohmic thermal sterilization
PPO	Polyphenoloxidase
PUFA	Polyunsaturated fatty acid
R	Universal gas constant
SD	Standard deviation
SFA	Saturated fatty acid
SLAB	Starter lactic acid bacteria
T	Temperature
TA	Titrable acidity
TFA	Trifluoroacetic acid
TI	Index of thrombogenicity
TPC	Total polyphenolic content
Trolox	(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid
TSS	Total soluble solids
UHP	Ultra-high pressure
UHPH	Ultra-high pressure homogenization
UHT	Ultra-high-temperature
US	Ultrasound
USD	United States dollar
USDA	United States Department of Agriculture
UV	Ultraviolet
UV-VIS	Ultraviolet–visible
V	Volume
VRBGA	Violet red bile glucose agar
WHO	World Health Organization
n-3	Omega-3 fatty acid
n-6	Omega-6 fatty acid
ΔE*	Total colour change variation

Contextualization

This thesis is composed of five chapters. **Chapter I** comprises the state of the art concerning (1) functional foods with focus on functional beverages; (2) conventional and emergent processing methods available for food industry today; (3) high pressure processing (HPP), the technology used in this master's thesis work, highlighting the most recent studies on the effects of this technology on microbiological and quality parameters in plant-based functional beverages; and (4) *Quercus* acorn, the raw material of the this study, emphasizing its high content regarding bioactive compounds and potential health benefits. In **Chapter II** a detailed description of the materials and methods used in this work is provided, followed by the **Chapter III** that presents and discusses the results regarding the microbiological, physicochemical, and sensorial parameters over storage on untreated, heat-treated, and HPP-treated acorn beverage. The main conclusions of this thesis are provided on the **Chapter IV**, followed by the proposed future work on the **Chapter V**, and the list of the consulted literature. 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 compiled literature review regarding functional foods, high pressure processing as an emerging technology in food processing, and *Quercus* acorn, the raw material of this master's thesis.

1. Functional foods

The increased production of processed food, rapid urbanization, and modern lifestyles lead to a shift in dietary patterns worldwide. People have started to consume more energetic foods rich in fats, sugars, and salt/sodium, and consequently many have stopped eating enough fresh fruit and vegetables or even whole grains as sources of important compounds, such as the dietary fibres [1]. According to World Health Organization (WHO), obesity, cancer, diabetes and cardiovascular diseases have been increasing mainly due the dietary patterns changes, which also have a major impact on health care costs, quality of life and in general public health. In 2016, more than 1.9 billion adults worldwide were overweight and of these, over 650 million were obese [2]. This problem is of such high magnitude that obesity is even considered the epidemic of the 21st century [3].

For these reasons, the production and consumption of functional foods has been extended in many parts of the world [4]. Consumers are taking a more holistic view of health and nutrition and start noticing that functional foods can play an important role in supporting their health, longevity and well-being. Furthermore, the concern about the potential side effects from modern-day medications provided by the pharmaceutical industry leads to the beginning of a search for more natural forms of prevention and cure [5]. Like Hippocrates wrote 2400 years ago "Let food be thy medicine and medicine be thy food" [6].

Nowadays, an extensive research is directed towards the understanding of how functional foods and food ingredients might help prevent chronic disease or optimize health [7]. As result, the number of published works on functional foods skyrocketed and it is one of the fastest expanding areas of food research today [8].

Following the trends observed in consumer demand, the food industry develops products that bring science and high-technology into everyday life by "promising" certain health benefits [9]. According to a report by Grand View Research published in 2016, the global functional foods market size was of 129.39 billion USD in 2015 and food industry is expecting that continue to grow over the next nine years (Figure 1) [10]. Consequently, it is estimated that an annual reduction of 20 % in health-care expenditure is possible through widespread consumption of functional foods [11].

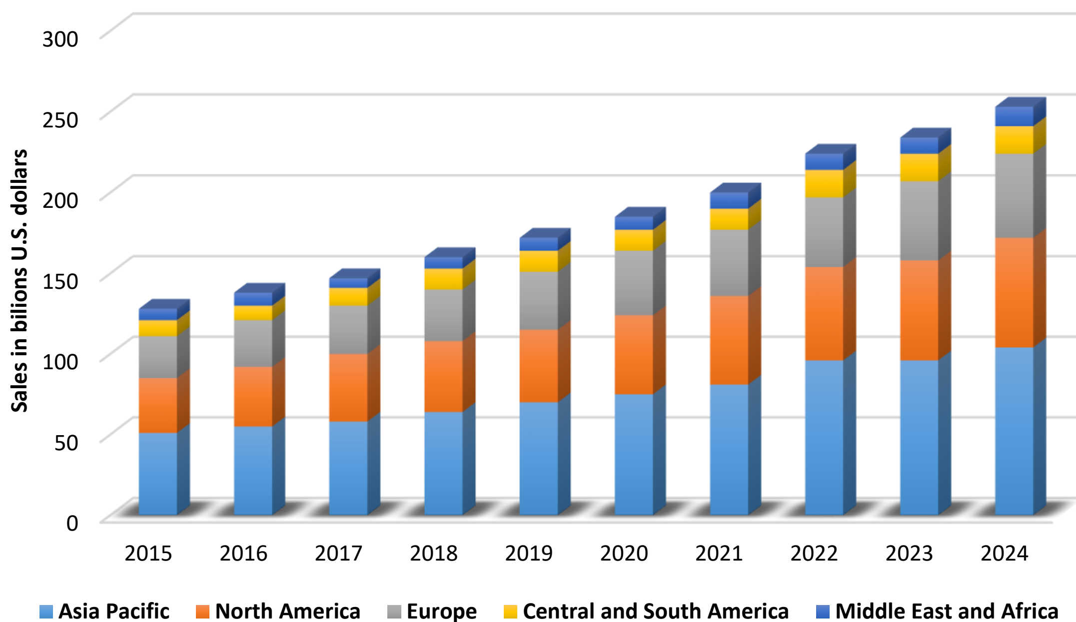


Figure 1: Expected total turnover of functional foods worldwide from 2015 to 2024, by region (in billions U.S. dollars). Data from Grand View Research (2016) [10].

1.1. Definitions and types of functional foods

The term "functional foods" was first introduced in Japan during the eighties, since then numerous definitions appeared and there is no one universally accepted definition [12]. Moreover, FAO (Food and Agriculture Organization) / WHO Codex Alimentarius System does not provide clear guidelines or specifications for functional foods [13]. However, to clarify and distinguish functional foods, four concepts were identified: nature of the food, health benefits, functionality, and consumption pattern, which should be included in a broadly accepted "functional food" definition [14]. In Table 1 can be seen some examples of definitions that contain these four concepts. The definition proposed by Doyon and Labrecque (2008) resulted from an extensive literature review and consultation with a group of North American and European experts. It is important to highlight that this type of analysis is important due the a large number of definitions, as well as great variations within definitions that make it difficult to provide industry partners with robust information on market trends and market potential, or to appropriately protect consumers through legislation [14]. The last definition, proposed by the Functional Food Center (2014), is unique because of its acknowledgement of "bioactive compounds", molecules that improve health through physiological mechanisms. Furthermore, this definition notes that bioactive

compounds must be taken in non-toxic amounts because, bioactive compounds have upper limits before they become dangerous [15].

Table 1: Examples of "functional foods" definitions containing the four concepts previously identified. Adapted from Doyon and Labrecque (2008) [14].

Authors	Definition
Diplock *et al.* (1999)	"A food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either improved stage of health and well-being and/or reduction of risk of disease. A functional food must remain food and it must demonstrate its effects in amounts that can normally be expected to be consumed in the diet: it is not a pill or a capsule, but part of the normal food pattern."
Lajolo (2002)	"A food that is a food and not a drug, that is part of a normal diet and that can produce benefits beyond basic nutrition."
Jansen and Krijger (2003)	"Foodstuffs mostly similar in appearance to conventional foods that fit daily in the diet and consumption pattern but that, in addition to their basic nutritional value, contain specific additives or properties achieved by processing or otherwise for which a physiological/health benefit beyond basic nutrition is claimed."
Health Canada (2006)	"A functional food is a conventional food or a food similar in appearance to a conventional food, it is part of a regular diet and has proven health-related benefits and (or) reduces the risk of specific chronic diseases above its basic nutritional functions."
Doyon and Labrecque (2008)	"A functional food is, or appears similar to, a conventional food. It is part of a standard diet and is consumed on a regular basis, in normal quantities. It has proven health benefits that reduce the risk of specific chronic diseases or beneficially affect target functions beyond its basic nutritional functions."
Functional Food Center (2014) [15]	"Natural or processed foods that contains known or unknown biologically-active compounds; which, in defined, effective non-toxic amounts, provide a clinically proven and documented health benefit for the prevention, management, or treatment of chronic disease."

From a product point of view, the functional property can be included in numerous distinct ways. Functional foods can be foods fortified, enriched, altered, non-altered, and enhanced in order to promote benefits to health and well-being (Table 2). Furthermore, it should be noted that some functional foods may only provide beneficial effects in individuals with specific risk factors. In other words, not all functional foods are functional for the entire population. Another matter to be taken into account is the threshold level of intake that is

necessary for a physiological effect. Many functional foods may thus be functional only under specific conditions of usage [16]. Moreover, functional foods offer potential health benefits, but consumers should also consider the overall quality of their diet.

Table 2: Types and examples of functional foods. Adapted from Lau *et al.* (2012) and Kotilainen *et al.* (2006) [13,16].

Types of functional food	Description	Example	Benefit to health and well-being
Fortified	A food fortified with nutrients usually found in lower amounts or modified to increase their bioavailability in humans by means of specialized food processing technologies.	Milk fermentation with specific bacteria to yield bioactive peptides	For example, lowers blood pressure
Enriched	A food with added new nutrients or components not normally found in a food.	Margarine enriched with plant sterols	Improve cholesterol levels
Altered	A food from which a deleterious component has been removed, reduced or replaced with another substance with beneficial effects.	Chewing gum sweetened with xylitol instead of sugar	Helps prevent dental caries
Non-altered	A food that naturally contains enough amounts of a beneficial nutrient or non-nutrient component.	Oats (beta-glucan)	Heart health
Enhanced	A food in which one of the components has been naturally enhanced through special growing conditions, new feed composition, genetic manipulation or otherwise.	Eggs with increased omega-3 content achieved by altered chicken feed	Heart health

The diversity of these foods suggests that a variety of bioactive components may be involved. Some main groups of functional components include carotenoids, dietary fibres, fatty acids, vitamins, prebiotics and probiotics, and minerals. Phytochemicals, enzymes and antioxidants also are commonly include (Table 3) [10,13]. However, to make functional food it is necessary to check the interactions between food components and body functions and/or pathological processes [17]. Foods comprised of different types of bioactive compounds may be considered "multifunctional foods" [18,19].

Table 3: Some examples of potential benefits of some popular food components. Adapted from Lau *et al.* (2012) [13].

Functional components	Natural sources	Potential benefits
Lycopene (carotenoid)	Tomato	Reduce the risk of prostate cancer
Beta-glucan (dietary fibre)	Oats, barley	Reduce cardiovascular disease, lower LDL and total cholesterol risks
Omega-3 – DHA and EPA (fatty acids)	Fish oils	Reduce cardiovascular disease and improve mental functions
Lactibacillus (probiotic)	Yoghurt	Improve quality of intestinal microflora
Isoflavone (phytochemical)	Soy-based products	Reduce cardiovascular disease, lower LDL and total cholesterol risks
Catechin (antioxidant)	Tea	Neutralize free radicals and reduce cancer risks

LDL (low-density lipoprotein); DHA (eicosapentaenoic acid); EPA (docosahexaenoic acid).

Functional food products have been developed in all food categories and are usually similar in appearance to conventional foods. Bakery/cereals, dairy products, meat/fish/eggs, soy products and fats/oils are among the more popular applications. Beverages, nutritional bars, baby food, and various snack types are also other options [10,13].

The more common end-use perspective of these products includes sports, nutrition, weight management, immunity, digestive health, clinical nutrition, and cardiovascular health related problems. Different segments consist of lowered cholesterol levels, obesity and appetite control, osteoporosis prevention, among others [10].

1.2. Consumption and acceptance in Europe and worldwide

Consumer's acceptance and attitude towards functional food products determine the market's size (see Figure 1) and the success. Regarding to health message of functional foods, markets for these products are very large and have been growing steadily in many parts of the world [13,20].

A number of factors can be responsible for consumer positive attitudes towards foods, such as advancing scientific evidence that diet can alter disease prevalence and progression, advances in food science and technology, and changes in food regulations [9]. However, the

current lack of legal definition and regulation for functional foods could negatively affect the consumer attitude to functional foods [16]. The consumers most likely to have a positive attitude towards functional foods are the ones who have faced illness among relatives or have experienced illness themselves. These consumers generally have a higher health motivation and perceived diet effectiveness of products [9]. However, today more and more people are aware that their diet can prolong life expectancy and improve the quality of life in the long term.

Thereby, the consumer awareness can be the most important factor determining demand for functional food and may explain the higher demand for functional foods in developed countries. In developed countries, it was found that the Americans accept and consume functional foods more easily, and the Europeans' that are more critical and question the functional foods efficiency [12,20].

The biggest functional foods markets are in Japan and the US. European markets are far behind them but are the Germany, France, United Kingdom and Netherlands that have higher consumption of functional foods than other European countries. In Europe, functional foods only have become very popular in recent years and the countries differ in their nutrition and health claims of functional foods and also the popularity of these products differs from country to country [20].

In the developing countries the demand for functional foods is small, but with prevalent poverty and high rates of malnutrition and diet-related diseases, growing functional foods markets provide opportunities for developing countries through improvements in public health, and for generating employment and incomes [16,21].

In terms of gender, age, levels of education and levels of incomes, the literature is conflicting with respect to the profile of functional food consumers [9].

1.3. From idea to market

The development and marketing of functional foods require significant research efforts, such as: (i) identify functional compounds and assess their physiological effects; (ii) develop a suitable food matrix, taking into account bioavailability and potential changes during processing and food preparation; and (iii) clinical trials on product efficacy in order to gain approval for health-enhancing marketing claims [16]. Foar the ingredients selection is necessary to assess their physiological effects, consider the interactions between the

bioactive compounds and other food components during food processing and storage, and their effect on the stability of the resulting product [11]. At the end of the formulation, it is necessary to do some controlled and regulated clinical trials in order to prove product functionality [5]. In the analysis should be take into account that exposing cells in a cell line to various combinations of bioactive compounds and the formulated beverages is not equivalent to a clinical trial in which humans consume these beverages [22]. While the findings are often interesting, they represent a very preliminary step in establishing the usefulness of these compounds in human health [23].

The approach to regulating functional foods and their marketing is heterogeneous due to the difficulties in classifying these products and to the varying views on what is considered sufficient scientific evidence to determine functionality. Common concerns in all legislation are ensuring product safety and public health, but regulation of functional foods differs from that of conventional foods mainly with respect to labelling and advertising [16]. In some European countries, a health benefit can be conveyed to the consumer using "nutrient-function claims" or "health claims" and it is regulated by EC Regulation 1924/2006 on "Nutrition and health claims made on foods." [6,16].

Regarding the target group, functional foods should be designed for niche markets, rather than being developed for the whole marketplace, because consumers seek healthy foods for a variety of reasons and have distinct preference patterns. From a marketing perspective, it is important to understand that consumers weigh different factors when making a purchase decision and these include not only health and nutrition but also price, convenience, and taste [9].

Besides the strategic identifying of the target markets, companies need to concentrate their efforts on educating consumers and communicating the health benefits of their products in a way that consumers understand and find credible. The consumer understanding of the health claims of functional foods plays an essential role in helping to guide their food choices. Since the consumers place a high level of trust in information sourced from government agencies, opportunities exist to partner and collaborate with other stakeholders [9].

Changing consumer perceptions and food choices will ultimately present challenges for food manufacturers who want to maintain their innovative edge. Innovation has become an imperative for food companies to create new products with high consumer acceptance or popularity. Understanding consumer needs should be a high priority strategic objective in new product development [11].

1.4. Trends, opportunities, and future perspectives

Functional foods are the "it" health trend today. This diffusion of functional foods has been due to several critical factors recognized as the key factors, such us: awareness of personal health deterioration, due to busy lifestyles with poor choices of convenience foods and insufficient exercise; increased incidence of self-medication; increased awareness of link between diet and health due to information by health authorities and media on nutrition; and a crowded and competitive food market [24].

Posted on *Food Technology*, Elizabeth Sloan reports the trends in the US functional foods market for 2018. According to the report published in April 2018, instant nutrition (quick, easy and healthy snacking), everyday performance foods, and health-enhancing food processing, are among the trends defining the functional food and beverage sector. New, comparing with previous years, are the rise of sports-type nutrition products, foods to help build strength; a resurgence of "full fat" foods, as a result of the popularity of fat rich weight loss diets; and the rapidly increasing sales of plant-based meat and milk alternatives [25]. Plant-based dining now means more than just swapping meats for vegetables, it represents a strategy that includes zero waste policies and a wider focus on sustainability [26]. The demand for organic foods and beverages; foods claiming to be excellent sources of protein; probiotics and fermented food is also big. Beyond that, clean labels, packaged food, ready to eat food and minimally processed food continue to be trends [25,27].

The latter of these referred trends of minimally processed food, is due to the fact that the term "processed foods" is usually equated to "bad foods" and many consumers try to avoid them. Although there are a few different levels of processed food, and some of them are actually good for a healthy diet, manufacturers are opting for minimal processing to improve nutritional benefits, such as filtered milks and cold pressed oils, for example, matching consumer demands [25]. Further, one challenge in the market is high sugar content in functional foods. The high sugar content acts as a challenge for the market as diabetes and obesity have become major health concerns across the globe [16]. Therefore, the brands are also focusing on sugar-free and low-calorie formulations as these are in high demand [28].

Food waste or by-products conversion to functional food ingredients is also a healthy trend in the food industry today. The food industry generates large amounts of waste or by-products annually around the world from a variety of sources, which are excellent sources of bioactive compounds. The efficient utilization of these products can help in reducing the

negative cost, reduce environmental pollution, demonstrate sustainability in food industry and have direct impact on the economy of the country [29].

Innovations in flavours and functional ingredients are boosting the market too. Manufacturers are introducing new flavours in the market to match the changing consumer preferences [28]. Furthermore, advances in technology have enabled the development of products that mask the unpleasant taste of some functional ingredients. For example, effective microencapsulation allows manufacturers to hide the bitter taste of amino acids and other ingredients [16].

Apart from all the above trends, it is necessary to take into account that the future viability and success of functional foods in the marketplace depend on several elements. The key issue is consumer acceptance of such products. For consumers to agree to pay the cost associated with functional foods, they must be convinced by its health claims through clear, truthful, and unambiguous messages [24].

Despite emerging from the trends of the common consumer, the application of biotechnology techniques for the development of functional food plants with higher levels of bioactive components or increased availability of nutrients is an important approach to benefit most populations in developing countries and improve the health and nutritional status overall [30].

In the next section, the issue of functional beverages will be deeply discussed, since this is the focus of this monography.

1.5. Functional beverages: The emerging side of functional foods

Among the different types of functional foods available, beverages represent one of the largest functional food markets. They are popular because of convenience and possibility to meet consumer demands for size, shape, appearance, and container contents, as well as ease of distribution and storage for refrigerated and shelf-stable products. Besides that, beverages are an excellent delivering means for nutrients and bioactive compounds such us: vitamins, minerals, antioxidants, fatty acids, plant extracts, fibre, prebiotics and probiotics [6].

Dairy-based beverages, vegetable and fruit beverages, sports drinks, and energy drinks are examples of functional beverages categories. Some of the commercially available beverages separated by categories are shown in Table 4. Globally, due to sociodemographic and sociocultural differences in consumer perceptions and acceptance, the trends relating to

functional beverages are also heterogeneous, evolving and growing at distinct rates, both within and across countries [6].

Dairy-based beverages contain proteins (essential amino acids), fat (unsaturated fatty acids), vitamins (mainly, A and E), carotenoids (mainly β-carotene), and minerals. Vegetable and fruit beverages are considered as the main dietary sources of bioactive substances, such as vitamins, phenolic compounds, and carotenoids [31]. Sports drinks are designed to be consumed before or during exercise to prevent dehydration, supply carbohydrates, provide electrolytes (such as sodium, potassium, calcium, magnesium), and sometimes, vitamins or other nutrients. Energy drinks have as their main goal to provide sustenance and improve performance, concentration, and endurance, being caffeine the most common ingredient, often combined with taurine, glucuronolactone, guarana, and B vitamins [6].

Table 4: Some examples of commercially available functional beverages. Adapted from Corbo *et al.*(2014) [6].

Brand	Producer	Active Compounds
Dairy Beverages		
Yakult®	Yakult Honsha Co, Japan	*L. casei* Shirota
Actimel®	Danone, France	*L. casei* Immunitas™
Verum®	Essum AB, Sweden	*Lactococcus lactis* L1A, *Lactobacillus rhamnosus* LB21
Heart Plus®	PB Food, Australia	Omega-3
Danacol®	Danacol, Belgium	Phytosterol
Zen®	Danone, Belgium	Magnesium
Vegetable and Fruit Beverages		
Proviva®	Skane Dairy, Sweden	*L. plantarum* 299v
Biola®	Tine BA, Norway	*L. rhamnosus* GG
Rela®	Arla Ingman Ou Ab., Finland	*L. acidophilus, L. reuteri, B. lactis*
Tropicana Farmstand®	Tropicana, U.S.A.	Vitamins A and C, potassium
Daily Greens®	Bolthouse Farms, U.S.A.	Vitamins A and C, manganese, iron, zinc
Oasis Health Break®	Lassonde Inc., U.S.A.	Omega-3
Sport Drinks		
Gatorade®	PepsiCo Inc., U.S.A.	Sodium, potassium
Powerade®	Coca-Cola Co., U.S.A.	Sodium, iron
Accelerade®	Pacific Health Laboratories Inc., U.S.A.	Sodium, potassium, vitamin E, calcium, protein
Energy Drinks		
Monster Energy®	Hansen Natural Corp., U.S.A.	Sodium, caffeine, B-vitamins and vitamin C, taurine, guarana, inositol, ginseng, L-carnitine, glucuronolactone
Red Bull®	Red Bull GmbH, Austria	Sodium, caffeine, B-vitamins, taurine, inositol, glucuronolactone
Rockstar Original®	Rockstar Inc., U.S.A.	Sodium, caffeine, B-vitamins, taurine, guarana, ginseng, inositol, ginkgo, L-carnitine

Besides the naturally present properties, beverages are a great opportunity to incorporate desirable nutrients and bioactive compounds, with the objective of promoting certain benefits. However, the beverage formulation should be taken into consideration. It is important to define the “optimal dosage” of each compound, namely the content should be high enough to exert health benefits, without hazardous effects or undesirable interactions [6]. Moreover, it is important to define the bioaccessibility and bioavailability of bioactive compounds to effectively improve beverage functionality [31]. While bioaccessibility refers to the fraction of bioactive substance that is released from the food matrix after digestion and solubilized into the gut for the uptake in the intestinal mucosa, the bioavailability is the fraction of nutrient secreted into circulation that is available for tissue uptake and metabolism.

A lot of research has been developed in this area and a large number of scientific papers has been published, making this one of the most interesting areas of research and innovation in the food field. Table 5 shows recent publications (since 2016) about some functional beverages, their bioactive compounds and possible beneficial effects.

Table 5: Recent publications (since 2016) on the state of the art in research about functional beverages.

Product	Bioactive compounds/ Ingredients	Beneficial effects	Reference
Green wheat juice beverage	- High concentrations of chlorophyll, active enzymes, vitamins, and other nutrients from green wheat juice	- Detoxifying, facilitating the elimination of toxins and fats from body	Salanţă *et al.* (2016) [32]
Plant extracts beverage	- With red betel, cinnamon, and ginger extracts - Flavonoids, tannins, and alkaloids	- Antioxidant and antidiabetic properties	Safithri *et al.* (2016) [33]
Fermented lactic beverage	- Use of concentrated tofu whey - Isoflavones and oligosaccharides	- No specific health data provided - Minimize the environmental impact	Benedetti *et al.* (2016) [34]
Whey-based beverage	- Supplemented with soy isoflavones or phytosterols - Probiotic (*Lactobacillus acidophilus* LA-5 or *Lactobacillus casei* LBC-81)	- No specific health data provided	Seyhan *et al.* (2016) [35]
Whey beverage	- Made from curd whey - Calcium lactate, vitamin D3, and prebiotic dietary fibre	- Significant decrease in low density LDL-cholesterol and triglycerides concentrations	Liutkevičius *et al.* (2016) [36]
Wheat-fermented milk beverage	- Probiotic that produce folic acid and riboflavin	- Protects against iron deficiency anaemia.	El-Azeem *et al.* (2016) [37]
Ku-jin tea beverage	- Phenolic compounds (gallic acid, ginnalin A, ginnalin B, ginnalin C, and 3,6-di-O-galloyl-1,5-anhydro-D-glucitol)	- Antioxidant, anti-viral, anti-tumor, and anti-diabetic activities	Bi *et al.* (2016) [38]

Data compiled from Web of Science and Scopus (2018).

Table 5: Recent publications (since 2016) on the state of the art in research about functional beverages (continued).

Product	Bioactive compounds/ Ingredients	Beneficial effects	Reference
Pineapple beverage	- Blending pineapple juice with cheese whey and paneer whey - Protein and minerals	- Can help in countering protein energy malnutrition and mineral deficiency among children in developing countries	Baba *et al.* (2016) [39]
Fruit juices with hibiscus extract beverage	- Phenolic compounds, vitamic C and carotenoids	- Highest antioxidant potential	Ogundele *et al.* (2016) [40]
Fermented soymilk beverage	- Three strains of β-glucosidase-producing *Bacillus subtilis* that produces a largest increase in aglycone content	- Aglycones are absorbed more easily and can have oestrogenic action and antioxidant capacity	Lee *et al.* (2016) [41]
Meadowsweet tea beverage	- Phenols (flavonols and ellagitannins), bioactive volatiles (methyl salicylate and salicylaldehyde), and water-soluble polysaccharide	- Antioxidant activity; anti-diabetic activity and anti-complement activity - Possible immune-modulating properties	Olennikov *et al.* (2016) [42]
Pluchea Indica Less tea beverage	- Phytochemical compounds (such as saponin, tanin, fenol hidrokuinon, and flavonoids)	- Antidiabetic effect (can reduce the random blood glucose in diabetic patients, eliminate tingling in the extremities, and bring improvement in physical fatigue)	Werdani *et al.* (2017) [43]
Coconut water beverage	- Probiotic (*Lactobacillus plantarum* DW12) - Supplemented with monosodium glutamate, ɣ-aminobutyric acid, vitamin B12, potassium, calcium, and sodium	- Provides not only probiotic but also other beneficial compounds for health	Kantachote *et al.* (2017) [44]
Fermented goat milk beverage	- High dietary fibre, oleic acid, and phenolic compounds - Probiotics (*L. rhamnosus* and *S. thermophiles*)	- Positive effect on gut microbiota metabolism, increasing the antioxidant capacity and the production of short-chain fatty acids, and decreasing the ammonium concentration	Freire *et al.* (2017) [18]
Blue corn and black beans beverage	- High quality protein, dietary fibre, and starch resistant	- High antihypertensive, antioxidant, and antidiabetic activity	Milán-Carrillo *et al.* (2017) [45]
Goat's milk-based beverage	- Enrichment with medicinal plant extracts derived from the Lamiaceae family - Rosmarinic acid, hydroxycinnamic acid derivatives, and luteolin derivatives	- Antioxidant capacity	Komes *et al.* (2017) [46]
Yerba mate beverage	- Phenolic compounds - salvianolic acid I, 5-*O*-caffeoiquinic acid, 4-*O*-caffeoylquinic acid, 3-*O*-caffeoylquinic acid, and 3,5-*O*-dicaffeoyquinic acid	- Antioxidant, antimicrobial, and anti-tumour activities	Correa *et al.* (2017) [47]

Data compiled from Web of Science and Scopus (2018).

Table 5: Recent publications (since 2016) on the state of the art in research about functional beverages (continued).

Product	Bioactive compounds/ Ingredients	Beneficial effects	Reference
Pigeon pea beverage	- Dietary fibre, vitamin C, tocopherol, and phenolic compounds	- Show hypoglycemic and hypocholesterolemic activities, and could improve the antioxidant status of diabetic hypercholesterolemia	Ariviani *et al.* (2018) [48]
Herbal green tea fermented beverage	- Phenolics, flavonoids, and condensed tannins	- Antihyperuricemic activity	Hardoko *et al.* (2018) [49]
Quercus based coffee-like beverage	- Phenolics (mainly ellagic acid) and fatty acids (oleic, linoleic, palmitic, stearic, and *cis* vaccenic)	- Antioxidant and anti-mutagenic capacity - Caffeine and gluten-free - Promising coffee alternative	Coelho *et al.* (2018) [50]
Cretan tea beverage	- Flavones, flavonols, and hydroxycinnamic acid derivatives	- Reduce the accumulation of advanced glycation end products (AGEs) associated with chronic and degenerative disorders	Maietta *et al.* (2018) [51]
Fermented pineapple beverage	- Probiotic from pineapple peel (*M. caribbica* 9 D) - Residual glucose and fructose and higher total phenolic compounds (catechin, chlorogenic acid, vanillin and ferulic acid)	- No specific health data provide	Amorim *et al.* (2018) [52]
High protein dairy beverage	- Oat flour, oat-β-glucan and milk protein	- Reduction of cardiovascular disease risk	Vasquez-Orejarena *et al.* (2018) [53]
Soymilk Kefir-based beverage	- Mixed probiotic - Bioactive soy compounds	- Consumption recommended to low income population with little access to products with high nutritional value and health benefits	Silva *et al.* (2018) [54]
Orange-flavoured beverage	- Soluble dietary fibre (β-glucan) and vitamin-like antioxidant (CoQ10) - Stevia extract	- Helps reduce cholesterol and maintain cardiovascular health - Natural sweetener	Liu *et al.* (2018) [55]
Orange-whey based beverage	- Probiotic (*Lactobacillus fermentum* PH5)	- Probiotic proven for cholesterol lowering profile - No addition of any preservatives and synthetic flavoured	Thakkar *et al.* (2018) [56]
Cranberry beverage	- Bioactive compounds present in cranberry and apple juice and ground cinnamon	- Anti-inflammatory, antioxidative, antimicrobial and immunomodulatory activity - Reduces gingival index and plaque index in patients with gingivitis	Woźniewicz *et al.* (2018) [57]
Star fruit juice fermented beverage	- Probiotic (*Lactobacillus helveticus* L10, *Lactobacillus paracasei* L26 or *Lactobacillus rhamnosus*)	- Non-dairy functional beverage with different flavour notes	Lu *et al.* (2018) [58]
Cornelian cherry juice fermented beverage	- Probiotic (*Lactobacillus plantarum* ATCC 14917) - High nutritive value	- No health data provided	Mantzourani *et al.* (2018) [59]

CoQ10 (Coenzyme Q10); ATCC (American Type Culture Collection).
Data compiled from Web of Science and Scopus (2018).

Table 5: Recent publications (since 2016) on the state of the art in research about functional beverages (continued).

Product	Bioactive compounds/ Ingredients	Beneficial effects	Reference
Blackberry and soursop beverage	- Antioxidant compounds from blackberry (anthocyanins) - Cytotoxic compounds from soursop (acetogenins)	- Highly effective consequences for the inactivation of breast and prostate tumour cells	Zambrano *et al.* (2018) [22]
Okara beverage	- Okara (soybean residue) - Probiotics (*Lactobacillus paracasei* and/or yeast *Lindnera saturnus*), soluble fibre, free amino acids, isoflavone aglycones, and fruity esters	- Probiotic - Minimize the environmental impact by reusing a by-product of industry	Vong *et al.* (2019) [60]
Soy whey beverage	- Use of kombucha consortium (tea fungus, a natural symbiotic consortium of bacteria, and yeast) - Isoflavone aglycones	- Antioxidant activity and anti-microbial activity - Minimize the environmental impact	Tu *et al.* (2019) [60]

Data compiled from Web of Science and Scopus (2018).

Analysing the summary of the most recent publications (Table 5), it can be concluded that plant-based beverages are becoming more popular. They represent an easy and convenient way of consuming fruits, vegetables, whole grains, nuts and seeds, which are important sources of health-promoting compounds [6]. Nowadays, cow milk allergy, lactose intolerance, calorie concern, prevalence of hypercholesterolemia and more preference to vegan diets has influenced consumers towards choosing cow milk alternatives. For that reason, non-dairy milk is a fast growing segment in newer food product development category of functional and specialty beverages worldwide [61].

Though numerous types of innovative food beverages, mainly from plant sources, are being exploited for cow milk alternative, many of these face some type of technological issues, related either to processing or preservation. Thus, some papers focus and propose applications of novel technologies to improve the production of beverages without compromise their sensory and functional properties [62]. The application of novel technologies can be possible and this will be the focus of the following sections (2 and 3).

2. Conventional versus emergent processing methodologies

With 25 million foodborne illnesses occurring annually around the world, food safety is a major concern of consumers, the food industry, and governments [63]. In a consumer-driven market, industry is constantly challenged to develop food products with characteristics

desired by the consumer, like high quality and freshness, at affordable costs without compromising food safety [64].

The food preservation methodologies are mainly designed with the aim of delaying the inevitable alteration of quickly perishable foods between production and consumption. Generally, microbial spoilage with the additional presence of potential pathogen agents are the principal food alterations considered. In addition, when a certain amount of bioavailable water is present, enzymatic reactions have an important role on food alterations. As a result, food preservation should be designed and performed with three basic aims at least: (i) destruction of pathogen agents; (ii) reduction of spoilage microorganisms; and (ii) inactivation of microbial enzymes, if necessary [65]. Furthermore, methodologies should satisfy the following conditions: (i) low numbers of microbial counts and high freshness; (ii) treatments carried on as early as possible to avoid the onset of microbial or enzymatic alterations; (iii) minimization of manipulation and general processing steps because each step or sub-step without a clear preservation effect may be cause of microbial contamination, recontamination or spoilage; (iv) performance of preliminary washing operations, only needed if high-level superficial contamination is demonstrated and/or forecasted [65].

This section has as its main goal to provide a brief description of the most varied conventional (2.1.) and emergent (2.2.) methodologies that are applied in the food industry currently.

2.1. Conventional processing

There are several conventional methods of food preservation divided into two main categories: (i) physical methods, such as heating, freezing, dehydration, freeze-drying, packaging, *etc*; and (ii) chemical methods, such as acidification, salting, fermentation, use of preservatives, *etc.* [63,66,67].

The heating processing is the most used methodology by food industry. Generally, this process involves the transfer of heat energy using conduction, convection and/or radiation. Two different food preservation procedures are performed through the heat treatment: pasteurisation and sterilisation. The first is able to destroy pathogenic life forms and the most of the vegetative microorganisms. Moreover, enzymes are reported to be inactivated with this treatment. In detail, pasteurisation can be described as a microbiostatic and partially microbicidal treatment. For this reason, it has to be combined with other preservation methods such as refrigeration, addition of chemical preservatives or vacuum packaging. On

the other hand, sterilisation is a more drastic treatment and has as the main difference the destruction of all microbial forms, including spores. With regard to the conditions applied, thermal pasteurization is generally done in what are called high-temperature short-time (HTST), which consist of temperatures between 70 °C and 100 °C, while thermal sterilization is done in ultra-high-temperature (UHT), applied at temperatures higher than 100 °C to create products with longer shelf-lives. The microbial reduction effectiveness of each treatment depends on the process temperature and treatment time. It should be also noted that treatments can differ according to the pH of preserved foods [68].

Despite the wide and easy use to extend food shelf life, the thermal or any other conventional method have several drawbacks, including degradation of bioactive compounds, undesired physicochemical changes, and alteration of bioactivity and organoleptic qualities [23]. For this reason, there is a search for viable methodologies to ensure the microbiological safety of foods while maintain the nutritional value and organoleptic properties like colour, aroma, texture and flavour [69,70]. Innovative non-thermal processing technologies have been studied and the following sub section (2.2.) provides a brief description of them.

2.2. Emergent processing

Food contains many heat sensitive nutrients which include vitamins, minerals, and nutrients with functional properties such as pigments, antioxidants and other bioactive compounds. However, the most common processing methodologies cause detrimental effects on these nutrients. Avoiding this requires innovative approaches and the industry needs methods that offer value-added products and new market opportunities, with satisfactory safety margins [67].

Nowadays, the most investigated preservation methodologies are non-thermal or very soft thermal technologies [67]. Unlike conventional methodologies, these do not involve increased temperatures or require them only during brief periods and at lower intensity[23]. High pressure processing (HPP), pulsed electric fields (PEF), oscillating magnetic fields (OMF), membrane filtration (MF) and ultraviolet (UV) radiation are some examples of these non-thermal innovative technologies [23,67,68], while ultrasound (US), ohmic eating and microwave are example of some emerging technologies that use a mild temperature . Furthermore, there are other technologies that protect the final product against deterioration,

like new packaging systems, modified atmosphere packaging (MAP) and active packaging. A more targeted approach, the molecular entrapment (e.g. encapsulation and nanoemulsions), can also be used for this purpose [23].

In spite of the intensive research efforts and investments, very few of these new preservation methods are until now implemented by the food industry, due to the fact that it made large investments in processing facilities relying mostly on conventional thermal processing technologies with well-established reliability and efficacy [67]. Even so, due to their high potential, non-thermal methods are gradually being inserted by companies and a lot of work can be done with the goal that these will come to dominate the food processing industry. Section 3 shows a detailed review regarding high pressure processing, since this technology is already well implemented industrially and was the chosen food processing technology for the experimental work of this thesis.

3. High pressure processing

Currently, the most successful non-thermal processing technology, and one that responds to many of the problems associated with conventional processing, is the high pressure processing (HPP), also referred to as ultra-high pressure (UHP) or high hydrostatic pressure (HHP) [67]. The main areas of interest for the application of this technology in food processing include the inactivation of microorganisms, the modification of biomacromolecules (protein denaturation, starch gelatinization, etc.) and the retention and improvement of food quality (taste, colour and functionality) [66,69–71]. Another application is the so-called hyperbaric storage, a new food preservation method under high pressure, as a possible energetic costless process, that inhibits microbial growth, similarly to freezing and refrigeration, but at room temperature [72]. Pressure-shift freezing is another form to use high pressure technology, that can efficiently produce much smaller and numerous ice crystal and also helps in reduction of microbial load [73,74]. The potential of the technology has been also confirmed for the extraction of valuable compounds from food wastes [75].

High pressure is also applied in non-food industries, including production of plastics, ceramics, metal-forming and pharmaceutical tablet manufacture [63,66]. A promising example of this non-food use is the development of vaccines, since the pressure causes inactivation of viruses and other infectious agents, as already tested for the virus of yellow fever and foot-and-mouth virus [76,77].

3.1. History and current days

The treatment of food using HPP dates back to 1899 (in West Virginia, USA), when Bert Hite reported that a pressure treatment of 1 hour at 600 MPa (at room temperature) could prolong the raw milk shelf life for four days, being the first to prove the effectiveness of pressure in inactivating spoilage bacteria [78]. A few years later, in 1914, Hite and Giddings showed that HPP pre-treated fruits (400 – 820 MPa) remained commercially stable at least for 5 years [79]. Among other important investigations, one with high relevance was carried out in 1917, by Larson *et al.* [80], which showed that a pressure of 607 MPa can kill non-spore-forming bacteria in 14 hours, and that a pressure of 1214 MPa, for the same length of time, is required to kill spores [80].

During the 1980s and the beginning of the 1990s, numerous studies were performed regarding the use of HPP to inactivate food microorganisms. Over the last decades, significant advances enabled the scale-up of pilot units to commercial systems, in which industrial HPP treatment of food may be carried out. The first food products introduced onto market processed by HPP (strawberry, apple, and kiwi jams) appeared in Japan in 1990, manufactured by Meidi-ya Food Factory Co [81].

The HPP method is considered one of the most promising non-thermal food preservation techniques, since it has an increasingly high number of equipment operating worldwide (Figure 2) [82]. Nowadays, the HPP treated product spectrum ranges the most varied products: plant-based products (e.g., apple juice, pomegranate juice, aloe vera gel), egg-dairy products (e.g., cheddar cheese, cow milk), seafood and meat products (e.g., atlantic mackarel, rainbow trout, beef), and alcoholic beverages (beer and wine) [83]. Figure 2 also shows the parcelling of commercially available HPP treated products by some categories.

Currently, more than 350 active HPP equipment exist worldwide, with capacities that reach 525 L - 3000 kg/h. Over the last decades, the commercial HPP market reached 9.8 billion USD and a projection of 12 billion USD has been made for 2018. The equipment service sector that rents the equipment for a short period also boomed to 330 million USD due to high demand to overcome expensive fixed cost of HPP equipment [83]. The Visiongain organization predicts a strong revenue growth of HPP market through to 2027 [84].

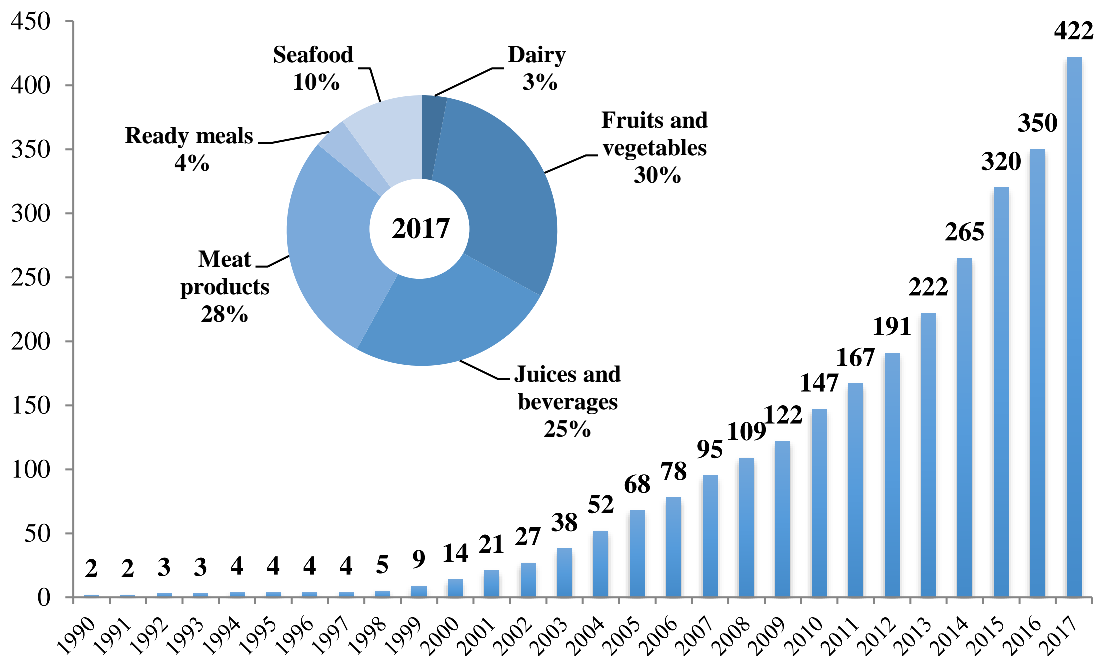


Figure 2: Evolution of the total number of high pressure industrial machines operating worldwide (1990 - 2017) and global HPP submarket share in 2017. Courtesy of Hiperbaric S.A. (Burgos, Spain).

3.2. General principles and equipment

The HPP technology is applied to sealed foods in a flexible vessel where range of pressures between 100 and 1200 MPa is applied at room temperature using a liquid (typically water) as a pressure transfer medium, subjecting the interior and surface of the food to the same pressure [85].

The application of HPP in foods is based on three principles: (i) the isostatic principle (Pascal's Principle), which assumes that the application of pressure is uniform and acts equally in all directions, in other words, it is homogeneous throughout the food regardless of its geometry and size; (ii) the principle of Le Chatelier's, which states that any phase transition, conformational change or chemical reaction which is accompanied by a reduction of volume is favoured by pressure and vice versa; and (iii) the principle of microscopic ordering, which states that constant temperature, an increase in pressure increases the degree of ordering of the molecules of a given substance [64,86].

The volume change (ΔV, $cm^3.mol^{-1}$), as previously mentioned by Le Chatelier's principle, can be related to the reaction equilibrium constant K as follows in (Equation 1):

$$\Delta V = RT\left(\frac{\partial \ln K}{\partial P}\right)_T \qquad \textbf{(Equation 1)}$$

where P is the pressure applied (MPa), T is the absolute temperature (K), R is the universal gas constant (8.314 cm^3 MPa mol^{-1} K^{-1}), and K is the equilibrium constant. So, since the biological processes are influenced by the application of pressure, it can be concluded that the biological processes are influenced by the application of pressure [87].

A typical HPP system (Figure 3) consists of a high pressure vessel, its top lid, a pressure generation system (piston/intensifier pump, low-pressure pump and tank), a control device and may have a cooling system (not shown in the diagram), since a mild increase in temperature (around 3 °C) occurs for each pressure change (100 MPa), because work is applied in adiabatic conditions [88,89]. Temperature should be supervised because it plays a quantifiable role [85]. Two types of compression processes can achieve generation of high pressures in the pressure vessels (Figure 3), A): (i) direct (represented on the left), used for laboratory studies, intrudes a piston into the vessel so as to reduce the volume and thus raise the pressure; or (ii) indirect (represented on the right), used for industrial applications, adopts exterior compression system which pressurizes the pressure medium using one or more pressure intensifiers and intrudes the pressurized medium into the vessel for compression. Beyond that, there are still two types of equipment orientation, vertical and horizontal (the last one being the most used currently) [90], and three types of operation systems, batch (for solid and liquid packed foods), continuous, and semi-continuous (both for unpacked pumpable foods) [86].

Common reported treatments to deal with hazardous microorganisms in vegetative form use from 300 to 600 MPa (at ambient temperature) for several min [85]. The maximum working pressure depends on the internal diameter of the vessel, wall thickness and the number of cycles the vessel is designed to accomplish [86]. In the case of higher pressures (> 600 MPa), pre-stressed vessel designs as multilayer vessels or wire-wound vessels are used [88].

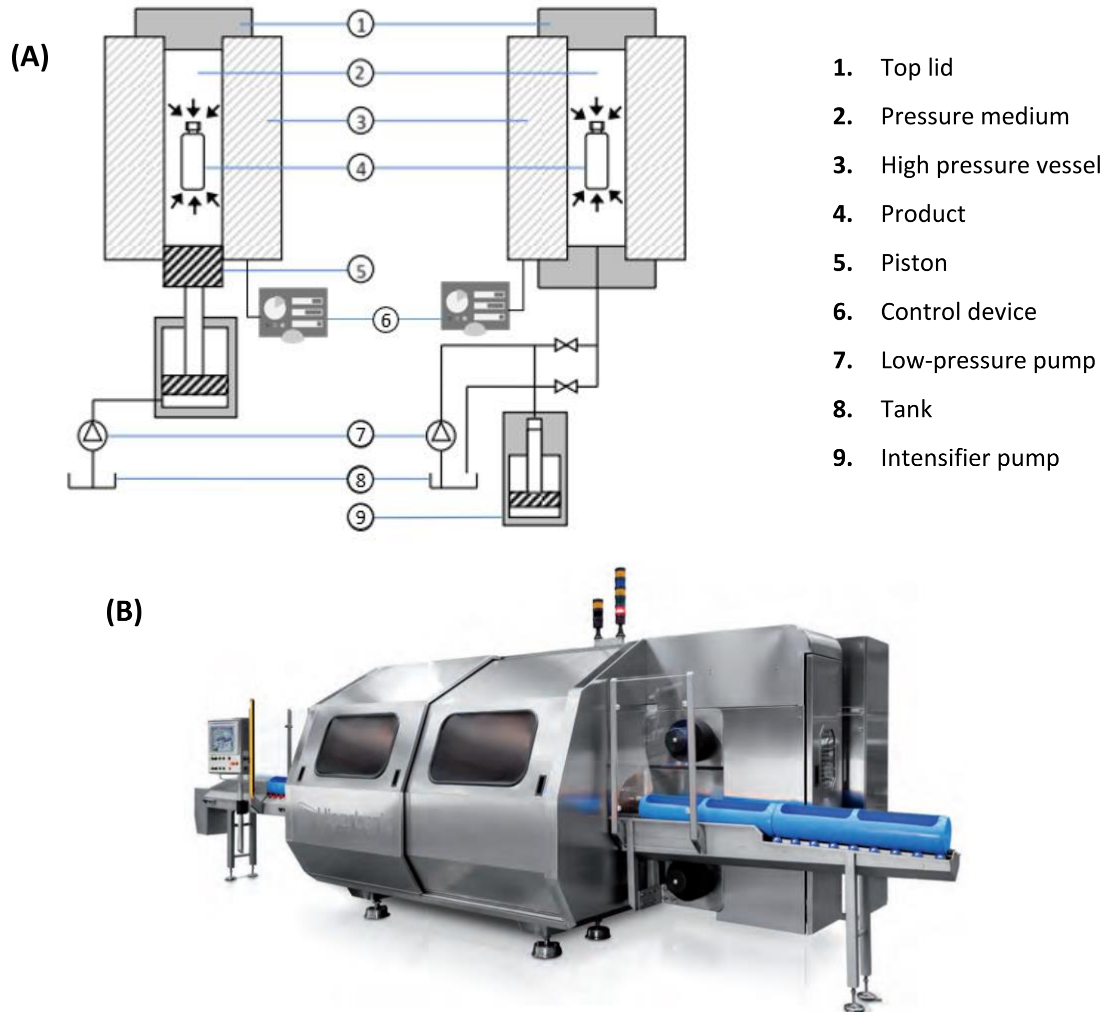


Figure 3: High-pressure processing system. (A) Schematics of direct (left) and indirect (right) compression high-pressure food processing techniques. Adapted from Elamin *et al.* (2015) [86]. (B) Hiperbaric 55, a typical horizontal equipment with indirect compression [91].

High pressure treatments include hydrodynamic treatment (high pressure homogenization, HPH) and hydrostatic treatments (high hydrostatic pressure, HHP). The HPH treatment applies pressures from 70 to 200 MPa in continuum to pumpable products, whereas HHP is applied in batch systems to both solid and fluid products that are already packaged, using a pressure between 150 and 900 MPa. Recently, ultra-high pressure homogenization (UHPH; 200 to 400 MPa) and pulsed-high hydrostatic pressure (p-HHP; combination of pressure, temperature, and pulses) have also been applied to preserve fruit and vegetable juices [62].

3.3. Efficiency of high pressure processing on food quality

One of the principal advantages of the HPP treatment is the extended shelf life and improvement of food safety due to large inactivation of microbial population [92]. Food safety and shelf life are often closely related to microbial quality, but phenomena such as enzymatic reactions, biochemical reactions, and structural changes can significantly influence consumers' perception of food quality [93]. Other of the main advantages of HPP is its minimal negative effect on the sensorial, functional, and nutritional attributes of food [94]. However, it is necessary to take into account that increasing treatment to increase microbial inactivation in shorter times, may also cause detrimental changes in food quality that could affect negatively the appearance and some properties of the food, compared to the unprocessed product [87]. In this section we will discuss some of the effects of pressure on the more common parameters that influence the final quality of the food product.

3.3.1. Inactivation of microorganisms

The HPP treatment exerts many effects that make it able to inactivate the pathogenic and deteriorating microorganisms. With increasing pressure, changes in cell morphology (including cell membrane permeability) and chemical reactions, protein denaturation, enzyme inhibition, inhibition of genetic mechanisms and destruction of ribosomes may occur [95]. However, HPP action depends on several key factors of the food product: (i) type of microorganisms; (ii) initial microbial population; (iii) their growth state; and (iv) the environment in which the microorganisms are present [63,92,96,97]. The first three factors are related to the characteristics of the microorganism itself. The HPP usually has a higher destructive effect in organisms with a greater degree of organization and structural complexity [92]. Thus yeasts are the most sensitive, followed by gram-positive vegetative bacteria, gram-negative vegetative bacteria, moulds and, lastly, the spores [63]. Due to their unique characteristics, bacterial spores are quite resistant to high pressures, they can survive even at 1000 MPa [98]. The initial microbial count in any matrix is also a primary factor that can reduce the effectiveness of any treatment. High initial microbial load always results in high survival counts after treatment. Sometimes pre-treatments and hygienic measures are practiced to improve the treatment action by HPP. Despite this, the microorganisms may react distinctively taking into account their growth phase. Cells in exponential growth are more sensitive than cells in the stationary phase, and the ability of cells to recover from

damage appears to be related to their resistance to pressure [92]. The last factor is relating to the food environment. The resistance of microorganisms to pressure is generally higher as the richer in nutrients the medium they are inserted into is. Factors like acidity/pH and the water activity also play a crucial role in determining cellular damage under pressure [92]. Thus, modifying the food environment, the treatments can be optimized to assure the microbiological safety. All microorganisms have a pH range in which they can grow and an optimum pH at which they grow best. If the pH of a food is not optimal for a species, it can enhance inactivation and/or inhibit outgrowth of sublethally injured cells. Generally, the degree of microbial inactivation increases when pH decreases, and bacterial spores are most resistant to pressure treatments at neutral pH. Curiously, the compression shifts the pH of the food as a function of applied pressure. In its turn, the water activity (a_w) of a food also can significantly influence the growth of microorganisms. However, the net effect of a_w on microbial inactivation by HPP treatment may be difficult to predict. Reducing the a_w appears to protect microbes against inactivation by HPP, while, on the other hand, the recovery of sublethally injured cells can be inhibited by low a_w [93].

With all of the mentioned factors it becomes necessary to adapt the critical limits, pressure values, the time, and maybe the treatment temperature to each food case, in order to ensure the microbiological safety of the products after processing and also during storage [99]. As previously mentioned, typical reported treatments to deal with hazardous microorganisms in vegetative form use from 300 to 600 MPa, at ambient temperature, for several min [85]. Due to their unique characteristics, bacterial spores, are quite resistant to high pressures at room temperature, as they survive 1000 MPa. Thus, it is unlikely that spores are inactivated by HPP alone. For these reasons, foods processed by HPP are considered to be only pasteurized and, on the market, are generally refrigerated or have low water and/or pH activity to prevent the germination of bacterial spores [63,98]. Favourable environmental conditions and/or a smooth treatment followed by a harder one could first activate spores to subsequently destroy them in a vegetative state [85].

Campylobacter spp., Salmonella spp., Listeria monocytogenes, Staphylococcus aureus, Escherichia coli and *Vibrio spp* are the main bacteria that cause food poisoning. Among these, *S. aureus* and *L. monocytogenes*, an important pathogen in acidified and other foods, are probably the two most intensively studied species in terms of use of HPP. As *L. monocytogenes* is moderately heat resistant and can grow anaerobically under refrigeration, it requires particular care for processing and storage. *S. aureus* and *E. coli* O157:H7 appears to have a high resistance to pressure (barotolerance) [93].

Besides destruction of microorganisms there are further influences of pressure on food materials to be expected, namely, effects on enzymes and nutritional, physicochemical and sensory parameters, which will be addressed in the following sub-sections.

3.3.2. Enzymes

Enzymes are proteins with an active site which results from a specific three-dimensional conformation that allow biological activity under the right conditions [100]. When under pressure, the main stabilizers of the three-dimensional conformation such us hydrogen bonds and hydrophobic, electrostatic, van der Waals interactions and disulphide bonds, can be disturbed [101]. Thus, HPP can have effects on enzymes divided in two classes: (i) reversible structural changes in enzymes at low pressures (100 - 200 MPa), that can result in stabilization or activation; and (ii) irreversible denaturation at high pressures (400 - 1000 MPa) by the loss of tertiary and quaternary structures, that result in changes in the active site with, consequently, enzyme inactivation [94,102]. The inactivation of enzymes is mostly irreversible and the efficacy is largely dependent on the enzyme types and sources [90]. If necessary, an effective means to accelerate the inactivation of enzymes is by increasing the temperature [102].

In products with some degree of processing, physiological and biochemical changes occur at a faster rate than in intact raw products, due to the rapid onset of enzymatic activity, for example browning and excessive tissue softening in based fruit products [103]. The major enzymes affecting the quality attributes in plant-based products include polyphenoloxidase (PPO), peroxidase (POD), pectinmethylesterase (PME), β-glucosidase (BGL), polygalacturonase (PG), actinidin, lipoxygenase (LOX), hydroperoxide lyase (HPL), among others [104].

The PPO is one of the main responsible for browning developed due to enzymatic oxidation of phenols (in the presence of oxygen) to quinones, which in turn condense and react non enzymatically with other substances such as phenolic compounds and amino acids to produce complex brown polymers. Sulphites are an antibrowning agent used as PPO inhibitors to prevent enzymatic browning in fruit product. However, sulphites are reported to induce adverse allergenic effects in certain sensitive individuals, and other additives able to reduce enzymatic browning are usually less effective. The HPP in combination with packaging of good barrier properties can avoid this problem in minimally processed products during storage. When opened, the products are exposed to air and it is not possible to prevent

browning due to only partial inactivation of the PPO by HPP [103]. Detailed information regarding PPO and other enzymes involved in fruit products can be consulted in a written review by Chakraborty *et al.* (2004) [104]. The enhancement of enzyme activity by HPP may be also achieved, particularly in some fruit products, although the reasons for increased activity are poorly understood. Treatment at pressures below 300 MPa can result in the activation of oxidative enzymes, thus increasing spoilage reactions in most fruit products (despite the fact that, in some cases, activation of these enzymes is also observed at higher pressures). When HPP alone is not sufficient to achieve complete inactivation of enzymes like PPO, a combined treatment may be adopted (see the sub section 3.5) [104].

As an alternative to thermal treatment, HPP can be used to inactivate enzymes in plant-based products by itself or by adding enzyme inhibitors (e.g. acidulates, reducing agents, etc.), combination of high pressure with anti-browning agents or use of packaging with good barrier properties in HPP-treated products. However, HPP disrupts the membranes of subcellular compartments in which the enzymes are usually sequestered, facilitating enzyme–substrate interaction [94].

3.3.3. Nutritional and functional parameters

The application of emerging technologies has as one of the main objective the improvement of the production of food products without compromising their nutritional and functional properties [6]. Recent evidences show that the total phenolic content of fruits or vegetables and their antioxidant properties can be preserved more effectively by HPP than by thermal treatment [105]. Over the last few years, studies have found that HPP treatment resulted in a higher retention of bioactive compounds from antioxidant components (for example, ascorbic acid, anthocyanins, and phenols), and hence higher antioxidant activity for HPP-treated fruits and vegetables, which provides added health benefits [6,106].

On other hand, HPP causes complex changes to the structure and reactivity of biopolymers such as starches, proteins and lipids [97]. Starch is a food component that contributes as energy source and gel-forming texture modifier. The HPP technique can induce gelatinization of starch, like heat treatments in the presence of water, increasing the susceptibility to enzymatic degradation and the visco-elastic characteristics. An interesting fact, which can trigger potential applications, is that retrogradation of HPP gelatinized starch

can be observed immediately after HPP process and heat gelatinized starch shows a slow retrogradation [90].

Various food proteins also have been studied in terms of HPP-induced physical changes [90]. Under the high-pressure environment, the mechanism of protein denaturation can be hypothesized similar to enzyme inactivation [104]. The pressure causes unfolding of the molecular structure and consequently aggregation with different proteins, resulting in changes to the texture of the food. Gel formation is observed in some proteins, such as proteins of soya, meat, fish and egg. Compared to heat treated gels, the pressure induced gels maintain their natural colour and flavour, and are described as smooth, glossy, soft, and having greater elasticity. Research is also being undertaken to unfold the structure of lower quality proteins using high pressures with the objective of improving their functional properties, like emulsifying and gelling capacity [97].

Regarding lipids, HPP can induce phase transitions: gel–liquid crystal transition of double layered membrane of phospholipids; and solid–liquid phase transition of oils, such as cacao butter and soybean oil [90]. During the HPP treatment, phospholipids from the membranes are compressed, favouring the passage from the liquid-crystalline phase into a gel state and, during decompression, the dual layer conformation is lost allowing the cytoplasmic leakage [63]. On the other hand, HPP decreases volume and entropy of a system and facilitates microscopic reordering of lipid molecules, leading to a phase transition from liquid to solid [107].

Pressure is a thermodynamic parameter whose unique effects on biological systems and the use of which has raised interest for several types of applications in biotechnology [95,108]. With the steadily growing knowledge and understanding about their effects, the potential for their use in several unique applications in bioscience (disaggregation of proteins, preparation of viral vaccines, modulation of food functionality, among others) has been rising over the past few years [108].

The incidence of food allergies is rapidly increasing, as is their severity and the number of foods involved. As previously mentioned, high pressure processed food can modify the activity of some enzymes and the structure of some proteins. Thus, the allergenicity of novel foods, such as HPP-treated foods, becomes a key concern in the safety food assessment. At the moment, there are no published reports available on the toxicity of high-pressure processed foods, so new studies on the putative allergenicity of high-pressure processed foods should be put into practice [96] . Conversely, several trials have been performed in order

to reduce the allergenicity of allergen proteins, naturally present in some foods, via extraction or alteration of their structure by application of HPP technology [109,110].

3.3.4. Sensory and physicochemical parameters

The sensory parameters (colour, flavour and texture) are important quality characteristics and one of the major driving forces affecting consumer perception and acceptance of foods. HPP processing (at low and moderate temperatures) could retain the sensory properties of food due to its minimal effect on the covalent bonds of low-molecular-mass compounds such as colour and flavour molecules and micronutrients [87,94].

Colour plays an important role in the appearance and consumer acceptance, especially fruit and vegetable beverages [111]. Factors such as non-enzymatic (Maillard reactions) and enzymatic browning, and process conditions like pH, acidity, oxidation, time and temperature, are responsible for the loss of colour during processing of foods [103]. The HPP treatment has been widely reported to keep colour properties closer to the fresh-made juice than thermal processes [111], due to its limited effect on low molecular weight food compounds like pigments (e.g. chlorophyll, carotenoids, anthocyanins, etc.) responsible for the colour of fruits and vegetables [112]. For some food products (like fresh meat products), it is important to take into account that depending on the pressure-time exposure, some degree of protein denaturation can take place during the HPP and this can result in a change in colour relative to raw products [93]. Furthermore, it is necessary to take into account that not only the colour changes caused by the treatment itself are important, but also the evolution of the product colour during storage [103].

New components related to colour and flavour can be produced when food is heated, due to the occurrence of browning reactions [90]. In its turn, HPP preserves the original colour and flavour [113], because HPP only affects the non-covalent bonds of the 3D structure of high molecular substances and, consequently, browning does not occur [114]. This is both an advantage and a disadvantage of HPP, because no specific aroma, such as that of thermal sterilization, is produced, and depending on the food product and consumer preference this may or may not be desired.

Textural characteristics of foods are also little affected by HPP, but only when the water content is high. This is because the physical structure of such foods is unchanged after pressurization since no shear forces are generated by hydrostatic pressure. On the other side,

foods with low water content (with large number of voids or air spaces), may undergo a permanent deformation due to gas displacement and liquid infiltration or compression and subsequent expansion of gas during the process [94]. Thus, the application of this treatment is limited to foods with a high water content [70]. For certain food products, HPP has an enormous potential as a technique to modify the texture. HPP induces changes in proteins and starches that can be used for the development of new products or to increase the functionality of some ingredients [93] (as mentioned in sub section 3.3.3).

In relation to other important physicochemical parameters such as pH/acidity, total soluble solids (TSS; measured by ºBrix; g/100 g) and titrable acidity (TA; g citric acid/100 mL), these are generally also unaffected by HPP [115–118].

3.4. Advantages, limitations, and possible solutions under study

In comparison with conventional thermal processing technology, HPP has numerous advantages: (i) the rapid, quasi-instantaneous and uniform action, throughout the mass independent of size, shape and food composition of the food/packaging (ii) the microbial safety of food without the addition of preservatives; (iii) the maintenance of the aroma and nutritional value of the original food; (iv) the realization at room temperature, which enables the reduction of energy consumption associated with heating and subsequent cooling; (v) generally short treatment times; (vi) creation of ingredients with new functional properties; (vii) increase of the shelf life, reduced defect rate, helping to expand the product target markets; (viii) the surface area of the food is not under processed; (ix) avoid secondary contamination of the food after pasteurization, because food does not directly contact the processing devices; (x) the pressure transfer medium can be recycled after processing, so there is not production of effluents; and (xi) once the pressure is raised, no energy consumption is required to hold the pressure without pressure leakage [63,66,70,83,90,114]. With all these advantages, HPP technology demonstrates its full potential and can be considered an "eco-friendly" technology.

At the molecular level, it is important to take into account that pressure does not affect the covalent bonds of food components, so sensory properties, nutrients or bioactive compounds do not suffer significant losses, in contrast to the high frequent effects of temperature. Molecular compression is only capable of affecting the weaker bonds and forces, such as hydrogen bonds, electrostatic interactions and Van der Walls forces, which

explains the preservation effects of this technique, because this changes the microbial membrane structures [66,67]. The ability to keep covalent bonds unaffected has been the central hypothesis for the preservation of biological activity of functional compounds, such as ascorbic acid, folates, antioxidants, anthocyanins, lycopene, and conjugated linoleic acid [64].

Despite all the advantages, HPP also has limitations, such us: (i) non-applicability to products with a low water content or containing a large number of air bubbles (as HPP requires the use of water as a pressure transfer medium the products containing air bubbles will be deformed under pressure); (ii) limited choices of food product containers because they must be equipped with deformability, restorability, heat integration, and low oxygen permeation; (iii) pressure alone cannot inactivate bacterial spores in low acid and high water activity products (in these cases storage under refrigeration is necessary); (iv) variable efficacy in enzyme inactivation, leading to possible enzymatic and oxidative degradation of food components during storage and distribution; (v) passive heating by adiabatic compression and passive cooling by adiabatic decompression are inevitable; (vi) still considered a high-cost technology, due to high implementation and maintenance costs; and (vii) higher price of the final product when compared to thermally processed products [64,69,70,81,90,114,119,120].

Nevertheless, some of these limitations can be overcome already or possible alternatives are under study. To begin, one should talk about the important topic of inactivation of bacterial spores. Spores, as usual, have enhanced resistance requiring distinct pressure and temperature procedures, comparatively to the treatments for hazardous microorganisms in vegetative form. An increase in food temperature above room temperature or, to a lesser extent, a decrease below room temperature, in some cases increases the inactivation rate of microorganisms during HPP treatment [67].

The price of HPP equipment can range anywhere between 500,000 and 2.5 million USD, excluding other costs like installation, utility, labour and facility [81,83]. Comparatively to other technologies, the initial investment to set-up a HPP system is higher, which represents a limiting factor to expand its use [83]. However, it is expected that implementation increase could reduce the final product price by decreasing the total processing costs [81]. In terms of energy efficiency, there are still ways to maximize the expenditure, such as the recovery of part of the decompression energy by synchronization of the compression and decompression phase in twin-vessel systems. Besides that, the vessel filling efficiency plays an important role, because it is beneficial to maximize the product load in the vessel to decrease the energy requirement per product unit [68].

3.5. Combined treatments with high pressure processing

As referred in the previous section, when used individually, HPP technique might not be effective and might require the use of combined treatments, which is expected to provide synergistic effects. So, different approaches have been tested, including: (i) use of intrinsic hurdles such as pH and TSS; (ii) combination with heat or application of heat before or after HPP process; (iii) use of other combined technologies; and (iv) combination with antimicrobials and bacteriocins. An overview of distinct approaches currently used to improve effectiveness of non-thermal processing technologies is reported in Bevilacqua *et al.* (2018) [62].

Recently, there have been new emerging applications that allow the food sterilization based on the conjugation of high pressures and thermal processes. These new applications are: Pressure Assisted Thermal Sterilization (PATS), Pressure Enhanced Thermal Sterilization (PETS) and Pressure Ohmic Thermal Sterilization (POTS) [121–123]. Of these technologies, PATS and PETS are already approved by the Food and Drug Administration (FDA) for the processing of low acid foods [122]. Other possibilities under study are a smooth HPP treatment followed by a harder one; and multiple pressurization stages. The objective is to activate sporulated forms to subsequently destroy them in a vegetative state [85].

3.6. The high pressure processing applied to plant-based functional beverages

Several polyphenol compounds have been identified in higher and edible plants. The more common ones are lutein and lycopene (non-vitamin A carotenoids) and certain groups of plant polyphenols, such as the anthocyanidins and procyanidins [6]. This generates the potential of some plants in the manufacture of functional beverages, that inserted in a balanced diet can decrease the risk of premature mortality, cardiovascular disorders, advancing age-induced oxidative stress, inflammatory responses and diverse degenerative diseases [112]. Unfortunately, some of these bioactive compounds are sensitive to atmospheric oxygen, light, pH, and temperature [23].

Traditionally, beverages have been preserved by thermal treatments such as pasteurization and sterilization. However, as mentioned before, they result in some disadvantages, such us loss of heat-labile nutrients mentioned above, which triggered a great increase of research on the HPP treatments [115]. In the beverages sector, the HPP conditions embrace pressures that range typically between 400 and 600 MPa, and are applied from a few seconds to 5 min, at refrigerated or at room temperature. Specifically, the most

frequently used treatment conditions for juices preservation in general involve 500 - 600 MPa during 2 - 3 min. In the case of low-acid juices, the sterilization is possible combining high pressure (500 - 900 MPa), and relatively mild temperature (90 - 120 ºC) during 5 min [99]. Table 6 shows a closer look at recent publications (since 2016) about HPP applied to plant-based beverages.

Table 6: Recent publications (since 2016) on the state of the art in research about HPP applied to plant-based beverages.

Product	HPP conditions	Key finding(s)	Reference
Aloe vera-litchi mixed beverage	600 MPa 15 min 56 °C	- Inactivation of PME (34%), PPO (65%) and POD (62 %) - Low ascorbic acid loss - Minimal changes in phenolics content - Natural microbiota present in samples was below the detection limit throughout the storage period (100 days,4 ºC) - Shelf life extended	Hulle *et al.* (2016) [124]
Pitaya-pineapple beverage	600 MPa 10 min 25 ºC	- At 600 MPa/10 min retentions of vitamin C of 133%, total phenolic of 87%, betacyanins and betaxanthins of 96% were obtained, so seem to be a good option to preserve antioxidants	Sandate-Flores *et al.* (2017) [125]
Red wine	400 MPa 5 s 25ºC	- Able to inactivate *B. bruxellensis* (>5 log reduction) - Produce a microbiologically stable wine, with no effect on the taste of the wine	Wyk *et al.* (2018) [126]
Tiger nuts' milk	200/300 MPa 40 ºC (UHPH)	- Shelf life extended from 30 and 57 days by supplying UHPH - Colour differentiated from heat treatment, with greater luminosity and whiteness	Codina-Torrella *et al.* (2018) [127]
Ripe persimmon beverage	400 MPa 5/10/30 min (UHP)	- Prevented the reduction in carotenoids - The microbial count was controlled to below the detection limit with more than 5 min - Increase in water-soluble tannin	Kim *et al.* (2018) [128]
Mixed fruit and vegetable smoothie	627.5 MPa 6.4 min 21 – 24 ºC	- Does not affect pH, total soluble solids, texture and total phenolic content - Leads to reductions of 85%, 45% and 10% on PME, POD and PPO - Increases antioxidant capacity by 75% - Maintains or slightly improves colour - Enhances microbial quality	Fernandez *et al.* (2018) [115]
Se-enriched kiwifruit juice	500 MPa 10 min 25 ºC	- Inactivate the total aerobic bacteria and yeasts and molds completely and guarantee microbiological safety in clear juices during storage (4 °C and 25 °C for 42 days) - Better preserved Se content, total phenols, chlorophyll, ascorbic acid, original colour and sensory property	Xu *et al.* (2018) [129]
Sweet whey fermented beverage	- 200 MPa, 10 min - 400 MPa, 1 min	- Both high hydrostatic pressure treatments preserved flavour, colour and texture attributes until 45 days - Treatments at 200 MPa/10 min maintained optimal amounts of SLAB	Pega *et al.* (2018) [130]
Sugarcane juice	600 MPa 20 min 30 ºC	- Enough to inactivate the load corresponding to indigenous and vegetative microflora completely (but survival of spores cannot be neglected) - Not show significant effect on the physicochemical parameters	Sreedevi *et al.* (2018) [117]

PME (Pectinmethylesterase); POD (Peroxidase); PPO (Polyphenol oxidase); SLAB (Starter lactic acid bacteria).
In some studies, more pressures than those presented in the table were tested.
Data compiled from Web of Science (2018).

The HPP technique has been used extensively on fruit purees, juices, jams, and many other fruit and vegetable products for pasteurization [73]. Over the last years, the application of this technology to the market has been carried out by several brands, three examples of which are Coldpress (London, United Kingdom), Evolution Fresh (California, EUA) and Copa (Alcobaça, Portugal) [99].

The next section presents a review of the literature on acorn, a potential product to produce a functional beverage, whose treatment for preservation within the scope of this thesis will be through the application of HPP technology.

4. Case study: Acorn

Many studies have been developed about bioactive compounds recovered from neglected/underutilized foods, food wastes, and their by-products [131]. A striking example of a neglected/underutilized food is the acorn from *Quercus genus*. According to the study "The economic potential of the acorn in Portugal", developed by Miguel Sottomayor (2015) [132], 55% of the acorns in Portugal, whose production is concentrated in the North and Alentejo regions, "is being wasted". Taking into account certain variables in the study, this surplus production may correspond to approximately 13.3 million euros [132]. Acorns and their by-products have an huge potential as alternative functional food, specifically considering their high nutritional value and richness in bioactive phytochemicals with biological action, contributing to the consumer well-being. Furthermore, they promote the use of a natural product in accordance with the principles of sustainability [133].

The acorns (Figure 4, left), as the fruits of oak trees (*Quercus genus*) are usually known, are abundant in the west and southwest of the Iberian Peninsula, since they grow in temperate areas like the Mediterranean region [134,135]. In Portugal, holm oak (*Quercus ilex*) and cork oak (*Quercus suber*) are the two main components of the cork oak forestland, commonly called Portuguese Montado (Figure 4, right), which is concentrated in the Alentejo [136] and occupying an area much higher than that dedicated to chestnut and almond [133]. Portugal concentrates the world's largest area of cork oak *Montado*, which corresponds to 34 % [135].

Figure 4: Acorns from *Quercus suber* (left) [134] and Portuguese Montado (right) [135].

The oak *(Quercus robur)*, sessile oak *(Quercus petraea)*, cork oak *(Quercus suber)*, holm oak *(Quercus ilex)*, pyrenean oak *(Quercus pyrenaica)*, and Portuguese oak *(Quercus faginea)* are trees of the Iberian Peninsula of the same genus *(Quercus)*, and family *(Fagaceae)* [137]. *Quercus suber* is the primary source of cork. Portugal produces 100,000 tonnes of cork per year, representing half of the cork harvested annually in the world [138]. In its turn, *Quercus ilex* is mainly used for firewood and charcoal [136]. Both species (*Q. suber* and *Q. ilex*) produce acorn, traditionally used for feeding the native black Iberian pig (*Sus scrofa mediterraneus*), which produces delicious and renowned hams and pork sausages [134,139]. In past times of scarcity, the acorns served for human consumption and to support the local economy [140]. Consumption as nuts (resembling chestnuts), flour (high starch contents), or cooking oil (high similarity with olive oil), are the most common forms of acorn consumption [131]. Nowadays, new products made from acorns are under development, and some are already on the market. Among the new uses, acorn chocolates, acorn flour and bread, acorn liquors, beer and other beverages, acorn caramels, and acorn honey stand out [134]. However, and despite their availability, acorns are currently far from being as widely used as other common nuts [131].

Since different species of *Quercus* exist, roughly 500 trees and shrubs, acorns come in a variety of shapes, sizes, colours and bitterness, depending on the phylogenetic as well as ecological factors [134,137,139,141,142]. Acorns are nuts whose mean width values are around 1.5 and 3 cm, while usually weighing in the range from 1.2 to 6.5 g [143]. Its morphological characteristics are illustrated and described in Figure 5.

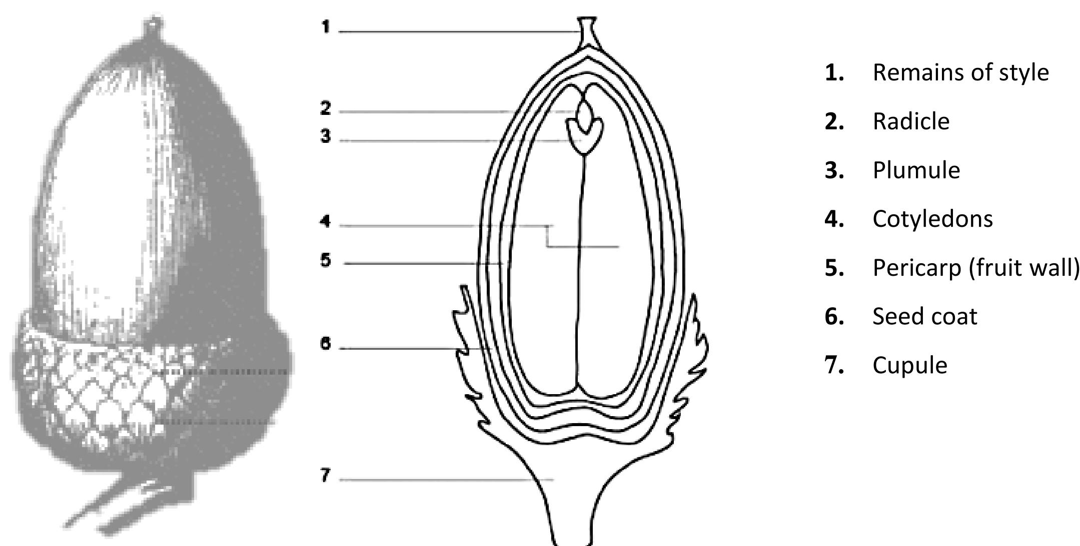


Figure 5: Schematic representation of a *Quercus* acorn and legend of morphological characters in longitudinal cross-section [131,141].

Table 7 compiles the nutritional composition of acorn from *Q. ilex* and *Q. suber*, reported by some authors and demonstrates the relevant nutritional value of both fruits. In addition to differences between the composition of building species, Viñas *et al.* (2003) [144] reported large differences in the components values in the shell (pericarp) and pulp fractions (radicle, plumule and cotyledons) of the acorns. Both could be considered as an added-value ingredient [131].

Table 7: Approximate nutritional composition established for *Quercus ilex* and *Quercus suber* acorns according to Afzal-Rafii *et al.* (1992) [145], Cañellas *et al.* (2003) [144], León-Camacho *et al.* (2004) [146] and Nieto *et al.* (2002) [147].

Source	*Quercus ilex*	*Quercus suber*
Crude Protein (%)	4.9 - 5.1	6.3 - 7.0
Lipid (%)	6.3 - 7.0	7.7 - 7.8
Crude Fibre (%)	2.68	__
Sugar (%)	5.73	__
Ash (%)	2.0 - 2.2	1.8 - 2.1
Na (%)	0.02	__
P (%)	0.08 - 0.15	0.12
Ca (%)	0.02 - 0.24	0.14
Mg (%)	0.04 - 0.07	0.06
K (%)	0.97	__
Fe (mg/kg)	2.71	2.56
Cu (mg/Kg)	2.20	2.79
Cholesterol (mg/Kg)	1.54	1.65

Acorns are described as being rich in water, carbohydrates (particularly starch) and lipids (particularly unsaturated fatty acids), and poor in proteins [146]. Acorns are also a source of minerals and important antioxidant compounds, namely phenolic compounds and sterols [148,149]. Acorn flours also possess an interesting nutritional profile, rich in fibre and lipids while possessing no gluten, which makes acorn flours an interesting matrix for the development of gluten free foodstuffs by employing a commonly disregarded and cheap alternative [150]. The acorn oil has high amounts of healthy fatty acids [137]. The acorn shell contains a great amount of fibre constituents, particularly lignin [147]. Despite this, the inclusion of acorns in human nutrition is still scarce.

Starch is the most important carbohydrate in the acorn, constituting approximately 50 % of the dry matter in acorn [137]. In the study of Correia *et al.* (2013) [140], isolated acorn starches from *Q. suber* and *Q. rotundifolia* presented high amylose content (53 - 59 %) and resistant starch content (30.8 - 41.4 %). The resistant starch functions as a substrate for bacterial fermentation (prebiotic) in the large intestine and has been reported in consequence of their many health benefits for humans [151]. Concretely, acorn flour has a low and slow glycemic response, compared to white bread, potatoes and processed cereals, which can make the release of glucose into the blood to occur evenly throughout the day, avoiding problems of diabetes, obesity and cardiovascular disease [137]. Despite not being as nutritionally rich as other common nuts, acorns represent a good alternative to other high-starch content products, such as chestnuts or potatoes [133].

Proteins represent 4 - 8 % of the dry matter of the acorn pulp [137]. More specifically, Gálvan *et al.* (2011) [152] studied the protein yield among *Q. ilex* acorn populations, and verified that it varied between 2.92 - 5.92 mg/g dry weight. In terms of amino acid content, the acorn contains all the essential amino acids except tryptophan. On the other hand, their potential nutritive value has a great importance as a relatively rich source of some amino acids. The levels of valine, threonine, isoleucine, leucine, phenylalanine and lysine in the acorn would meet the requirements for adults. Particular attention is paid to the lysine needs of adults, since this indispensable amino acid is more likely to be limiting in cereal-based diets, characteristic of the population in large areas of the third world. The small deficiencies in essential amino acids of acorns could probably be rectified with complementary legumes, dairy, eggs, fish or meats [137].

Lipid fraction of acorns shows elevated values of both monounsaturated and polyunsaturated fatty acids [150]. Oleic acid (> 63 % of total fatty acid content) is the main fatty acid, followed by linoleic and palmitic acids [153]. In general, acorns fatty acid profiles

are similar to those typically detected in sunflower, peanut, olive and avocado oils [133]. Comparing with olive oil, the amounts of the remaining fatty acids are significantly higher in the acorn oil, highlighting linolenic acid, of the family of omega 3, which is an important polyunsaturated fatty acid in the prevention of cardiovascular diseases [137]. Besides the potential effects of their direct consumption, the inclusion of acorns in animal feeding might improve the quality of their meat [131].

The mineral content in acorns is also noteworthy, since minerals are important for certain essential functions of the body [131,137]. To complement Table 7, which shows some results for acorns of *Q. suber* and *Q. ilex*, Rakić *et al.* (2006) [149] described considerable amounts of Fe, Cu, Zn, and Mn, besides Ca, Mg, P, and K in lower levels, in *Q. robur* acorn samples.

Tocopherols (vitamin E) are the main substances with antioxidant properties naturally present in seed oils. The antioxidant action of vitamin E has a double aspect: protection *in vivo*, protecting cellular lipids from oxidation, and *in vitro*, protecting the oil and food from rancidity oxidative. [137]. Acorn oil also shows high amounts of tocopherol (vitamin E), with a predominance of γ -tocopherol (90 % of total tocopherol content), although there is a wide variation between species [146]. In general, γ –tocopherol reaches levels 4.6 - 8.7 fold higher than those detected for α-tocopherol. Acorns are also an excellent source of provitamin A, since it has been reported that a small amount of acorns would guarantee the recommended daily requirements of vitamin A, which might be a great advantage in areas where vitamin A deficiency is a common problem, particularly in some African and Southeast Asian low-income countries [131].

Equally relevant are the detected levels of phytosterols, sterols that are found naturally in plants, among which β-sitosterol is the major compound (> 90 % of sterols) in acorns. The values reported for acorn sterols are higher than those obtained in almond, soybean, olive and pistachio oils [131,137]. β-sitosterol chemical structure is very similar to that of cholesterol and studies have also indicated that a diet high in this and other phytosterols can inhibit cholesterol absorption and reduce serum cholesterol levels by competing for intestinal absorption [137].

In addition to the tocopherols, phenolic compounds, such as phenolic acids, flavonoids and tannins, are considered as being the primary bioactive compounds in acorn fruits and that act as strong natural antioxidants, reducing rancidity [146,149,153]. Cantos *et al.* (2003) [153] reported 32 phenolic compounds isolated from *Q. rotundifolia*, *Q. ilex* and *Q. suber* acorns harvested in the south of the Iberian Peninsula and the vast majority correspond to hydrolysable tannins and their precursors (gallic and ellagic acid). The presence of these

types of compounds provides health benefits, which are mainly correlated to their high antioxidant activity, besides having important functions in decreasing the risk of cardiovascular and inflammatory illnesses, diabetes, cancer, microbial infection, human immunodeficiency virus (HIV), infection and other diseases, inclusively when evaluated under epidemiologic studies [131,133].

The acorns of all *Quercus* are edible. However, there are certain varieties of *Q. ilex* (*Azinheira,* as it is known in Portugal) that are sweet and eaten like chestnuts, but in general, the rest are more or less bitter, that is to say with greater or lesser concentration of tannins [137]. Tannins are responsible for the bitterness and astringency of the foods that contain them. To facilitate its palatability, and to lose the bitterness of its tannins, acorns can be leached in water, since tannins are water-soluble polyphenols, and then roasted or thoroughly boiled [134,148]. These compounds are reported as providing benefits to human health, specifically for their anticarcinogenic and antimutagenic properties. Thus, it is necessary to maintain as much as possible the nutritional and phytochemical profiles of acorns, whereby further studies should be performed to conclude the best processing practices to counteract astringency without compromising the acceptability of the food [131].

Several studies have been done to increase the potential uses of the acorn as a functional food. Custódio *et al.* (2013) [136] indicates that extracts from *Q. suber* and *Q. ilex* acorns are endowed with biocompounds that act synergistically in relieving symptoms associated with neurological disorders, by exhibiting antioxidant activity, inhibitory activity, and potential to prevent oxidative stress-induced cell damage. In another study, Custódio *et al.* (2015) [148] presented a comparative evaluation of the antioxidant potential and inhibitory activity of leaf and acorn extracts of *Q. suber* on key enzymes relevant for hyperglycemia and neurodegenerative diseases. The results of both studies suggest possible applications of acorns as functional foods, which could add value to an underutilized agricultural product [136,148].

From all the above, it can be concluded that acorn valorisation perfectly fits into current trends. It becomes obvious that acorns must be considered as functional foods or as alternative sources of several highly-valued food ingredients, improving the sustainability of the agro-food chain [131]. Even so, there is still a lot of research to be done on the acorn alone and its potential applications. Future studies should focus on: (i) the mechanisms of toxicity in acorns, in order to be fully understood; (ii) specific characteristics of different acorn species, because that may influence functional performance, palatability and variability of a given food product; (iii) each of the acorn tissues, such shell and cupule, to

determine a purpose for all parts of the acorn; among other studies to increase their value for further applications in the food and pharmaceutical industries.

Thereby, the development and characterization of an acorn beverage can be a convenient way of consuming this currently undervalued and underexploited fruit, and which is an important source of health-promoting compounds, including vitamins, minerals, antioxidants, fatty acids, protein and fibre. Beyond that, a beverage is an excellent delivering means for the acorn bioactive compounds.

Scope and Objectives

The main objective of this work was to valorise the *Quercus* acorn as a food product for human consumption through the development of a functional acorn beverage. Acorn was chosen for two major reasons: (i) high content in several bioactive compounds with potential health benefits for the consumer; and (ii) to valorise a food product that is left behind in the fields. A beverage was selected taking into account the growing evolution and trends of the functional food market and consumer demand for healthier and ready-to-eat foods. On the other hand, the HPP was applied as pasteurization methodology, since several studies have proved that this technology lead to maintaining quality and freshness of plant-based beverages compared to conventional thermal treatment. However, the effect of the HPP on the acorn beverage had never been studied before, not even in the whole acorn or any by-product of it.

In this work, the beverage was submitted to HPP at different pressure values (450 and 600 MPa), conjugated with different processing times (5, 12.5 and 20 min), at room temperature. For comparison purposes, the beverage was also thermally processed (85 °C, 30 min). Subsequently, the different acorn beverages were studied with the objective of proceeding to its microbiological, physicochemical and sensory characterization. These assays were conducted to attest the adequacy of HPP treatment as a pasteurization technique, preserving the nutritional and the sensory qualities, as well as the microbiological safety of the novel functional acorn beverage. A schematic representation of the whole work is presented in Figure 6.

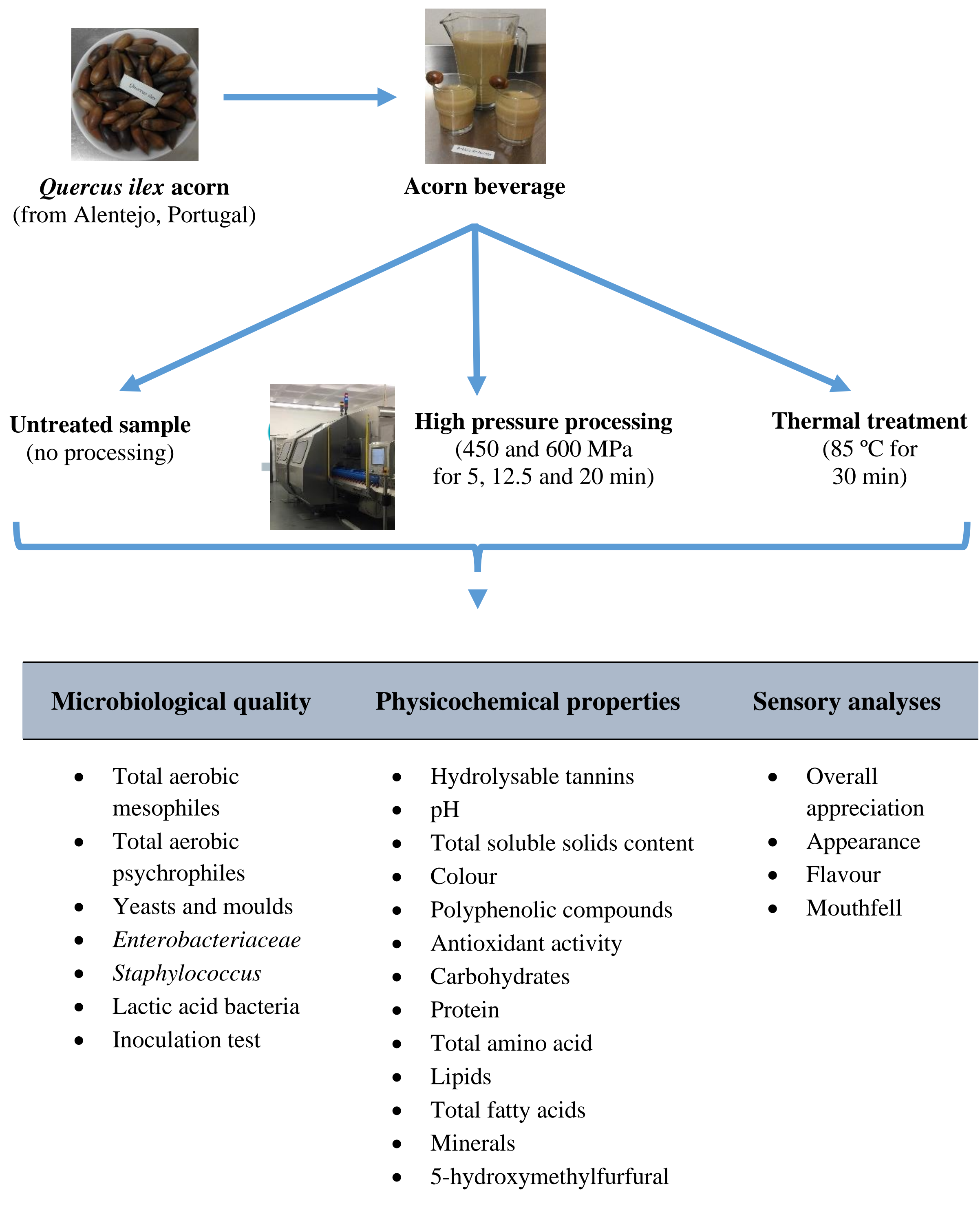


Figure 6: Schematic representation of this master's thesis work.

Chapter II – Materials and Methods

This section comprises all the methodologies employed on this work.

1. Chemicals and bacterial isolates

Chemicals purchased from Sigma-Aldrich (Missouri, USA) were peptone from animal tissue, chloramphenicol, potassium iodate, tannic acid, sodium carbonate, gallic acid, ellagic acid, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, phosphate buffered saline (PBS), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), fluorescein sodium salt, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), boric acid, bromocresol green, sodium phosphate dibasic dihydrate, propionic acid, dimethyl sulfoxide (DMSO), homoserine and norvaline, sodium tetraphenylborate, phthaldialdehyde (OPA), tritridecanoin, sodium methoxide, 5-hydroxymethylfurfural (5-HMF) and potassium dihydrogen phosphate. The chemicals acquired from Merck (Darmstadt, Germany) were macconkey agar, palcam agar, ethanol, Folin-Ciocalteu reagent, acetonitrile, trifluoroacetic acid (TFA), potassium persulfate, phenol, sulphuric acid, sodium hydroxide, hydrochloric acid (32%), *n*-hexane and nitric acid. Chemicals purchased from Biokar diagnostics (Allonne France) were plate count agar (PCA), rose bengal chloramphenicol agar base, mannitol salt agar, Man Rogosa and Sharpe (MRS) agar, violet red bile glucose agar (VRBGA), MRS broth, bacillus cereus agar and palcam selective supplement (6BS0040). The remaining chemicals were pseudomonas agar from Lab M (Lancashire, United Kingdom), buffer solutions for pH calibration from Hach (Colorado, USA), D-(+)-glucose anhydrous and mercaptoetanol form Fluka (New Jersey, USA), Kjeldahl catalyst (Cu) (0.3% in CuSO4.5H2O) from PanReac AppliChem (Darmstadt, Germany), bromothymol blue from May & Baker (London, United Kingdom), borate buffer (BBS) from Vaz Pereira (Benavente, Portugal) and N,N-dimethylformamide (DMF) from VWR (Pennsylvania, USA).

Clinical bacterial isolates, *Listeria monocytogenes* (13,562), *Bacillus cereus* (ATCC 2599), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 10145) were kindly provided by CHTMAD – Centro Hospitalar de Trás-os-Montes e Alto Douro (through, Maria José Alves, Ph.D.).

2. Acorn beverage preparation

The acorns (*Q. ilex*), supplied by Herdade do Freixo do Meio (Montemor-o-Novo, Portugal), were stored at 4 °C until be processed. The acorns were peeled, washed and then soaked in water (10 g of acorn per 100 mL of water) for 12 h at room temperature. The leaching water was then removed, and the acorns were homogenized, with water (200 mL per 10 g of acorns), using a food mixer (8010ES blender, Cole-Parmer, Vernon Hills, Illinois) and filtered through a linen cloth to eliminate the larger residues.

3. High-pressure processing, thermal treatment, and storage

Acorn beverage aliquots (50 mL) were placed in low permeability polyamide-polyethene bags, which were manually heat sealed. For pressurised beverages, the experiments were carried out on an industrial-scale high-pressure equipment (Model 55, Hyperbaric, Burgos, Spain) with a pressure vessel of 55 L. This equipment has a pressure vessel of 200 mm inner diameter and 2000 mm length and a maximum operation pressure of 600 MPa. It is connected to a refrigeration unit (RMA KH 40 LT, Ferroli, San Bonifacio, Italy) that allows to control the temperature of the input water used as a pressurizing fluid. To pressurise the beverages, the bags were placed in a cylindrical loading container at room temperature and pressurized at 450 and 600 MPa for 5, 12.5, and 20 min, as typically applied in the beverages sector [99]. For thermal treatment, the acorn beverage were heated in a water bath at 85 °C for 30 min [154] and then immediately chilled in a mixture of water and ice.

All samples were performed in duplicate and stored at 4 °C in the dark up to 56 days. For each different sample, several bags were prepared, so that new bags could be opened aseptically at each sampling time (0, 7, 14, 21, 35, 49, and 56 days of storage) in order to perform microbiological analyses and, afterwards, colour, pH, and total soluble solids (TSS) measurements. A 3 mL of each sample were centrifuged (Universal 320 R; Hettich, Tuttlingen, Germany) at 5000 rpm for 10 min at 4 ºC and the supernatant was collected to an Eppendorf tube and frozen at -20 °C to further evaluate the total polyphenolic content (TPC) and the antioxidant activity. The non-centrifuged beverage was also stored at -80 ° C until further analysis. Before each measurement, samples were equilibrated at an ambient temperature (20 ± 1 °C) and diluted if necessary.

4. Microbiological analyses

Microbial analyses were carried out by adding 1.0 mL of sample to 9.0 mL of sterile peptone water glass tube, followed by decimal dilutions and plated in a specific culture media, according to the microorganism to be analysed. Total aerobic mesophiles and total aerobic psychrophiles were enumerated in PCA, incubated at 30 ± 1 °C during 72 h and 7 ± 1 °C during 5 days, according to ISO 4833-2:2013 [155] and Berizi *et al.* (2016) [156], respectively. Yeasts and moulds were enumerated in rose bengal, previously supplemented with chloramphenicol (0.050 g/mL prepared in ethanol 50 % (v/v) per 1 L of culture media) that was sterilized with a 0.22 µm cellulose acetate syringe filter (VWR, Pennsylvania, USA), and Petri plates were incubated at 25 ± 1 °C during 72 h, according to ISO 21527-1:2008 [157]. *Enterobacteriaceae* were enumerated on VRBGA, incubated at 37 ºC for 24 h, according to ISO 21528-2:2017 [158]. *Staphylococcus* were enumerated on mannitol, incubated at 37 °C for 24 - 48 h according ISO 6888-1:1999 [159]. Lactic acid bacteria were enumerated on MRS agar, incubated at 37 ºC for 72 h under anaerobic conditions, according to ISO 21528-2:2017 [160]. All analyses were performed in duplicate using 25 µL of sample, or one of their serial dilutions, by surface plating technique described by Miles et *al.* (1938) [161], except the *Enterobacteriaceae* analysis which was performed by pour plate technique with 1 mL. The results were expressed as the decimal logarithm of colony forming units (CFU) per mL of acorn beverage as defined in (Equation 2):

$$\frac{\log_{10} \mathrm{CFU}}{\mathrm{mL}} = \log_{10}\left(\frac{\mathrm{CFU}}{\mathrm{V} \times \mathrm{DF}}\right) \quad \textbf{(Equation 2)}$$

where V corresponds to the plated volume and DF is the dilution in which it was possible to count the colonies.

Microbiological quality of beverages was monitored during their storage (0, 7, 14, 21, 35, 49, and 56 days), when microbiological counts exceed to the satisfactory microbial limit for their acceptance, 6.00 $\log_{10}$ CFU/mL [127], the maximum load considered in this study, the beverage was considered as expired.

4.1. Inoculation test

Four different food contaminant/pathogenic bacteria were used in this study, two Gram-positive: *Listeria monocytogenes* 13,562 and *Bacillus cereus* ATCC 2599; and two Gram-negative: *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 10145. These strains are stored in polypropylene tubes at the temperature of -80 °C in MRS broth containing 30 % (v/v) glycerol for later use. Afterwards, the frozen strains were reactivated in MRS broth at 37 °C for 24 h and the viable cell count was performed with streak plate technique in agar medium. The Gram staining was used to verify that cultures were pure. The pure strains were re-suspended in falcon tubes containing MRS broth that were incubated at 37 ± 1 °C during 24 h, together with a falcon tube of un-inoculated medium as a control tube. The next day, the optical density of the suspension of bacteria cells was determined on a UV-VIS spectrophotometer (1240 UV-visible spectrophotometer; Shimadzu, Kyoto, Japan) at 625 nm. Comparing the optical density with the McFarland turbidity scale (Table 8) [162], it was possible estimate the approximate cell density per mL of medium and adjust to 0.5 McFarland turbidity standard (as used for antimicrobial testing purposes [163]). A 0.5 McFarland Latex Standard is comparable to a bacterial suspension of 1.5×10^8 CFU/mL.

Table 8: McFarland turbidity scale by Hardy Diagnostics [162].

Latex Standard	0.5	1	2	3	4	5	6	7	8
Approximate Cell Density × 10^8 / mL	1.5	3	6	9	12	15	18	21	24
Absorbance Specification at 625 nm	0.08 to 0.10	0.14 to 0.17	0.27 to 0.31	0.38 to 0.42	0.51 to 0.55	0.67 to 0.70	0.74 to 0.77	0.83 to 0.88	0.94 to 0.98

Four tubes of acorn beverage were inoculated with the respective microorganism and incubated at 37 ± 1 °C during 24 h. After the incubation period, a sample of each beverage was processed at 450 MPa/5 min. *Listeria monocytogenes* were enumerated in palcam at 37 ± 1 °C during 24 h, according to ISO 11290-1 [164] method, but using a different culture medium [165]. Palcam was previously supplemented with palcam selective supplement (6BS0040), prepared by mixing 5 mL of water sterilized with a 0.22 µm cellulose acetate syringe filter. *Bacillus cereus* were enumerated in bacillus cereus agar, previously supplemented with polymyxin B selective supplement, and Petri plates were incubated at 37 ± 1 °C during 24 h, according to ISO 7932:2004 [166]. *Escherichia coli* were enumerated

in macconkey agar at 37 ± 1 °C during 24 h, according to ISO 16654:2001 [167]. *Pseudomonas aeruginosa* were enumerated in pseudomonas agar at 37 ± 1 °C during 24 h, according to ISO 16266-2:2018 [168]. All the analyses were conducted in duplicate but only for some selected samples (untreated, heat-treated, and pressurized samples at 450 MPa/5 min).

In addition, a test was performed in which the beverage was left to rot, exposed to air and at 37 °C. Microbial analyses for this beverage were performed as described in section 4 of this chapter (Chapter II).

5. Physicochemical analyses

5.1. Hydrolysable tannins content

Hydrolysable tannins content (HTC) was determined by the method of Willis *et al.* (1998) [169] with slight modifications, in order to evaluate the leaching efficiency of acorn tannins by water. The acorn beverage was centrifuged (M-240; Boeckel + Co, Hamburg, Germany) at 14000 rpm for 10 min at 4 ºC and the supernatant was collected to an Eppendorf tube. Then, 500 µL of sample were mixed with 2.5 mL of potassium iodate solution (25 g/L) in a sample tube. Optimum reaction time to gain maximum absorbance value was determined to be 8 min for samples and standard solutions of tannic acid by a preliminary experiment. The absorbance of the red colour was measured at 550 nm. A calibration curve (0.060 - 2.000 mg/mL) was previously established using tannic acid as standard and all the measurements were replicated twice. To evaluate the leaching rate the following equation (Equation 3) was considered:

$$\begin{matrix}\text{Loss of tannins}\\ \text{by leaching}\end{matrix}\ (\%) = \frac{\text{HTC (leaching water)}}{\text{HTC (leaching water)} + \text{HTC (acorn beverage)}} \times 100 \qquad \textbf{(Equation 3)}$$

where HTC (leaching water) is the tannin concentration in leaching water and HTC (acorn beverage) is the tannin concentration in the acorn beverage. HTC was expressed as mg of tannic acid equivalents in mL of acorn beverage.

5.2. pH and total soluble solids content

The pH was measured at room temperature (20 ± 1 °C) using a potentiometer equipped with a Crison 52-08 electrode (Crison Instruments, Barcelona, Spain). The equipment was calibrated using commercial solutions with pH 7.00 and 4.01, at each sampling time.

The TSS content was measured as ºBrix at room temperature (20 ± 1 °C) using a portable refractometer (Palette PR-32α; Atago Co., LTD, Tokyo, Japan). One drop was placed on the

refractometer glass prism, and the TSS was obtained as Brix percentage. Distillate water was used as a blank.

All analyses (pH and TSS) were performed in duplicate at all sampling times (0, 7, 14, 21, 35, 49, and 56 days of storage).

5.3. Colour

A Petri dish was filled with acorn beverage and a Konica-Minolta CR-400 chromameter (Osaka, Japan) equipped with a D65 illuminant and a CIE observer 2° was placed directly over the sample. The instrument was calibrated using white reference tiles (Y 93.9; x 0.3163, y 0.3327). The L^*, a^* and b^* CIE colour system was used to evaluate the colour. L^* defines the lightness (0 = black, 100 = white), a^* defines the red-greenness ($-a^*$ =greenness, $+a^*$ = redness) and b^* defines the blue-yellowness ($-b^*$ = blueness, $+b^*$ = yellowness) [170]. The values of each parameter were obtained in order to calculate the total colour change variation (ΔE^*), as shown in (Equation 4):

$$\Delta E^* = [(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]^{\frac{1}{2}} \qquad \textbf{(Equation 4)}$$

in which ΔE^* is the total colour change variation between a sample and the control (untreated acorn beverage values identified with the subscript "0").

Analyses for each sample were conducted in triplicate at all sampling times (0, 7, 14, 21, 35, 49, and 56 days of storage).

5.4. Determination of polyphenolic compounds

5.4.1. Total polyphenolic content

For the TPC determination, the Folin-Ciocalteu colorimetric method was performed in a 96-well microplate (Nunc, Roskilde, Denmark), following the method described by Coscueta *et al.* (2018) [171], with some modifications. The procedure consisted in mixing 20 µL of sample with 80 µL of the Folin-Ciocalteu reagent 10% (v/v) and then 100 µL of sodium carbonate solution (7.5 % w/v), always following the sequence described. After shaking thoroughly and incubating for 1 h in darkness at room temperature, the absorbance was measured at 765 nm. A calibration curve of gallic acid (0.0017 - 0.0213 mg/mL) was prepared on each assay in order to express the results as mg of gallic acid equivalents per mL of acorn beverage. Absorbance measurements were performed on the Multidetection plate reader (Synergy H1, Vermont, USA) operated using the Gen5 software (BioTek

Instruments). Analyses for each sample were conducted in triplicate at all sampling times (0, 7, 14, 21, 35, 49, and 56 days of storage).

5.4.2. Identification and quantification of individual polyphenolic compounds

Qualitative and quantitative profiles of polyphenols were carried out by high-performance liquid chromatography with a diode-array detector (HPLC-DAD), according to the method described by Oliveira *et al.* (2015) [172], with some modifications. Analysis was conducted on a High Performance Liquid Chromatograph (Waters Alliance e2695, Mildford MA, USA), separation module system interfaced with a photodiode array UV-Vis detector 2998 (Waters, Mildford MA, USA). The separation of the compounds was carried out in a reverse phase C18 column (COSMOSIL 5C1 8-AR-II Packed Column– 4.6 mm I.D. × 250 mm; Dartford, UK). The mobile phase was composed of solvent A: water/acetonitrile/TFA (94.8/5/0.2) and solvent B: acetonitrile/TFA (99.8/0.2) and the following elution gradient was used: 0-1 min 0 % of B; 1–30 min 21 % of B; 30-42 min 27 % of B; 45-55 min 58 % of B; 55-60 min 0 % of B and kept another 1 min 0 % of B. The flow rate was 1 mL/min, the oven temperature was set as 25 ºC and the injection volume was 20 µL.

The gallic acid and ellagic acid were detected at 280 and 360 nm, respectively, while data acquisition and analysis were accomplished using Software Empower 3. These compounds were identified and quantified by external calibration curve by comparison to pure standards (in the range of concentrations of 7.8 – 250.0 µg/mL). Analyses were conducted in triplicate and only for some samples (untreated, heat-treated, and pressurized at 450 MPa/5 min) at 0, 21, and 56 days of storage. All the acorn beverage samples were filtered using 0.45 µm syringe filters (Macherey-Nagel, Düren, Germany) and the results expressed as mg of gallic/ellagic acid per mL of acorn beverage.

5.5. Antioxidant activity (ABTS, DPPH, and ORAC assay)

The **ABTS scavenging assay** was performed in a 96-well microplate, following the method described by Gonçalves *et al.* (2009) [173], with some modifications. The radical cation $ABTS^{\bullet+}$ was produced by reacting ABTS aqueous solution (7 mM) with potassium persulfate (2.45 mM) in the ratio 1:1 (v/v) during 16 h in darkness at room temperature. Immediately before the procedure, the $ABTS^{\bullet+}$ solution was filtered using a 0.45 µm syringe filter and adjusted to an absorbance of 0.700 ± 0.020 at 734 nm with ultra-pure water. Then, a mixture of 15 µL of sample with 200 µL of diluted $ABTS^{\bullet+}$ solution was incubated for 5 min at 30 °C, and the absorbance at 734 nm was measured. A calibration curve of ascorbic acid

(0.0088 - 0.0881 mg/mL) was prepared on each assay in order to express the results as mg of ascorbic acid equivalents per mL of acorn beverage. Incubation and absorbance measurements were performed on the Multidetection plate reader. Analyses for each sample were conducted in triplicate at all sampling times (0, 7, 14, 21, 35, 49, and 56 days of storage).

In data processing, the scavenging activity is expressed as % reduction in absorbance in relation to control (Equation 5), and it is possible to define regression equations between net ABTS scavenging and ascorbic acid concentration.

$$\text{ABTS scavenging (\%)} = \frac{\text{Abs}_{734}(\text{control}) - \text{Abs}_{734}(\text{sample})}{\text{Abs}_{734}(\text{control})} \times 100 \qquad \textbf{(Equation 5)}$$

where Abs_{734}(control) is the absorbance at 734 nm of the diluted $ABTS^{\bullet+}$ solution and Abs_{734}(sample) is the absorbance at 734 nm of the sample.

The **DPPH$^{\bullet}$ free radical assay** was performed in a 96-well microplate according to the method described by Jing *et al.* (1995) [169] with some modifications. Quantification was done by mixing 25 µL of sample with 175 µL of methanolic DPPH$^{\bullet}$ (60 µM; if necessary, one should adjust the absorbance to 0.600 ± 0.100 at 515 nm). After 30 min in darkness at room temperature, the absorbance was read at 515 nm. A calibration curve of Trolox (0.0075 - 0.0751 mg/mL) was prepared on each assay in order to express the results as mg of Trolox equivalents per mL of acorn beverage. Absorbance measurements were performed on the Multidetection plate reader. Analyses for each sample were conducted in triplicate at all sampling times (0, 7, 14, 21, 35, 49, and 56 days of storage).

In data processing, the scavenging activity is expressed as % reduction in absorbance regarding the control (Equation 6), and it is possible to define regression equations between net DPPH scavenging and Trolox concentration.

$$\text{DPPH scavenging (\%)} = \frac{Abs_{515}(control) - Abs_{515}(sample)}{Abs_{515}(control)} \times 100 \qquad \textbf{(Equation 6)}$$

where Abs_{515}(control) is the absorbance at 515 nm of the DPPH$^{\bullet}$ solution and Abs_{515}(sample) is the absorbance at 515 nm of the sample.

The **oxygen radical absorbance capacity (ORAC) assay** is performed in a black 96-well microplate, following the method described by Dávalos *et al.* (2004) [174], with some modifications. All the solutions were carried out in phosphate buffer (75 mM, pH 7.4) prepared with ultrapure water. To perform this test, 20 µL of sample were mixed with 120 µL

of fluorescein (70 nM) and pre-incubated for 10 min at 37 °C. Then, 60 µL of AAPH solution (12 mM) was rapidly added and the final reaction mixture was incubated at 40 °C for 140 min. Throughout this period, the fluorescence was recorded in a Multidetection plate reader with 485 nm excitation and 520 nm emission filters. A blank using phosphate buffer instead of the antioxidant solution and eight solutions for a calibration curve of Trolox (10-80 µM) was prepared on each assay. The Gen5 software processed the raw data and the results were expressed as mg of Trolox equivalents per mL of acorn beverage. Analyses were conducted in duplicate and only for some samples (untreated, heat-treated, and pressurized at 450 MPa/5 min) at 0, 21, and 56 days of storage.

5.6. Total carbohydrate content

The total carbohydrate content was determined with the ultraviolet–visible (UV-VIS) spectrophotometric phenol/sulphuric acid method, firstly described by Dubois *et al.* (1956) [175], with some modifications. The procedure consisted in mixing 2 mL of sample with 1 mL phenol solution (5 % w/v) and 5 mL of H_2SO_4 (95 %). The mixture was then stirred rapidly and left to cool to room temperature for about 10 min before the absorbance was measured at 490 nm on a UV-VIS spectrophotometer. Quantification was performed by a calibration curve made with glucose (20 – 100 mg/mL). Total carbohydrate content was expressed in mg of glucose per mL of acorn beverage. Analyses were conducted in duplicate and only for the samples with 0 days of storage.

5.7. Total protein content

The total protein was determined through the Kjeldahl method as described in ISO 1871 [176], using 6.25 as converting factor to protein [133]. This method is divided into three steps: (i) digestion, (ii) distillation, and (iii) titration. In the first step, the digestion, 2 mL of sample, 1 g of Kjeldahl Catalyst (Cu) (0.3 % in CuSO4.5H2O) previously crushed with mortar and pestle, and 4 mL of sulphuric acid 95 – 97 % (v/v) were placed into a glass digestion tube. The digestion tube with the mixture was placed into the digestion unit (Foss Tecator, 2012 Digestor) heated to 416 ºC for about 150 min. The vapours of water and sulphuric acid were bubbled through a solution of sodium hydroxide (scrubber) to neutralize them. The digestion was finished when the sample was totally transparent with a slightly blue colour due to the Cu from the catalyst. Then, the sample was allowed to cool to room temperature, and 20 mL of distilled water was cautiously added. In the distillation step, the glass tube with the digested sample was transferred to the distillation unit (VELP

SEIENTIFICIA, UDK 129) and an Erlenmeyer flask with a mixture of 25 mL of boric acid (4 %) and pH indicators, bromothymol blue and bromocresol green, was placed at the opposite end of the distiller with the collecting tube immersed in the solution. When the default program has been run on the device (which takes approximately 5 min), 30 mL of sodium hydroxide solution 10 N was added to the sample to neutralize the pH and to convert NH_4^+ into NH_3 (solution turns blue/black). At the same time, a stream of water vapour was bubbled into the sample to entrain the NH_3 formed. Lastly, the NH_3 was condensed and captured by the Erlenmeyer solution, which turns from red violet to green. In the last step, the titration, the solution was titrated to a slightly violet colour with HCl 0.01 M. Analyses were conducted in duplicate and only for some samples (untreated, heat-treated, and pressurized at 450 MPa/20 min and 600 MPa/20 min) at 0 days of storage.

In data processing, the total protein content is determined using the (Equation 7). A conversion factor is used to convert percentage of nitrogen into percentage of total protein. Since most of the proteins typically contain 16 % nitrogen, a conversion factor of 6.25 is used (100/16 = 6.25) [133].

$$Protein\ content\ (\%) = [HCl] \times \frac{V(HCl\ corrected)}{V(sample)} \times 14 \times 100 \times 6.25 \qquad \textbf{(Equation 7)}$$

where [HCl] is the HCl concentration (M), V(HCl corrected) is the volume spent to titrate the solution minus the volume spent to titrate the blank (mL), 14 is the atomic mass of nitrogen (g/mol), and 6.25 is the conversion factor.

5.8. Total lipid content

Total lipid content was determined by gravimetric Soxhlet method according to ISO 659:2009 [177] and using *n*-hexane as solvent as in the work presented by Lopes *et al.* (2005) [178]. Soxhlet extractions were performed using 50 mL of acorn beverage previously lyophilized. The sample was transferred into a cellulose thimble and then placed into the main chamber of the Soxhlet extractor. A 100 mL volume of *n*-hexane was added and the whole assembly was heated for 6 h using a heating jacket. The solvent (heated to reflux) travelled into the main chamber and the partially soluble components were slowly transferred to the solvent. Finally, the extracts were concentrated using a speed-vacuum evaporator (RVC 2-18; Martin Christ Gefriertrocknungsanlagen, Osterode am Harz, Germany) at 40 °C and 335 mbar. The lipid fraction extracted dried in an oven for 1 h at

100 ºC and the total lipid content of beverages was expressed in sample dry weight percentage. Analyses were conducted in duplicate and only for the samples with 0 days of storage.

5.9. Identification and quantification of total amino acid content

Qualitative and quantitative profiles of total amino acid were carried out by HPLC with a High Resolution Fluorescence Detector according to Pripis-Niculau (2000) [179], with some modifications. Lyophilized samples (20 mg) were hydrolysed in a SPME vial with 3 mL of HCl 6 M under vacuum at 105 °C during 20 h. Then, the samples cooled to room temperature and 4 mL of borate buffer was added. The pH was corrected to 9.5 with NaOH 10 M and the volume was made up to 10 mL before filtering the hydrolysate with 0.45 µm syringe filters for further analysis.

The chromatographic analysis was carried out using two distinct eluents, A and B. Eluent A consisted of 10 g/L sodium phosphate dibasic dihydrate, 7.4 g/L propionic acid, 20 mL/L DMSO, 65 mL/L acetonitrile and ultrapure water, with the final pH value being adjusted to 6.65 using NaOH 4 M. Eluent B was composed of 330 mL/L methanol, 70 mL.L^{-1} DMSO, 400 mL/L acetonitrile and ultra-pure water. Both eluents were filtered under vacuum and degassed by an ultrasonic bath. The reagent A (25 mL) consisted of 3 mL of a previously prepared internal standard solution (20 mg/mL of homoserine and norvaline in 0.1 M HCl), 120 µL mercaptoetanol, 500 mg of sodium tetraphenylborate and 25 mL of borate buffer. Reagent B (100 mL) was comprised of 3.5 g of iodoacetic acid, 50 mL of borate buffer, adjusted to a pH value of 9.5 with NaOH 4 M, and the volume was completed with borate buffer. Finally, 50 mL of reagent C were prepared by mixing 225 mg of OPA, 5 mL of methanol, and completed to 50 mL with borate buffer. Then, 0.5 mL of mercaptoethanol were added and the solution was bubbled with N_2.

The characterization and quantification of the total amino acids was performed using a liquid chromatography apparatus (HPLC Gold 128 Solvent module, Beckman Coulter, Brea, USA) with a High Resolution Fluorescence Detector ($\lambda_{excitation}$ 356 nm; $\lambda_{emission}$ 445 nm; Waters 474, Milford, USA) and an autosampler (model 410 Varian prostar, Agilent technologies, Santa Clara, USA). The system was connected to a Chromolith® Performance RP18 (4.6 × 100 mm) (Merck, Darmstadt, Germany) column, operating at a flow rate of 0.8 mL/min. From the filtered samples, 100 µL were used by the autosampler, mixing with 250 µL of reagent A, and 250 µL of reagent B. After 3 min, 250 µL of reagent C was added and 10 µL of the mixture was injected into the HPLC system. Analyses were conducted in

duplicate and only for some samples (untreated, heat-treated, and pressurized at 450 MPa/5 min) at 0, 21, and 56 days of storage. Tryptophan was not determined.

5.10. Identification and quantification of total fatty acids

Qualitative and quantitative profiles of fatty acids (FA) were carried out by gas chromatography-flame ionization detector (GLC-FID), according to the method described by Pimentel *et al*. (2015) [180], with some modifications. 200 µL of tritridecanoin was added to 500 mg of sample. Then, 2.26 mL of methanol were added, followed by 800 µL of hexane and 240 µL of sodium methoxide (5.4 M). Samples were vortexed and incubated at 80 ℃ for 10 min. After cooling in ice, 1.25 mL of DMF and 1.25 mL of sulphuric acid/methanol (3 M) were added. Samples were vortexed and incubated at 60 ℃ for 30 min. Finally, after cooling, they were vortexed and centrifuged (1250 g, 18 ℃, 5 min). Upper layer containing FA methyl esters (FAME) was collected for further analysis. Samples were analysed in a gas chromatograph HP6890A (Hewlett-Packard, Avondale, PA, USA), equipped with a flame ionization detector and a BPX60 capillary column (60 m x 0.25 mm x 0.25 µm; SGE Europe Ltd, Courtaboeuf, France). Analysis conditions were as follows: injector (split 25:1; injection volume 1 µL) and detector temperatures were 250 ℃ and 275 ℃, respectively; flow rate was of 1 mL/min. The oven temperature initially was 60 ℃ and then increased to until a final temperature of 225 ℃. Supelco 37, FAME from CRM-164 was used for identification of fatty acids. GLC-Nestlé36 was assayed for calculation of response factors and detection and quantification limits (LOD: 0.79 ng FA/mL; LOQ: 2.64 ng FA/mL). Analyses were conducted in duplicate and only for some samples (untreated and pressurized at 450 MPa/5 min) at 0, 21, and 56 days of storage.

Nutritional quality indices of acorn beverage were also analysed from FA composition data. The indices of atherogenicity (AI) and thrombogenicity (TI) were calculated as proposed by Ulbricht and Southgate (1991) [181]. AI and TI indices were calculated using (Equation 8) and (Equation 9), respectively:

$$AI = \frac{[C12:0 + 4 \times C14:0 + C16:0]}{[\sum MUFA + \sum PUFA]}$$ **(Equation 8)**

$$TI = \frac{[C14:0 + C16:0 + C18:0]}{\left[0.5 \times (\sum MUFA + \sum \omega 6) + 3 \times \sum \omega 3 + \frac{\sum \omega 3}{\sum \omega 6}\right]}$$ **(Equation 9)**

where MUFA is the monounsaturated FA, PUFA is the polyunsaturated FA and ω6 and ω3 are omega-6 and omega-3 FA, respectively.

Other indicators included the ratios ω6/ω3, MUFA/PUFA, and the PUFA/saturated fatty acids (SFA), which were also calculated.

5.11. Identification and quantification of individual minerals

Qualitative and quantitative profiles of minerals were carried out by inductively coupled plasma optical emission spectroscopy (ICP-OES), according to the method described by Omohimi *et al.* (2017) [180], with some modifications. Prior to the mineral analysis, the samples underwent a microwave assisted digestion. For this, a weighed amount of each sample (2 mL) was mixed with 5 mL of HNO_3 65 % in a Teflon reaction vessel and heated in a Speedwave^{TM} MWS–3 + (Berghof, Germany) microwave system. Digestion procedure was conducted in five steps: (i) 160 ºC/5 min, (ii) 190 ºC/20 min, (iii3) 100 ºC/2 min, and (iv) 100 ºC/2 min. The resulting clear solutions after the digestion procedure were transferred into 50 mL tubes and then brought to 20 mL with deionised water. The elemental composition was determined using an ICP-OES equipment, model OptimaTM 7000 DV ICP-OES (Dual View, PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) with radial plasma configuration. Standard plasma conditions were used, namely 1300 W for radio-frequency power, 1.5 mL/min pump rate, and 15.0, 0.2, and 0.8 L/min for plasma, auxiliary and nebulizer gas flows, respectively. Analyses were conducted in duplicate and only for some samples (untreated, heat-treated, and pressurized at 600 MPa/20 min) at 0, 21, and 56 days of storage.

5.12. Quantification of 5-hydroxymethylfurfural

The 5-hydroxymethylfurfural (5-HMF) was measured by HPLC with an UV detector, at the CINATE (Laboratório de análises e ensaios a alimentos e embalagens) from School of Biotechnology of Catholic University of Portugal, Porto, Portugal. The samples were homogenized, filtered with a 0.45 µm membrane and injected directly into the Beckman Coulter HLPC, equipped with a Beckman UV detector (model 168). The separation of 5-HMF was carried on a C18 Hypersil ODS column (250 mm x 4.6 mm, 5 µm particle size) and the detection was achieved with a wavelength of 284 nm. The analysis was performed by the external standard method and the mobile phase used was potassium dihydrogen phosphate (0.01 mol/L)/Methanol (85:15 v/v). Analyses were conducted in duplicate and

only for some samples (untreated, heat-treated, and pressurized at 450 MPa/5 min) at 0 days of storage.

6. Sensory analysis

The sensory analysis used in this work was based on the work performed by Monteiro *et al.* (2016) [182]. Sessions took place in the ISO 8589:2007 [183] compliant sensory testing facilities of School of Biotechnology of Catholic University of Portugal, Porto, Portugal.

The trial test was conducted with sixty non-experienced participants. Twenty millilitres of each sample (pressurized and heat-threated samples) were served in cups, identified by a random 3-digit code (741 to pressurization at 450 MPa/5 min and 285 to heat-treated sample) balanced the first-order effects and the carry-over effects [183]. Water was supplied to clean the palate between tastings. Participants were then asked to indicate the sample they liked the most and the one they liked the least. All samples were produced and prepared according to good hygiene and manufacturing practices and no information about the samples was provided, except for safety and hygiene considerations related to their preparation. Regarding scoring scales to evaluate the results of the questionnaires, for overall appreciation, appearance, flavour, and mouthfeel, the attribute "I dislike extremely" meant 0 points, while "I like extremely" meant 10 points. Afterward, the participants were asked about their preference between the two samples, indicating how much they like one sample over the other. In this issue, "much worse" meant -2 points, "similar" meant 0 points and "much better" meant 2 points. In the end, the participants answered the question of whether they would buy the most enjoyed vegetable beverage.

7. Statistical analysis

Statistical analysis was performed with IBM SPSS® statistics for windows (2012), version 21.0 (Armonk, NY: IBM Corp.). To analyze statistical differences, when a normal distribution was observed, the one-way analysis of variance (ANOVA) test was employed in association with Tukey's post hoc test. To perform statistical analysis of the sensorial tests, t-student test for paired samples was used. The differences were considered statistically significant at a 5 % confidence degree level ($p \leq 0.05$), though differences significant at a 1 % level were also marked. The results were expressed as mean ± standard deviation (SD).

Chapter III – Results and Discussion

This section comprises all the obtained results and the respective discussion.

1. Microbiological analyses

Microbiological quality of vegetable beverages determines their shelf life and also affects the biochemical changes that occur during their storage [127]. The total counts of aerobic mesophiles, aerobic psychrophiles, yeasts and moulds, *Enterobacteriaceae*, *Staphylococcus,* and lactic acid bacteria on untreated acorn beverage throughout storage time (56 days) at 4° C are shown in Table 9. Immediately after each treatment by both HPP and thermal processing, microbial counts were lower than the detection limit, which was also verified during subsequent storage of the acorn beverage until 56 days. Furthermore, there were no *Staphylococcus* and lactic bacteria counts in any of the samples tested, including the control samples (untreated).

On day 7, the *Enterobacteriaceae* counts were above the established maximum load (6.00 log_{10}CFU/mL) meaning that *Enterobacteriaceae* were the main microorganisms responsible by the untreated beverage expiration in less than a week. The composition and the low-acid pH of this beverage may have strongly promoted this fast microbiological growth [184]. As far as we know, this is the first work reporting data about the shelf life of an acorn beverage, but similar results were reported by Corrales *et al.* (2012) [185], when studied the microbiological shelf life of a similar beverage of raw tiger nuts'. These authors concluded that the shelf life generally was not higher than 2 or 3 days when the beverages were stored at 4 ºC. On the other hand, counts of mesophiles, psychrophiles, yeasts and moulds, and *Enterobacteriaceae* showed some oscillations over time probably due to the loss of viability and the recovery of these microorganisms as a result of the low storage temperature (4 ºC) during a long storage time [186]. Despite the lack of a European legislation, it is essential to assess the microbiological quality of this beverage because of its short shelf life and high microbial load since harvesting. This may be because much of the acorn is harvested from the ground.

The HPP and thermal treatments had a significant effect ($p < 0.05$) on the microbial counts, since the initial counts of mesophiles, psychrophiles, yeasts and moulds, and *Enterobacteriaceae* were reduced from 4.70 ± 0.09, 4.46 ± 0.08, 5.37 ± 0.10, and 5.58 ± 0.30 log_{10}CFU/mL, respectively, to undetectable levels after the treatments and during the entire storage period of 56 days. Thus, the microbiological shelf life of the beverage was improved from less than a week to 56 days, but it was not possible to observe differences between HPP and thermal treatments. Similar results were obtained by Xu *et al.* (2018) [187], when an clear Se-enriched kiwifruit juice was processed by HPP at 500 MPa/10 min and the total aerobic bacteria and yeasts and molds were completely inactivated, ensuring the microbiological

safety during storage (4 and 25 °C) for 42 days. However, in both treatments, it has been reported that the microbial spoilage tends to increase throughout storage, most likely due to the growth of surviving bacteria and the recovery of injured ones [186,188]. This could possibly happen to acorn beverage if the storage time was prolonged or if the treatments intensity was reduced.

Usually HPP has been more effective in delaying microbial growth when compared to the thermal treatment. Picouet *et al.* (2015) [189] reported that in processed carrot juice (600 MPa/5 min/10 °C) after 29 days of storage at 5 ºC, the mesophiles and yeasts and moulds counts were, respectively, 3.0 ± 0.4 and 0.8 ± 1.3 for HPP samples and 5.7 ± 0.7 and 3.9 ± 0.6 for mild heated samples (80 ºC/7 min). However, for the acorn beverage was used a more intense heat treatment of 85 ºC applied for 30 min. The Codina-Torrella *et al.* (2018) [127] studied the tiger nuts' milk beverage self-life but using UHPH finding better results for UHPH samples comparing with thermal treatments. Also, several studies have been done using the same technology applied to soymilk and almond milk beverage with promising results [190–193]. Nevertheless, the results of these articles cannot be compared, since the pressure is applied by a different technology than that used in this work and with temperature associated.

Table 9: Evolution of total aerobic mesophiles, total aerobic psychrophiles, yeasts and moulds, *Enterobacteriaceae*, *Staphylococcus,* and lactic acid bacteria counts during 56 days of storage at 4 °C on untreated acorn beverage.

	log_{10}CFU/mL ± SD						
Storage time (days)	**0**	**7**	**14**	**21**	**35**	**49**	**56**
Mesophiles	4.70 ± 0.09^{c}	4.12 ± 0.16^{a}	4.34 ± 0.16^{ab}	4.87 ± 0.24^{c}	4.76 ± 0.06^{c}	4.60 ± 0.14^{bc}	4.68 ± 0.04^{c}
Psychrophiles	4.46 ± 0.08^{ab}	4.96 ± 0.24^{c}	4.38 ± 0.15^{a}	4.64 ± 0.32^{abc}	4.80 ± 0.12^{bc}	4.31 ± 0.08^{a}	4.43 ± 0.08^{ab}
Yeasts and Moulds	5.37 ± 0.10^{d}	4.69 ± 0.02^{ab}	4.52 ± 0.13^{a}	5.15 ± 0.15^{cd}	4.89 ± 0.07^{bc}	4.93 ± 0.22^{bc}	5.11 ± 0.04^{cd}
Enterobacteriaceae	5.58 ± 0.30^{a}	6.72 ± 0.08^{b}	6.45 ± 0.15^{b}	7.96 ± 0.03^{c}	6.65 ± 0.27^{b}	6.61 ± 0.70^{b}	6.78 ± 0.15^{b}
Staphylococcus	< LoD	< LoD	< LoD	< LoD	< LoD	< LoD	< LoD
Lactic bacteria	< LoD	< LoD	< LoD	< LoD	< LoD	< LoD	< LoD

Results are expressed as the mean ± SD (standard deviation). LoD (Limit of detection). CFU (Colony-forming unit). Different lowercase letters in the same row indicate significant statistical differences ($p < 0.05$) between different days.

1.1. Inoculation test

Following the analyses of all microbiological results, as well as all physicochemical results presented in the next sections, the HPP treatment at 450 MPa/5 min was selected for inoculation tests because it was enough to assure the microbiological safety and the retention of the physicochemical properties of the acorn beverage.

After the inoculation and subsequent treatment by HPP, the inoculated bacteria (*B. cereus*, *E. coli,* and *P. aeruginosa*) were below the detection limit. However, all bacteria were quantified in control being achieved 5.8, 8.1, and 7.1 log reductions for *B. cereus*, *E. coli* and *P. aeruginosa,* respectively, after the pressurization. Although the HPP treatment was an effective method for reducing these pathogenic bacteria, the metabolic repair from injury of these bacterial cells during storage should be considered, since some researchers have demonstrated that this can occur [194–196].

In the case of *L. monocytogenes,* no conclusions could be drawn, as no counts were observed in the untreated sample.

Table 10: Inoculation test. Bacterial counts on untreated and HPP-treated (450 MPa/5 min) acorn beverage.

	log_{10}CFU/mL	
Inoculated bacteria	**Untreated**	**HPP-treated (450 MPa, 5 min)**
***Listeria monocytogenes* 13,562 ***	< LoD	< LoD
***Bacillus cereus* ATCC 2599 ***	5.8	< LoD
Escherichia coli* ATCC 25922 *	8.1	< LoD
Pseudomonas aeruginosa* ATCC 10145 *	7.1	< LoD

* Gram-positive bacteria. ** Gram-negative bacteria. LoD (Limit of detection).

The results obtained are in agreement with the findings of Bozoglu *et al.* (2004) [195]. In this study, after milk treatment at 450 MPa/10 min, *L. monocytogenes* CA and *E. coli* O157:H7 were inactivated or injured. In another study, Liepa *et al*. (2018) [194] reported that the milk treatment at 550 MPa/15 min proved to be effective for inactivation of *L. monocytogenes* ATCC 7644 and *E. coli* ATCC 25922. In its turn, Yang *et al.* (2012) [197] showed that a good condition for vegetative pathogens inactivation in milk by using HHP was under 300 MPa/30 min at 25 ºC. Since the pH of milk (6.65) [195] is slightly higher than the pH of acorn beverage (5.09, data shown in section 2.2.), this beverage may require less processing to achieve the same log reduction as milk, which can explain the total absence of

colonies in selective medium, only with processing at 450 MPa/5 min performed in the present study. Unfortunately, the HPP treatment used as a conventional approach has been ineffective against bacterial spores, particularly *B. cereus* spores [198]. Possibly, the absence of counts of this microorganism after the HPP treatment is due to the exclusive inactivation of the vegetative form, once HPP inactivates vegetative *B. cereus*, rather than the endospores. To inactivation of *B. cereus* spores, Demazeau *et al.* (2018) [198] reported that a program with compression and decompression cycles applied to human milk with microbial contamination was well succeeded. Regarding *P. aeruginosa*, Shigehisa *et al.* (1991) [199] reported the inactivation of this microorganism inoculated into pork slumes at pressures higher than 300 MPa/10 min at 25 ºC.

Further research is needed to establish better the minimum processing conditions (pressure and time at room temperature) that results in a reliable inactivation and un-recovery of pathogens in acorn beverage. Besides that, it is imperative that shelf life studies are conducted over a period for potential repair of injury, to ascertain the microbiological safety of low acid food products, like this beverage. The description of high pressure effects on inactivation of microorganisms was reviewed in Chapter I, section 3.3.1.

2. Physicochemical analyses

2.1. Hydrolysable tannins content

Tannins are phenolic compounds that have the ability to combine with proteins and other polymers such as polysaccharides, causing the sensation of astringency, which is no more than the loss of the lubricating effect of saliva [200]. Furthermore, excessive tannin consumption can be associated with digestibility problems and reduce the nutritional value of foods. An example is that tannins chelate with minerals in digestive tract, which means they bind to the essential minerals and reduce their absorption [201]. To reduce these effects, an acorn leaching step was performed and the process efficiency was evaluated by calculating the rate of leached tannins. *Quercus ilex* acorns are nuts especially rich in hydrolysable and condensate tannins, but the vast majority correspond to hydrolysable tannins, that are compounds containing a central core of glucose or another polyol esterified with gallic acid [153,202]. Thus, the concentration of hydrolysable tannins in the acorn beverage and the leaching water was 0.64 ± 0.04 and 0.47 ± 0.04 mg of tannic acid equivalents per mL, respectively. From these values, the rate of tannin loss by leaching was calculated, being 26.9 %. Although the astringency of the acorn beverage has not been evaluated, almost 27 %

of the tannins have been eliminated, which will certainly have an impact on a sensory analysis. Furthermore, *Q. ilex* acorns are known to be sweeter than other acorn varieties [137]. On the other hand, there is also no interest in completely eliminating the tannins present in the acorn beverage because they also have health benefits. The hydrolysable tannins are associated with antimutagenic, anticarcinogenic, and antimicrobial properties [201].

2.2. pH and total soluble solids content

The initial pH (day 0) of acorn beverage was 5.09 ± 0.07, 5.89 ± 0.01, and 6.20 ± 0.04 for untreated, heat-treated, and HPP-treated samples, respectively. The acorn beverage processing increased significantly the pH values ($p < 0.05$), with the HPP-treated samples showing significantly higher values than the heat-treated samples (Figure 8). This trend continued over the storage time, although the differences between the heat and the HPP-treated samples were not significant ($p > 0.05$) from day 35 of storage until the end of storage. The pH of treated samples decreases significantly ($p < 0.05$) with the storage time.

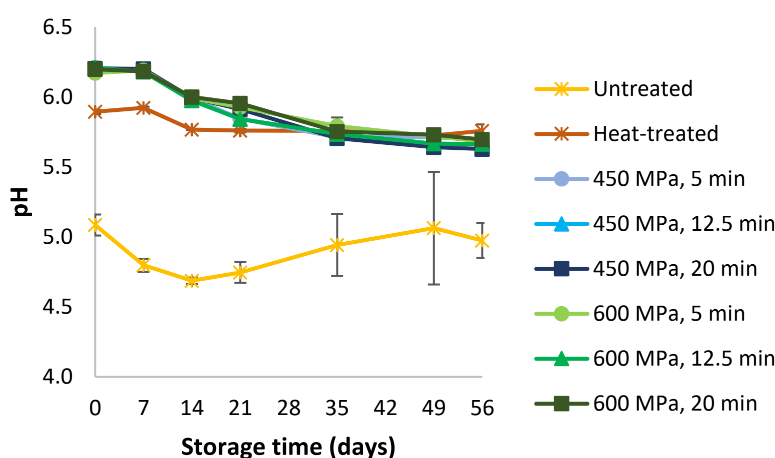


Figure 7: Evolution of pH during 56 days of storage at 4 °C on untreated, heat-treated, and HPP-treated (450 and 600 MPa for 5, 12.5, and 20 min) acorn beverage samples.

The pH is one of the main factors affecting the shelf-life of foods [203]. All microorganisms have a pH range in which they can grow and an optimum pH at which they grow best. The pH of a food, if not optimal for a particular species, can thus not only enhance inactivation during treatment but also inhibit outgrowth of sublethally injured cells [93]. The lower the pH is, the higher the shelf-life stability will be [203]. The compression of foods during HPP treatment may change the pH of the food as a function of applied pressure, and the direction of pH changes and its magnitude must be determined for each food treatment process [93]. In the present study, the HPP treatment caused the pH increases. Similar to this finding,

previous studies treated meat with HPP and reported an increase in ultimate pH values [203–205]. However, these results are not in agreement with other studies on the effect of HPP on different fruits and/or vegetable based beverages, that show that pH is generally unaffected by the treatment [124,170,206–210].

The TSS content in acorn beverage was significantly higher ($p < 0.05$) for heat-treated samples (Figure 8) probably due the conversion of starch to soluble sugars [211]. However, no significant differences ($p > 0.05$) were obtained between untreated and HPP-treated samples meaning that HPP did not influence the TSS content. This is in agreement with a study reported by Jayachandran *et al.* (2015) [209] about the effect of HPP (500 MPa/20 min and 600 MPa/15 min) on a litchi based mixed fruit beverage, that showed that TSS was not affected by the treatment.

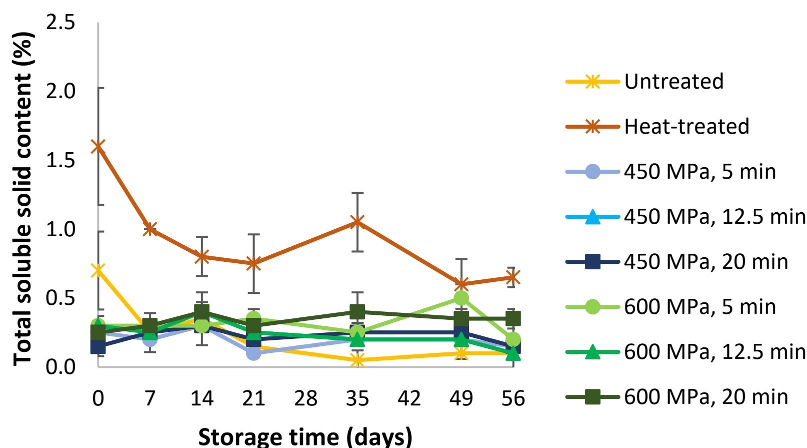


Figure 8: Evolution of TSS during 56 days of storage at 4 °C on untreated, heat-treated, and HPP-treated (450 and 600 MPa for 5, 12.5, and 20 min) acorn beverage samples.

In shelf-life studies on pasteurized fruit beverages, a decrease in TSS content during the storage time is one of the most important factors for grading the quality of the product. Microorganisms cause fruit beverage spoilage by fermentation of sugars and can therefore change the degree Brix [212]. In the present study, it was hard to see this happening over storage time due to low content of soluble sugars in the acorn, since the carbohydrates in the acorn are in starch form [137], but a significant decrease ($p < 0.05$) in the untreated sample TSS can be observed over the storage time.

2.3. Colour

The colour parameters are a key indicator of physicochemical changes and they are vital to assess the quality of the acorn beverage, given that the colour may be affected during a pasteurization process [185]. Colour is one of the first appreciations of the consumer, conditioning their preferences and influencing their choice. Besides that, this characteristic is also related to chemical composition and physical changes produced during processing [193]. Thus, a change in the shade of foods as a result of food processing can be perceived by the consumers as a low quality product, and consequently rejected [185].

The evolution of the colour parameters (L*, a*, b*, and ΔE*) is presented in Figure 9. Globally, the lightness (L*; Figure 9-A) of acorn beverage were not significantly affected by the treatment type ($p > 0.05$), but the L* values of some samples decreased significantly ($p < 0.05$) from first (day 0) to the last day of storage (day 56). Regarding the red-greenness parameter (a*; Figure 9-B), it was not observed significant changes ($p > 0.05$) due the treatments or during storage time, excepted at days 35 and 49, between untreated (higher values) and processed samples. The blue-yellowness parameter (b*; Figure 9-C) was the most affected parameter by the nature of the treatment, with heat-treated samples showing significantly lower values ($p < 0.05$) than all the other samples. a* and b* colour parameters in all acorn beverages had positive values, indicating that red and yellow were the primary contribution to the colour of that beverage.

Total colour difference (ΔE*), is a comprehensive indicator of colour change, where larger values indicate more severe colour changes. It has been considered that the casual viewer can notice a difference between two colours only when the ΔE* value is greater than 2.0 – 3.5. A trained eye is capable of differentiating two colours that differ by an ΔE* value equal or greater to 2.0 [213]. So, in relation to the ΔE* values (Figure 9-D), variations between heat and HPP-treated samples were not significant ($p > 0.05$), except on day 21, where the heat-treated samples showed higher values ($p < 0.05$) than the HPP-treated samples, indicating that heat conduced at higher colour changes. In general, perceptible colour differences (ΔE* > 2.0) between the untreated acorn beverage and the pasteurized one were detected and the highest value of ΔE* (8.1 ± 1.5) was obtained for the thermal treatment on day 7. Nevertheless, the ΔE* values for HPP-treated samples were lower than those for heat-treated samples until the day 21, which indicates less noticeable differences. Furthermore, the treated samples at 450 MPa had the lowest ΔE* values. Although some specific samples

show significant differences in some parameter over the storage time, in general the degradation of all samples between the start and the end of storage time was not visible.

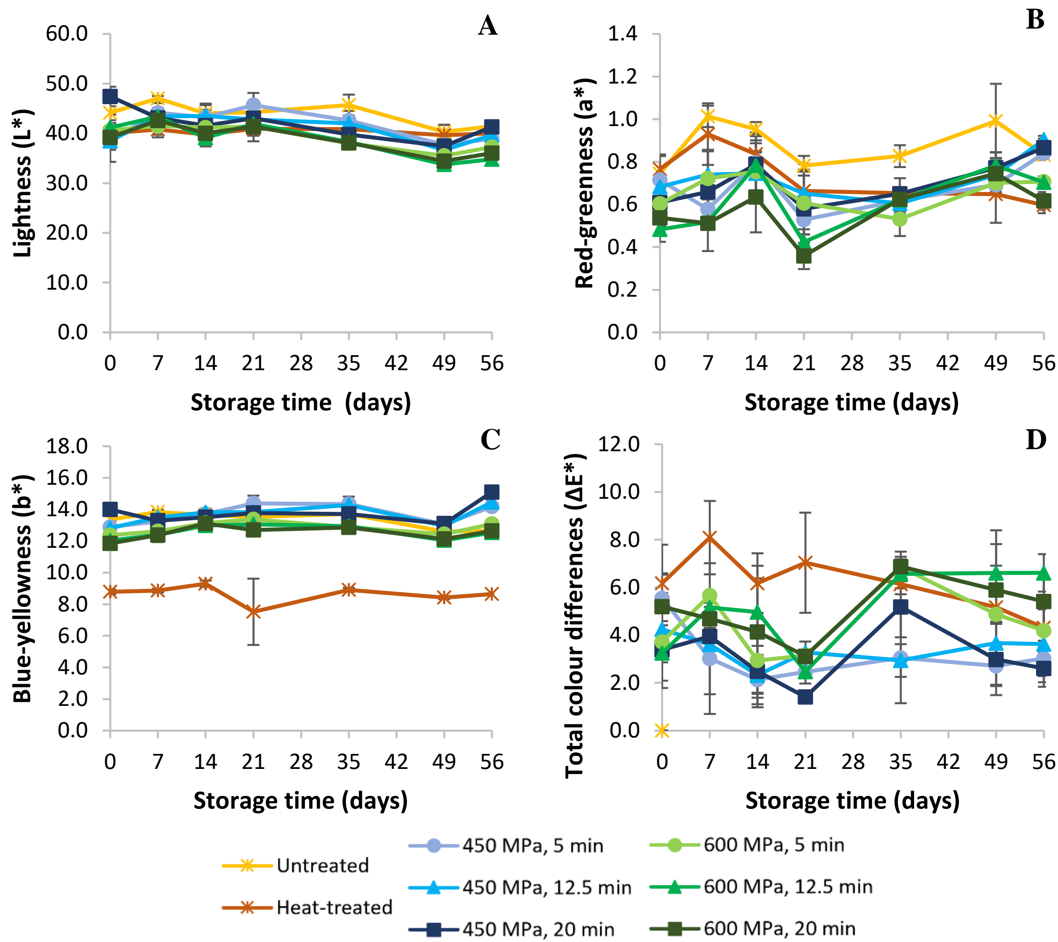


Figure 9: Evolution of L* (A), a* (B), b* (C), and ΔE (D) during 56 days of storage at 4 °C on untreated, heat-treated, and HPP-treated (450 and 600 MPa for 5, 12.5, and 20 min) acorn beverage samples.

The colour of HPP-treated products also depends on the inhibition of browning related enzymes, the stability of the pigments as affected by HPP treatment and the storage conditions [103]. In the literature, the colour change in food products after HPP and conventional thermal treatments have been frequently discussed. Xu *et al.* (2018) [129], in a study with Se-enriched kiwi fruit beverages, showed that there was a significant increase in ΔE* value and all values were greater than 2.0 during the 42 days of storage for the heat-treated samples. In its turn, HPP-treated samples showed no significant differences in ΔE* value until the late storage stage, indicating that HPP had no noticeable effects on the overall colour quality in comparison to the thermal treatment. Similar outputs were reported in cucumber, bayberry, carrot, and tomato beverages as reported by Liu *et al.* (2016) [214], Zhu *et al.* (2015) [215], and Dede *et al.* (2007) [216], respectively.

2.4. Determination of polyphenolic compounds

2.4.1. Total polyphenolic content

The results obtained (Figure 10) showed that, immediately after the HPP and thermal treatments no significant effects ($p > 0.05$) on the preservation of TPC were observed. However, the value for untreated samples is significantly higher ($p < 0.05$) than heat and HPP-treated samples from day 21 to the end of storage time (day 56), which demonstrates that any processing was efficient in TPC preservation. The highest TPC values were 0.0754 ± 0.0017, 0.0555 ± 0.0023, and 0.0629 ± 0.0045 mg of gallic acid equivalents per mL of the untreated, heat-treated, and HPP-treated acorn beverage samples, respectively. Throughout storage time (day 0, 21, and 56), TPC values of heat-treated samples remained constant ($p > 0.05$), while values for untreated and HPP-treated samples increased significantly from the beginning (day 0) of the storage time to the end (day 56). At the end of the storage time, the lowest TPC value was observed in heat-treated samples compared to HPP-treated, although the difference was not statistically significant ($p > 0.05$).

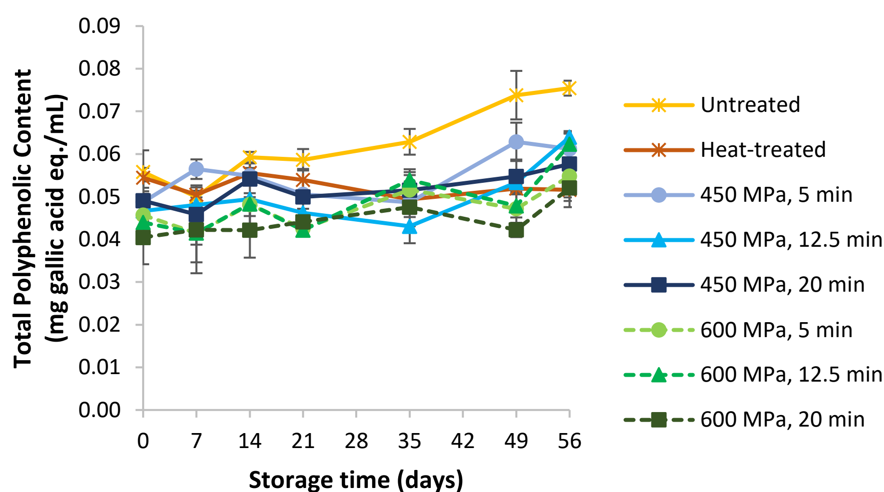


Figure 10: Evolution of TPC during 56 days of storage at 4 °C on untreated, heat-treated, and HPP-treated (450 and 600 MPa for 5, 12.5, and 20 min) acorn beverage samples.

In the literature, some researchers reported that HPP can increase the TPC values significantly ($p < 0.05$) [187,217–219], which can be related to an increased extractability of some antioxidant components, such as anthocyanins, amino acids and protein with a phenolic hydroxyl group [220]. In fact, Patras *et al.* (2009) [217] reported a slight but significant increase in TPC values of strawberry and blackberry purées after HPP (600 MPa/15 min/20 ºC), which they attributed to improved extraction due to tissue disruption by HPP. The opposite, a decrease in TPC values after HPP, is also reported by some authors [221,222], such as Cao *et al.* (2011) [221] for strawberry pulps pressurized at 400 MPa. The authors speculate to be due to

higher residual activity of PPO and POD, since these enzymes catalysed the oxidation of the phenols. In addition, it is still possible that the action of HPP, in certain food matrices and at certain pressures and times, may not significantly alter the TPC values, preserving the quality of a fresh food product. This can be concluded analysing the work of Patras *et al.* (2009) [217], in which the HPP at 500 MPa/15 min produced a non-significant increase on TPC values of strawberry purée.

Regarding the heat-treated samples, it has been reported that the TPC values can decrease more than the values obtained for HPP-treated samples. Ali *et al.* (2019) [223] reported that the TPC values of wheatgrass beverage were significantly decreased ($p < 0.05$) by 36.0 % and 7.5 %, after thermal (75 ºC/15 s) and HHP (500 MPa/60 s) treatments, respectively. Regarding the effect of storage time, some studies have shown that a longer storage time significantly promoted the degradation of phenolic compounds compared to fresh product [187,224]. Vieira *et al.* (2018) [225] reported no significant changes between the TPC of fresh and processed orange juices (550 MPa/70 s and 70 ºC/30 s) on the day of treatment. However, during storage, the authors observed that TPC of both processed orange juices decreased, being that behaviour more significant in heat-treated juices, for which a decrease of about 25 % ($p < 0.05$) was observed after 36 days.

2.4.2. Identification and quantification of individual polyphenolic compounds

Only two different phenolic compounds were identified and quantified in the acorn beverage samples, the gallic and the ellagic acid. In day 0, the gallic concentrations were 0.028 ± 0.004, 0.074 ± 0.014, and 0.064 ± 0.03 mg per mL for untreated, heat-treated and HPP-treated acorn beverage, respectively, and ellagic acid was detected only in trace amounts. Untreated samples had significantly lower gallic acid concentration than heat and HPP-treated samples. The gallic acid exists in different forms, one being the esterified to glucose form, as in hydrolysable tannins [202]. With the heat treatment, the hydrolysable tannins can hydrolyse to gallic acid, which is why heat-treated samples may have significantly higher concentrations than untreated samples [50]. For HPP-treated samples, the significantly higher concentrations of gallic acid can be attributed to improved extraction due to tissue disruption by HPP [217]. All the samples of the day 21 and 56 of storage showed some noise at baseline and the amounts of these compounds were taken as traces, which indicates a possible degradation of gallic and ellagic acid with storage time.

Cantos *et al.* (2003) [153] distinguished thirty-two different phenolic compounds in the methanolic extracts obtained from *Q. rotundifolia*, *Q. ilex,* and *Q. suber* acorns, all of them

gallic and ellagic acid derivatives. Some of these compounds are hydrolysable tannins as they can be hydrolysed to yield gallic acid and/or ellagic acid. The presence of these types of compounds provides health benefits, which are mainly correlated to their high antioxidant activity, besides having important functions in decreasing the risk of some diseases as referred in Chapter I, section 4.

2.5. Antioxidant activity (ABTS, DPPH, and ORAC assay)

The Figure 11 summarizes the ABTS and DPPH results obtained during storage for acorn beverage. For the ABTS assay (Figure 11-A), no significant differences ($p > 0.05$) were detected between treatments on day 0 and 56 of storage. However, on day 21 the antioxidant activity by this assay was significantly higher ($p < 0.05$) for untreated samples when compared with both processed samples. Differences over storage time (day 0 to day 56) were also not significant ($p > 0.05$).

When the DPPH method was used (Figure 11-B), the antioxidant activity was significantly higher ($p < 0.05$) for untreated samples on 21 and 56 days of storage, compared to the processed samples, and significantly higher ($p < 0.05$) for heat-treated samples on 0 days of storage. The differences over time for this method were inconclusive.

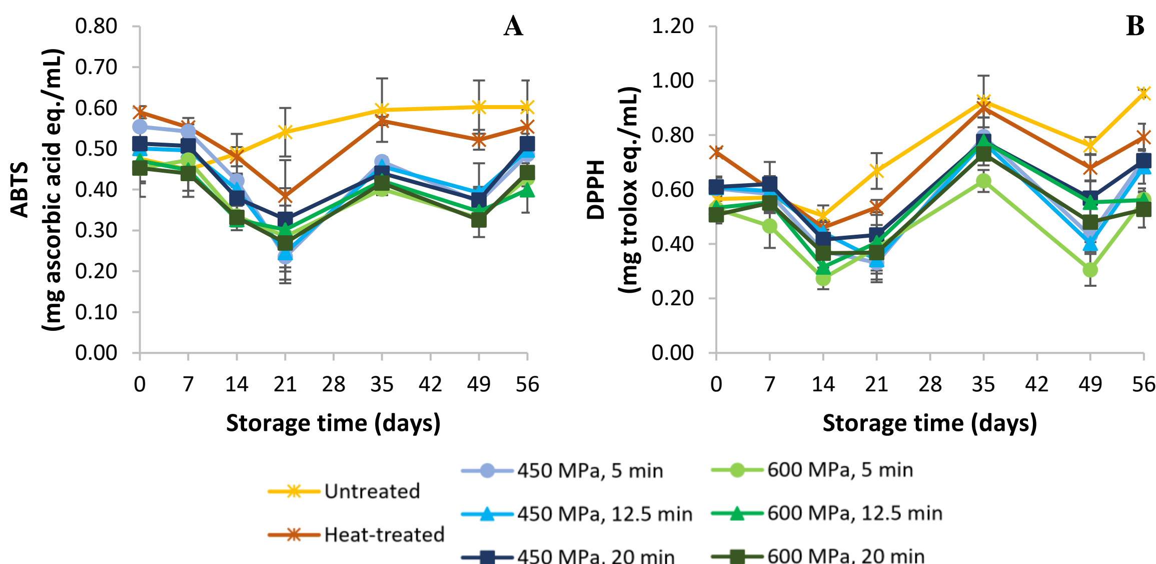


Figure 11: Evolution of ABTS (A) and DPPH (B) values during 56 days of storage at 4 °C on untreated, heat-treated, and HPP-treated (450 and 600 MPa for 5, 12.5, and 20 min) acorn beverage samples.

Among the treatments it can be observed that the antioxidant activity values of both tests, ABTS and DPPH, for heat-treated samples are generally higher than the values of HPP-treated samples, which in turn are similar between different pressurizations and processing times.

There are several studies in the literature reporting that the HPP treatment produces a better retention of antioxidant activity in fruits and juices than the thermal treatment [216,223,226]. Ali *et al.* (2019) [223] reported that antioxidant activity by DPPH assay of wheatgrass beverage were significantly decreased ($p < 0.05$) by 13.2 % and 6.6 %, after thermal (75 ºC/15 s) and HPP (500 MPa/60 s) treatments, respectively. However, in the case of acorn beverage, there is a possible explanation for the higher values obtained in heat-treated samples compared to HPP-treated ones. It is reported by Coelho *et al.* (2018) [50] that roasting acorn in an oven (225 - 230 ºC/15 min) promotes the partial thermal hydrolysis of existing hydrolysable tannins present and effectively increases the antioxidant capacity. Terán-Hilares *et al.* (2017) [227], on the other hand, reported the enhanced antioxidant properties of tara (*Caesalpinia spinosa*) gallotannins by thermal hydrolysis at 121 ºC/15 min. Thus, it can be predicted that the same occurs in the acorn beverage during thermal processing (85 ºC/30 min). Regarding the impact of storage time, Changa *et al.* (2019) [228] reported that the antioxidant capacity of pressurized white grape juice decreased throughout the storage time, but the extent of these differences was substantially lower than that in heat-treated juice, indicating that HPP treatment can better retain the quality of grape juice. However, in this study, with acorn beverage, no significant changes were observed between the beginning and the end of storage time.

Regarding ORAC method (Figure 12), heat-treated samples show a significantly ($p < 0.05$) higher retention of antioxidant activity, what can be explained by the partial thermal hydrolysis of hydrolysable tannins. However, the results reported in the literature demonstrate that HPP treatment produces better retention (as previously mentioned). Ali *et al.* (2019) [223] reported that the values of wheatgrass beverage significantly decreased ($p < 0.05$) after thermal treatment, whereas the values after HPP treatment did not decrease significantly ($p > 0.05$).

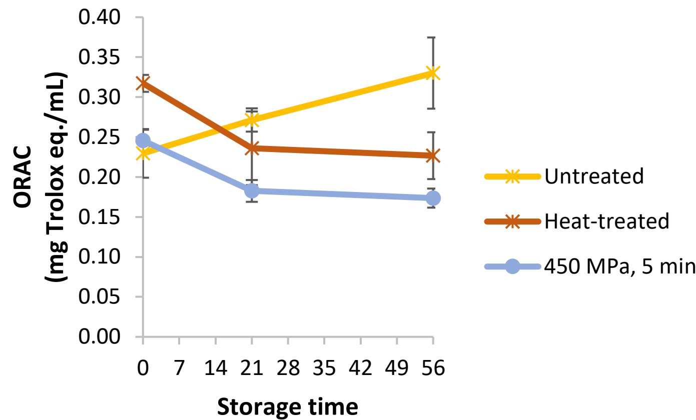


Figure 12: Evolution of ORAC values during 56 days of storage at 4 °C on untreated, heat-treated, and HPP-treated (450 MPa/5 min) acorn beverage samples.

Depending on their chemical structure, the phenolic compounds or their degradation products may have different activities in different antioxidant capacity assays. The used assays in the present study are based on two different chemical principles: ABTS and DPPH assays are based on an electron transfer reaction, whereas the ORAC assay represents a hydrogen atom transfer mechanism. This may explain the different results in terms of antioxidant capacity between assays [222].

2.6. Total carbohydrate, lipid, and protein content

The results presented demonstrate the stability of the acorn carbohydrates, proteins, and lipids, expressed in terms of total content after the thermal and HPP treatments, once no significant differences ($p > 0.05$) were observed between treatments.

The *Q. ilex* acorn flour (dry) was described by Silva *et al.* (2016) [150] as being rich in carbohydrates, particularly starch, which corresponds to approximately 57 %. Although the HPP has not shown impact on total carbohydrate content (Figure 13), the HPP can affect the functionality of carbohydrate molecules in often unique ways, which may allow the optimization of food manufacturing processes and the production of novel food [223].

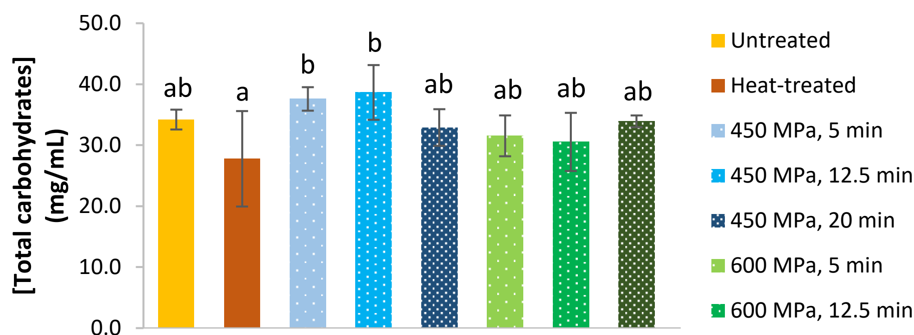


Figure 13: Total carbohydrate content on untreated, heat-treated, and HPP-treated (450 and 600 MPa for 5, 12.5, and 20 min) acorn beverage samples. Different lowercase letters on the bars indicate significant statistical differences ($p < 0.05$) between different treatments.

Regarding protein, Viñas *et al.* (2003) [144] reported that *Q. ilex* acorn presented a value of 5.06 %, so the acorn protein content values presented in acorn beverage (Figure 14) were within the expected range, taking into account the homogenization and the filtration process to produce an acorn beverage. However, the HPP can modify the secondary, tertiary, and quaternary structures of proteins and alter some of the epitope structures, which can reduce the allergenicity and improve the functional properties, allowing the optimization of food manufacturing processes and the production of novel food [223,229]. Proteins are stable within a certain pressure-temperature domain, but exceeding these limits disturbs the three-dimensional protein structure, causing unfolding and denaturation of the molecule and hence inactivation [230].

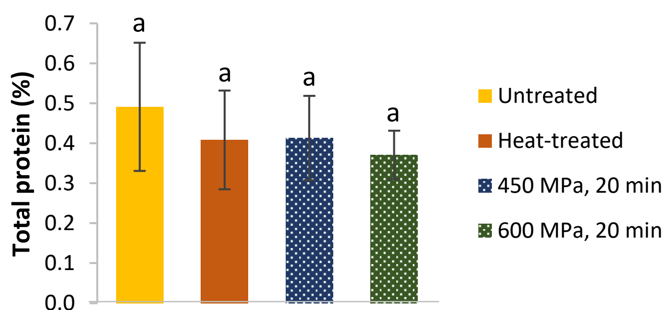


Figure 14: Total protein content on untreated, heat-treated, and HPP-treated (450 and 600 MPa for 5, 12.5, and 20 min) acorn beverage samples. Different lowercase letters on the bars indicate significant statistical differences ($p < 0.05$) between different treatments.

Concerning the lipid content, Viñas *et al.* (2003) [144] reported that *Q. ilex* acorn shown a value of 7.03 %, so the acorn lipid content values presented (Figure 15) in acorn beverage were within the expected range, taking into account the homogenization and the filtration process to produce an acorn beverage. The lipids are the most pressure-sensitive biological components, since lipid assemblies are governed by hydrophobic interactions. Several studies investigate the physics and chemistry of lipids under high pressure, and efforts have

been made to understand the effect of pressure on lipids' phase transformation behaviour, structure, physiology of biomolecules, lipid oxidation, and how the lipids interact with other food components (including proteins, carbohydrates) under high pressure [107].

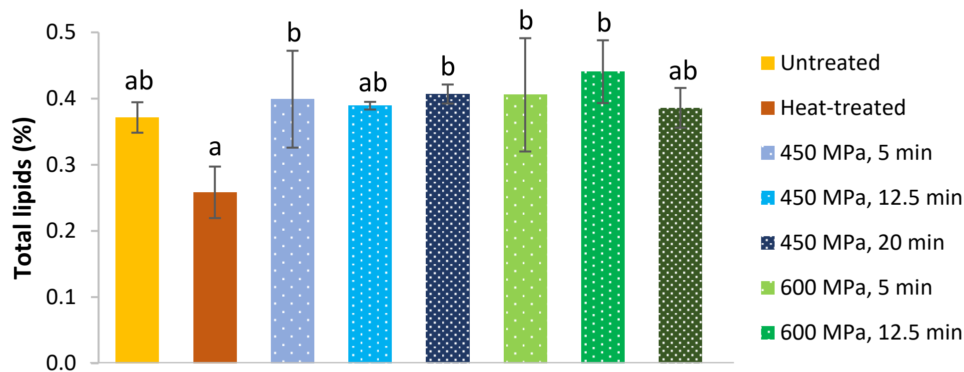


Figure 15: Total lipid content on untreated, heat-treated, and HPP-treated (450 and 600 MPa for 5, 12.5, and 20 min) acorn beverage samples. Different lowercase letters on the bars indicate significant statistical differences ($p < 0.05$) between different treatments.

2.7. Identification and quantification of total amino acid content

Table 11 shows the mean values for each amino acid present in acorn beverage (free amino acids and protein constituent amino acids). The acorn beverage has a great importance as a source of amino acids, since this beverage contains all the essential amino acids determined. Although tryptophan has not been determined in the present work due to extensive degradation during acid hydrolysis performed to analyse the other essential amino acids, an alkaline hydrolysis could be used specifically to quantify tryptophan [231]. However, this amino acid is present in acorn in very low concentrations (0.074 g/100g of acorn), as reported to *Quercus spp.* by United States Department of Agriculture (USDA) [232], being the probability to be detected in the beverage really very low.

The acorn amino acids composition obtained in this study, it is in agreement with the studies reported by Nieto *et al.* (2002) [147] and Özcan *et al.* (2006) [233]. The essential amino acid present at higher concentrations in this study was the histidine, however, in the study by Nieto *et al.* (2002) [147] with *Q. ilex*, leucine and lysine were the essential amino acids detected in higher concentration. Particular attention is paid to the lysine content, since this indispensable amino acid is more likely to be limiting in cereal-based diets [137]. It is not possible to determine the concentration of free amino acids in the acorn beverage due to the very low concentration present in this beverage and no conclusions were drawn about the effect of treatments on amino acid content. In the literature, some studies reported that the protein unfolding promoted by HPP enhances the protein hydrolysis, so the HPP treatment

may increase the concentration of free amino acids [234]. However, this was not observed in this study.

Table 11: Total amino acid composition of untreated, heat-treated, and HPP-treated (450 MPa/5 min) acorn beverage samples.

		Untreated		Heat-treated		HPP-treated (450 MPa, 5 min)	
	Storage time (days)	**0**	**56**	**0**	**56**	**0**	56
Amino acid (mg per g of beverage dry weight)	**Aspartic acid**	5.0	4.4	3.5	5.1	4.0	3.8
	Glutamic acid	6.2	5.5	< LoQ	5.3	4.7	4.1
	Cysteine	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ
	Asparagine	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ
	Serine	1.7	1.7	1.0	1.4	1.4	1.0
	Histidine*	5.9	5.1	5.1	5.2	5.1	5.1
	Glutamine	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ
	Glycine	0.7	0.7	0.7	0.7	0.7	0.7
	Threonine*	2.0	1.7	1.1	1.4	1.6	1.1
	Arginine	2.8	2.9	1.3	2.0	2.6	1.6
	Alanine	2.3	2.0	1.5	2.1	1.7	1.5
	Tyrosine	1.1	1.1	0.7	0.4	0.9	0.6
	Valine*	2.9	2.6	1.8	2.3	2.2	1.8
	Methionine*	0.1	< LoQ	0.1	0.1	< LoQ	< LoQ
	Tryptophan*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Phenylalanine*	1.8	1.7	1.7	1.3	1.4	1.0
	Isoleucine*	1.8	1.6	1.6	1.4	1.3	1.1
	Leucine*	3.7	3.2	3.2	2.8	2.6	2.1
	Lysine*	2.5	0.8	0.8	0.2	0.7	0.1

Values represent the mean of duplicate analyses of two individual samples. *Essential amino acids for humans [235]. n.d. (not determined).

2.8. Identification and quantification of total fatty acids

Twenty-eight FA were identified and quantified at the untreated and HPP-treated (450 MPa/5 min) samples at day 0, 21, and 56 of storage. The most abundant FA found in acorn beverage were oleic (~62 %) and linoleic (~16 %) acids, which together constituted of about 78% of the total FA (Table 12). In addition, a high amount of palmitic acid was also found (~14 %), whereas stearic acid was low (~3 %), however these four acids corresponds to approximately 95 % of the lipophilic fraction. The mean percentage of these main fatty acids in acorn beverage was very similar to that reported previously on healthy acorns. León-Camacho *et al.* (2004) [146] reported the values 62.88, 17.35, 13.57 and 2.33 % for oleic,

linoleic, palmitic, and stearic acids, respectively, in fresh *Q. ilex* acorns. In addition, more 24 molecules were also detected in acorn beverage, but in percentages below to 1 % or in traces, as can be seen in Appendix I.

Table 12: Changes in fatty acid (FA) group profile along storage time (day 0, 21, and 56), expressed in percentage (%) on untreated and HPP-treated (450 MPa/5 min) acorn beverage samples.

		Untreated			HPP-treated (450 MPa, 5 min)		
	Storage time (day)	**0**	**21**	**56**	**0**	**21**	**56**
Fatty acids (% in total FA)	**Palmitic (C16)**	14.23	14.33	14.23	14.06	14.23	14.13
	Stearic (C18)	3.25	3.27	3.26	3.23	3.28	3.25
	Oleic (C18:1 c9)	62.30	62.20	62.51	62.43	62.51	62.63
	Linoleic (C18:2 c9c12)	16.35	16.16	16.17	16.30	16.27	16.30
	Gamma-linolenic, GLA (ɣ C18:3 c6c9c13)	0.04	0.04	0.04	0.03	0.03	0.03
	Alpha-linolenic, ALA (α C18:3 c9c12c15)	0.64	0.62	0.62	0.63	0.62	0.62
	SFA	18.61	18.77	18.62	18.42	18.62	18.48
	MUFA	63.52	63.41	63.63	63.61	63.64	63.75
	PUFA	17.71	17.56	17.52	17.79	17.61	17.64
	Trans FA	0.64	0.70	0.65	0.78	0.64	0.64
	ω3 PUFA	0.64	0.62	0.62	0.63	0.62	0.62
	ω6 PUFA	16.39	16.20	16.21	16.33	16.31	16.34

Values represent the mean of duplicate analyses of two individual samples. SFA (Saturated fatty acid). MUFA (Monounsaturated fatty acids). PUFA (Polyunsaturated fatty acids). ω3 (omega−3). ω6 (omega−6).

Although the lipid fraction in an acorn beverage is very low as it is diluted (1:21), this fraction has been characterized in order to determine its potential and the impact of HPP treatment. The oleic acid (C18:1 c9) is a MUFA with a double bond at the n-9 position. Like other FA, MUFA are almost completely absorbed from the intestine and are oxidised (for energy production), converted into other fatty acids, or incorporated into tissue lipids. The linoleic acid (C18:2 c9c12), an ω6, cannot be synthesised by the body and is therefore an essential FA. The intake of linoleic acid is beneficial to regulate the blood low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol concentrations [236]. Alpha-linolenic acid (ALA) belongs to the family of ω3 PUFA that have important functions in the body, such as prevention of cardiovascular diseases. This FA is commonly present in western diets in small quantities and new potential dietary sources are of interest for the functional foods industry [237]. The gamma-linolenic acid (GLA), an ω6 detected in trace amounts, has potential applications as an anti-inflammatory nutrient or adjuvant [238]. The ω3 and ω6 PUFA play a significant role in health and disease by generating potent modulatory molecules for

inflammatory responses and affecting the gene expression of various bioactive molecules [238]. SFA and *trans* FA have been implicated in obesity, heart disease, diabetes, and cancer, while MUFA and PUFA generally have a positive effect on health [236]. In general, the lipid fraction of the acorn beverage shows elevated values of MUFA (63.52 %) and PUFA (17.71 %), which have been reported to reduce cholesterol levels.

The nutritional quality indices are presented in Table 13. According to Ulbricht and Southgate (1991) [181], AI and TI are measurements of the influence of diet on coronary heart disease (CHD). AI relates the risk of atherosclerosis and it is based on FA, which can increase (C12, C14, and C16) or decrease (ΣMUFA, ΣPUFA) the level of blood cholesterol. C12, C14, and C10 are considered pro-atherogenic and MUFAs and PUFAs, antiatherogenic. TI values relate to the tendency to form clots in the blood vessels, defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic FA (MUFAs, ω6 PUFAs, and ω3 PUFAs). The authors recommend the consumption of food products with very low values for AI and TI, indicating positive health benefits [181]. The lipid fraction of the untreated acorn beverage has shown low AI and TI values, 0.31 and 0.46, respectively. Comparatively to other foods, such as coconut oil, which was reported to be a highly atherogenic food with an AI value of 13.63 [239]. The PUFA/SFA ratio is another good indicator of the nutritional value of dietary fat. According to nutritional recommendations [240], acorn beverage would be considered a healthy food because the PUFA/SFA ratio (0.95) was above 0.45. However, excessive PUFA intake also has undesirable effects, therefore the intake of PUFA and SFA must be balanced [150]. The obtained MUFA/PUFA ratio was 3.59. It is relevant because a diet with a high MUFA/PUFA ratio provides better protection against heart diseases that a diet only rich in PUFA [241]. Also, it is important to balance ω3 and ω6 PUFA. A low ω6/ω3 ratio decreases the risk of cardiovascular diseases and some types of cancer when it is around 2 [242]. This parameter is very high in acorn beverage and other acorn products [150]. Nevertheless, the lipid fraction of this beverage is very low, so this index should not denote a risk.

All the fatty acids' percentages and the nutritional quality indices in the present work were similar between the untreated and HPP-treated acorn beverage samples, which is in agreement with the other studies with HPP application. In a study with human milk, Moltó-Puigmartí *et al.* (2011) [243] reported no statistically significant differences between fatty acid proportions in pressurised (400, 500, and 600 MPa for 5 min) and untreated samples. In other study, Rodríguez-Alcalá (2014) [244] reported the total concentration of SFA, MUFA, and PUFA remained stable when cow milk were processed by HPP (400 MPa/15 min at 25 °C).

As far as the authors know, this is the first time that the AI and TI indices have been calculated for HPP treated acorn.

Table 13: Lipid quality indices of untreated and HPP-treated (450 MPa/5 min) acorn beverage samples along storage time (day 0, 21, and 56).

		Untreated			HPP-treated (450 MPa, 5 min)		
Storage time (day)		0	21	56	0	21	56
Quality parameter	AI	0.31	0.34	0.33	0.35	0.26	0.28
	TI	0.46	0.54	0.52	0.50	0.39	0.41
	ω6/ω3	25.76	26.20	26.21	25.85	26.17	26.19
	MUFA/PUFA	3.59	3.61	3.63	3.58	3.61	3.61
	PUFA/SFA	0.95	0.94	0.94	0.97	0.95	0.95
	(MUFA+PUFA)/ SFA	4.37	4.31	4.36	4.42	4.36	4.40

AI (Index of atherogenicity). TI (Index of thrombogenicity). ω6 (omega-6). ω3 (omega-3). MUFA (Monounsaturated fatty acids). PUFA (Polyunsaturated fatty acids). SFA (Saturated fatty acids)

2.9. Identification and quantification of individual minerals

Figure 16 shows mean values for each mineral present in acorn beverage. The main mineral quantified was potassium (5 ppm), as referred in the literature by Sekeroglu *et al.* (2017) [245] but for raw acorn. The rest of the minerals, potassium, phosphorus, magnesium, manganese, iron, copper, molybdenum, and zinc were found in minor amounts (0 to 0.8 ppm). Heavy metal concentrations, lead and nickel, were found in trace amounts, much lower than the acceptable limits for herbs and spices by WHO [246]. Thus, this study showed that acorn beverage has useful minerals and lower composition by hazardous heavy metals. Regarding processing, there were no significant differences between the mineral content of the untreated samples and the heat and HPP-treated samples. HPP exerted different effects on the mineral bioaccessibility, depending on the mineral species.

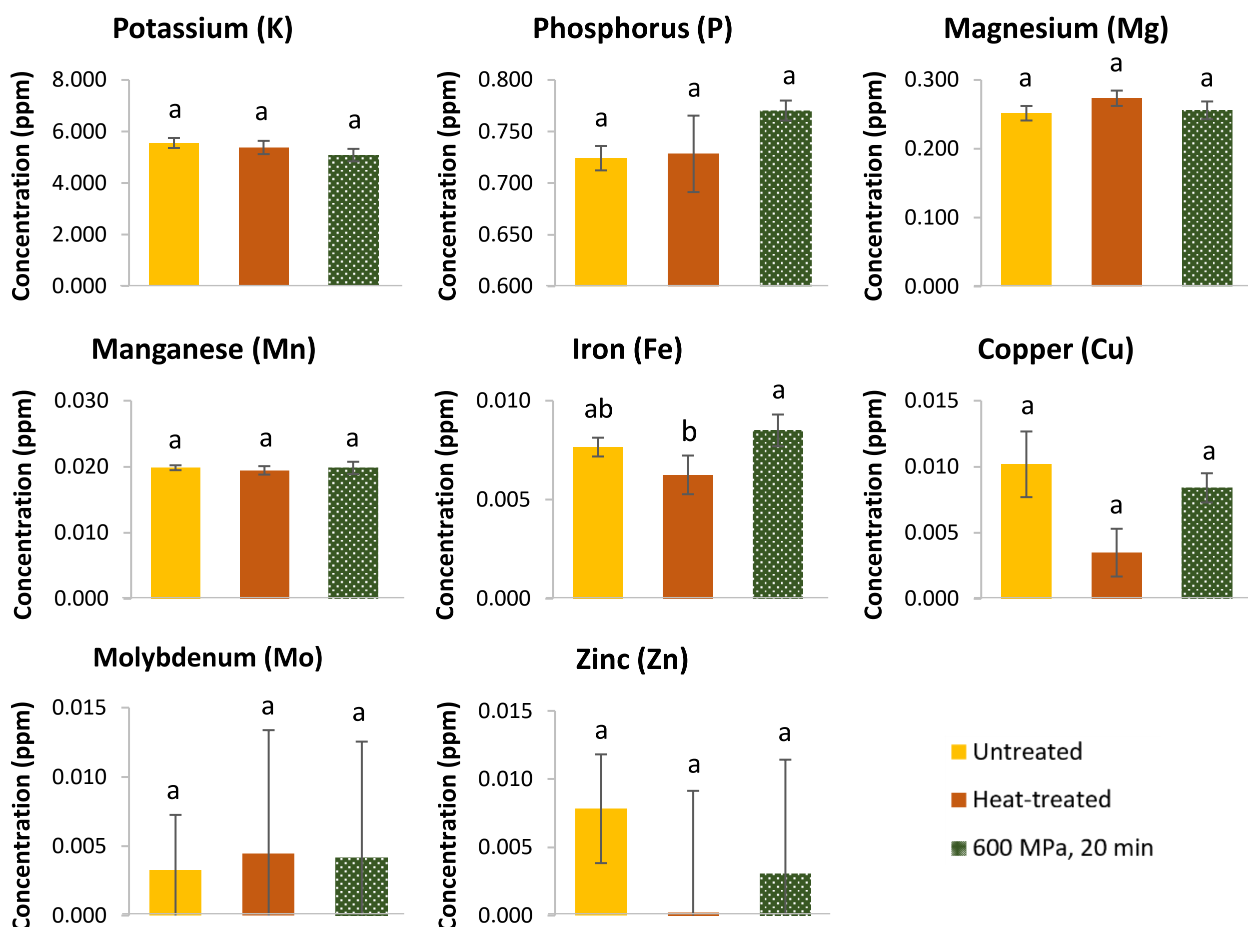


Figure 16: Mineral content on untreated, heat-treated, and HPP-treated (600 MPa for 20 min) acorn beverage samples. Different lowercase letters on the bars indicate significant statistical differences ($p < 0.05$) between different treatments.

2.10. Quantification of 5-hydroxymethylfurfural

The 5-hydroxymethylfurfural (5-HMF) was not quantified (LoQ < 0.5 mg/L) in any of the samples analysed, not even in the thermally processed samples, indicating that the pasteurization and pressurization conditions were not too severe to produce this compound and browning did not occur, at least due to the formation of this compound. In a study with cashew apple juice, Damasceno *et al.* (2008) showed that 100 ºC more than 100 min was needed to detect formation of HMF [247]. Another possibility for the absence of 5-HMF in the acorn beverage is its low content in low-molecular-weight sugar, such as fructose or glucose, since most carbohydrates in this beverage are in starch form and the basic substrate for HMF formation are monosaccharides [248].

It is necessary to include the analysis of HMF in the nutritional assessment of some foods and beverages, since recent findings have established that the compound is metabolized by humans being to 5-sulfoxymethylfurfural (5-SMF), a derivate with demonstrated nephrotoxicity and mutagenic activity [249,250]. HMF is a breakdown product formed during the Maillard reaction from Amadori compounds, but it is also formed from sugars when they are heated at high temperatures in slightly acidic media [251]. The initial pH of the acorn beverage was between 5.0 and 6.5, so this parameter makes the beverage susceptible to 5-HMF formation.

Due to incomplete data regarding the content of 5-HMF in foods, it is not possible to evaluate the consumption of this compound with the diet. Only a few articles about the presence of 5-HMF in soft beverages have been published [252], and due to high consumption of this group of products, beverages may have an important share in the daily intake of 5-HMF.

3. Sensory analysis

Sixty non-experienced participants evaluated two different acorn beverages (Figure 17), the heat-treated and HPP-treated ones (450 MPa/5 min). In this test, untreated acorn beverage was not analysed. The samples were similar ($p > 0.05$) in overall appreciation and flavour, but the participants had preference ($p < 0.05$) for heat-treated samples in terms of mouthfell and HPP-treated samples in terms of appearance. At the end of the test, the panel preferred the HPP-treated sample. Nonetheless, the improvement of the acorn beverage recipe for better consumer acceptance is necessary.

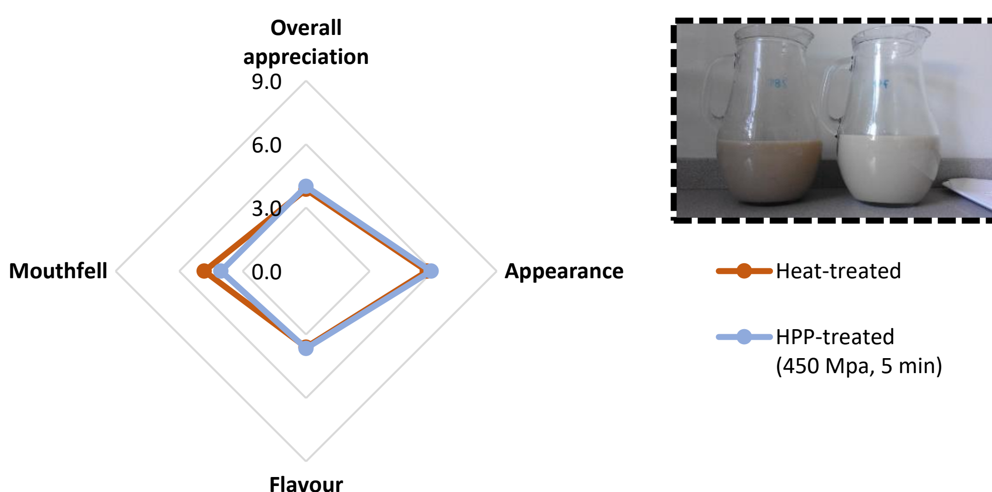
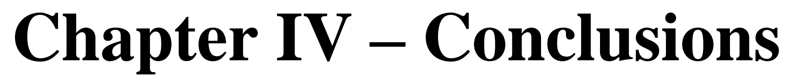


Figure 17: Acorn beverage sensorial analysis by a non-trained panel of 42 participants. Heat-treated (left) and HPP-treated at 450 MPa/5 min (right).



Chapter IV – Conclusions

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

The main objectives of this research work were valorize the *Quercus* acorn as a food product for human consumption through the development of a functional beverage, and evaluate the effect of HPP on the quality and shelf life extension of this beverage during storage.

In general, the acorn beverage showed indications of being a good source of amino acids, fatty acids, and minerals, as well as present very low values of AI and TI compared to other similar food products. In its turn, the HPP applied treatment preserved the colour and nutritional attributes of the beverage. Although heat-treated samples have mostly superior antioxidant activity compared to HPP-treated samples, this can be due to the partial thermal hydrolysis of hydrolysable tannins present in acorn, as postulated by some authors, which effectively increases the antioxidant capacity. Regarding microbiological safety, the HPP tested treatment (450 MPa/5 min) showed to be enough to inactivate several microorganisms (*Enterobacteriaceae*, mesophiles, psychrophiles, molds and yeasts, *Staphylococcus*, and lactic bacteria) over ten weeks of storage. Furthermore, the inactivation of *Bacillus cereus*, *Escherichia coli,* and *Pseudomonas aeruginosa* after the selected HPP treatment was also observed. In the sensory analysis, a slight but significant ($p < 0.05$) preference for the HPP-treated acorn beverage was observed. The acorn leaching step was important to reduce the tannins responsible for astringency.

Briefly, this study showed that HPP is a potential non-thermal technology to be applied on acorn functional beverage, not only to increase its shelf life, but also to improve their quality after treatment. The developed acorn beverage, being free of lactose or gluten, can be a good alternative as a source of bioactive compounds even for lactose intolerant or gluten intolerant consumers.

To the best of the author's knowledge, this was the first study about an acorn functional beverage and about the application of HPP to an acorn product.

This section proposes the essential future work regarding the application of HPP on pasteurization of an acorn functional beverage.

Considering both, the literature revision and the experimental work in this thesis, there are still many areas that need further exploitation. Therefore, it would be relevant to study:

- Improvement of the acorn beverage recipe for better consumer acceptance and enrich the beverage with calcium and/or added bioactive compounds.
- A better development of the processing conditions (pressure and time at room temperature) that result in a reliable inactivation and un-recovery of pathogens in acorn beverage. Lower pressures and less processing time should be looked in to, as well as shelf life studies with pathogens conducted over a period of time for potential repair of injury, to ascertain the microbiological safety.
- The toxicological aspects or even the exact functional value of acorn beverage.
- The direct effect on gut microbiota through human faeces inoculum. The impact of the digestive process upon the beverage should also be evaluated, as reductions in the total viable counts are somewhat expected during a regular digestive process and this could change the impact upon gut microbiota.
- Develop a beverage without residues. It could be interesting to determine their potential usage in the development of functional animal feed or other starchy products for humans.
- Characterization of the chemical composition of the studied acorns should be of interest, as to better understand their fermentability and to better correlate with prebiotic effects.

References

(1) World Health Organization (WHO). Healthy Diet. https://www.who.int/nutrition/publications/nutrientrequirements/healthydiet_factsheet/en/ (accessed Oct 29, 2018).

(2) World Health Organization (WHO). Obesity and overweight. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (accessed Nov 1, 2018).

(3) Akram, D. S.; Astrup, A. V.; Atinmo, T.; Boissin, J. L.; Bray, G. A.; Carroll, K. K.; Chitson, P.; Chunming, C.; Dietz, W. H.; Hill, J. O.; et al. *Obesity: Preventing and Managing the Global Epidemic*; 2000.

(4) Küster-Boluda, I.; Vidal-Capilla, I. Consumer Attitudes in the Election of Functional Foods. *Spanish Journal of Marketing - ESIC* **2017**, *21*, 65–79.

(5) London, C. Functional Foods That Boost the Immune System. In *Functional Food Product Development*; 2010; pp 293–321.

(6) Corbo, M. R.; Bevilacqua, A.; Petruzzi, L.; Casanova, F. P.; Sinigaglia, M. Functional Beverages: The Emerging Side of Functional Foods: Commercial Trends, Research, and Health Implications. *Comprehensive Reviews in Food Science and Food Safety* **2014**, *13* (6), 1192–1206.

(7) Hasler, C. M. Functional Foods: Benefits, Concerns and Challenges—A Position Paper from the American Council on Science and Health. *The Journal of Nutrition* **2002**, *132* (12), 3772–3781.

(8) European Commission. NUTRAHEALTH - A boost for functional foods research https://cordis.europa.eu/project/rcn/105533/brief/en (accessed Nov 2, 2018).

(9) Agriculture and Agri-Food Canada (AAFC). *Market Analysis Report: Consumer Trends - Functional Foods*; 2009.

(10) Grand View Research. Functional Foods Market Analysis By Product (Carotenoids, Dietary Fibers, Fatty Acids, Minerals, Prebiotics & Probiotics, Vitamins), Application, End-Use (Sports Nutrition, Weight Management, Immunity, Digestive Health) And Segment Forecasts, 2018 To 2024 https://www.grandviewresearch.com/industry-analysis/functional-food-market.

(11) Sun-Waterhouse, D. The Development of Fruit-Based Functional Foods Targeting the Health and Wellness Market: A Review. *International Journal of Food Science and Technology* **2011**, *46* (5), 899–920.

(12) Ozen, A. E.; Pons, A.; Tur, J. A. Worldwide Consumption of Functional Foods: A Systematic Review. *Nutrition Reviews* **2012**, *70* (8), 472–481.

(13) Lau, T. C.; Chan, M. W.; Tan, H. P.; Kwek, C. L. Functional Food: A Growing Trend among the Health Conscious. *Asian Social Science* **2012**, *9* (1), 198–208.

(14) Doyon, M.; Labrecque, J. A. Functional Foods: A Conceptual Definition. *British Food Journal* **2008**, *110* (11), 1133–1149.

(15) Martirosyan, D. M.; Singh, J. A New Definition of Functional Food by FFC: What Makes a New Definition Unique? *Functional Foods in Health and Disease* **2015**, *5* (6), 209–223.

(16) Kotilainen, L.; Rajalahti, R.; Ragasa, C.; Pehu, E. Health Enhancing Foods - Opportunities for Strengthening the Sector in Developing Countries. *Agriculture and Rural Development Discussion* **2006**, *30*, 1–82.

(17) European Commission Concerted Action on Functional Food Science in Europe (FUFOSE). Scientific Concepts of Functional Foods in Europe Consensus Document. *British Journal of Nutrition* **1999**, *81*, S1–S27.

(18) Campos, F.; Angela, M.; Adorno, T.; Kimiko, I.; Antoniassi, R.; Carolina, A.; Dória, S.; Maria, K.; Sivieri, K. Impact of Multi-Functional Fermented Goat Milk Beverage on Gut Microbiota in a Dynamic Colon Model. *Food Research International* **2017**, *99* (May), 315–327.

(19) Barclay, D. Multiple Fortification of Beverages. *Food and Nutrition Bulletin* **1998**, *19* (2), 168–171.

(20) Özen, A. E.; Bibiloni, M. del M.; Pons, A.; Tur, J. A. Consumption of Functional Foods in Europe; a Systematic Review. *Nutricion Hospitalaria* **2014**, *29* (3), 470–478.

(21) Williams, M.; Pehu, E.; Ragasa, C. Functional Foods: Opportunities and Challenges for Developing Countries. *Agriculture and Rural Development* **2006**, No. 19 Set, 1–4.

(22) Zambrano, A.; Raybaudi-massilia, R.; Arvelo, F.; Sojo, F. Cytotoxic and Antioxidant Properties in Vitro of Functional Beverages Based on Blackberry (Rubus Glaucus B.) and Soursop (Annona Muricata L.) Pulps. *Functional Foods in Health and Disease* **2018**, *8* (11), 520–536.

(23) Domínguez Avila, J. A.; Wall Medrano, A.; Ruiz Pardo, C. A.; Montalvo González, E.; González Aguilar, G. A. Use of Nonthermal Technologies in the Production of Functional Beverages from Vegetable Ingredients to Preserve Heat-Labile Phytochemicals. *Journal of Food Processing and Preservation* **2018**, *42* (2), 1–12.

(24) Granato, D.; Branco, G. F.; Nazzaro, F.; Cruz, A. G.; Faria, J. A. F. Functional Foods and Nondairy Probiotic Food Development: Trends, Concepts, and Products. *Comprehensive Reviews in Food Science and Food Safety* **2010**, *9* (3), 292–302.

(25) Sloan, A. E. Trends in functional foods - USA 2018 http://www.ift.org/food-technology/past-issues/2018/april/features/top-10-functional-food-trends-2018.aspx (accessed Dec 4, 2018).

(26) Harvey, A. Technomic's Take: 7 Key Trends for 2019 https://www.technomic.com/newsletters/technomics-take/7-key-trends-2019 (accessed Dec 4, 2018).

(27) Technavio Research. Top 8 Vendors in the Functional Food and Beverage Market in the US From 2017 to 2021: Technavio https://www.businesswire.com/news/home/20170828005480/en/Top-8-Vendors-Functional-Food-Beverage-Market (accessed Dec 4, 2018).

(28) Global Functional Foods and Beverages Market 2017-2021 - Increase in the Use of Natural Ingredients and Botanicals in Functional Drinks - Research and Markets https://www.businesswire.com/news/home/20170110005663/en/Global-Functional-Foods-Beverages-Market-2017-2021 (accessed Dec 4, 2018).

(29) Helkar, P. B.; Sahoo, A. K.; Patil, N. J. Review: Food Industry By-Products Used as a Functional Food Ingredients. *International Journal of Waste Resources* **2016**, *6* (3), 1–6.

(30) Niba, L. L. The Relevance of Biotechnology in the Development of Functional Foods for Improved Nutritional and Health Quality in Developing Countries. *African Journal of Biotechnology* **2003**, *2* (12), 631–635.

(31) Rodríguez-Roque, M. J.; Rojas-Graü, M. A.; Elez-Martínez, P.; Martín-Belloso, O. In Vitro Bioaccessibility of Health-Related Compounds as Affected by the Formulation of Fruit Juice- and Milk-Based Beverages. *Food Research International* **2014**, *62*, 771–778.

(32) Salanta, L.; Tofana, M. Development of Functional Beverage from Wheat Grass Juice. *Food Science and Technology* **2016**, *73* (2), 155–156.

(33) Safithri, M.; Kurniawati, A.; Syaefudin. Formula of Piper Crocatum, Cinnamomum Burmanii, and Zingiber Officinale Extracts Extracts as a Functional Beverage for Diabetics. *International Food Research Journal* **2016**, *23* (3), 1123–1130.

(34) Benedetti, S.; Schwinden, E.; Maria, C.; Müller, O.; Gontijo, M.; Santos, R. Utilization of Tofu Whey Concentrate by Nano Fi Ltration Process Aimed at Obtaining a Functional Fermented Lactic Beverage. *Journal of Food Engineering* **2016**, *171*, 222–229.

(35) Seyhan, E.; Yaman, H.; Özer, B. Production of a Whey-Based Functional Beverage Supplemented with Soy Isoflavones and Phytosterols. *Dairy Technology* **2016**, *68*, 1–8.

(36) Liutkevičius, A.; Speičienė, V.; Kaminskas, A.; Alenčikienė, G.; Mieželienė, A.; Bagdonaitė, L.; Vitkus, D.; Garmienė, G. Development of a Functional Whey Beverage, Containing Calcium, Vitamin D, and Prebiotic Dietary Fiber, and Its Influence on Human Health. *CyTA - Journal of Food* **2016**, *14* (2), 309–316.

(37) El-azeem, A. S. A.; Hegazy, A. M.; Badawy, I. H.; Ibrahim, G. A.; El-, K.; Sayed, H. S. E.-; Sharaf, O. M.; June, M.; June, M. Effectiveness of Functional Wheat - Fermented Milk Beverage against Tannic Acid Induced Anemia. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* **2016**, *7* (2622), 2622–2632.

(38) Bi, W.; Shen, J.; Gao, Y.; He, C.; Peng, Y.; Xiao, P. Ku-Jin Tea (Acer Tataricum Subsp. Ginnala or A. Tataricum Subsp . Theiferum), an Underestimated Functional Beverage Rich in Antioxidant Phenolics. *Journal of Functional Foods* **2016**, *24*, 75–84.

(39) Baba, W. N.; Din, S.; Punoo, H. A.; Wani, T. A. Comparison of Cheese and Paneer Whey for Production of a Functional Pineapple Beverage: Nutraceutical Properties and Shelf Life. *Journal of Food Science and Technology* **2016**, *53* (June), 2558–2568.

(40) Ogundele, O. M. A.; Awolu, O. O.; Badejo, A. A.; Nwachukwu, I. D.; Fagbemi, T. N. Development of Functional Beverages from Blends of Hibiscus Sabdariffa Extract and Selected Fruit Juices for Optimal Antioxidant Properties. *Food Science & Nutrition* **2016**, *4* (5), 679–685.

(41) Lee, K. H.; Kim, S. H.; Woo, K. S.; Kim, H. J.; Choi, H. S.; Kim, Y. H. Functional Beverage from Fermented Soymilk with Improved Amino Nitrogen, β -Glucosidase Activity and Aglycone Content Using Bacillus Subtilis Starter. *Food Science and Biotechnology* **2016**, *25* (5), 1399–1405.

(42) Olennikov, D. N.; Kashchenko, N. I.; Chirikova, N. K. Meadowsweet Teas as New Functional Beverages: Comparative Analysis of Nutrients, Phytochemicals and Biological Effects of Four Filipendula Species. *Molecules* **2016**, *22* (1), 1–23.

(43) Dwi, Y.; Werdani, W.; Widyawati, P. S. Antidiabetic Effect of Pluchea Indica Less Tea as a Functional Beverage in Diabetic Patients. *Atlantis Press.* **2018**, *98*, 164–167.

(44) Kantachote, D.; Ratanaburee, A.; Hayisama-ae, W.; Sukhoom, A. The Use of Potential Probiotic Lactobacillus Plantarum DW12 for Producing a Novel Functional Beverage from Mature Coconut Water. *Journal of Functional Foods* **2017**, *32*, 401–408.

(45) Milán-Carrillo, J.; Gutiérrez-Dorado, R.; Cuevas-rodriguez, E. O.; Sánchez-Magaña, L. M.;

Rochín-Medina, J. J.; Reyes-Moreno, C. Functional Beverage Produced with Bioprocessed Blue Corn and Black Beans with Antihypertensive and Antidiabetic Potential. *Revista Fitotecnia Mexicana* **2017**, *40* (4), 451–459.

(46) Komes, D.; Bušić, A.; Belščak-cvitanović, A.; Brnčić, M.; Vojvodić, A.; Dujmić, F. Novel Approach to the Development of Functional Goat's Milk-Based Beverages Using Medicinal Plant Extracts in Combination with High Intensity Ultrasound Treatment. *Food Technology and Biotechnology* **2017**, *55* (4), 484–495.

(47) Correa, V. G.; Gonçalves, G. A.; Sá-nakanishi, A. B. De; Ferreira, I. C. F. R.; Barros, L.; Dias, M. I.; Koehnlein, E. A.; Souza, C. G. M. De; Bracht, A.; Peralta, R. M. Effects of in Vitro Digestion and in Vitro Colonic Fermentation on Stability and Functional Properties of Yerba Mate (Ilex Paraguariensis A. St. Hil.) Beverages. *Food Chemistry* **2017**, *237*, 453–460.

(48) Ariviani, S.; Affandi, D. R.; Listyaningsih, E.; Handajani, S. The Potential of Pigeon Pea (Cajanus Cajan) Beverage as an Anti-Diabetic Functional Drink. *Earth and Environmental Science* **2018**, *102* (1), 1–9.

(49) Hardoko; Tanudjaja, Y.; Mastuti, T. S.; Halim, Y. Utilization of Soursop Leaves as Antihyperuricemic in Functional Beverage "Herbal Green Tea." *International Food Research Journal* **2018**, *25* (1), 321–328.

(50) Coelho, M.; Silva, S.; Rodríguez-Alcalá, L. M.; Oliveira, A.; Costa, E. M.; Borges, A.; Martins, C.; Rodrigues, A. S.; Pintado, M. M. E. Quercus Based Coffee-like Beverage: Effect of Roasting Process and Functional Characterization. *Journal of Food Measurement and Characterization* **2018**, *12* (1), 471–479.

(51) Maietta, M.; Colombo, R.; Corana, F.; Papetti, A. Cretan Tea (Origanum Dictamnus L.) as a Functional Beverage: An Investigation on Antiglycative and Carbonyl Trapping Activities. *Food and Function* **2018**, *9* (3), 1545–1556.

(52) Cunha, J.; Hilsdorf, R.; Ferreira, W. Probiotic Potential of Yeasts Isolated from Pineapple and Their Use in the Elaboration of Potentially Functional Fermented Beverages. *Food Research International* **2018**, *107*, 518–527.

(53) Vasquez-Orejarena, E.; Simons, C. T.; Litchfield, J. H.; Alvarez, V. B. Functional Properties of a High Protein Beverage Stabilized with Oat-β-Glucan. *Journal of Food Science* **2018**, *83* (5), 1360–1365.

(54) Silva, C. F. G. da; Santos, F. L.; Santana, L. R. R. de; Silva, M. V. L.; Conceição, T. de A. Development and Characterization of a Soymilk Kefir-Based Functional Beverage. *Food Science and Technology* **2018**, *38* (3), 543–550.

(55) Liu, N.; Nguyen, H.; Wismer, W.; Temelli, F. Development of an Orange-Flavoured Functional Beverage Formulated with Beta-Glucan and Coenzyme Q10-Impregnated Beta-Glucan. *Journal of Functional Foods* **2018**, *47*, 397–404.

(56) Thakkar, P.; Vaghela, B.; Patel, A.; Modi, H. A.; Prajapati, J. B. Formulation and Shelf Life Study of a Whey-Based Functional Beverage Containing Orange Juice and Probiotic Organisms. *International Food Research Journal* **2018**, *25* (4), 1675–1681.

(57) Woźniewicz, M.; Nowaczyk, P. M.; Kurhańska-Flisykowska, A.; Wyganowska-Świątkowska, M.; Lasik-Kurdyś, M.; Walkowiak, J.; Bajerska, J. Consumption of Cranberry Functional Beverage Reduces Gingival Index and Plaque Index in Patients with Gingivitis. *Nutricion Research* **2018**, *8*, 36–45.

(58) Lu, Y.; Tan, C. W.; Chen, D.; Liu, S. Q. Potential of Three Probiotic Lactobacilli in Transforming Star Fruit Juice into Functional Beverages. *Food Science and Nutrition* **2018**, *6* (July), 1–10.

(59) Mantzourani, I.; Nouska, C.; Terpou, A.; Alexopoulos, A.; Bezirtzoglou, E.; Panayiotidis, M.; Galanis, A.; Plessas, S. Production of a Novel Functional Fruit Beverage Consisting of Cornelian Cherry Juice and Probiotic Bacteria. *Antioxidants* **2018**, *7* (11), 163.

(60) Vong, W. C.; Liu, S. The Effects of Carbohydrase, Probiotic Lactobacillus Paracasei and Yeast Lindnera Saturnus on the Composition of a Novel Okara (Soybean Residue) Functional Beverage. *LWT - Food Science and Technology* **2019**, *100*, 196–204.

(61) Sethi, S.; Tyagi, S. K.; Anurag, R. K. Plant-Based Milk Alternatives an Emerging Segment of Functional Beverages: A Review. *Journal of Food Science and Technology* **2016**, *53* (9), 3408–3423.

(62) Bevilacqua, A.; Petruzzi, L.; Perricone, M.; Speranza, B.; Campaniello, D.; Sinigaglia, M.; Corbo, M. R. Nonthermal Technologies for Fruit and Vegetable Juices and Beverages: Overview and Advances. *Comprehensive Reviews in Food Science and Food Safety* **2018**, *17* (1), 2–62.

(63) Baptista, I.; Rocha, S. M.; Cunha, Â.; Saraiva, J. A.; Almeida, A. Inactivation of Staphylococcus Aureus by High Pressure Processing: An Overview. *Innovative Food Science and Emerging Technologies* **2016**, *36*, 128–149.

(64) Balasubramaniam, V. M. (Bala); Martínez-Monteagudo, S. I.; Gupta, R. Principles and Application of High Pressure–Based Technologies in the Food Industry. *Annual Review of Food Science and Technology* **2015**, *6* (1), 435–462.

(65) Micali, M.; Fiorino, M. Thermal Processing in Food Industries and Chemical Transformation.

In *The Chemistry of Thermal Food Processing Procedures*; 2016; pp 7–41.

(66) Sousa, S. G.; Delgadillo, I.; Saraiva, J. A. Human Milk Composition and Preservation: Evaluation of High-Pressure Processing as a Nonthermal Pasteurization Technology. *Critical Reviews in Food Science and Nutrition* **2016**, *56* (6), 1043–1060.

(67) Awsi, J.; Monika, S.; SA, S.; Tsering, N. Non-Thermal Processing in Food Applications: A Review. *International Journal of Food Science and Nutrition* **2017**, *2* (6), 171–180.

(68) Rodriguez-Gonzalez, O.; Buckow, R.; Koutchma, T.; Balasubramaniam, V. M. Energy Requirements for Alternative Food Processing Technologies - Principles, Assumptions, and Evaluation of Efficiency. *Comprehensive Reviews in Food Science and Food Safety* **2015**, *14* (5), 536–554.

(69) Zhang, H.; Mittal, G. S. Effects of High-Pressure Processing (HPP) on Bacterial Spores: An Overview. *Food Reviews International* **2008**, *24* (3), 330–351.

(70) Huang, H. W.; Wu, S. J.; Lu, J. K.; Shyu, Y. T.; Wang, C. Y. Current Status and Future Trends of High-Pressure Processing in Food Industry. *Food Control* **2017**, *72* (12), 1–8.

(71) Correia, I.; Nunes, A.; Saraiva, J. A.; Barros, A. S.; Delgadillo, I. High Pressure Treatments Largely Avoid/Revert Decrease of Cooked Sorghum Protein Digestibility When Applied before/after Cooking. *LWT - Food Science and Technology* **2011**, *44* (4), 1245–1249.

(72) Duarte, R. V; Moreira, S. A.; Fernandes, P. A. R.; Fidalgo, L. G.; Santos, M. D.; Queirós, R. P.; Diana, I.; Delgadillo, I.; Saraiva, J. A. Preservation under Pressure (Hyperbaric Storage) at 25 °C, 30 °C and 37 °C of a Highly Perishable Dairy Food and Comparison with Refrigeration Conservación Bajo Presión (Almacenamiento Hiperbárico) a 25 °C , 30 °C y 37 °C de Un Producto Lácteo. *CyTA – Journal of Food* **2015**, *13* (3), 321–328.

(73) Siddiq, M.; Ahmed, J.; Lobo, G. M.; Ozadali, F. *Tropical and Subtropical Fruits: Processing and Packaging Tropical and Subtropical Fruits*; 2012.

(74) Otero, L.; Sanz, P. D. High-Pressure-Shift Freezing: Main Factors Implied in the Phase Transition Time. *Journal of Food Engineering* **2006**, *72* (4), 354–363.

(75) Shouqin, Z.; Junjie, Z.; Changzhen, W. Novel High Pressure Extraction Technology. *International Journal of Pharmaceutics* **2004**, *278* (2), 471–474.

(76) Ishimaru, D.; Sá-Carvalho, D.; Silva, J. L. Pressure-Inactivated FMDV: A Potential Vaccine. *Vaccine* **2004**, *22* (17–18), 2334–2339.

(77) Gaspar, L. P.; Mendes, Y. S.; Yamamura, A. M. Y.; Almeida, L. F. C.; Caride, E.; Gonçalves, R. B.; Silva, J. L.; Oliveira, A. C.; Galler, R.; Freire, M. S. Pressure-Inactivated Yellow Fever

17DD Virus: Implications for Vaccine Development. *Journal of Virological Methods* **2008**, *150* (1–2), 57–62.

(78) Hite, B. H. The Effect of Pressure in the Preservation of Milk - A Preliminary Report. *Agricultural Experiment Station* **1899**, *58*, 13–35.

(79) Hite, B. H.; Giddings, N. J.; Weakley Jr., C. E. The Effect of Pressure on Certain Micro-Organisms Encountered in the Preservation of Fruits Ans Vegetables. *West Virginia Agric Exp Stn Bull* **1914**, *146*.

(80) Larson, W. . P. .; Hartzell, T. . B. .; Diehl, H. S. . The Effect of High Pressures on Bacteria. *The Journal of Infectious Diseases* **1918**, *22* (3), 271–279.

(81) Barba, F. J.; Orlien, V.; Mota, M. J.; Lopes, R. P.; Pereira, S. A.; Saraiva, J. A. Implementation of Emerging Technologies. In *Innovation Strategies in the Food Industry: Tools for Implementation*; Elsevier Inc., 2016; pp 117–148.

(82) Huang, H.-W.; Wu, S.-J.; Lu, J.-K.; Shyu, Y.-T.; Wang, C.-Y. Current Status and Future Trends of High-Pressure Processing in Food Industry. *Food Control* **2017**, *72* (12), 1–8.

(83) Misra, N. N.; Koubaa, M.; Roohinejad, S.; Juliano, P.; Alpas, H.; Inácio, R. S.; Saraiva, J. A.; Barba, F. J. Landmarks in the Historical Development of Twenty First Century Food Processing Technologies. *Food Research International* **2017**, *97*, 318–339.

(84) Visiongain. Food High Pressure Processing (HPP) Technologies Market 2016-2026 https://www.visiongain.com/report/food-high-pressure-processing-hpp-technologies-market-2016-2026/ (accessed Dec 14, 2018).

(85) Martín-Belloso, O.; Soliva-Fortuny, R.; Elez-Martínez, P.; Robert Marsellés-Fontanet, A.; Vega-Mercado, H. Non-Thermal Processing Technologies. In *Food Safety Management: A Practical Guide for the Food Industry*; Elsevier Inc., 2014; pp 443–465.

(86) Elamin, W. M.; Endan, J. B.; Yosuf, Y. A.; Shamsudin, R.; Ahmedov, A. High Pressure Processing Technology and Equipment Evolution: A Review. *Journal of Engineering Science and Technology Review* **2015**, *8* (5), 75–83.

(87) Nunes, C.; Santos, M. C.; Saraiva, J. A.; Rocha, S. M.; Coimbra, M. A. Influence of High Hydrostatic Pressure Technology on Wine Chemical and Sensorial Characteristics: Potentialities and Drawbacks. In *Advances in Food and Nutrition Research*; 2017; Vol. 82, pp 205–235.

(88) Ferrentino, G.; Asaduzzaman, M.; Scampicchio, M. M. Current Technologies and New Insights for the Recovery of High Valuable Compounds from Fruits By-Products. *Critical*

Reviews in Food Science and Nutrition **2018**, *58* (3), 386–404.

(89) Patazca, E.; Koutchma, T.; Balasubramaniam, V. M. Quasi-Adiabatic Temperature Increase during High Pressure Processing of Selected Foods. *Journal of Food Engineering* **2007**, *80* (1), 199–205.

(90) Yamamoto, K. Food Processing by High Hydrostatic Pressure. *Bioscience, Biotechnology and Biochemistry* **2017**, *81* (4), 672–679.

(91) Hiperbaric. Hiperbaric 55. https://www.hiperbaric.com/en/hiperbaric55 (accessed Dec 10, 2018).

(92) Syed, Q.; Buffa, M.; Guamis, B.; Saldo, J. Factors Affecting Bacterial Inactivation during High Hydrostatic Pressure Processing of Foods: A Review Factors. *Food Science and Nutrition* **2016**, *8398*, 474–483.

(93) Hogan, E.; Kelly, A. L.; Sun, D. High Pressure Processing of Foods: An Overview. In *Emerging technologies for food processing*; 2005; pp 3–32.

(94) Neetoo, H.; Chen, H. Application of High Hydrostatic Pressure Technology for Processing and Preservation of Foods. In *Progress in Food Preservation*; 2012; pp 247–276.

(95) Mota, M. J.; Lopes, R. P.; Delgadillo, I.; Saraiva, J. A. Microorganisms under High Pressure - Adaptation, Growth and Biotechnological Potential. *Biotechnology Advances* **2013**, *31* (8), 1426–1434.

(96) Taylor, P.; Rastogi, N. K.; Raghavarao, K. S. M. S.; Balasubramaniam, V. M.; Niranjan, K. Opportunities and Challenges in High Pressure Processing of Foods Opportunities and Challenges in High. *Critical Reviews in Food Science and Nutrition* **2007**, *47*, 69–112.

(97) Fellows, P. Processing Using Electric Fields, High Hydrostatic Pressure, Light or Ultrasound. In *Food Processing Technology: Principles and Practice*; 2000; pp 210–227.

(98) Reddy, N. R.; Solomon, H. M.; Tetzloff, R. C.; Rhodehamel, E. J. Inactivation of Clostridium Botulinum Type A Spores by High-Pressure Processing at Elevated Temperatures. *Journal of Food Protection* **2003**, *66* (8), 1402–1407.

(99) Daher, D. Effect of High Pressure Processing on the Microbial Inactivation in Fruit Preparations and Other Vegetable Based Beverages. *Agriculture* **2017**, *7* (9), 1–18.

(100) Hendrickx, M.; Ludikhuyze, L.; Broeck, I. Van Den; Weemaes, C. Effects of High Pressure on Enzymes Related to Food Quality. *Food Science and Technology* **1998**, *9*, 197–203.

(101) Terefe, N. S.; Buckow, R.; Versteeg, C. Quality-Related Enzymes in Fruit and Vegetable

Products: Effects of Novel Food Processing Technologies, Part 1: High-Pressure Processing. *Critical Reviews in Food Science and Nutrition* **2014**, *54* (1), 24–63.

(102) Mújica-Paz, H.; Valdez-Fragoso, A.; Samson, C. T.; Welti-Chanes, J.; Torres, A. High-Pressure Processing Technologies for the Pasteurization and Sterilization of Foods. *Food and Bioprocess Technology* **2011**, *4* (6), 969–985.

(103) Perera, N.; Gamage, T. V; Wakeling, L.; Gamlath, G. G. S.; Versteeg, C. Colour and Texture of Apples High Pressure Processed in Pineapple Juice. *Innovative Food Science and Emerging Technologies* **2010**, *11* (1), 39–46.

(104) Chakraborty, S.; Kaushik, N.; Rao, P. S.; Mishra, H. N. High-Pressure Inactivation of Enzymes: A Review on Its Recent Applications on Fruit Purees and Juices. *Comprehensive Reviews in Food Science and Food Safety* **2014**, *13*, 578–596.

(105) Jeża, M.; Wiczkowskia, W.; Zielińskab, D.; Białobrzewskic, I.; Błaszczak, W. The Impact of High Pressure Processing on the Phenolic Profile, Hydrophilic Antioxidant and Reducing Capacity of Purée Obtained from Commercial Tomato Varieties. *Food Chemistry* **2018**, *261*, 201–209.

(106) Tokuşoğlu, Ö. Effect of High Hydrostatic Pressure Processing Strategies on Retention of Antioxidant Phenolic Bioactives in Foods and Beverages - A Review. *Polish Journal of Food and Nutrition Sciences* **2016**, *66* (4), 243–251.

(107) Zulkurnain, M.; Maleky, F.; Balasubramaniam, V. M. High Pressure Processing Effects on Lipids Thermophysical Properties and Crystallization Kinetics. *Food Engineering Reviews* **2016**, *8* (4), 393–413.

(108) Aertsen, A.; Meersman, F.; Hendrickx, M. E. G.; Vogel, R. F.; Michiels, C. W. Biotechnology under High Pressure: Applications and Implications. *Trends in Biotechnology* **2009**, *27*, 434–441.

(109) Somkuti, J.; Smeller, L. High Pressure Effects on Allergen Food Proteins. *Biophysical Chemistry* **2013**, *183*, 19–29.

(110) Huang, H.; Hsu, C.; Yang, B. B.; Wang, C. Potential Utility of High-Pressure Processing to Address the Risk of Food Allergen Concerns. *Comprehensive Reviews in Food Science and Food Safety* **2014**, *13*, 78–90.

(111) Gatius, F.; Ibarz, A.; Barbosa-ca, G. V. Changes on Colour Parameters Caused by High-Pressure Processing of Apple Juice Made from Six Different Varieties. *Food Science and Technology* **2012**, *47* (10), 2158–2164.

(112) Gopal, K. R.; Kalla, A. M.; Srikanth, K. High Pressure Processing of Fruits and Vegetable Products: A Review. *International Journal of Pure & Applied Bioscience* **2017**, *5* (5), 680–692.

(113) Huang, H.-W.; Lung, H.-M.; Yang, B. B.; Wang, C.-Y. Responses of Microorganisms to High Hydrostatic Pressure Processing. *Food Control* **2014**, *40* (31), 250–259.

(114) Wang, C.-Y.; Huang, H.-W.; Hsu, C.-P.; Yang, B. B. Recent Advances in Food Processing Using High Hydrostatic Pressure Technology. *Critical Reviews in Food Science and Nutrition* **2016**, *56* (4), 527–540.

(115) Fernandez, M. V; Denoya, G. I.; Agüero, M. V; Jagus, R. J.; Vaudagna, S. R. Optimization of High Pressure Processing Parameters to Preserve Quality Attributes of a Mixed Fruit and Vegetable Smoothie. *Innovative Food Science and Emerging Technologies* **2018**, *47*, 170–179.

(116) Raj, A. S.; Chakraborty, S.; Rao, P. S. Thermal Assisted High-Pressure Processing of Indian Gooseberry (Embilica Officinalis L.) Juice – Impact on Colour and Nutritional Attributes. *LWT - Food Science and Technology* **2019**, *99*, 119–127.

(117) Sreedevi, P.; Srinivasa Rao, P. Microbial Destruction Kinetics of High-Pressure-Processed Sugarcane Juice (Saccharum Officinarum). *Food Process Engineering* **2018**, *41* (7), 1–7.

(118) Barba, F. J.; Esteve, M. J.; Frigola, A. Physicochemical and Nutritional Characteristics of Blueberry Juice after High Pressure Processing. *Food Research International* **2013**, *50* (2), 545–549.

(119) Sevenich, R.; Rauh, C.; Knorr, D. A Scientific and Interdisciplinary Approach for High Pressure Processing as a Future Toolbox for Safe and High Quality Products: A Review. *Innovative Food Science and Emerging Technologies* **2016**, *38*, 65–75.

(120) Kadam, P. S.; Jadhav, B. A.; Salve, R. V; Machewad, G. Review on the High Pressure Technology (HPT) for Food Preservation. *Food Processing & Technology* **2012**, *3*, 1–5.

(121) Wang, Y.; Ismail, M.; Farid, M. Processing of Baby Food Using Pressure-Assisted Thermal Sterilization (PATS) and Comparison with Thermal Treatment. *High Pressure Research* **2017**, *37* (4), 579–593.

(122) IFSH. *IFSH Receives FDA Acceptance of Pressure Enhanced Sterilization Process for Commercial Production of Multi- Component Shelf-Stable Foods*; 2015.

(123) Park, S. H.; Balasubramaniam, V. M.; Sastry, S. K.; Lee, J. Pressure-Ohmic-Thermal Sterilization: A Feasible Approach for the Inactivation of Bacillus Amyloliquefaciens and

Geobacillus Stearothermophilus Spores. *Innovative Food Science and Emerging Technologies* **2013**, *19* (2013), 115–123.

(124) Hulle, N. R. S.; Rao, P. S. Effect of High Pressure and Thermal Processing on Quality Changes of Aloe Vera-Litchi Mixed Beverage (ALMB) during Storage. *Journal of Food Science and Technology* **2016**, *53*, 359–369.

(125) Sandate-flores, L.; Rostro-alanis, M. de J.; Mancera-Andrade, E. I.; Esquivel-Hernandez, D. A.; Brambila-Paz, C.; Parra-Saldívar, R.; Welti-Chanes, J.; Escobedo-avellaneda, Z.; Rodríguez-Rodríguez, J. Using High Hydrostatic Pressures to Retain the Antioxidant Compounds and to Reduce the Enzymatic Activity of a Pitaya-Pineapple (Stenocereus Sp .– Fragaria Ananassa) Beverage. *Food Science and Technology* **2017**, *54*, 611–619.

(126) Wyk, S. Van; Farid, M. M.; Silva, F. V. M. SO2, High Pressure Processing and Pulsed Electric Field Treatments of Red Wine: Effect on Sensory, Brettanomyces Inactivation and Other Quality Parameters during One Year Storage. *Innovative Food Science and Emerging Technologies* **2018**, *48*, 204–211.

(127) Codina-Torrella, I.; Guamis, B.; Zamora, A.; Quevedo, J. M.; Trujillo, A. J. Microbiological Stabilization of Tiger Nuts' Milk Beverage Using Ultra-High Pressure Homogenization. A Preliminary Study on Microbial Shelf-Life Extension. *Food Microbiology* **2018**, *69*, 143–150.

(128) Kim, G.; Kim, S.; Kim, J.; Kim, K.; Choi, S. Changes in Microbial Growth, Carotenoids, and Water- Soluble Tannin Content of Ripe Persimmon Beverage after Ultra-High Pressure Treatment. *Food Science and Technology International* **2018**, *24*, 351–360.

(129) Xu, X.; Deng, J.; Luo, D.; Bao, Y.; Liao, X.; Gao, H.; Wu, J. Comparative Study of High Hydrostatic Pressure and High Temperature Short Time Processing on Quality of Clear and Cloudy Se-Enriched Kiwifruit Juices. *Innovative Food Science and Emerging Technologies* **2018**, *49*, 1–12.

(130) Pega, J.; Denoya, G. I.; Castells, M. L.; Sarquis, S.; Aranibar, G. F.; Vaudagna, S. R.; Nanni, M. Effect of High-Pressure Processing on Quality and Microbiological Properties of a Fermented Beverage Manufactured from Sweet Whey Throughout Refrigerated Storage. *Food and Bioprocess Technology* **2018**, *11*, 1101–1110.

(131) Vinha, A. F.; Barreira, C. M.; Costa, A. S. G.; Oliveira, M. B. P. P. A New Age for Quercus Spp . Fruits: Review on Nutritional and Phytochemical Composition and Related Biological Activities of Acorns. *Comprehensive Reviews in Food Science and Food Safety* **2016**, *15* (6), 947–981.

(132) Sottomayor, M. Potencial Económico Da Bolota Em Portugal: Análise Exploratória. In *Symposium A Bolota. Montemor-o-Novo, Portugal. 20 de março 2015*; 2015; p 14.

(133) Vinha, A. F.; Costa, A. S. G.; Barreira, J. C. M.; Pacheco, R.; Oliveira, M. B. P. P. Chemical and Antioxidant Profiles of Acorn Tissues from Quercus Spp.: Potential as New Industrial Raw Materials. *Industrial Crops and Products* **2016**, *94*, 143–151.

(134) García-Gómez, E.; Pérez-Badia, R.; Pereira, J.; Puri, R. K. The Consumption of Acorns (from Quercus Spp.) in the Central West of the Iberian Peninsula in the 20th Century. *Economic Botany* **2017**, *71* (3), 256–268.

(135) APCOR. Montado Forest. http://www.apcor.pt/en/montado/forest/ (accessed Oct 17, 2018).

(136) Custódio, L.; Patarra, J.; Alberício, F.; Neng, N. R.; Nogueira, J. M. F.; Romano, A. Extracts from Quercus Sp. Acorns Exhibit in Vitro Neuroprotective Features through Inhibition of Cholinesterase and Protection of the Human Dopaminergic Cell Line SH-SY5Y from Hydrogen Peroxide-Induced Cytotoxicity. *Industrial Crops and Products* **2013**, *45*, 114–120.

(137) Costas, C. *Manual de Cocina Bellotera Para La Era Post Petrolera*; 2013.

(138) Allard, G.; Berrahmouni, N.; Besacier, C.; Boglio, D.; Briens, M.; Brizay, A.; Camia, A.; Colletti, L.; Conigliaro, M.; D'annunzio, R.; et al. *State of Mediterranean Forests 2013*; 2013.

(139) Rodriguez-Estevez, V.; Sanchez-Rodriguez, M.; Arce, C.; R., A.; M., J.; Gustavo, A. Consumption of Acorns by Finishing Iberian Pigs and Their Function in the Conservation of the Dehesa Agroecosystem. *Agroforestry for Biodiversity and Ecosystem Services - Science and Practice* **2012**.

(140) Correia, P. R.; Nunes, M. C.; Beirão-da-Costa, M. L. The Effect of Starch Isolation Method on Physical and Functional Properties of Portuguese Nut Starches. II. Q. Rotundifolia Lam. and Q. Suber Lam. Acorns Starches. *Food Hydrocolloids* **2013**, *30* (1), 448–455.

(141) Bakiş, Y.; Babaç, M. T. Morphological Variability of Acorns and Its Taxonomic Significance in Quercus L. from Turkey. *Bangladesh Journal of Botany* **2014**, *43* (3), 293–299.

(142) Aldrich, P. R.; Cavender-Bares, J. Quercus. In *Wild Crop Relatives: Genomic and Breeding Resources: Temperate Fruits*; 2011; pp 1–247.

(143) Gea-Izquierdo, G.; Cañellas, I.; Montero, G. Acorn Production in Spanish Holm Oak Woodlands. *Investigación agraria. Sistemas y recursos forestales* **2006**, *15* (3), 339–354.

(144) Viñas, C. R.; Gómez, S. R.; Ayanz, Y. A. S. M. *Caracterización y Evolución Anual Del Valor Bromatológico de Las Quercíneas Mediterráneas*; 2003.

(145) Afzal-Rafii, Z.; Dodd, R. S.; Pelleau, Y. Mediterranean Evergreen Oak Diversity: Morphological and Chemical Variation of Acorns. *Canadian Journal of Botany* **1992**, *70* (7), 1459–1466.

(146) León-Camacho, M.; Viera-Alcaide, I.; Vicario, I. M. Acorn (Quercus Spp.) Fruit Lipids: Saponifiable and Unsaponifiable Fractions: A Detailed Study. *Journal of the American Oil Chemists' Society (JAOCS)* **2004**, *81* (4), 447–453.

(147) Nieto, R.; Rivera, M.; Garcıa, M. A.; Aguilera, J. F. Amino Acid Availability and Energy Value of Acorn in the Iberian Pig. *Livestock Production Science* **2002**, *77*, 227–239.

(148) Custódio, L.; Patarra, J.; Alberício, F.; Neng, N. da R.; Nogueira, J. M. F.; Romano, A. Phenolic Composition, Antioxidant Potential and in Vitro Inhibitory Activity of Leaves and Acorns of Quercus Suber on Key Enzymes Relevant for Hyperglycemia and Alzheimer's Disease. *Industrial Crops and Products* **2015**, *64*, 45–51.

(149) Rakić, S.; Povrenović, D.; Tešević, V.; Simić, M.; Maletić, R. Oak Acorn, Polyphenols and Antioxidant Activity in Functional Food. *Journal of Food Engineering* **2006**, *74* (3), 416–423.

(150) Silva, S.; Costa, E. M.; Borges, A.; Carvalho, A. P.; Monteiro, M. J.; Pintado, M. M. E. Nutritional Characterization of Acorn Flour (a Traditional Component of the Mediterranean Gastronomical Folklore). *Journal of Food Measurement and Characterization* **2016**, *10* (3), 584–588.

(151) Man, J.; Yang, Y.; Huang, J.; Zhang, C.; Zhang, F.; Wang, Y.; Gu, M.; Liu, Q.; Wei, C. Morphology and Structural Properties of High-Amylose Rice Starch Residues Hydrolysed by Amyloglucosidase. *Food Chemistry* **2013**, *138* (4), 2089–2098.

(152) Galván, J. V.; Valledor, L.; Cerrillo, R. M. N.; Pelegrín, E. G.; Jorrín-Novo, J. V. Studies of Variability in Holm Oak (Quercus Ilex Subsp. Ballota [Desf.] Samp.) through Acorn Protein Profile Analysis. *Journal of Proteomics* **2011**, *74* (8), 1244–1255.

(153) Cantos, E.; Espín, J. C.; López-Bote, C.; Hoz, L. D. LA; Ordóñez, J. A.; Tomás-Barberán, F. A. Phenolic Compounds and Fatty Acids from Acorns (Quercus Spp.), the Main Dietary Constituent of Free-Ranged Iberian Pigs. *Journal of Agricultural and Food Chemistry* **2003**, *51*, 6248–6255.

(154) Westerik, N.; Wacoo, A. P.; Sybesma, W.; Kort, R. Novel Production Protocol for Small-Scale Manufacture of Probiotic Fermented Foods. *Journal of Visualized Experiments* **2016**, No. September, 1–8.

(155) International Organization for Standardization (ISO). Microbiology of the Food Chain -

Horizontal Method for the Enumeration of Microorganisms - Part 2: Colony Count at 30 ºC by the Surface Plating Technique. ISO 4833-2:2013, Geneva.

(156) Berizi, E.; Shekarforoush, S. S.; Hosseinzadeh, S. Effects of Methanolic Pomegranate Peel Extract on the Chemical, Sensory, Textural, and Microbiological Properties of Gutted Rainbow Trout (Oncorhynchus Mykiss) during Frozen Storage. *Journal of Food Protection* **2016**, *79* (10), 1700–1706.

(157) International Organization for Standardization (ISO). Microbiology of Food and Animal Feeding Stuffs - Horizontal Method for the Enumeration of Yeasts and Moulds - Part 1: Colony Count Technique in Products with Water Activity Greater than 0,95. ISO 21527-1:2008, Geneva.

(158) International Organization for Standardization (ISO). Microbiology of the Food Chain - Horizontal Method for the Detection and Enumeration of Enterobacteriaceae - Part 2: Colony-Count Technique. ISO 21528-2:2017. Geneva.

(159) Biokar diagnostics. Mannitol Salt Agar - Technical Data sheet https://dongnamlab.com/publics/product/856/files/Mannitol_salt_agar__TDS_BK030_BM148_v9.pdf (accessed Aug 6, 2019).

(160) International Organization for Standardization (ISO). Milk Products - Enumeration of Presumptive Lactobacillus Acidophilus on a Selective Medium - Colony-Count Technique. ISO 20128:2006, Geneva.

(161) Miles, A. A.; Misra, S. S. The Estimation of the Bactericidal Power of the Blood. *J. Hyg. (Lond).* **1938**, *38* (6), 732–749.

(162) Hardy diagnostics. MCFARLAND LATEX STANDARDS - IFU Instructions for use https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/McFarlandStds.htm.

(163) Kesici, U.; Demirci, M.; Kesici, S. Antimicrobial Effects of Local Anaesthetics. *International Wound Journal* **2019**, *16*, 1029–1033.

(164) International Organization for Standardization (ISO). Microbiology of the Food Chain - Horizontal Method for the Detection and Enumeration of Listeria Monocytogenes and Listeria Spp. - Part 1: Detection Method. ISO 11290-1:2017. Geneva, 2017.

(165) Biokar diagnostics. Palcam agar - Technical Data sheet https://dongnamlab.com/publics/product/880/files/PALCAM_agar_TDS_BK145_BM020_v6.pdf (accessed Aug 8, 2019).

(166) International Organization for Standardization (ISO). Microbiology of Food and Animal

Feeding Stuffs - Horizontal Method for the Enumeration of Presumptive Bacillus Cereus - Colony-Count Technique at 30 °C. ISO 7932:2004. Geneva, 2004.

(167) International Organization for Standardization (ISO). Microbiology of Food and Animal Feeding Stuffs - Horizontal Method for the Detection of Escherichia Coli O157. ISO 16654:2001. Geneva, 2006.

(168) International Organization for Standardization (ISO). Water Quality - Detection and Enumeration of Pseudomonas Aeruginosa - Part 2: Most Probable Number Method. ISO 16266-2:2018. Geneva, 2018.

(169) Jing, L.; Ma, H.; Fan, P.; Gao, R.; Jia, Z. Antioxidant Potential, Total Phenolic and Total Flavonoid Contents of Rhododendron Anthopogonoides and Its Protective Effect on Hypoxia-Induced Injury in PC12 Cells. *BMC Complementary and Alternative Medicine* **2015**, *15*, 1–12.

(170) Barba, F. J.; Esteve, M. J.; Frigola, A. Physicochemical and Nutritional Characteristics of Blueberry Juice after High Pressure Processing. *Food Research International journal* **2013**, *50* (2), 545–549.

(171) Coscueta, E. R.; Malpiedi, L. P.; Nerli, B. B. Micellar Systems of Aliphatic Alcohol Ethoxylates as a Sustainable Alternative to Extract Soybean Iso Flavones. *Food Chemistry* **2018**, *264* (October 2017), 135–141.

(172) Oliveira, C. M.; Barros, A. S.; Ferreira, A. C. S.; Silva, A. M. S. Influence of the Temperature and Oxygen Exposure in Red Port Wine: A Kinetic Approach. *Food Research International* **2015**, *75*, 337–347.

(173) Gonçalves, B.; Falco, V.; Moutinho-Pereira, J.; Balcelar, E.; Peixoto, F.; Correia, C. Effects of Elevated CO2 on Grapevine (Vitis Vinifera L.): Volatile Composition, Phenolic Content, and in Vitro Antioxidant Activity of Red Wine. *Journal of Agricultural and Food Chemistry* **2009**, *57* (1), 265–273.

(174) Dávalos, A.; Gómez-Cordovés, C.; Bartolomé, B. Extending Applicability of the Oxygen Radical Absorbance Capacity (ORAC - Fluorescein) Assay. *Journal of Agricultural and Food Chemistry* **2004**, *52* (1), 48–54.

(175) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry* **1956**, *28* (3), 350–356.

(176) International Organization for Standardization (ISO). Food and Feed Products - General Guidelines for the Determination of Nitrogen by the Kjeldahl Method. ISO 1871:2009.

Geneva, 2009.

(177) International Organization for Standardization (ISO). Oilseeds - Determination of Oil Content (Reference Method), 2009.

(178) Bernardo-gil, M. G. Characterisation of Acorn Oils Extracted by Hexane and by Supercritical Carbon Dioxide. *European Journal Lipid Science Technology* **2005**, *107*, 12–19.

(179) Pripis-nicolau, L.; Revel, G. De; Bertrand, A.; Maujean, A. Formation of Flavor Components by the Reaction of Amino Acid and Carbonyl Compounds in Mild Conditions. *Journal of Agricultural and Food Chemistry* **2000**, *48* (9), 3761–3766.

(180) Omohimi, C. I.; Piccirillo, C.; Roriz, M.; Ferraro, V.; Vasconcelos, M. W.; Sanni, L. O.; Tomlins, K.; Pintado, M. M.; Abayomi, L. A. Study of the Proximate and Mineral Composition of Different Nigerian Yam Chips, Flakes and Flours. *Journal of Food Science and Technology* **2017**, *55* (1), 42–51.

(181) Ulbricht, T. L. V; Southgate, D. A. T. Coronary Heart Disease: Seven Dietary Factors The. *The Lancet* **1991**, *338* (OCT 19), 985–992.

(182) Monteiro, M. J. P.; Costa, A. I. A.; Fliedel, G.; Cissé, M.; Bechoff, A.; Pallet, D.; Tomlins, K.; Pintado, M. M. E. Chemical-Sensory Properties and Consumer Preference of Hibiscus Beverages Produced by Improved Industrial Processes. *Food Chemistry* **2016**, *225*, 202–212.

(183) International Organization for Standardization (ISO). Sensory Analysis - General Guidance for the Design of Test Rooms. ISO 8589:2007. Geneva. **2007**.

(184) Park, S. H.; Balasubramaniam, V. M.; Sastry, S. K. Quality of Shelf-Stable Low-Acid Vegetables Processed Using Pressure-Ohmic-Thermal Sterilization. *LWT - Food Science and Technology* **2014**, *57* (1), 243–252.

(185) Corrales, M.; Souza, P. M. De; Stahl, M. R.; Fernández, A. Effects of the Decontamination of a Fresh Tiger Nuts' Milk Beverage (Horchata) with Short Wave Ultraviolet Treatments (UV-C) on Quality Attributes. *Innovative Food Science and Emerging Technologies* **2012**, *13*, 163–168.

(186) Kimura, K.; Morimatsu, K.; Inaoka, T.; Yamamoto, K. Injury and Recovery of Escherichia Coli ATCC25922 Cells Treated by High Hydrostatic Pressure at 400 e 600 MPa. *Journal of Bioscience and Bioengineering* **2017**, *123* (6), 698–706.

(187) Xu, X.; Deng, J.; Luo, D.; Bao, Y.; Liao, X. Comparative Study of High Hydrostatic Pressure and High Temperature Short Time Processing on Quality of Clear and Cloudy Se-Enriched Kiwifruit Juices. *Innovative Food Science and Emerging Technologies* **2018**, *49* (99), 1–12.

(188) Bull, M. K.; Hayman, M. M.; Stewart, C. M.; Szabo, E. A.; Knabel, S. J. Effect of Prior Growth Temperature, Type of Enrichment Medium, and Temperature and Time of Storage on Recovery of Listeria Monocytogenes Following High Pressure Processing of Milk. *International Journal of Food Microbiology* **2005**, *101*, 53–61.

(189) Picouet, P. A.; Sárraga, C.; Cofán, S.; Belletti, N.; Guàrdia, M. D. Effects of Thermal and High-Pressure Treatments on the Carotene Content, Microbiological Safety and Sensory Properties of Acidified and of Non-Acidified Carrot Juice. *LWT - Food Science and Technology* **2014**, *62* (1), 920–926.

(190) Cruz, N.; Capellas, M.; Hernández, M.; Trujillo, A. J.; Guamis, B.; Ferragut, V. Ultra High Pressure Homogenization of Soymilk: Microbiological, Physicochemical and Microstructural Characteristics. *Food Research International* **2007**, *40*, 725–732.

(191) Poliseli-scopel, F. H.; Hernández-Herrero, M.; Guamis, B.; Ferragut, V. Comparison of Ultra High Pressure Homogenization and Conventional Thermal Treatments on the Microbiological, Physical and Chemical Quality of Soymilk. *LWT - Food Science and Technology* **2012**, *46* (1), 42–48.

(192) Briviba, K.; Gräf, V.; Walz, E.; Guamis, B.; Butz, P. Ultra High Pressure Homogenization of Almond Milk: Physico-Chemical and Physiological Effects. *Food Chemistry* **2016**, *192*, 82–89.

(193) Ferragut, V.; Valencia-flores, D. C.; Pérez-González, M.; Gallardo, J.; Hernández-Herrero, M. Quality Characteristics and Shelf-Life of Ultra-High Pressure Homogenized (UHPH) Almond Beverage. *Foods* **2015**, *4*, 159–172.

(194) Liepa, M.; Baltrukova, S.; Safety, F.; Health, A.; Bior, E.; Zagorska, J.; Galoburda, R. Survival of Pathogens in High Pressure Processed Milk. *Research for Rural Development* **2018**, *1*, 215–221.

(195) Bozoglu, F.; Alpas, H.; Kaletunç, G. Injury Recovery of Foodborne Pathogens in High Hydrostatic Pressure Treated Milk during Storage. *FEMS Immunology and Medical Microbiology* **2004**, *40* (3), 243–247.

(196) Tomasula, P. M.; Renye, J. A.; Hekken, D. L. Van; Tunick, M. H.; Kwoczak, R.; Toht, M.; Leggett, L. N. Effect of High-Pressure Processing on Reduction of Listeria Monocytogenes in Packaged Queso Fresco. *Journal of Dairy Science* **2014**, *97* (3), 1281–1295.

(197) Yang, B.; Shi, Y.; Xia, X.; Xi, M.; Wang, X.; Ji, B.; Meng, J. Inactivation of Foodborne Pathogens in Raw Milk Using High Hydrostatic Pressure. *Food Control* **2012**, *28* (2), 273–278.

(198) Demazeau, G.; Plumecocq, A.; Lehours, P.; Martin, P.; Couëdelo, L.; Billeaud, C. A New High Hydrostatic Pressure Process to Assure the Microbial Safety of Human Milk While Preserving the Biological Activity of Its Main Components. *Frontiers in Public Health* **2018**, *6* (NOV), 1–8.

(199) Shigehisa, T.; Ohmori, T.; Saito, A.; Taji, S.; Hayashi, R. Effects of High Hydrostatic Pressure on Characteristics of Pork Slurries and Inactivation of Microorganisms Associated with Meat and Meat Products. *International Journal of Food Microbiology* **1991**, *12* (2–3), 207–215.

(200) Prinz, J. F.; Lucas, P. W. Saliva Tannin Interactions. *Journal of Oral Rehabilitation* **2008**, *27* (11), 991–994.

(201) Chung, K. T.; Wong, T. Y.; Wei, C. I.; Huang, Y. W.; Lin, Y. Tannins and Human Health: A Review. *Critical Reviews in Food Science and Nutrition* **1998**, *38* (6), 421–464.

(202) Brglez Mojzer, E.; Knez Hrnčič, M.; Škerget, M.; Knez, Ž.; Bren, U. Polyphenols: Extraction Methods, Antioxidative Action, Bioavailability and Anticarcinogenic Effects. *Molecules* **2016**, *21* (7), 1–38.

(203) Kim, Y. A.; Ba, H. Van; Dashdorj, D.; Hwang, I. Effect of High-Pressure Processing on the Quality Characteristics and Shelf-Life Stability of Hanwoo Beef Marinated with Various Sauces. *Korean Journal for Food Science of Animal Resources* **2018**, *38* (4), 679–692.

(204) Morton, J. D.; Pearson, R. G.; Lee, H. Y.-Y.; Smithson, S.; Mason, S. L.; Bickerstaffe, R. High Pressure Processing Improves the Tenderness and Quality of Hot-Boned Beef. *Meat Science* **2017**, *133*, 69–74.

(205) Souza, C. M.; Boler, D. D.; Clark, D. L.; Kutzler, L. W.; Holmer, S. F.; Summer, J. W.; Cannon, J. E.; Smit, N. R.; Mckeith, F. K.; Killefer, J. The Effects of High Pressure Processing on Pork Quality, Palatability, and Further Processed Products. *Meat Science* **2011**, *87*, 419–427.

(206) Tsai, M.; Cheng, M.; Chen, B.; Wang, C. Effect of High-Pressure Processing on Immunoreactivity, Microbial and Physicochemical Properties of Hazelnut Milk. *International Journal of Food Science and Technology* **2018**, *53* (7), 1672–1680.

(207) Wang, H.; Chen, B.; Wang, C. Comparison of High Pressure and High Temperature Short Time Processing on Quality of Carambola Juice during Cold Storage. *Journal of Food Science and Technology* **2018**, *55* (12), 1716–1725.

(208) Chen, X.; Qin, W.; Ma, L.; Xu, F.; Jin, P.; Zheng, Y. Effect of High Pressure Processing and Thermal Treatment on Physicochemical Parameters, Antioxidant Activity and Volatile

Compounds of Green Asparagus Juice. *LWT - Food Science and Technology* **2014**, *62* (1), 927–933.

(209) Jayachandran, L. E.; Chakraborty, S.; Rao, P. S. Effect of High Pressure Processing on Physicochemical Properties and Bioactive Compounds in Litchi Based Mixed Fruit Beverage. *Innovative Food Science and Emerging Technologies* **2015**, *28*, 1–9.

(210) Varela-santos, E.; Ochoa-martinez, A.; Tabilo-munizaga, G.; Reyes, J. E.; Pérez-won, M.; Briones-labarca, V.; Morales-castro, J. Effect of High Hydrostatic Pressure (HHP) Processing on Physicochemical Properties, Bioactive Compounds and Shelf-Life of Pomegranate Juice. *Innovative Food Science and Emerging Technologies* **2012**, *13*, 13–22.

(211) Wei, S.; Lu, G.; Cao, H. Effects of Cooking Methods on Starch and Sugar Composition of Sweetpotato Storage Roots. *PLoS ONE* **2017**, *12* (8), 1–10.

(212) Rivas, A.; Rodrigo, D.; Martínez, A.; Barbosa-Cánovas, G. V; Rodrigo, M. Effect of PEF and Heat Pasteurization on the Physical - Chemical Characteristics of Blended Orange and Carrot Juice. *LWT - Food Science and Technology* **2006**, *39*, 1163–1170.

(213) Krapfenbauer, G.; Kinner, M.; Gossinger, M.; Schonlechnet, R.; Berghofer, E. Effect of Thermal Treatment on the Quality of Cloudy Apple Juice. *Journal of Agricultural and Food Chemistry* **2006**, *54*, 5453–5460.

(214) Liu, F.; Zhang, X.; Zhao, L.; Wang, Y.; Liao, X. Potential of High-Pressure Processing and High-Temperature/Short-Time Thermal Processing on Microbial, Physicochemical and Sensory Assurance of Clear Cucumber Juice. *Innovative Food Science and Emerging Technologies* **2016**, *34*, 51–58.

(215) Zhu, S.; Yu, Y. Storage Stability of Chinese Bayberry Juice after High Pressure or Thermal Treatment. *Journal of Food Processing and Preservation* **2015**, *39*, 2259–2266.

(216) Dede, S.; Alpas, H.; Bayındırlı, A. High Hydrostatic Pressure Treatment and Storage of Carrot and Tomato Juices: Antioxidant Activity and Microbial Safety. *Journal of the Science of Food and Agriculture* **2007**, *782* (June 2006), 773–782.

(217) Patras, A.; Brunton, N. P.; Da Pieve, S.; Butler, F. Impact of High Pressure Processing on Total Antioxidant Activity, Phenolic, Ascorbic Acid, Anthocyanin Content and Colour of Strawberry and Blackberry Purées. *Innovative Food Science and Emerging Technologies* **2009**, *10* (3), 308–313.

(218) Corrales, M.; Toepfl, S.; Butz, P.; Knorr, D.; Tauscher, B. Extraction of Anthocyanins from Grape By-Products Assisted by Ultrasonics, High Hydrostatic Pressure or Pulsed Electric Fields: A Comparison. *Innovative Food Science and Emerging Technologies* **2008**, *9* (1), 85–

91.

(219) Jiménez-Martínez, M. C.; Montalvo-González, E.; Sáyago-Ayerdi, S. G.; Mercado-Mercado, G.; Ramírez-de León, J. A.; Paz-Gamboa, E.; Vivar-Vera, M. A. Effect of High Hydrostatic Pressure on the Polyphenols and Antioxidant Activity of Plantain Pulp (Musa Paradisiaca AAB). *Journal of the Science of Food and Agriculture* **2017**, *97* (8), 2508–2515.

(220) Weltin-Chanes, J.; Vélez-Ruiz, J. F.; Barbosa-Canovas, G. V. *Transport Phenomena in Food Processing*; 2003.

(221) Cao, X.; Zhang, Y.; Zhang, F.; Wang, Y.; Yi, J.; Liao, X. Effects of High Hydrostatic Pressure on Enzymes, Phenolic Compounds, Anthocyanins, Polymeric Color and Color of Strawberry Pulps. *Journal of the Science of Food and Agriculture* **2011**, *91* (5), 877–885.

(222) Terefe, N. S.; Kleintschek, T.; Gamage, T.; Fanning, K. J.; Netzel, G.; Versteeg, C.; Netzel, M. Comparative Effects of Thermal and High Pressure Processing on Phenolic Phytochemicals in Different Strawberry Cultivars. *Innovative Food Science and Emerging Technologies* **2013**, *19*, 57–65.

(223) Ali, N.; Popović, V.; Koutchma, T.; Warriner, K.; Zhu, Y. Effect of Thermal, High Hydrostatic Pressure, and Ultraviolet-C Processing on the Microbial Inactivation, Vitamins, Chlorophyll, Antioxidants, Enzyme Activity, and Color of Wheatgrass Juice. *Journal of Food Process Engineering* **2019**, No. February, 1–11.

(224) Marszałek, K.; Szczepańska, J.; Starzonek, S.; Woźniak, Ł.; Trych, U.; Skąpska, S.; Rzoska, S.; Saraiva, J. A.; Lorenzo, J. M.; Barba, F. J. Enzyme Inactivation and Evaluation of Physicochemical Properties, Sugar and Phenolic Profile Changes in Cloudy Apple Juices after High Pressure Processing, and Subsequent Refrigerated Storage. *Journal of Food Process Engineering* **2019**, *42* (4), 1–8.

(225) Vieira, F. N.; Lourenço, S.; Fidalgo, L. G.; Santos, S. A. O.; Silvestre, A. J. D.; Jerónimo, E.; Saraiva, J. A. Long-Term Effect on Bioactive Components and Antioxidant Activity of Thermal and High-Pressure Pasteurization of Orange Juice. *Molecules* **2018**, *23* (10), 1–15.

(226) Zhang, Y.; Liu, X. C.; Wang, Y.; Zhao, F.; Sun, Z.; Liao, X. Quality Comparison of Carrot Juices Processed by High-Pressure Processing and High-Temperature Short-Time Processing. *Innovative Food Science and Emerging Technologies* **2016**, *33*, 135–144.

(227) Terán-Hilares, R.; Chirinos, R.; Pedreschi, R.; Campos, D. Enhanced Antioxidant Properties of Tara (Caesalpinia Spinosa) Gallotannins by Thermal Hydrolysis and Its Synergistic Effects with α-Tocopherol, Ascorbyl Palmitate, and Citric Acid on Sacha Inchi (Plukenetia Volubilis) Oil. *Journal of Food Process Engineering* **2017**, *41* (1), 1–10.

(228) Changa, Y.-H.; Wub, S.-J.; Chenc, B.-Y.; Huangb, H.-W.; Wang, C.-Y. Effect of High Pressure Processing and Thermal Pasteurization on Overall Quality Parameters of White Grape Juice. *Journal of the Science of Food and Agriculture* **2019**, *56* (8), 623–626.

(229) Zhou, H.; Wang, C.; Ye, J.; Chen, H.; Tao, R.; Cao, F. Effects of High Hydrostatic Pressure Treatment on Structural, Allergenicity, and Functional Properties of Proteins from Ginkgo Seeds. *Innovative Food Science and Emerging Technologies* **2016**, *34*, 187–195.

(230) Sila, D. N.; Duvetter, T.; De Roeck, A.; Verlent, I.; Smout, C.; Moates, G. K.; Hills, B. P.; Waldron, K. K.; Hendrickx, M.; Van Loey, A. Texture Changes of Processed Fruits and Vegetables: Potential Use of High-Pressure Processing. *Trends in Food Science & TechnologyTrends in Food Science and Technology* **2008**, *19* (6), 309–319.

(231) Williams, A. P. AMINO ACIDS | Determination. In *Encyclopedia of Food Sciences and Nutrition*; 2003; pp 192–197.

(232) United States Department of Agriculture (USDA). Nuts, acorns, raw https://fdc.nal.usda.gov/fdc-app.html#/food-details/170157/nutrients (accessed Nov 29, 2019).

(233) Özcan, T. Total Protein and Amino Acid Compositions in the Acorns of Turkish Quercus L. Taxa. *Genetic Resources and Crop Evolution* **2006**, *53* (2), 419–429.

(234) De Maria, S.; Ferrari, G.; Maresca, P. Effect of High Hydrostatic Pressure on the Enzymatic Hydrolysis of Bovine Serum Albumin. *Journal of the Science of Food and Agriculture* **2017**, *97* (10), 3151–3158.

(235) Chapter 6 - Protein and Amino Acids. In *Recommended Dietary Allowances*; 1990; Vol. 10th Editi, pp 13–21.

(236) European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition, and A. (NDA). Scientific Opinion on Dietary Reference Values for Fats, Including Saturated Fatty Acids, Polyunsaturated Fatty Acids, Monounsaturated Fatty. *European Food Safety Authority (EFSA) Journal* **2010**, *8* (3), 1–107.

(237) Petrovic, S.; Sobajic, S.; Rakic, S.; Tomic, A.; Kukic, J. Investigation of Kernel Oils of Quercus Robur and Quercus Cerris. *Chemistry of Natural Compounds* **2004**, *40* (5), 347–348.

(238) Kapoor, R.; Huang, Y.-S. Gamma Linolenic Acid: An Antiinflammatory Omega-6 Fatty Acid. *Current Pharmaceutical Biotechnology* **2006**, *7* (6), 531–534.

(239) Alba, M. De; Pérez-andrés, J. M.; Harrison, S. M.; Brunton, N. P.; Burgess, C. M.; Tiwari, B. K. High Pressure Processing on Microbial Inactivation, Quality Parameters and Nutritional

Quality Indices of Mackerel Fi Llets. *Innovative Food Science and Emerging Technologies* **2019**, *55* (March), 80–87.

(240) British Department of Health. *Nutritional Aspects of Cardiovascular Disease. Report on Health and Social Subjects No. 46. London: HMSO*; 1994.

(241) Naydenova, N.; Kaishev, I.; Iliev, T.; Mihaylova, G. Fatty Acids Profile, Atherogenic and Thrombogenic Health Indices of White Brined Cheese Made from Buffalo Milk. *Agricultural Science and Technology* **2014**, *6* (August), 1–43.

(242) Simopoulos, A. P. The Importance of the Omega-6/Omega-3 Fatty Acid Ratio in Cardiovascular Disease and Other Chronic Diseases. *Experimental Biology and Medicine* **2008**, *233* (6), 674–688.

(243) Moltó-Puigmartí, C.; Permanyer, M.; Castellote, A. I.; López-Sabater, M. C. Effects of Pasteurisation and High-Pressure Processing on Vitamin C, Tocopherols and Fatty Acids in Mature Human Milk. *Food Chemistry* **2011**, *124* (3), 697–702.

(244) Rodríguez-Alcalá, L. M.; Alonso, L.; Fontecha, J. Stability of Fatty Acid Composition after Thermal, High Pressure, and Microwave Processing of Cow Milk as Affected by Polyunsaturated Fatty Acid Concentration. *Journal of Dairy Science* **2014**, *97* (12), 7307–7315.

(245) Sekeroglu, N.; Ozkutlu, F.; Kilic, E. Mineral Composition of Acorn Coffees. *Indian Journal of Pharmaceutical Education and Research* **2017**, *51* (3), S504–S507.

(246) World Health Organization (WHO). *WHO Monographs on Selected Medicinal Plants*; 1999; Vol. 1.

(247) Damasceno, L. F.; Fernandes, F. A. N.; Magalhães, F. A. N.; Brito, E. S. Evaluation and Optimization of Non Enzymatic Browning of "Cajuina" during Thermal Treatment. *Brazilian Journal of Chemical Engineering* **2008**, *25* (2), 313–320.

(248) Kowalski, S.; Lukasiewicz, M.; Duda-chodak, A.; Zięć, G. 5-Hydroxymethyl-2-Furfural (HMF) – Heat-Induced Formation, Occurrence in Food and Biotransformation – a Review. *Polish Journal of Food and Nutrition Sciences* **2013**, *63* (4), 207–225.

(249) Bakhiya, N.; Monien, B.; Frank, H.; Seidel, A.; Glatt, H. Renal Organic Anion Transporters OAT1 and OAT3 Mediate the Cellular Accumulation of 5-Sulfooxymethylfurfural, a Reactive, Nephrotoxic Metabolite of the Maillard Product 5-Hydroxymethylfurfural. *Biochemical Pharmacology* **2009**, *78*, 414–419.

(250) Glatt, H.; Sommer, Y. Health Risks of 5-Hydroxymethylfurfural (HMF) and Related

Compounds. In *Acrylamide and other hazardous compounds in heat-treated foods*; Woodhead Publishing Limited, 2006; pp 328–357.

(251) Rufían-Henares, J. A.; Delgado-Andrade, C.; Morales, F. J. Analysis of Heat-Damage Indices in Breakfast Cereals: Influence of Composition. *Journal of Cereal Science* **2006**, *43*, 63–69.

(252) Czerwonka, M.; Opiłka, J.; Tokarz, A. Evaluation of 5-hydroxymethylfurfural Content in Non-alcoholic Drinks. *European Food Research and Technology* **2017**, *244*, 11–18.

Appendices

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

Appendix I – Fatty acids profile

Table 14: Changes in all the fatty acid (FA) along storage time (day 0, 21 and 56), expressed in percentage (%) on untreated and HPP-treated (450 MPa/5 min) acorn beverage samples.

		Untreated			HPP-treated (450 MPa, 5 min)		
	Storage time (day)	**0**	**21**	**56**	**0**	**21**	**56**
Fatty acids (% in total FA)	**C10**	0.01	0.01	0.01	0.01	0.01	0.01
	C10:1	0.03	0.03	0.03	0.01	0.01	0.01
	C12	0.07	0.08	0.06	0.06	0.05	0.04
	C14	0.12	0.16	0.14	0.15	0.12	0.13
	C14:1	0.11	0.11	0.06	0.11	0.08	0.09
	C15	0.03	0.03	0.03	0.03	0.03	0.03
	C16 i	0.02	0.02	0.02	0.02	0.02	0.02
	C15:1	0.11	0.09	0.06	0.11	0.07	0.08
	C16	14.23	14.33	14.23	14.06	14.23	14.13
	C16:1 c7	0.06	0.06	0.06	0.06	0.06	0.06
	C16:1 c9	0.10	0.10	0.09	0.09	0.10	0.08
	C17 i	0.01	0.01	0.01	0.01	0.01	0.01
	C17 ai	0.05	0.05	0.04	0.10	0.03	0.03
	C 17	0.12	0.12	0.12	0.12	0.13	0.12
	C17:1 c10	0.09	0.18	0.17	0.06	0.09	0.07
	C18	3.25	3.27	3.26	3.23	3.28	3.25
	C18:1 c9	62.30	62.20	62.51	62.43	62.51	62.63
	C18:1 c11	0.81	0.81	0.80	0.80	0.81	0.80
	C18:2 t9t12	0.02	0.07	0.02	0.16	0.01	0.01
	C18:2 c9c12	16.35	16.16	16.17	16.30	16.27	16.30
	C18:3 t9t12c15	0.04	0.04	0.04	0.04	0.04	0.04
	ɣ C18:3 c6c9C13	0.04	0.04	0.04	0.03	0.03	0.03
	α C18:3 c9c12c15	0.64	0.62	0.62	0.63	0.62	0.62
	C20	0.45	0.45	0.46	0.45	0.46	0.45
	C18:2 c9t11	0.59	0.59	0.59	0.59	0.59	0.59
	C22	0.23	0.23	0.22	0.22	0.23	0.23
	C20:2 c13c16	0.04	0.05	0.04	0.04	0.05	0.04
	C24	0.09	0.09	0.09	0.09	0.09	0.08

Values represent mean of duplicate analyses of two individual samples.

Appendix II - Questionnaire presented in the sensory analysis

Teste sensorial a uma bebida de vegetal

Por favor preencha o questionário seguinte:

Idade:__________ anos Sexo: ☐F ☐M

Profissão:

- ☐ Quadro Superior / Técnico Superior
- ☐ Quadro Médio / Técnico
- ☐ Auxiliar
- ☐ Desempregado à procura do primeiro emprego
- ☐ Estudante universitário
- ☐ Estudante de Liceu
- ☐______________ (outro)

Frequência com que consome bebidas vegetais:

- ☐ Mais de uma vez por semana
- ☐ Cerca de uma vez por semana
- ☐ Cerca de uma vez por mês
- ☐ 8 a 10 vezes por ano
- ☐ Nunca

Agora, por favor, prove a amostra 285 e seguidamente a 741 e assinale a sua apreciação relativamente à **APRECIAÇÃO GLOBAL, ASPECTO**, **SABOR** e **TEXTURA NA BOCA**.

Entre cada prova beba um pouco de água ou aguarde cerca de 30 segundos.

APRECIAÇÃO GLOBAL

Amostra nº 285

- ☐ Gosto extremamente
- ☐ Gosto muito
- ☐ Gosto
- ☐ Gosto ligeiramente
- ☐ Não gosto nem desgosto
- ☐ Desgosto ligeiramente
- ☐ Não gosto
- ☐ Desgosto muito
- ☐ Desgosto extremamente

Gostei:

____

____

Não gostei:

____

Amostra nº 741

- ☐ Gosto extremamente
- ☐ Gosto muito
- ☐ Gosto
- ☐ Gosto ligeiramente
- ☐ Não gosto nem desgosto
- ☐ Desgosto ligeiramente
- ☐ Não gosto
- ☐ Desgosto muito
- ☐ Desgosto extremamente

Gostei:

____

____

Não gostei:

____

ASPECTO

Amostra nº 285
- ☐ Gosto extremamente
- ☐ Gosto muito
- ☐ Gosto
- ☐ Gosto ligeiramente
- ☐ Não gosto nem desgosto
- ☐ Desgosto ligeiramente
- ☐ Não gosto
- ☐ Desgosto muito
- ☐ Desgosto extremamente

Gostei:

____

____

Não gostei:

____

____

Amostra nº 741
- ☐ Gosto extremamente
- ☐ Gosto muito
- ☐ Gosto
- ☐ Gosto ligeiramente
- ☐ Não gosto nem desgosto
- ☐ Desgosto ligeiramente
- ☐ Não gosto
- ☐ Desgosto muito
- ☐ Desgosto extremamente

Gostei:

____

____

Não gostei:

____

____

SABOR

Amostra nº 285
- ☐ Gosto extremamente
- ☐ Gosto muito
- ☐ Gosto
- ☐ Gosto ligeiramente
- ☐ Não gosto nem desgosto
- ☐ Desgosto ligeiramente
- ☐ Não gosto
- ☐ Desgosto muito
- ☐ Desgosto extremamente

Gostei:

____

____

Não gostei:

____

____

Amostra nº 741
- ☐ Gosto extremamente
- ☐ Gosto muito
- ☐ Gosto
- ☐ Gosto ligeiramente
- ☐ Não gosto nem desgosto
- ☐ Desgosto ligeiramente
- ☐ Não gosto
- ☐ Desgosto muito
- ☐ Desgosto extremamente

Gostei:

____

____

Não gostei:

____

____

TEXTURA NA BOCA

Amostra nº 285

- ☐ Gosto extremamente
- ☐ Gosto muito
- ☐ Gosto
- ☐ Gosto ligeiramente
- ☐ Não gosto nem desgosto
- ☐ Desgosto ligeiramente
- ☐ Não gosto
- ☐ Desgosto muito
- ☐ Desgosto extremamente

Gostei:

____

____

Não gostei:

____

____

Amostra nº 741

- ☐ Gosto extremamente
- ☐ Gosto muito
- ☐ Gosto
- ☐ Gosto ligeiramente
- ☐ Não gosto nem desgosto
- ☐ Desgosto ligeiramente
- ☐ Não gosto
- ☐ Desgosto muito
- ☐ Desgosto extremamente

Gostei:

____

____

Não gostei:

____

____

Na sua opinião a amostra 285 é ☐ ☐ ☐ ☐ ☐ **do que a amostra 741**

muito pior similar muito melhor

Compraria a bebida vegetal que gostou mais?

☐ Sim ☐ Não ☐ Talvez

Se não ou talvez, porquê? ____

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Muito obrigada pela sua colaboração!