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Food Proteins: Sustainable Sources of Proteins Responsible for Emulsions Stability in Food Products

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Food Proteins: Sustainable Sources of Proteins Responsible for Emulsions Stability in Food Products

By
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Abstract

One of the major challenges manufactures are facing is the fact that consumer's personal health standards are changing constantly, modern consumers are willing to know more about the ingredients used in their food. The relationship with ethics and sustainability is leading to changes in the food industry. Seen this, manufactures are opting for other sources of ingredients, such as plant-based alternatives. However, the use of these sort of products need to be developed due to of their unknown properties. The use of sustainable plant proteins provides a variety of benefits to the product, such as added nutritional value as fiber, proteins and antioxidants, emulsion formation, product stability and extended shelf-life. There is a wide variety of plant-based proteins used in the food industry. The most common protein from animal origin used in the food industry are whey protein (Concentrate/Isolate) and casein, egg protein and egg lecithin which provide a strong emulsion thickness. Sustainable plant based proteins are being widely used, such as pea protein, tea saponin, potato protein, soy beans and microalgae and they provide similar or even better emulsion stability and nutritional benefits. This research was conducted with 281 people, the aim is to identify behaviours, knowledge, attitudes, preferences, and awareness levels associated with sustainable sources of plant-based proteins used as ingredient in the food industry versus animal origin proteins of people living in Ireland. The findings identified that consumers are demanding for more accurate information on product ingredients description and benefits, the food industry is developing new methods for the better usage of plant-based proteins and improvement of their natural properties, providing improved sensory, physical and nutritional properties to the final product. This study observed that consumers are not fully aware of plant-based proteins used as product ingredient, neither are aware of their nutritional benefits. The sensory test exposed that consumers showed to still prefer whey protein sensory characteristics, when compared to plant-based proteins. The majority confirmed to be aware of the relationship of the use of more sustainable sources of food proteins and the development of a fully sustainable food chain and with the future health of the planet. Moreover, we can relate these facts with consumers' willingness to pay a small increase of value for a sustainable food product, in contradiction, when considering purchase attributes, sustainability was the last considered, the firstly considered were nutrition and cost. The results from this study highlight the need for more research in this area, with the aspiration to enforce the usage of more plant-based proteins as product ingredient, improving nutrition and emulsification properties.

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I hereby certify that this material, which I now submit in part fulfilment of the requirement for the award of MSc in Food Safety Management, is entirely my own work and has not been taken from the work of others save and to the extent such work has been cited and acknowledged within the text of my work.

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Abbreviations

ACN – Anthocyanin

AST – Astaxanthin

ASPI - Algae Soluble Protein Isolate

CATA - Check-All-That-Apply Analysis

EAI - Emulsifying Activity Index

EC - Emulsion capacity

EG - Ester gum

ESI - Emulsifying Stability Index

ES - Emulsion stability

GA - Gum Arabic

GF - Gluten-free

GMO - Glycerol monooleate

GMOs – Genetically Modified Organisms

GS - Ginseng saponins

HC-MCC - Highly concentrated micellar casein concentrated

HHP - High hydrostatic pressure

HIUS - High intensity ultrasound

IPSP - Isoelectrically precipitated sweet potato

LGC - Least gelation concentration

MCC - Micellar casein concentrate

MCT – Medium chain triglycerides

MFGM - Milk fat globule membrane

MPC - Milk protein concentrate

MS - Modified starch

MWT - Microwave radiation treatment

PFJ - Potato fruit juice

PPC - Pea protein concentrate

PPI - Pea protein isolate

PWPS - Polymerized whey protein solution

QS - *Quillaja* saponins

RCM - Recombined concentrated milk

SDS - Dodecyl sulphate solution

SN - Sodium caseinate

SPP - Sweet potato protein

SPI - Soy protein isolate

TSC - Tri sodium citrate

UDSP - Ultra-filtered/diafiltered sweet potato

WPC - Whey protein concentrates

WPI - Whey protein isolate

YWPS - Polymerized liquid whey protein

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SECTION ONE

LITERATURE REVIEW

1.0 LITERATURE REVIEW:

Introduction

Nowadays, changes in personal health standards, ethics and sustainability has led to the increased demand for plant-based food or ingredients in food products. Seen this, manufacturers are looking for other plant-based alternatives to use in the food industry to redesign these products formula and serve these new consumers (Chung, et al., 2019).

Emulsifiers are substances with an active surface, their role is the creation of emulsions, facilitating the emulsion formation and promoting emulsion stability. Emulsions are considered to be a dilution of two or more liquids of difficult miscible, some of the food industry type of emulsions are either oil-in-water (O/W) examples of mixtures are milk, creams, salad dressings, mayonnaise and soups; water-in-oil (W/O) margarine and butter as good examples; multiple emulsions in high up systems (W/O/W or O/W/O) or nanoemulsions (used in drug/nutraceutical industry) (Lam & Nickerson, 2013).

In the food industry there are many types of emulsifiers, they can be in a low molecular weight – synthetic (e.g. monoglycerides, sucrose esters, polyglycerol esters) (Lam & Nickerson, 2013) or natural (derived from milk proteins, polysaccharides, phospholipids and surfactants). The choice of an appropriate emulsifier is one of the most important aspect when formatting an emulsion-based product, a better understanding of the molecular basis of the emulsifier is of vital importance for the emulsions creation (Ozturk & McClements, 2016).

An important feature for an effective emulsifier is that proteins need to be quick enough to move to the interface and have the ability to unfold and be absorbed, and consequently form a viscoelastic film to encircle oil droplets, slowing down emulsions coalescence and flocculation (Shevkani, 2015). Another important feature for an effective emulsifier is a low molecular weight, in either synthetic (e.g., monoglycerides, sucrose esters, polyglycerol esters) or natural (e.g., soy or egg lecithin) (Lam & Nickerson, 2013).

Soybean and wheat are historically considered to be the main sources of plant based protein ingredients, meanwhile, milk powder (casein, whey protein) are considered to be the major source of animal based protein used to emulsify mixtures. However, nowadays, all these protein emulsifiers are considered to cause allergic reactions in sensitive human being. The need for more researches on plant based emulsifiers are needed because of the increased concern to dietary restrictions linked to milk and egg protein. Allergic reactions, diseases such as bovine spongiform encephalitis and multidrug-resistant food-borne pathogens are some of the concerns mentioned (Burger & Zhang, 2019). A study carried out for Lam at al. (2013) studied other natural sources of proteins and its possible emulsifying properties. It was analysed ingredients from legumes, such as faba bean, lentil, pea and chickpea, also oilseed and flaxseed crops emulsifying properties.

Table 1

Examples of plant and animal proteins emulsifiers used in mechanistic studies (Lam & Nickerson, 2013)**.**

Protein	Factors investigated	Measuring parameters	References
Soy and wheat gluten	Protein-type, pH, temperature, alteration to pH post-emulsification	Rheology, droplet size	Bengoechea, Romero, Aguilar, Cordobés, and Guerrero (2010)
Wheat (gliadins)	Hydrolysis, pH, ionic strength	Surface hydrophobicity, emulsion activity index, emulsion stability index, foam capacity, foam drainage	Agyare, Addo, and Xiong (2009)
Wheat (gluten), bovine serum albumin, casein	Hydrolysis, ionic strength, pH	Foam stability, foam capacity, creaming kinetics, droplet size, resistance to coalescence	Popineau, Huchet, Larré, and Bérot (2002)
Soy and corn germ protein fluor	Protein type (soy isolate vs. fluor vs. concentrate), pH, temperature	Emulsifying capacity, emulsion stability	Wang and Zayas (1992)
Soy	Temperature	Protein aggregate size, droplet size, colorimetry, interfacial composition, emulsion viscosity, TEM	Keerati-u-rai and Corredig (2009)
Egg	Protein-type (egg yolk vs. whole egg), temperature, addition of sugar, protein concentration, addition of salt (ionic strength)	Turbidity, droplet size, aggregate size (Native-PAGE electrophoresis)	Campbell, Raikos, and Euston (2005)
Egg	Protein-type (egg yolk vs. egg white), addition of xanthan gum, storage time	Droplet size, creaming test, optical microscopy, emulsion viscosity, SDS–PAGE	Drakos and Kiosseoglou (2006)
Egg	Addition of polysaccharide, pH	Surface charge, protein adsorption, droplet size	Padala, Williams, and Philips (2009)
Milk (whey)	Oil concentration, protein concentration, emulsion viscosity, addition of salt, heat treatment	Accelerated creaming test, emulsion viscosity, droplet size	Djordjevic, Kim, McClements, and Decker (2004)
Milk	Protein-type (casein vs. whey), pH modification post-emulsification, addition of salt, heat treatment	Droplet size	Hunt and Dalgleish (1995)
Milk (caseinate)	Preparation method (single step vs. two step), addition of surfactant	Creaming stability, emulsion activity index, droplet size	Einhorn-Stoll, Weiss, and Kunzek (2002)
Lentil	Protein concentration, ionic strength	Interfacial tension, surface hydrophobicity, emulsion stability index, emulsion activity index	Joshi et al. (2012)
Pea	Protein type (commercial preparations vs. isolate) pH, protein concentration	Emulsion stability, foam stability, droplet size	Aluko, Mofolasayo, and Watts (2009)
Pea, fava bean, cowpea, French bean, and soybean	Protein type, protein concentration, ionic strength, comparison of subunits	Thermal stability, SDS–Page, hydrophobicity, protein solubility, emulsion stability	Kimura et al. (2008)
Soy, pea, lentil, chickpea, and faba bean	Protein-type, extraction (isoelectric vs. salt extraction)	Surface charge, surface hydrophobicity, protein solubility, interfacial tension, emulsion capacity, emulsion activity index, emulsion stability index, droplet size, creaming stability	Karaca, Low, and Nickerson (2011a)
Chickpea	Protein extraction (ultrafiltration vs. isoelectric), concentration, protein fractionation)	SDS–Page, interfacial tension, interfacial rheology, aggregate size, droplet size, concentration of protein at interface, emulsion stability, zeta-potential	Papalamprou, Doxastakis, and Kiosseoglou (2010)
Milk (whey), canola and flaxseed	Protein-type, extraction (isoelectric vs. salt extraction)	Emulsion capacity, emulsion stability, creaming stability, emulsion activity index, surface charge, surface hydrophobicity, interfacial tension, protein solubility, droplet size	Karaca, Low, and Nickerson (2011b)
Canola	Protein concentration, pH, ionic strength, addition of hydrocolloid	Emulsion activity index, emulsion stability index	Uruakpa and Arntfield (2005)
Gelatin	Hydrolysis, oil polarity, addition of surfactant, pH	Surface tension, interfacial tension, surface charge, emulsion stability	Olijve, Mori, and Toda (2001)
Gelatin and milk (whey)	Protein content (alone and in mixtures), pH, protein concentration	Surface charge, droplet size, surface protein concentration, surface protein content, emulsion stability, emulsion rheology, optical microscopy, oil oxidative stability	Taherian, Britten, Sabik, and Fustier (2011)

1.1 Protein structure

Proteins are formed by blocks, and twenty different types of amino acids are responsible to build these blocks structure. Nineteen of these amino acids have the same general structure H_2N-CαH(R)-CO_2H, having only group R (side chain) as a divergence between each type of protein. These 20 amino acids are divided in five subclasses, which took into consideration their side chain type. However, some amino acids classification's is not considered absolute, because some amino acids did not fit perfectly in a group, such as Pro. Subclass aromatic is composed by (Phe, Trp, Tyr), nonpolar & aliphatic (Ala, Gly, Leu, Met, Pro, Val), polar & uncharged (Asn, Cys, Gln, Ser, Thr), positively charged (Arg, His, Lys) and negatively charged (Asp, Glu) (Yada, 2017).

Table 2

Properties and occurrence of the common amino acids found in dietary proteins

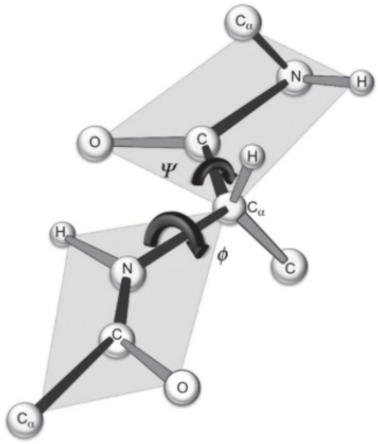
Type of side chain (R)	Amino acid	Abbreviation	Symbol	Mass (Da)[a]	pI[b]	pK_a (R group)[c]	Residue nonpolar surface area (Å^2)[d,e]	Estimated hydrophobic effect, side chain burial (kcal/mol)[d]	Percentage with solvent exposed area[d,f]		Frequency in proteins[g] (%)
									>30Å^2	<10Å^2	
Aromatic	Phenylalanine	Phe	F	147.18	5.48		39+155	2.3	42	42	4.2
	Tryptophan	Trp	W	186.12	5.89		37+199	2.9	49	44	1.1
	Tyrosine	Tyr	Y	163.18	5.66	9.7	38+116	1.6	67	20	3.2
Nonpolar, aliphatic	Alanine	Ala	A	71.09	6.00		86	1.0	48	35	8.1
	Glycine	Gly	G	57.05	5.97		47	0.0	51	36	7.2
	Isoleucine	Ile	I	113.16	6.02		155	2.7	39	47	6.8
	Leucine	Leu	L	113.16	5.98		164	2.9	41	49	10.3
	Methionine	Met	M	131.19	5.74		137	2.3	44	20	2.5
	Proline	Pro	P	97.12	6.30		124	1.9	78	13	5.1
	Valine	Val	V	99.14	5.96		135	2.2	40	50	4.3
Polar, uncharged	Asparagine	Asn	N	115.09	5.41		42	−0.1	82	10	4.0
	Cysteine	Cys	C	103.15	5.07	9.0–9.5	48	0.0	32	54	1.2
	Glutamine	Gln	Q	128.14	5.65		66	0.5	81	10	3.8
	Serine	Ser	S	87.08	5.68		56	0.2	70	20	6.2
	Threonine	Thr	T	101.11	5.60		90	1.1	71	16	5.1
Positively charged	Arginine	Arg	R	156.19	10.76	12.0	89	1.1	84	5	5.1
	Histidine	His	H	137.14	7.59	6.0–7.0	43+86	1.3	66	19	2.2
	Lysine	Lys	K	128.17	9.74	10.4–11.1	122	1.9	93	2	5.9
Negatively charged	Aspartic acid	Asp	D	114.11	2.77	3.9–4.0	45	−0.1	81	9	5.2
	Glutamic acid	Glu	E	129.12	3.22	4.3–4.5	69	0.5	93	4	6.5

[a] Average mass of the amino acid (from National Institute of Standards and Technology Chemistry WebBook, 2016) minus the mass of a water molecule (18.00 Da).
[b] From Haynes (2015).
[c] From Creighton (1993).
[d] From Karplus (1997) and the Leibniz Institute on Aging—Fritz Lipmann Institute (2016a).
[e] The aliphatic and aromatic surface areas are reported separately for the amino acids with an aromatic side chain.
[f] The percentages of each residue with solvent exposed area >30Å^2 or <10Å^2 were calculated based on 55 proteins in the Brookhaven database using solvent accessibility data from Bordo and Argos (1991).
[g] From Jordan et al. (2005).

The primary protein structure relates to the sequence of amino acids within the protein chain, starting from the N-terminus to the C-terminus, as shown below. Proteins diversity arises because each site in the primary sequence can be occupied by one of the 20 amino acids (Yada, 2017).

Fig 1

Represents a segment of a peptide chain (Cα-CO-NH-Cα), the grey area highlights the peptide plane and the backbone of the chain is represented by the black links (Yada, 2017).



The distinctive and interesting properties of recently discovered naturally occurring amino acid, selenocysteine, has been mentioned Yada., (2017) because of the improvement it can provide to some food systems. Selenocysteine is a coded amino acid, mainly identified in proteins or peptides such as glutathione peroxidase and iodothyronine deiodinase.

Whey Protein

Whey protein isolate (WPI) is a product of cheese production and has been used as a natural emulsifier in the food and beverages industry, improving the formation of oil-in-water emulsions (Shiroodi, et al., 2015). It basically constitutes β-lactoglobulin, α-lactalbumin, bovine serum albumin and immunoglobulin. These amino acids form an amphiphilic protein, which in emulsions are responsible for emulsion stabilization and prevention against flocculation. Bai, et al. (2019) showed that emulsions stabilized by WPI are largely affected by changes in pH and ionic strength, as well as, thermal treatment.

Soy Protein

Soybean is basically composed 40% protein and 20% oil. Soy protein isolate molecule is obtained by removing oil at lower temperatures, its natural structure is mainly formed by a mixture of proteins. These proteins are classified according to their sedimentation levels, known as globulins and albumins, which have a globular structure (Nishinari, et al., 2014). This globular structure is directly related to low molecular flexibility, insufficient interfacial and emulsifying properties. However, it's well known that many different technologies are used in soy protein to modify their structure and molecule aggregation, aiming to improve the functional properties (Huang, et al., 2019).

Pea Protein

Pea seeds are a great source of protein, pea protein concentrates are normally composed by 3-10% carbohydrate, 0.5-3,5% lipid and 4-9% moisture. They contain approximately 20% - 30% proteins of different types, which mainly include globulins (65%-80%) and albumins (10%-20%). Legumin and vicilin are the two main globulin's portion. Globulins are also considered to be the storage proteins for the seeds. Legumin protein has a hexameric structure, with a higher molecular weight (320-400kDa) when compared to vicilin (150-200kDa), composed six subunits, each subunit containing an acid and a basic chain linked for a disulphide bond. Albumins are considered to be metabolic and enzymatic proteins, an easy to dissolve in water emulsions. Differences between the species and methods of productions can lead to a variation in proportions of globulins to albumins and or legumin to vicilin, and this can cause differences in functionality. An important example in this case is, a higher content of globulins can lead to improved emulsifying properties (Burger & Zhang, 2019).

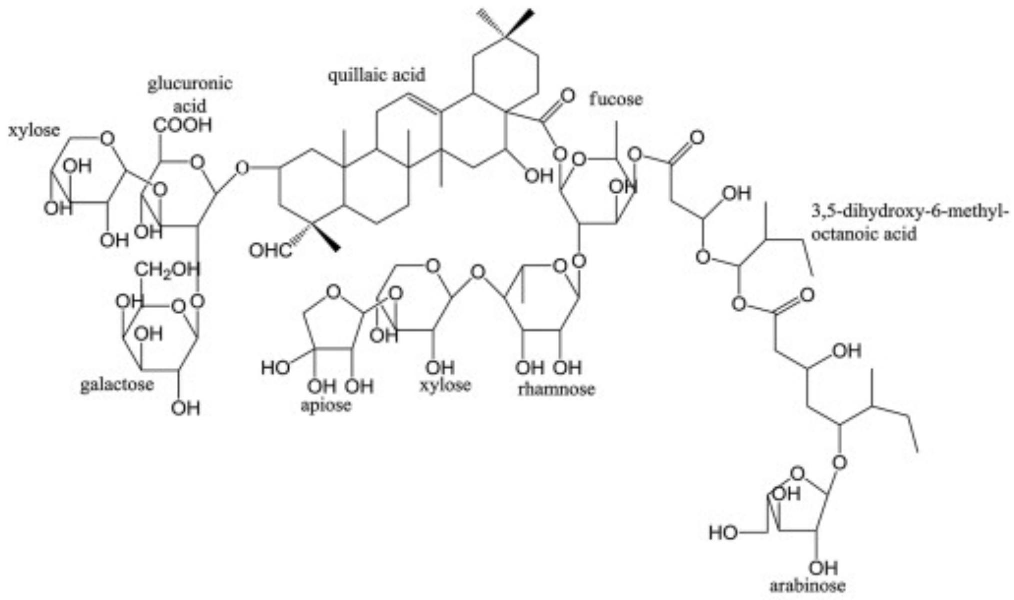
Tea saponin/ Quillaja saponin

Saponins are glycosides of high molecular weight, they are extracted from the bark of the Quillaja saponaria Molina tree. It's active surface components is able to form surfactant micelles and to stabilize oil-in-water emulsions. Saponin's surface structure are considered active because it consists of both hydrophilic (such as rhamnose, xylose, arabinose, galactose, fucose, glucuronic acid) and hydrophobic chemical groups (such as quillaic acid and hypsogenic acid) on the same molecule.

The commercial form of Quillaja saponin is Q-NaturaleÒ, and it has been widely studied for the use in the food industry. This ingredient contains a variety of surface active components which will lead to different structure and functionalities when compared to other molecules (Yang, et al., 2013).

Fig 2

Structural representation of Quillaja saponin. Natural saponins have a wide variety of chemical structure, and different surface activity (Yang, et al., 2013).



Potato Protein

Food security is being confronted by the rapidly growth of the world population, the need for a broaden variety of food proteins in the food industry has led to exploration of new and sustainable sources of food proteins. Potato protein isolate is found as a by-product of potato starch production, named potato fruit juice (PFJ), which is usually composed by solid matter of which other substances such as protein, peptides and amino acids. There are three main groups of potato proteins, representing up to 40% is the glycoproteins patatins, the second group representing up to 50% of the potato proteins is the protease inhibitors. The last group is formed by oxidative enzymes, such as lipoxygenase, polyphenol oxidase and enzymes associated with starch synthesis (Schmidt, et al., 2018).

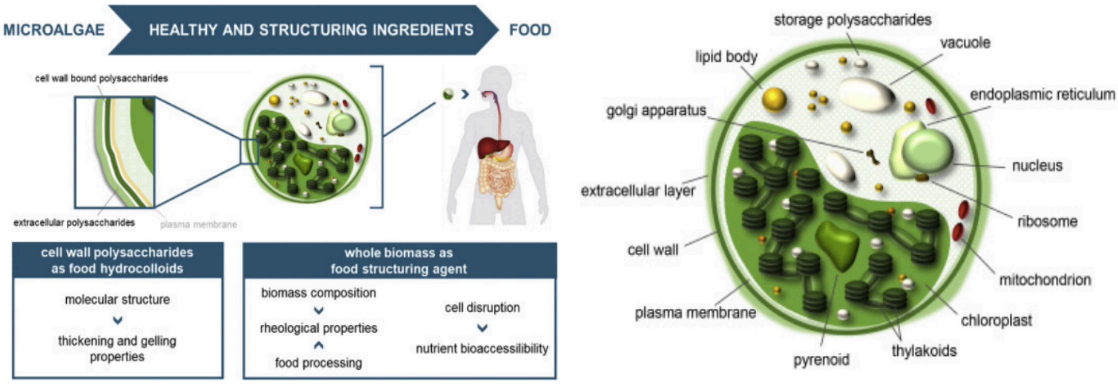
Potato protein is considered higher quality compared to other vegetable and cereal proteins sources as they contain a greater amount of lysine, which is considered of major importance and is not highly found in such crops. However, the industrial extraction methods have been a challenge because of the aqueous nature of PFJ and its multifaceted composition. Thermal coagulation and acidic precipitation are the industrial methods used in the food industry, the result in a high yield of protein recovery, though often leading to a large loss of protein functionality, which difficult their application (Waglay, et al., 2014)

Microalgae

Microalgae structure is composed by biopolymers, such as proteins and carbohydrates. These biopolymers might have potential role as texturizer, stabilizer or emulsifier in food products. Microalgae is considered to be a sustainable source of food thickeners. Polysaccharides are considered to be a common thickening and gelling agent used in the food industry, however, there is a lack of studies on the microalgal cell wall functionality related to polysaccharides, and this is due to the large diversity and complexity of their cell molecular structures (Bernaerts, et al., 2019).

Fig 3

Representation of a eukaryotic microalgal cell. In certain microalgae species the organelles can be differently organized, or even absent (Bernaerts, et al., 2019).



A wide diversity of microalgae biochemical composition has been identified, this is due to drastic changes on the biomass profiles. Hence, a variety of literature data is found approaching microalgae composition. Microalgae possess a range of 9-77% of proteins, 6-54% carbohydrates and 4-74% lipids. It is also mentioned that their biomass profiles can be affected by changing cultivation conditions, such as light intensity, temperature and nutrient availability (Bernaerts, et al., 2019).

1.3 Proteins emulsifying properties

Proteins are known in the food industry also because of its emulsifying properties, which are highlighted due to its amphiphilic nature. Having an amphiphilic structure, means that they contain hydrophobic and hydrophilic groups along the chemicals compounds chain. These groups are responsible for embracing the molecules that usually wouldn't format a stable emulsion. The understanding of the term "emulsion capacity" is defined as the ratio in grams of oil needed per grams of protein that can be helped before a phase inversion (Lam & Nickerson, 2013) Emulsion stability (ES) is a measure of the amount of creaming over time (Burger & Zhang, 2019).

Proteins are high molecular structure, if compared to other emulsifiers, such as phospholipids, they tend to diffuse to the interface in a much slower rate. This characteristic interfere to their emulsion forming stability. However, once they get to the interface a partial denaturation occurs in order to expose buried hydrophobic amino acids, in sequence, the hydrophobic amino acids position their surface within the oil phase and the hydrophilic amino acids re-align with the aqueous phase. Seen this, the emulsion capacity lead to be lower than with the smaller weight molecules (Lam & Nickerson, 2013).

When determining protein's emulsifying characteristics, a main aspect that needs to be taken in consideration is their physiochemical properties. An important factor that influences proteins absorption in the oil side of the interface, is the surface hydrophobicity. This will lead to a better molecule integration, hence a higher emulsion stability. Surface charge of the protein is another factor that influences protein's solubility in the aqueous phase, because a high protein solubility is required for a better diffusion ratio to the interface (Lam & Nickerson, 2013).

1.4 Methods to measure emulsifying properties

Burger & Zhang (2019) refers to the large variety of methods used to measure emulsifications properties of proteins, such as turbidimetric techniques, conductivity and droplet size measurements, and relates to the reason why they were never comparable in studies. Turbidimetric techniques, which include Emulsifying Activity Index (EAI) and Emulsifying Stability Index (ESI) is responsible to measure the ability of the protein be absorbed to the interface, meanwhile EAI measures the absorbed layer's stability within a determined period of time.

Dynamic light scattering is another method to measure emulsion particle size or droplet diameter. It's known that the smaller the droplet size is and the narrower the size distribution, the better the emulsifying properties. The system which the droplet size distribution is measured is based on water, buffer systems or within 1% w/v sodium dodecyl sulphate solution (SDS). SDS has the ability to interrupt oil droplets flocculation by moving protein molecules from the interface, this allows the measurement of each droplet size (Ettoumi, et al., 2016).

It was concluded that this large variety of parameters are not comparable between studies because of the various existing measuring protocols, emulsions preparation/processing techniques and different proteins processing standards, which lead to the lack of compatibility (Burger & Zhang, 2019).

1.5 Protein absorption process

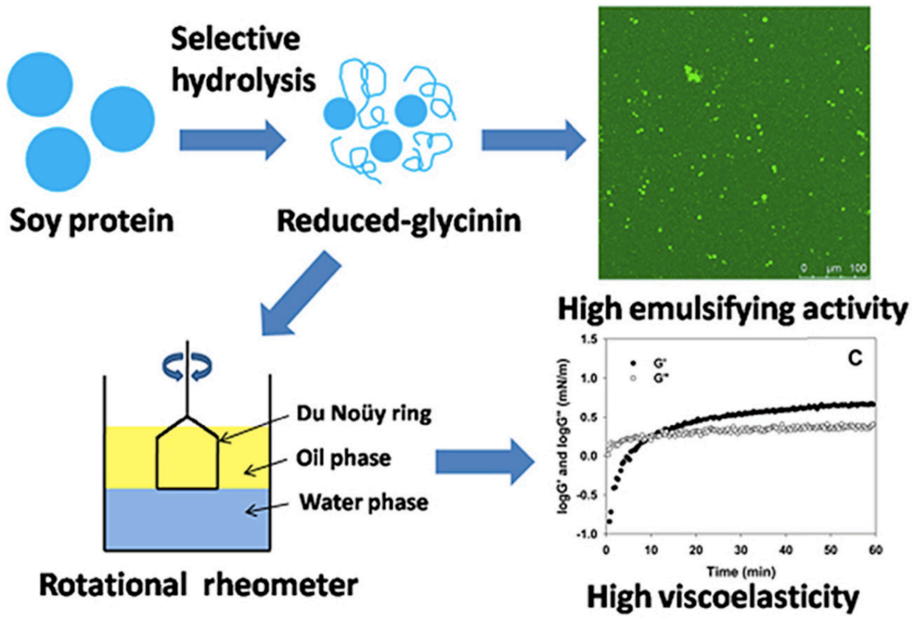
Protein absorption is a complex process, it's of vital importance in the study of protein stabilized emulsion, to analyse the interfacial viscoelastic behaviour and understand protein charge, hydrophobicity, structural flexibility or molecular weight, these aspects are of most importance for interfacial absorption (Li, et al., 2016).

Li et al., (2016) describes the protein absorption process of soy protein and specify three main steps, protein diffusion, formation of the protein monolayer and interfacial gelification. Protein diffusion is a process where the interfacial tension and complex viscosity does not go under changes for a period of time and can only be identified for a dilute protein solution. The following step is when the formations of the protein monolayer occurs, which include the processes of structural deformation, interfacial tension reduction and the rapid increase in complex viscosity. Third and last step of protein absorption, specially for globular proteins is the interfacial gelification. This step is when the multilayer is formed, with the aim to increase the complex viscosity a slight reduction in the surface tension is applied.

Li et al., (2016) also describes other processes that have been confirmed to be efficient for protein absorption.

Fig 4

Represents the process of soy protein hydrolysis, improving emulsifying and viscoelastic properties (Li, et al., 2016).



1.6 Protein-stabilised emulsions

-Milk protein

The nutritional and functional properties of the milk proteins are very important factors for many different processed foods. Their chemical structure, configuration, and aggregation state in milk protein food products has the capacity to stabilize the oil droplets in oil-in-water emulsions (A. Taneja, 2015).

Milk provides the ideal nutrition to newborns because it is a natural fluid produced by mammalians. It contains many functional proteins, such as casein, whey protein immunoglobulins, lactoferrin and α-lactalbumin which help infants to have a stronger immune system and prevent infectious diseases. Whey protein, in the past was produced as a waste from cheese production, and plays an important role in the immune system especially because of amyloid A protein, and fatty acid-binding protein acting as a transporter plasma fatty acids. Another component important milk component is Lysozyme, a protective antimicrobial factor which improves intestinal health of people with low immunity system and inhibitory effect on the proliferation of tumour cells (Weixuan, et al., 2019).

There are many different types of milk protein products currently available as food ingredients, whey protein concentrates and isolates (WPCs, WPIs), milk protein concentrate (MPCs), caseins and caseinates (A. Taneja, 2015). Other frequently used proteins in the food industry as emulsifiers also include ovalbumin and bovine serum albumin(Lam & Nickerson, 2013).

Milk proteins are composed of polypeptide chains with amphiphilic nature, they contain hydrophilic and hydrophobic amino acids, for this reason they can be absorbed by oil-water emulsions and help the homogenization process encompassing oil droplets. Protein molecules are dense and it tend to be thick and diffuse in slower proportion if compared to other small molecular emulsifiers (Lam & Nickerson, 2013).

Fig 5

Represent (A) the migration of globular proteins to a water-oil interface, (B) shows the reorientation process and (C) the formation for the viscoelastic film.

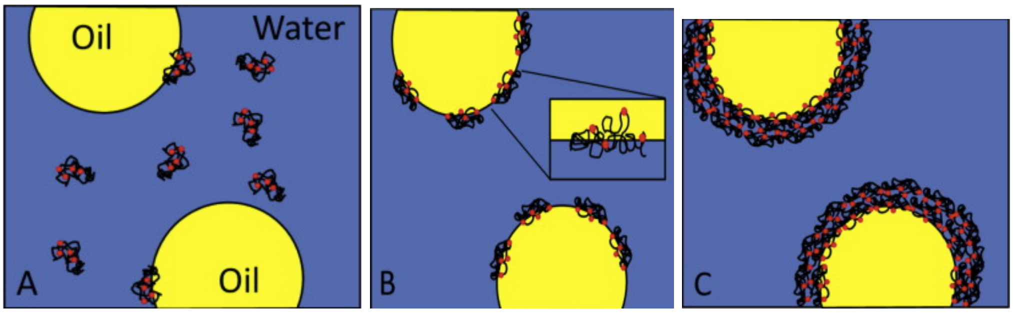
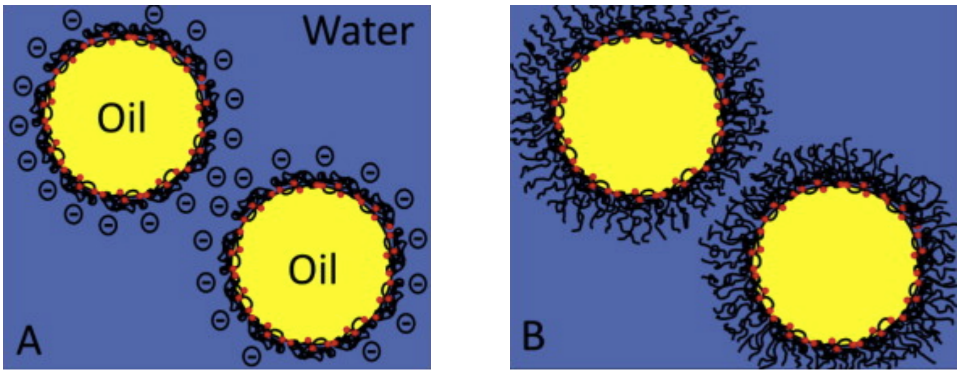


Fig 5.1

Expose in (A) the mechanisms for emulsion stability and eletrostatic repulsion and in (B) steric stabilisaton.



Red dots in bths figures 4 and 4.1 represent hydrophobic moieties found in proteins.

The emulsifying properties of these proteins derived from milk rely on their physiochemical properties, the hydrophobic group influences the oil side absorption, leading to a better interaction rate and higher emulsion ability. Whereas, surface charge with in the aqueous phase, is another important aspect that influences milk protein solubility and stability. It is noted that a high emulsion solubility is needed for an efficient diffusion rate (Lam & Nickerson, 2013).

The interest of the food industry on the use of partial hydrolysed protein to enhance emulsions stability, are increasing researches on the field. Proteins derived from milk, soy and wheat have been studied, and also the partial hydrolysis properties of these ingredients. The main purpose to study the effects of partial hydrolysis is to evaluate if these proteins are providing better emulsifying properties and increasing emulsions solubility. The functionality improvement of hydrolysed protein increased the use in the food industry, this is because of reappearance of hidden hydrophobic groups which increases the surface hydrophobicity, reducing its molecular weight, enabling better oil-water interface adherence (Lam & Nickerson, 2013).

A study conducted by Loi et al., (2019) analysed the effects different milk protein compositions would give on creaming stability, other aspects such as physicochemical properties and oxidative stability of the emulsions were also investigated. Five different protein compositions were prepared, and individualities such as droplet size, zeta potential, viscosity and creaming index were used to characterise physicochemical properties of sodium caseinate, whey protein concentrate (WPC) and glycerol monooleate (GMO). Results revealed that emulsions with only sodium caseinate showed better emulsions stability and viscosity when compared to emulsions with only WPC, however protein composition had no significant relationship with creaming stability. Emulsions with mixed proteins types, such is sodium caseinate and WPC provided better oxidative stability when compared to emulsions with only a single type. GMO mixed with other proteins showed stable emulsions formation, effective physicochemical properties and oxidative stability.

The rheological properties of fresh and reconstituted milk protein concentrates were compared by Kieferle et al., (2019), as the processability of fresh and reconstituted milk essentially depend on their rheological properties, it's important to investigate the differences in viscosity prior usage. The results showed that reconstituted milk protein concentrates had higher viscosities and particularly more elastic properties than fresh milk samples, at the same protein concentration. Furthermore, it's important to consider the intense shearing because it showed a decrease in the viscosity and on the particle size of the reconstituted milk protein concentrate. These facts confirm the hypothesis that thermal burden during milk protein powder production, as well as, storage conditions and duration can significantly influence the reconstituted milk protein concentrate microstructure and hence, rheological properties.

An investigative study was conducted by Fang & Guo (2019), this study aims to evaluate the usage of polymerized whey protein solution (PWPS) prepared from cheese production on providing stability on yogurts and analyse the physicochemical, texture properties and microstructure. The four yogurt samples were evaluated by 14 quantitative descriptive analysis method and 14 sensory attributes. The results indicated that polymerized liquid whey protein (YWPS) showed significant improvement in viscosity, texture and syneresis when compared with the control yogurt sample, proving that polymerized whey protein solution (PWPS) prepared from cheese production may be an effective thickening agent for yogurt solution.

CASEIN EMULSIFYING PROPERTIES

The food industry has a great interest on casein's functional properties, however, casein's micelle capacity to emulsify and stabilize oil-in-water emulsions is of most significance on the food industry interests. Casein's micelle is a highly aggregated colloid of 150-200 nm diameter and its structure comprises of phosphoproteins (molecules of αs_1, αs_2, β, κ) and also minerals (calcium phosphate) which are responsible to ensure their colloidal stability. Casein's micelles are known to be responsible, especially in dairy products, to contribute to their functional properties, an important role is the ability to form or to stabilize gels, foams and emulsions samples (Lazzaro, et al., 2017).

Chemical nature, conformation at the interface and aggregation state of caseins are directly linked with its emulsifying and stabilizing capacity. Caseins are known to be absorbed at the interface, in individual or aggregated form and are able to strongly perform emulsifying agent role. Sodium caseinate is an example of poorly aggregated casein system, which are less effective for the stabilization of emulsions than highly aggregated casein micelles (Lazzaro, et al., 2017).

The emulsifying property and packing behaviour of casein gel particles at oil-water interface study was accomplished by Wang et al., (2018) with the aim to create a food-grate soft gel particles that stabilize oil-in-water emulsions and compare to caseins performance as a Pickering stabilizer properties. Casein gel particles ability to stabilize the oil droplet was assessed with Turbiscan analysis, taking in consideration creaming, coalescence and flocculation. Sodium caseinate ((CN) was used as the control. The gel particles were casein sub-micelles (CN-GP) and calcium-induced casein micelles (CN-Ca-GP) with genipin. The microscopy results observed that the oil droplets stabilized by CN flocculated, whereas the ones stabilized by CN-GP or CN-Ca-GP were stable against flocculation. Oil droplets stabilized by CN-GP or CN-Ca-GP showed higher stability against creaming when compared to emulsions stabilized by CN. To summarize, casein gel particles are considered to be encouraging ingredients for food emulsion-based products.

Milk fat globule membrane (MFGM) act as an effective natural emulsifier because of its polar lipids and proteins structure, its amphiphilic properties will provide efficiency as stabilizer of fat globules membranes and act as an effective food emulsifier. MFGM can bring technological, nutritional, biological functions and bring antimicrobial and antiviral activities, contributing with gut physiology health and with the development of central nervous system in newborns. Seen this, Jukkola et al., (2019) evaluated the effectiveness of MFGM derived from buttermilk as a food emulsifier, and the interactions with calcium and casein phosphoproteins. The results revealed that MFGM fragments proved to be an efficient substitute of a commercial available phospholipids (lecithin), providing similar functional and emulsifying properties. MFGM showed better emulsion stability on pH above 6, when compared to lecithin in the pH range between 5.8 and 7.8. When casein was added in the emulsion, the emulsion stability increased forming droplet flocs. MFGM carries calcium-binding ability and it inhibit protein (casein) flocculation, providing an effective tool to prevent phase separation and stable MFGM-based food emulsions.

Research conducted by Lazzaro et al., (2017) investigated and compared the effects of gradual disaggregation of the casein micelle on their structure, composition, charge, hydration, emulsifying and stabilizing capacity in dairy emulsions samples. Tri sodium citrate (TSC) was used to gradually demineralize CA, taking calcium and inorganic phosphate from pure casein micelles. Four suspensions of different demineralized casein aggregates (CAs) were used, and to control the diffusible phase, dialysis was the method used on each suspension. The demineralization process is progressively done, from 24 to 81% calcium reduction and associated to TSC increased concentration. CAs after been physico-chemically characterized were used to generate two types of milkfat-in-suspension (30:70 v/v), in order to finally analyse, separately, their emulsification and stabilization properties.

The results were divided in two stages, first focused on the physico-chemical and colloidal characteristics of the CA's and second functional properties, highlighting their use as emulsifying agents in two types of dairy emulsions samples. Three different types of micelle-like aggregates particles were identified in different proportions in every suspension, they are micelle-like aggregates, sodium caseinate-like aggregates and casein monomers. The results revealed differences in emulsifying and emulsion-stabilizing properties. The presence of small casein aggregates influenced the formation of emulsions with smaller droplet sizes, hence presenting better emulsifying capacity. The evaluation of surface activity of the four CA revealed similarity and the only variations observed was related to their aggregation state, which influences their emulsifying capacity. The stabilizing capacity was influenced by unstable storage conditions (during 21 days, at 50 °C), however all emulsions kept stable, with no occurrence of coalescence during storage. Large droplets influenced the occurrence of creaming and flocculation (which was also influenced by the presence of small demineralized CAs). The formation of emulsions was not influenced by the aggregation state of the CAs and neither by the properties of the interfacial casein layers. Finally, the different levels of emulsions instability were related to the nature of the non-absorbed CAs and storage conditions (Lazzaro, et al., 2017).

The impact of high intensity ultrasound (HIUS) pre-treatment on the functional and structural properties and characteristics of micellar casein concentrate (MCC) was studied by Zhang et al., (2018). The aspects analysed were conductivity, solubility, emulsifying, gelling and structural aspects. The results revealed that ultrasound pre-treatment significantly improved functional properties of MCC, such as conductivity, solubility, emulsifying, gelling properties increased as the ultrasonic time extended, without significant changes on pH. In addition, an increase in surface hydrophobicity, reduction in particle size and structural characteristics of MCC was observed, compared to the control samples. To sum-up, when HIUS is used on MCC as a treatment, is confirmed the potential be used in food industry application.

- Emulsifying properties of plant-based protein

- **Pea protein**

Pea is part of the legumes family, it's rich in starch and protein and contain about 50% of starch and 20% to 30% of protein. Peas are largely used to produce pea starch products and as a by-product pea protein products are produced, which is nutritionally rich in essential amino acids (Chen, et al., 2019). It's rich nutritional value, provides a good source of yield bioactive peptides, which offer antioxidant activity and angiotensin I providing health benefits. Pea protein isolate have been comparable to soy protein because of its comparable emulsification properties. However, pea protein brings the advantage of being non-allergenic (Burger & Zhang, 2019).

The pea *Pisum sativum L.* protein specie it's been largely studied because of its nutritional functionality properties, readily availability and low allergen conditions. A study conduct by Burger & Zhang (2019) focused on a review of the current understanding and characterizing pea protein's use as an emulsifier. Pea proteins emulsifications properties were compared to other protein emulsifiers, conditions such as, temperature, pH and ionic strength were taken in consideration. Also some physicochemical pea protein's properties were analysed, as solubility, surface activity, hydrophobicity and composition.

A study conducted in 2019 analysing the emulsifying and stability properties of pea proteins, highlighted some extraction methods to obtain less denatured proteins with better solubility, emulsifying and foaming properties. The most used methods to extract and produce pea proteins are alkali extraction, acid precipitation and spray drying, and to obtain less denatured protein methods such as salt extraction-dialysis and micellar precipitation are used (Chen, et al., 2019).

The emulsifying characteristics of pea protein is one of their most important functional properties, and its need in the food industry as a plant-based emulsifier will broaden research. Many factors can influence pea protein emulsifying properties, such as heat treatment which can expose the hydrophobic groups in the protein molecules, hence leading to an increased protein aggregation, and better emulsifying properties of soy protein isolate. Protein molecule structure, chemical composition, pH, environmental conditions, ionic strength and the volume fraction of oil phase also contribute with the emulsions stability and absorption (Chen, et al., 2019). Thus, the commercial utilization of pea protein as emulsifier is still limited. The lack of knowledge on its structural and functional properties, and a large gap between commercially used pea proteins and the pea proteins prepared in laboratory (Burger & Zhang, 2019).

Vicilin is a globulin associated with legumin and in peas is used for protein storage. Studies have identified Vicilin and legumin important effects on the emulsifying properties of peas, however the pea protein richer in vicilin revealed better emulsifying properties (Chen, et al., 2019).

Chen et al., (2019) evaluated and compared two different pea protein, its concentration in relation to emulsifying ability and stability. In addition, the percentage and composition of absorbed proteins was also evaluated, the aim was to understand the competitive relationship of interfacial absorption between the two different types of pea proteins.

Chen et al., (2019) concluded that higher protein concentration improves emulsifying ability and stability, and that temperature also influences on the emulsions solubility. When the temperature was under 50°C, both proteins showed poor solubility, however when the temperature increased to 90°C, pea protein I (Nutralys, S85F) had better solubility than pea protein II (F85M). Another element observed is the effects of heating on the surface hydrophobicity value, which is important because it improves the protein absorption to the interface and is directly related to better emulsifying properties. The result revealed that the protein that was extracted at 90°C had the highest surface hydrophobicity value.

Tamanak et al., (2016) mentioned that pea proteins when compared to non-soy legume proteins (i.e. chickpea, faba bean), has poor solubility, limiting its emulsifying properties. It happens because of its low surface charge density, a more hydrophobic surface structure, leading to a relative poor solubility. Tamn et al., (2016) connects pea proteins poor solubility with their complex molecular structure, their globular form which may limit emulsification power and for having a compact tertiary structure, restricting the molecular flexibility.

Table 3

Shows the emulsifying properties of pea protein compared with other protein source (Burger & Zhang, 2019).

Comparison Material	Homogenization method	Pea protein source	Core material	Pea protein results	Reference
Soy	HSM 20,000rpm	Commercial	Canola oil 10%	Smaller droplets	Aluko et al. (2009)
	Microfluidizer 1000bar	Commercial	MCT 10%	Smaller droplets	Amine et al. (2014)
	Microfluidizer 2070bar	Commercial	Rapeseed 10%	Smaller droplets	O'Sullivan et al. (2016)
	Homogenizer 550bar	Commercial	CLA 20%	Larger droplets	Fernandez-Avila et al. (2016)
	Ultrasound 1min	7S Vicilin	Soybean oil 14%	Larger droplets	Kimura et al. (2008)
	HSM 12,000rpm	Commercial	Sunflower oil 25%	Lower EAI	Fuhrmeister and Meuser (2003)
	HSM 12,000rpm	AE-IP	ND 25%	Lower EAI	Tömösközi et al. (2001)

Legumes	HSM 20,000rpm	AE-IP and UF	Corn oil 25%	Similar EAI	Boye, Aksay et al. (2010)
	Homogenizer 400bar	Commercial	Sunflower oil 10%	Similar droplet size	Ladjal-Ettoumi, Boudries, et al. (2016)
	Homogenizer 400bar	AE-IP	Sunflower oil 10%	Similar droplet size	Ladjal-Ettoumi, Boudries, et al. (2016)
	HSM 7200rpm	AE-IP and SE	Flaxseed oil 50%	Similar EAI	Karaca et al. (2011)
	HSM 9500rpm	AE-IP and UF	Corn oil 50%	Similar droplet size	Makri et al. (2005)
	Homogenizer 500bar	Flour	Corn oil 2.5%	Similar droplet size	Tsoukala et al. (2006)
	HSM 20,000rpm	AE-IP	Sunflower oil 25%	Higher ESI, lower EAI	Shevkani et al. (2015)
Egg, whey	HSM 7200rpm	Commercial	Canola oil	Lower EC	Stone, Avarmenko, et al. (2015)
Potato, milk	Homogenizer 1000bar	Commercial	MCT 10%	Larger droplet size	Amine et al. (2014)
Casein	Homogenizer 1380bar	Commercial	Canola oil 5%	Lower stability	Yerramilli et al. (2017)
Casein, albumin	HSM 20,000rpm	Pea Vicilin	n-dodecane 33%	Higher EAI	Rangel et al. (2003)

*HSM: high speed mixer; MCT: medium chain triglyceride; CLA: conjugated linoleic acid; EAI: emulsion activity index; AE-IP: alkaline extraction isoelectric precipitation; ND: core material wasn't disclosed; UF: ultrafiltration; SE: salt extraction; ESI; emulsion stability index.

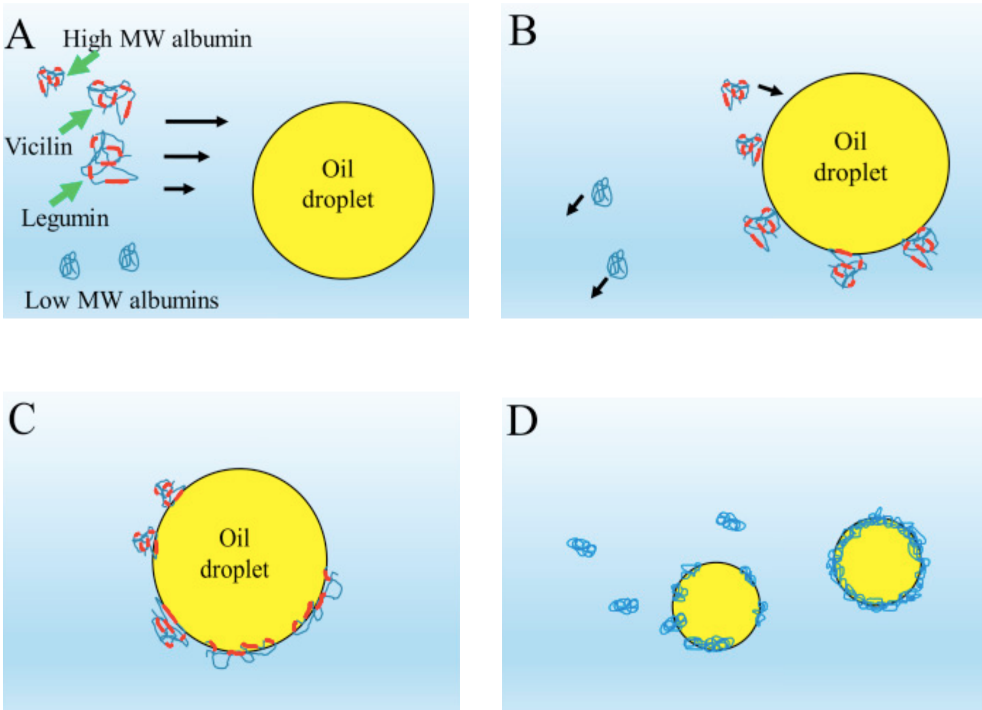
Researches have been done with the aim to modify physical, chemical and enzymatic modifications in pea proteins structure, so their emulsifier properties would be increased. A common modification successfully done was to add a polysaccharide to the emulsion, which caused the increase of the surface net charge and created a steric hindrance, stabilizing the emulsion (Liu et al., 2012).

Stone et al., (2015) demonstrate a comparative study between pea protein and non-legume protein sources, stablishing emulsion stability (ES) and emulsion capacity (EC) values. The results were: pea protein compared to soy protein had similar emulsion capacity (EC), and both were better than wheat’s protein emulsion capacity (EC).

Nevertheless, egg protein and whey protein had a better performance relating to emulsion capacity (EC). The emulsion stability of pea protein was also reduced when compared to egg, soy, and whey protein. Based on the results above, pea protein is inferior to traditional emulsifiers.

Fig 6

Characterization of the behaviour of pea protein at the oil-water interface, where Fig (A) represents migration, Fig (B) adhesion, Fig (C) partial denaturation and reorientation and Fig(D) viscoelastic film formation at the interface.



The incubation of pea protein isolate with gum Arabic was studied by Zha et al., (2019) and the investigation of the impact of incubation time on the properties and functionalities between pea protein isolate (PPI) and gum Arabic (GA). To evaluate conjugation reaction degree of PPI-GA conjugated, the formation of Maillard reaction products, the loss of free amino groups and colour changes were taken in consideration. The results showed that emulsions stabilized by PPI and gum Arabic conjugates provided improved physical stability, and enhanced protein solubility. In addition, samples of corn oil-in-water emulsions stabilized by PPI-GA conjugates with one day incubation showed better stability against environmental stress, when compared to the ones prepare with PPI or PPI-GA mixture. PPI-GA conjugated emulsions resulted in a smaller particle size, higher surface charge and stronger steric hindrance to stabilize the emulsion droplets against environmental stress and lipid oxidations. To sum-up, poor soluble plant proteins can be modulated, improving their solubility and functionality if the Maillard reaction with polysaccharides is controlled.

The emulsifying properties and interfacial properties of mildly fractioned yellow pea were evaluated and compared to regular pea protein isolate by Geerts et al., (2017). Oil-in-water emulsions were kept under acidic pH, acceleration forces and freeze-thaw treatment to maintain stability. It was found that mildly fractioned proteins were able to form strong and viscoelastic layer on the interface, hence giving protection against disruption and high compressive forces. Emulsions stabilized by mildly fractioned proteins showed less emulsion flocculation and coalescence. To summarize, the study concluded that yellow pea mild fractionation leads to a rich fraction of protein, and this fractions will improve its emulsification properties, therefore, the proteins state rather than the purity directly influences emulsification properties.

A study conducted by Walia & Chen (2020) analyse pea protein nanoemulsions properties with the aim to evaluated vitamin D absorption and emulsion stability using Caco-2 cells, with the aim to develop novel non-dairy functional foods for vitamin D fortifications. Nanoemulsions were prepared using high-pressure homogenizer. Caco-2 cells studied the influences that surfactants, such as pea protein, soy lecithin and protein-lecithin combination on emulsions particle size and cell acceptance. The results exposed that protein-based nanoemulsions exhibited higher cellular acceptance than emulsions prepared with a mixed of protein and lecithin. Overall, nanoemulsions stabilized by pea proteins have demonstrated potential to enhance vitamin D absorption and a cell uptake comparable to emulsions stabilized with lecithin.

Foaming and structure properties of pea protein isolate (PPI) was analysed after the use of high intensity ultrasound (HIUS) by Xiong et al., (2018). The effects of HIUS used in 20kHz at varying amplitude 30%, 60% 90% for 30 min was studied, and the results revealed HIUS induced protein molecular unfolding, decreased PPI particle size and increased exposed hydrophobicity, hence decreasing surface tension at air-water interface. Furthermore, HIUS increased PPI foaming ability and foaming stability, suggesting that HIUS treatment provide a potential to be used to improve on foaming properties of PPI.

The study of blending soluble pea proteins with dairy proteins with the aim to evaluate emulsion stability was conducted by Hinderink et al., (2019). Blends of pea protein isolate (PPI) with whey protein isolate (WPI) or sodium caseinate (SC) were tested to physically stabilise emulsions prepared by high pressure homogenization. The samples were evaluated on droplet size, emulsion stability, surface load and interfacial compositions. The droplet size and surface load were the lowest for SC and WPI-stabilised emulsions, with PPI having the highest. Whereas emulsions that were stabilized by protein blends, revealed intermediate droplet size values and surface loads. Emulsions stabilized by WPI-PPI or SC-PPI blends produced stable emulsions, implying synergistic reactions reflecting stability over a longer period of time, if compared to emulsions stabilized with only PPI or SC.

Partial replacement of sodium caseinate with pea protein isolate was assessed by Yerramilli et al., (2017) with the aim to evaluate possible improvements on emulsion stabilization. Emulsions were created with mixed pea protein isolate (PPI) and sodium caseinate (SC). The presence of only SC- stabilized nanoemulsions lead to creaming because of depletion flocculation by protein excess, also PPI by itself also failed when producing stable nanoemulsions. However, the presence of both proteins at the oil droplet interface did not display any creaming and remained stable for more than 6 months. When pea protein aggregate interacted with SC, under high-pressure homogenization, occurred prevention of flocculation. Seen this, the study confirmed that the interaction between SC and PPI in the aqueous phase is confirmed, because of the generation of changes in surface hydrophobicity and intrinsic fluorescence of the homogenised mixed-protein solutions.

-Tea saponin/ Quillaja saponin

Recently many studies have been interested in the use of saponins as a plant-based emulsifier. Saponins are extracted from the *Quillaja Saponaria Molina tree*, as a secondary metabolite. Saponins, when compared to other natural emulsifiers, can be used at low levels and be able to form fine oil droplets and keep the emulsion stable over different environmental changes. These metabolites are already available for the use in the food industry as some previous researches have reported quillaja saponin as an efficient emulsifier for forming and stabilizing oil-in-water (O/W) emulsions and nanoemulsions (Zhu, et al., 2019).

Seen the potential of saponins as effective emulsifiers and that overexploitation may not be environmentally-friendly. *Camellia* Lutchensis seeds has been used for years as a source of tea oil in China, and its emulsifying properties increased the utilization of *Camellia* seed oil press-cake as a by-product. *Camellia* seed cake utilization has also been highly used because of economic and sustainability reasons, the use of the waste after extracting the oil can be done because it's still rich in tea saponins. *Camelia* seed disposal waste can also be used to improve the nutritional value of food products, supplements, cosmetics, personal care, detergent and in pharmaceutical industries (Zhu, et al., 2019).

A study conducted by Zhu et al., (2019) compared the use of natural emulsifier Tea saponins and Quillaja saponins to a synthetic emulsifier (Tween 80) with the aim to evaluate the performance of formation and stabilization of nanoemulsions using high-pressure homogenization method. The research also compared the interfacial properties of the three emulsifiers and evaluated the impact of environmental conditions, such as pH, ionic strength, temperature and long-term storage on the stability of the emulsions. The results identified that tea saponins could make nanoemulsions at relatively low surfactant-to-oil proportion, the oil droplets were stable over a variation of temperature between 30- 90 °C, salt concentration ≤ 200 mM NaCl, pH between very basic and acidic level (3-8) (when subordinate to highly acidic pH (pH 2) or ionic strength droplet flocculation occurred), the storage temperature range at 5 - 37°C and 55 °C proved a good long-term stability. Overall, the tea saponins had similar results to commercially available quillaja saponins, and that they are effective to be used as natural surfactant, stabilizing emulsions, to be used in food and other industries. In addition, it provides advantages over other plant-based emulsifiers, such as proteins, polysaccharides or phospholipids for certain food industry application.

Saponins are phytochemicals that provide interfacial active compounds derived from plants, which consists of hydrophobic alglycone structure and hydrophilic sugar remains. Saponins are a varied class of natural substances, differences in the molecular structure and interfacial properties is not completely understood, so Böttcher & Drusch (2017) evaluated saponins self-assembly and behaviour at aqueous interfaces. A fundamental understanding on interfacial structure of individual molecules at the interface was carried out and the results related that interfacial configuration may distinguish depending on botanical origin and structure. The presence of hydrogen bonds was associated to the formation of strong viscoelastic interfacial films, saponins with triterpenoid structure are more prone to form strong viscoelastic films, resulting in stable foams and emulsions, specially at the air/water-interface. The amount of sugar chains slightly affected interfacial properties. To summarize, saponins are considered to be a promising class of natural surfactants that can be used in the food industry as a dispersant, taking in consideration their molecular structure to understand their interfacial properties.

McClements & Gumus (2016) reviewed the replacement of synthetic surfactants to natural emulsifiers as an alternative to functional ingredients in novel products. The study valuated physicochemical basis of emulsion formation and stabilization by natural emulsifiers, and the comparison of its benefits and limitations. The results exposed that natural emulsifiers, such as biosurfactants, phospholipids, proteins, polysaccharides and colloidal particles are efficient on forming oil-in-water emulsions with small droplets at low levels that are stable over different environmental conditions, being useful for the production of commercially available food products.

A comparison of modified starch (MS) and *Quillaja* saponins (QS) properties in the formation and stabilization of orange oil nanoemulsions using microfluidization was analysed by Zhang et al., (2016). The results revealed ideal viscosity ratios of dispersed to continuous phase to QS as emulsifier, and QS was also identified to be superior to MS on nanoemulsion fabrication and better turbidity. When Ester gum (EG) was added in the oil phase, nanoemulsions stabilized by QS were stable for two weeks, and prevented Ostwal ripening and modified phase viscosity ratio. QS introduced high interfacial charge and low interfacial tension which resulted in the formation and stabilization of nanoemulsions. However, MS resulted destabilization of nanoemulsions by coalescence.

A study conducted by Shu et al., (2018) analysed the usage of ginseng saponins as a natural emulsifier in the formulation and characterization of astaxanthin-enriched nanoemulsions. Astaxanthin (AST) is a lipid-soluble carotenoid, naturally present in some microorganisms and aquatic animals, its strong antioxidant activity is due to the presence of conjugated double bonds and hydroxyl groups. Ginseng is also known to be rich in saponins, ginseng saponins (GS) provide functional benefits and health benefits, aside of their surface-active properties. The results of GS characterization revealed that, these natural emulsifiers exhibit strong surface activity, giving to AST nanoemulsions pronounced stability, providing stable droplets against heat treatment (30-90°C for 30 minutes), and nanoemulsions kept at low temperature (5°C) presented excellent stability if compared to samples stored at higher temperatures, resulting a certain thermal instability of AST.

Natural surfactants, such as Quillaja saponin and Lecithin were used to evaluate the formation and stabilization of vitamin E based nanoemulsions. Ozturk et al., (2014) examined the effect of oil compositions (0-100% vitamin E acetate), surfactant type and concentration (0,0005-5%) on the particle diameter and vitamin loading capacity. The study showed that when increasing both surfactant concentration, the mean particle diameter decreased, with lower levels of quillaja saponin needed to form small droplets. Physical stability was also evaluated, Ozturk et al., (2014) took in consideration pH effects, ionic strength and temperature. Quillaja saponin samples were stable over a pH variation (3-8) and salt addition (concentration 0-300 mM NaCl), however showing flocculation at lower pH and higher salt concentrations. Both surfactants formed stable droplets over heating variation (30-90 °C for 30 minutes), in no salt added conditions. This study proved that the use of quillaja saponin in the food industry will provide similar surfactants properties when to lecithin, which is known as an efficient surfactant.

Several studies have identified Q-Natural®, a natural food-grade surfactant, which is based on quillaja saponin, as a highly surface active substance. A study performed by Yang et al., (2013) analysed the formation and stability of emulsions using molecule of Quillaja saponin (Q-Natural®). Tween 80, Q-Natural® 200, having respectively molecular weights, 1310 and 1650 g mol^{-1} and a medium chain triglyceride (MCT) were used to form emulsion samples. Many factors had to be taken in consideration, such as, pH and salt, emulsion stability (environmental stresses tests), thermal processing (variation of 30-90°C for 30 min), long-term storage evaluation (emulsions samples were subordinated to different and controlled temperature conditions, between 5, 37 and 55°C, for 30 days), particle characterization (the droplet sizes were measured using a static light scattering instrument) and interfacial tension measurements X surfactant concentration (analysed at the MCT oil-water interface using a drop shape analysis instrument).

Emulsions were prepared by using 10 wt% lipid phase (MCT) with 90wt% aqueous phase. The aqueous phase consisted of either Tween 80, or Q-Natural® and a buffer solution (10mM sodium phosphate buffer, pH 7.0). The results revealed that interfacial activity is an important factor when settling their ability to form and stabilize emulsions. For both surfactants, the interfacial tension was reduced when surfactant concentration increased, this indicated that surfactants were absorbed to the oil-water interface. Overall, this study found out that Q-Natural® have a high surface activity and had similar interfacial properties as Tween 80. Q-Natural® was able to form oil-in-water emulsions with relatively small droplet sizes ($d<200$ nm) at low surfactant-to-oil rates, which was stable to a wide range of thermal treatments (30-90°C), salt concentration (≤300mM NaCl) and pH conditions between 3 and 8. Moreover, they had good long-term stability when stored at different range of temperature conditions. However, this study found out that the samples were unstable when stored at highly acidic pH (2) or high ionic conditions (400 or 500 mM NaCl), leading to flocculation and creaming. Nevertheless, the overall results stablished Q-Naturale as an effective natural surfactant that may be suitable to use in food and beverage companies. It is also mentioned that for its use in food and beverage industries, is important to investigate factors such as, taste, potential toxicity, reliability of supply and cost (Yang, et al., 2013).

The emulsifying properties of mixed surfactant sources, such as Quillaja saponins with Na-caseinate, pea protein, rapeseed lecithin and egg lecithin were examined with the aim to analyse what kind of textures these mixtures may build thus enhancing emulsions stability. Reichert et al., (2018) prepared oil-in-water emulsions (10%) at pH 7with co-surfactants concentration varying (0.1-5%) alone and mixed with Quillaja saponins concentration varying (0.05-0.5%). The results showed the behaviour of mixed surfactants systems, food-grade binary surfactant systems provide an attractive effect to stabilize matrices with tailored characteristics. It was observed various phase structures, changes in surface activity and absorption behaviour, also an improvement synergistic on emulsifying properties for Quillaja saponin. The interfacial tension reduction, as well as, reduction of emulsion droplet size was observed at high Quillaja saponin concentration, even in different type and concentration of co-surfactant. Even though, there is the need for further studies to investigate interfacial properties of mixed saponins, the results demonstrated that food-grade binary surfactant carries a potential to be developed and used as new type of emulsifier.

-Potato protein Isolate

In the past years, potato has been noticed as an innovative protein source for human consumption, and as novel plant based protein offering more sustainable and cost effective alternatives. Potato protein just as other natural sources of proteins possess high nutritional values, assuring food functional properties, forming structural networks such as gels, foams and emulsions. In food products where the formation of a final set gel is required, proteins are of great importance for gel building by binding water, as well as, for the textural properties of the final sensory perception of the food gel (Schmidt, et al., 2018).

Many factors influence negatively potato gel formation properties, heat treatment such as pasteurisation and spray drying may affect heat unstable proteins such as patatin, whey protein and ovalbumin. Potato protein is probably going to be spray dried into a powder to be used in food products, alternatives to heat processing technologies may be interesting to ensure potato protein high solubility and improved foam stability. High pressure treatment has been used instead of traditional heat treatment to preserve protein quality and potato protein functional properties (Schmidt, et al., 2019).

Aside of the nutritional and functional properties, potato protein carries a particular feature, being gluten-free (GF). The development of new GF products is emerging because of changes in the dietary requirements for celiac disease, for people looking for non-allergenic ingredients, and because of the industry needs for product variation. These new requirements are leading to a new category of products in the food market, expanding the variety of products offered to this public (Calero, et al., 2013).

Calero et al., (2013) conducted a study evaluating the use of chitosan in emulsions formulated with high-oleic sunflower oil and potato protein isolate (PPI) and relating the concentration of chitosan with the stability, microstructure and rheological properties. PPI was used as emulsifier and chitosan as stabiliser, the impact of chitosan concentration on the physical stability of emulsions was evaluated in a range of visual inspection, rheological and microstructural techniques. The results declared that oil-water emulsions in the presence of PPI as emulsifier provided poor solubility, however after the addition of chitosan to PPI-based emulsions the stability of this emulsions have enriched.

A study conducted by Villanueva et al., (2018) analysed the effect of decreasing the pH of starch dispersions to 4.5 on the thermal properties of rice, potato and tapioca starches and protein fortification (egg white and soybean protein isolate) on the rheological properties of the gels prepared at 90°C and 120°C. This study aimed to improve the texture of new gluten-free food products. This study concluded that the impact of acidification, non-gluten protein fortification and improvement of viscoelastic properties on thermal transitions of rice, potato and tapioca starches has influenced rheological behaviour of starches depended on their botanical origin and were induced by the presence and type of protein added, was also influenced by the pH conditions. The acidification of rice starch to pH 4.5 increased the gelation temperature, having the addition of albumin and soy protein, while it as reduced in the presence of tapioca starch.

Moreover, the addition of proteins gave a structuring effect on tapioca gels, improving viscoelasticity and reducing tanδ values. To summarise, Villanueva et al., (2018) concluded that, in general, the samples acidification led to feebler gel structures, demonstrating more effects for potato starch, and this is directly related to higher phosphate content. However, protein incorporation strengthened gel structure, nevertheless, lower temperatures were identified to be more efficient to modulate the functional and textural properties of gel-like starch-based gluten-free formulations.

Another sort of study to analyse microwave radiation effects and protein addition to rice and potato starch was conducted by Villanueva et al., (2018), the supplement of protein added in the samples were 5% of calcium caseinate (CA) or soy protein isolate (SPI). To analyse the improvements that may have occurred in the samples, hydration, pasting properties and viscoelastic features were taking in consideration. The present study aimed to evaluate the physical modification of the systems and the possibility to develop ingredients with new functionalities. The results revealed that the microwave radiation treatment (MWT) was influenced by the botanical origin of the starch, the presence and the type of protein added. The key changes were notated when SPI was added, in addition, potato starch blends showed an increased water absorption capacity and decreased water solubility index. To sum-up, MWT on potato-protein blends supported gel stability, reduced pasting profile and improved viscoelastic moduli, seem this, Villanueva et al., (2018) could confirm that the combination of MWT with protein addition allows to develop new food products with tailored properties.

Potato protein extraction from pilot plant was processed with the aim to evaluate their structural and functional properties. Waglay et al., (2019) used three processes for potato protein isolation from by-products of potato fruit juice and potato pulp. The results revealed that protein profile of all extracts showed similar patatin recovery, all isolation used processes showed effect on the secondary structure, hence leading to higher carbohydrates level and prevention of denaturation after heat treatments. Multi-enzymatic system treatment proved enhancement of emulsifying activity index. The results on emulsion and foam stability, in all isolation techniques, provided comparable results in all the samples. The prepared PPI and potato protein concentrate (PPC) revealed potential to be used in food systems because of their enhanced foaming and emulsifying properties.

Physicochemical and functional properties of whole potato flour from four Maori potato varieties were compared to two other modern potato varieties by Zhu & He (2020). Aspects such as chemical composition, *in vitro* antioxidant activity, swelling power, water solubility index, thermal and rheological properties, gelling and in vitro starch digestibility were taken in consideration when evaluating each sample. The results revealed that dietary fiber, lipids, minerals and phenolic compounds were higher in Maori potatoes. In relation to rheological and textural properties, there was an association with the starch content of each potato, and this is due to properties diversity. Relatively high moisture content of Nad flour may be due to high sugar concentration.

A study conducted by Reyniers et al., (2019) aiming to evaluate the impact of sodium, calcium and aluminium chloride on the physicochemical properties of potato flakes in relation to water dynamics and oil uptake in the production of deep-fried crisps. The results exposed that for gelation enhancement of potato flakes was reached with potato starch amylopectin binding. Ionic crosslinking of potato starch amylopectin its recognized to directly improve gel forming capacity and dough sheet strength during production of potato-based snacks products.

Heat-induced gelation treatment on sweet potato proteins and their gel forming potential and structure were studied by Arogundade et al., (2012). The solubility of ultra-filtered/diafiltered sweet potato (UDSP) and isoelectrically precipitated sweet potato (IPSP) proteins gels, when in different aqueous solvent, counting or not denaturing agent, revealed hydrophobicity. Taking in consideration the least gelation concentration (LGC) as the gelation capacity index, IPSP protein showed better gelling capacity than UDSP. The levels of covalent interactions were higher than physical interaction in both protein gels. IPSP exposed better mechanical properties, such as hardness and springiness, when compared to UDSP protein gel. These rheological and gelation properties of sweet potato protein confirm their great prospect as functional ingredient added in food products.

The effects of high hydrostatic pressure (HHP) conjoint the aim to correlate with structure and gelation properties of sweet potato protein (SPP) was investigated by Zhao et al., (2019). The results revealed that by the inter-molecular cross-linking, MTGase promoted the formation of high molecular mass aggregates of SPP and promoted structure changes. High hydrostatic pressure improved gelation properties and enzymatic catalysis promoted better textural behaviour of sweet potato protein.

-Soy lecithin

Different plant-based alternatives are widely being used instead of milk products, such as soy, almond, coconut, rice and oat milks. However, there is a lack on their physical and sensory properties when compared to dairy-based creamers (Chung, et al., 2019).

Soy lecithin is a relevant natural surfactant that has been used in the food industry as an effective emulsifier. It's emulsifier properties are due to its amphiphilic molecular structure, which contain a lipophilic group formed of fatty acids and a hydrophilic group formed of phosphoric based esters, which are responsible for its great emulsification potential (Wang, et al., 2017).

A study conducted by Chung et al., (2019) investigated the properties of oil-in-water emulsions stabilized by sodium caseinate or soy lecithin in black coffee solutions. The aim was to verify the use of soy lecithin as a potential emulsifier in order to fully or partially substitute sodium caseinate in oil-in-water emulsions. The test identified that either caseinate or soy lecithin, in all model emulsions, had similar whitish appearances. In addition, blends stabilized by both stabilizers at the same time were able to produce O/W emulsions with similar colours, particle sizes, and electrical properties, indeed, it was also able to whiten a black coffee slightly higher whitening power than those samples with only caseinate. Overall, was concluded that soy lecithin can be successfully used to replace sodium caseinate, fully or partially in O/W emulsions.

A review on possible ways to use mixed emulsifiers on improvement of emulsion formation, stability and performance was done by McClements & Jafari (2018). This study aimed to put together all the information available on the usage of the combination of two or more different emulsifiers to enhance emulsion properties, rather than isolated emulsifiers. The mixtures involved proteins, polysaccharides, phospholipids, surfactants and etc. The type of emulsifiers and levels added was evaluated on the impact of emulsion formation and stability. The results revealed that the effect of using combinations of sodium caseinate and soy lecithin showed to procedure thyme oil-in-water nanoemulsions containing smaller droplets than when using individual emulsifier or when using a high-shear mixer. The same group revealed that a mixture of a cationic surfactant and soy lecithin provided smaller droplet sizes of thyme oil nanomeulsions than when individual emulsifiers were used. The results should help the selection of emulsifiers combinations, hence improving their performance in emulsion-based food products.

SOY PROTEINS

Soy proteins has been used in the food industry for many years, the reason for a wide use is its large possibilities in emulsifying properties of food products. Products such as beverages, infant formulas, ice cream and dairy products are examples of its usage. However, due to their large molecular weight and globular structure, improvements need to be made regarding soy protein poor surface activity, such as proteolysis which may improve their emulsifying properties (Li, et al., 2016).

A constant increasing interest on the development of food pickering stabilizers has occurred in the food science environment, the development of novel non-thermal process to produce soy protein isolate (SPI) nanoparticles with the aim to perform as effective emulsions stabilizers. A study conducted by Ou et al., (2017) analysed the effects of the addition of Ca^{2+} would induce. The use of Ca^{2+} on a determined protein concentration (2%) induced aggregation and crosslinking, when the Ca^{2+} concentration was increased, a progressive turbidity and particle size was observed this indicates aggregation and formation of nano-sized particles. The results revealed that the addition of Ca^{2+} at SPI samples, seen the finding of larger particle sizes, lower surface charge, stronger internal integrity, lower emulsification performance and higher emulsion stability against coalescence, confirm that the fabricated SPI nanoparticles could perform as an effective pickering stabilizers.

A study conducted by Mozafarpour et al., (2019), analysed the structured of soy proteins and methods to improve interfacial activity and emulsifying property. Many different methods have been used in soy proteins such as chemical, thermo mechanical and enzymatic modifications with the aim to modify their physical properties. Also the use of hydrogen and disulphide bonds to stabilize their compact structure of globular soy proteins is mentioned as tactic to enhance their absorption.

Partial denaturation under controlled heating and shear conditions followed by unfolding of their rigid globular structure was identified as responsible to improve protein surface activity, consequently improving absorption of soy proteins at air/water and oil-water interfaces. Heat treatment makes structural modifications, leading to a more flexible protein and the improvement of the molecule surface activity (Mozafarpour, et al., 2019).

Li et al., (2016), in order to investigate ways to improve soy protein isolate (SPI) emulsifying properties and behaviour of absorption at the oil/water emulsions, a selected hydrolysis method was used in different soy proteins and sodium caseinate was used as reference. According to the tests results, the emulsifying properties of (7S) and (RG) were comparable with the sodium caseinate, known as an efficient emulsifier. However, even through RG droplet size of the emulsions was considered small, flocculation still occurred. Emulsion viscosity was also analysed between the samples, and according to the shear-thinning behaviour (7S) and (SPH) ended up revealing similar conditions, according to the graph curves. The use of interfacial shear rheology allows globular proteins, such as (7S), to form high viscoelastic interfacial films, protecting the oil droplet against flocculation and coalescence. This study also provided information on reduced-glycinin (RG) structures, which contains globular and flexible structures, allowing them to create a high viscoelastic interfacial film, improving absorption and the formation of stable emulsions.

A study conducted by Lopes-da-Silva & R. Monteiro (2019) analysed gelling and emulsifying properties of soy protein hydrolysates with the presence of neutral polysaccharide. The commercial functionality of soy protein isolate is implicated due their large molecular weight, and the need to use denaturation process for better emulsifying properties. Seen this, this study evaluated the use of controlled enzymatic hydrolysis of soy protein and the changes caused by the interactions between proteins/peptides and other food components, called polysaccharides.

Aggregation of soy proteins were observed after many different proteases, these hydrophobic interactions occurred during aggregation process under controlled conditions may be favourable to improve gelling and viscoelastic properties on the interfacial proteins films, hence the improvement on functionality and many other bioactive properties.

Soy protein isolate (SPI) and anthocyanin (ACN) were studied for its great potential to be used as a novel Pickering emulsion. Ju et al., (2020) aim to study the effects of the combination of these two compounds using the characteristics to fabricate a novel emulsifier. The evaluated properties taken in consideration were particle size, zeta-potential, emulsion stability, surface hydrophobicity and oxidant capacity. The mixture of nanoparticles revealed effective emulsion stability, improved oxidative stability and resistance to in vitro digestion. In addition, the emulsions provide polyphenols, which are related to health benefits.

Ji et al., (2015) used sodium caseinate (SC) and soy protein isolate (SPI) mixed together, aiming to analyse its storage stability and improvements in its functional properties. These mixed proteins emulsions were produced through high pressure homogenization. The results exposed that both SC and SPI were absorbed on the oil-water interface, meanwhile, the system stability were influenced by electrostatic repulsion and steric stabilization. The results also proved an efficient retention of vitamin A after three months of storage at room temperature, demonstrating efficiency in emulsion protection and a significant capacity on the development of liposoluble nutrition delivery system, with satisfactory storage stability and physiological benefits.

-Microalgae proteins

Microalgae are microscopic algae, utilized by humans for many years. They are considered one of the oldest plant species in the world, their diversity is estimated around 200,00 to 800,000 number of species, they are responsible for photosynthesis and are

constituted of multi-cellular structure. Microalgae referred importance is because of its rich nutritional value, efficiency and productivity
(Koyande, et al., 2019).

Microalgae is considered a novel functional ingredient, which provide scientifically prove of health benefits to human body and reduce the risk to develop disease. Aside to their multiple nutritional, health benefits and balanced composition, microalgae has also a structuring role acting as an effective texturizing ingredient in food. The specie Photoautotrophic microalgae is rich in proteins, storage polysaccharides and cell wall related polysaccharides, and these components are known as structural biopolymers. The structural biopolymers carry the benefit of isolating cell wall related polysaccharides to be used as food hydrocolloids. In addition, red microalgae and cyanobacteria could also be a source of novel food hydrocolloids, because of its extracellular polysaccharides, which contains intrinsic viscosity, similar to the commercial available thickening agents. Nevertheless, even with the limited study on microalgae functionalities, food processing operations have demonstrated success in providing required microstructural and rheological properties (Bernaerts, et al., 2019).

Microalgae nutritional value is emphasized because of their similar quantities of protein, when compared to other protein sources like milk, soybean, egg and meat. Moreover, microalgae have naturally developed protective systems, due to extreme changes in the environmental conditions, this lead for the production of antioxidants and pigments, which are important for human consumptions as supplements, because it isn't naturally internally produced. In addition, in an environmental point of view, microalgae protein extraction is more efficient and requires less water sources, when compared to terrestrial crops production, such as wheat, pulse legumes and soybeans (Koyande, et al., 2019).

Microalgae biomolecules influences their emulsifiers and surfactants functionality. In the extracellular microalgae structure there are polymeric substances, like polysaccharides, proteins, nucleic acids and lipids, in the intracellular side there are cytosolic proteins, membrane lipids and molecules such as DNA (Law, et al., 2018).

It's known that ruptured algae can clearly act as efficient surfactants and emulsifiers, however there is a gap about their real role when stabilizing emulsions, and to help to fill this gap a systematic study conducted by Law et al., (2018) was developed with the aim to understand emulsification and demulsification processes in the wet lipid extraction. Knowing the demulsification processes, will be easier to develop strategies to feasibly solvent recovery.

The results revealed that proteins are great emulsifiers and the microalgae protein extracts have the same level of emulsification properties. Polar lipids, such as phospholipids and glycolipids, which are normally surface-active, can reduce the interfacial tension, however its properties were not successful when the task was to stabilize emulsions. Neither serum nor the microalgae cell debris had effective surfactants effects and they required polar lipids fractions for better emulsion stability. In addition, this study concluded that the components together negatively influenced emulsions stability, because they competed for interfacial absorption (Law, et al., 2018).

Microalgae and cyanobacteria protein isolate has been recently a current study subject, because of its wide protein variety, high protein content and emulsifying properties (Teuling, et al., 2019). Teuling's research analysed microalgal and cyanobacterial protein isolates with the aim to characterize the different emulsion behaviour, according to the amount of protein present in the samples and variation. Taking advantage of the analysis, also evaluated the non-proteinaceous components emulsifying properties.

Teuling et al., (2019) research findings revealed that Algae Soluble Protein Isolate - ASPI, concentration 66-77% w/w protein, had similar or higher emulsifying capacities (9mg protein/mL oil), when compared to other proteins such as, whey protein isolate (WPI), concentration 93% w/w protein, (18mg protein/mL oil), soy protein (10mg protein/mL oil) and sodium caseinates. ASPI and WPI could form stable emulsions against creaming, having droplet sized of 0.2-0.3m, at the same pH conditions (pH8.0). The level needed of each ASPI to stabilize oil fractions emulsions varied within the range between similar proteins isolates (9-74mg protein/mL oil). However, compared to other derived proteins sources such as, sugar beet leaf protein (21mg protein/mL oil), dairy proteins (12-50mg protein/mL oil), leguminous (10-15mg protein/mL oil) and eggs (100-250mg protein/mL oil) values varied to a greater range (Teuling, et al., 2019).

However, the algae proteins did not cause interfacial and molecular properties changes, as expected, hence no changes in the emulsions behaviour and emulsions stability (Teuling, et al., 2019). The presence of non-proteinaceous components in algae and cyanobacterial protein isolates, were analysed in another study, Schwenzfeier, et al., (2014) proved that charged carbohydrates can be responsible for stabilizing emulsions, hence preventing flocculation, under pH conditions between 3-7.

Spirulina is a term used for supplements made of two cyanobacteria species known as *Arthospira platensis* and *A. maxima,* they are formed of a polysaccharide cell wall, contains chlorophyll, phycocyanin, carotenoids, minerals, vitamins, essential fatty acids and other bioactive compounds. Proteins is naturally and highly found in Spirulina, and it's being widely used in bread and other bakery products, such as sweet bread, cassava cake and doughnuts, biscuits and pasta. Massoud et al., (2016) evaluated the physicochemical, microbiological and sensory properties of croissants fortified with Spirulina at concentrations varying from 0.5 to 1.5%. The founding of this study was that the addition spirulina protein, increased protein content of the final products and due to the non-starch polysaccharides the microalgae biomass is capable to improve water-retention capacity of bakery products, providing more stability and increasing product shelf life. To summarize, microalgae protein enrichment of bakery products is identified to improve textural and sensory properties, thus providing a novel opportunity for healthy bakery products in manufacturing. Besides the final product being rich in protein, was also identified to contain different sources of minerals, essential fatty acids, essential amino acids and vitamins, such as vitamin B_{12}.

Dairy products are of great importance in the role of human consumption partners, this is due to their nutritional effects on human health. Besides of the nutritional benefits, dairy products are not often considered high in bioactive substances. Fermentation of milk products have been mentioned to be one of the best ways to improve the overall dietary intake, especially if they are fermented using natural sources, such as microalgae proteins. A study conducted by Barkallah et al., (2017) analysed the effects caused by the addition of Spirulina on yogurt and the relationship with improvements on physicochemical, textural, antioxidant and sensory properties during fermentation and storage. Spirulina was incorporated into yogurt emulsions at four different concentrations (0.25, 0.5, 0.75 and 1%), characteristics such as fermentation process, texture, nutraceutical and sensory was analysed. The enhancement of 0.25% of Spirulina was enough to accelerate the end of fermentation and preserve the sensory appropriateness, hence providing better water holding capacity and lower whey syneresis during a period of 28 days of storage. Overall, the study proved that microalgae proteins can offer not only product stability but also nutritional added-value, showing considerably improvements of antioxidant activity and high content in natural pigments.

The selection of a specific microalgae specie to be used in the right food product is ideal for better structural properties, therefore, more information is requited on microalgae rheological characteristics. Seen this, Bernaerts et al., (2017) analysed the rheological properties of seven commercially available microalgae species in aqueous suspensions, before and after mechanical and thermal processing. The obtained results revealed cell wall polysaccharides and proteins are influenced by environmental conditions such as pH, furthermore, results evidenced that rheological properties of microalgae suspensions can be altered during mechanical and/or thermal processing. Suspensions of *P. cruentum, C. vulgaris and O. aurita* presented the largest structural properties. Biomass of *Nannochloropsis* sp., P. *tricornutum* and *Schizochytrium sp.* was identified to be interesting to enrich fluid food systems, such as fruit juices and smoothies without influencing product's structural properties. P. *cruentum* showed a wide structuring potential, being potentially used as a multifunctional ingredient in food products, improving nutritional value as well as thickening agent. *C. vulgaris* and A. *platensis* was also identified and as a multifunctional ingredient used in food products that go through mechanical and thermal processes, such as vegetable based soups or several dairy products.

This scientific study stretched the knowledge on the selection of microalgae species towards of application in the food industry, seeing that some microalgae species hardly influence the structural properties of a product and other show a great potential to be used as a structuring agent in food purposes (Bernaerts, et al., 2017).

The effect of gel setting on the addition of *Spirulina* and *Haematococcus* gels on novel food was analysed by Batista et al., (2012) with the aim to their effects on the linear viscoelastic behaviour vegetarian food gels prepared from pea protein, k-carrageenan and starch. The linear viscoelastic properties of pea, k-carrageenan and starch gel systems highly depended on the gel setting conditions, such as temperature/time of thermal processing and heating/cooling rates. The samples were kept in an increasing temperature range, the ones between 70°C -90°C kept for 5 minutes showed more structured gels, while the ones kept at 90°C in a time variation of 5 to 30 min resulted in a less pronounced gels. *Haematococcus* gels showed a highly structured and less dependent on gel setting conditions after the effects of heating and cooling rates. The addition of microalgae biomass promoted some modifications on the gel structure, although not showing improvements to gel setting conditions.

Ursu et al., (2014) studied the extraction, fraction and functional properties of proteins from the microalgae *Chlorella vulgaris.* This study aims to evaluate extraction and emulsifying properties of *Chlorella vulgaris* proteins. Extraction was done by pH shifting and ultrafiltration. Protein solubilisation was done using high pressure cell disrupter under pH = 7 or pH = 12, moreover, for a higher solubilisation yield a combination of alkaline conditions and mechanical treatments was utilized. Aspects such as proteins molecular weights, isoelectric points, amino acids composition and their emulsifying properties were studied and compared to commercially available ingredients. The results exposed that a better emulsifying capacity was delivered when protein solubilisation occurs at pH = 7 and in all cases, the emulsifying capacity and stability of microalgae proteins were found to be similar or higher than commercially available food ingredients, especially when compared to sodium caseinate.

1.7 Aim and objectives:

Overarching aim:

This paper will focus on assessing current functional and nutritional properties of plant proteins and the barriers inhibiting their wide spread use in the food industry.

Study Objectives:

1- Determine the functional properties of a number of plant proteins through a literature review.

2- To Obtain an insight into the use of sustainable proteins among Irish costumers

   a) Investigate consumer's awareness and perception of plant based protein as a food ingredient, regarding its benefits and relationship with sustainability.

3- To examine through a sensory analysis consumer's preferences:

   a) Descriptive analysis.

   b) Liking Test

   c) Triangle test

   d) Preference test

4- Analyse the results and verify different variables and their relation to the observations from literature and the questionnaires and analysis.

5- Original research: Fill the gaps found in the literature and provide accurate data to future researchers.

SECTION TWO

MATERIALS AND METHODOLOGY

2.0 MATERIALS & METHODOLOGY:

2.1 Introduction:

This chapter discuss the research method used in this study, the respondents, the data gathering procedures, and the treatment analysis of data.

2.2 The Research Design:

This study took place between August and December, 2019. It was conducted in order to understand the current functional and nutritional properties of plant proteins and to investigate the barriers inhibiting their wide spread usage by consumers.

Mixed research methods were implemented in this study, with the intention to collect, analyse and mix both qualitative and quantitative data, within a single study at some stage of the research process, aiming to understand the research problem precisely. The aim to capture the reasons consumers consider when purchasing food products, their preferences and knowledge and relate to a sensory test, which is going to tell us their preferences and main characteristics between an animal origin protein and two plant based proteins used in the food industry. Putting these results together, is going to give us a more accurate and effective result.

A common method to measure respondent's attitudes, beliefs and opinion was used in some questions. This is one of the most used methods for survey collection, "Likert Scale", providing a substantial advantage which the respondent can express their natural feelings, not forcing them to stand on a particular topic. It works with quantitative data, being easily understood and effective for data collection (Tullis & Albert, 2013).

2.3 The Sample Selection:

A total number of 221 people were selected to complete the customer's online questionnaire. The selection of respondents was random and in order to assess the extent of understanding and views from different customers and to obtain a wide variety. All participants are protein products consumers. The study did not use gender, age and level of education as a parameter to distinguish respondents, as the questions and answers provided would not be affected by that.

A second survey was conducted in accordance with the online customer's survey. A number of sensory analysis study was completed with a number of 60 participants, the selection of participants was also random with the aim to approach diverse fields. This second study took in consideration gender, age range, containing a descriptive analysis of tree different protein samples and evaluating their taste and characteristics preferences.

The online survey was distributed randomly to a total of 221 anonymous Irish participants, while the sensory analysis was applied in a face-to-face method in a particular population of 60 anonymous Irish respondents. The diversity of people and different age range and reality, give better inference as to a customer behaviour knowledge, and attitude toward plant-based proteins used in the food industry and its nutritional properties.

2.4 The Questionnaire Design:

An online questionnaire was developed comprising only one section and containing 8 quick questions to be answered. These questions were developed to gather information regarding costumer's knowledge about plant-based proteins used in the food industry as an ingredient, its functional properties and correlating with costumer's worries on sustainability. Each question was carefully developed by the authors, in a simple accessible language, in order to comprise the study objectives, and on contribute to in-depth and effective comparison and results to future researchers.

The outline of the questions and the rationale behind the development of each question is discussed below.

The online questionnaire form consisted of one section, composed of eight questions related to the perception of plan-based/sustainable proteins used as a food ingredient. The questionnaire started assessing the participant knowledge regarding microalgae as a food ingredient. Followed by a question that was designed in order to assess customer's awareness of microalgae nutritional benefits, this question allowed the relationship with question three, if the same amount of people, which know about their nutritional benefits also consider consume microalgae as a source of high protein nutrition.

Next question, the respondents were asked if they are familiar with the importance of sustainable food products for the future change of the planet, subsequently, they were asked if they would change to change to a plant-based diet if this led to improvements on the future health of the planet.

After that, a question was designed to gather information on participant attitude on how much they would be prepared to increase their spending on sustainable products, if this change would develop a fully sustainable food chain. The answers consisted in an increase of 0%, 10%, 30%, 50% and 70%. This question allowed an analysis on how much they cared for sustainable food chain that would make them invest less or more when purchasing a product. Finally, they were asked to consider the criteria when purchasing a product, from most important to least important. The options were cost, sensory, organic, brand, nutrition and sustainability. This question allows to evaluate what aspects they most take in consideration when purchasing food, and which one is the least important to them.

For the sensory analysis study, three protein samples and 4 different tests were used. The protein samples were whey protein, pea protein and chlorella protein (representing microalgae). It consisted of section 1, asking about their gender and age range, followed by Check-All-That-Apply (CATA) analysis, where the participants were asked to consume a portion of the solution and review all the sensory characteristics they thought did apply for each sample, separately. The test was designed to allow multi answers options from respondents. This test is a simple and fast sensory profiling tool, which allows researchers to analyse sensory similarities and differences between the samples.

The second test was the ranking or ranking test, the 60 consumers were asked to consume the three samples again, one each time, and mark with an "X" the point on the scale which correlates to their liking of the sample. Three main point were stablished on the scale: Extremely Dislike, Neither and Extremely like. This test allows to analyse what protein samples they preferred compared to the other two samples.

Third was the sensory triangle test with the aim to determine whether there is a sensory difference, they were given three sample, two were the same and one is different. They were asked to identify the sample which they felt was different. The samples used were whey protein (1) and pea protein (2). This test allows to evaluate if the participants could determine a difference between two similar products.

The last test in the sensory analysis was the preference test, designed to evaluate the acceptability of these protein samples among the participants. They were given three different protein samples (Whey, Pea and Chlorella) and asked to identify the one of preference.

2.5 Piloting the Questionnaire:

A pilot study is considered as an instrument of research to test in a small scale version or trial run the questionnaires, in preparation of the main study. Pilot studies have been identified as a miniature of full versions, undertaken to test if all the components of the questionnaire will work efficiently together and provide an accurate result (Donald, 2018).

Once the questionnaire was constructed and corrected, a pilot test was carried out to the first 20 random participants for the online customer survey and 3 for the sensory analysis. After ensuring the results of the pilot survey were accurate, and the questions were fully understood, it was sent for the next 201 respondents for the online survey and 60 respondents for the sensory analysis, increasing the likelihood of the survey.

The surveys were carried out between October and December of 2019, one-hundred percent of the customer survey was carried out online, while the sensory analysis was completely applicable face-to-face. All participants were given assurances that their data and views would remain anonymous.

2.6 Data Preparation and analysis:

Once the completion of all data collection has occurred, the findings from this data are then assessed. An online tool, Survey Monkey, is the online drive used to collect customer's opinions and to store all the data and convert to Excel tables for data analysis. For the sensory analysis, all the raw data was added on MS Excel tables and converted into graphs, providing evidences which supports or refuges the hypotheses drawn.

All data and insights provided in this study were collected during the survey and interviews, thus their accuracy and completeness is reliant on the honesty and openness of participants. The data collected from the questionnaires was imputed into the statistical software MS Excel 2019 to make analysis easier and more accurate. Frequencies were carried out on the data to generate percentages.

The data analysis section of this research attempted to answer all research questions put forward and the main objective of the research, to find out the main factors associated with issues evolving plant-based proteins usage in the food industry related with customer's awareness and acceptability in Ireland.

2.7 Data Collection:

The online customer survey questionnaires and the sensory analysis questions aimed to understand and discover consumer knowledge, behaviour and attitude toward plant-based proteins used as a food ingredient.

The online questionnaire was distributed through social networking site, such as, E-mail, Facebook, LinkedIn, etc. the entire survey took 48 days, from October 31 to December 18 of 2019, to be completed the results were collected directly through Survey Monkey. The data collection for sensory analysis was initially done with college students and lately with close friends, the results were put together in MS Excel table to be analysed and created results.

2.8 Limitations:

According to Szolnoki &Hoffmann (2013), the strength and limitations of different survey methods are numerous, such as, expense, interviewer bias, danger and source of disturbance, geographical limitations, quality of responses, time pressure on respondents, the inability to use visual help, the flexibility besides the amount of information that is provided. On the other hand, significant advantages of surveys are easy accessibility and in regard to its vast ability to collect a large variety of data on different topics.

In the case study, there were some significant weaknesses that must be considered. There was an aim to obtain an insight into the usage of sustainable proteins in the Irish food sector, a questionnaire was designed and when the data collection stage came, more than 150 emails were sent and only 4 were answered. The idea to go to restaurants and ask the head chefs for the possibility to answer it was declined, because they couldn't stop service. However, from around 30 restaurants visited, only 4 head chefs could answer the six questions questionnaires. Hence, it was decided to not use this data for its low response rates and timeframe.

Other limitations were identified when conducting the sensory analysis, such as 10 people dropping the survey due to having allergies or being vegan, and a lack of knowledge attributed to the topic of the study to some of the respondents. However, as the questions were made pretty simple for a better understanding, it did not affect the response rate.

The Triangle test wasn't conducted under controlled conditions, such as air, light, temperature and neutral decor. However, all the care to ensure each product sample was prepared and presented in a standardised and consisted manner to ensure that no sources of bias are introduced which could have a negative impact on the end test result.

Nonresponse bias was not an issue in our studies since the population of interest was diversified taking in consideration all kind of public and that provided varied insight. Hence, our samples were deemed appropriate in meeting our objectives.

SECTION THREE

RESULTS

3.0 Results:

3.1 SENSORY ANALYSIS STUDY

3.1.1 Respondents profile:

This research is based on a small scale that was specifically selected through Ireland's population. The sample size of the research was 60, which was made up of 34 females and 26 male respondent's giving a 57:43 females to male ratio.

For this question five age categories were given as possible answers. The lowest grouping category was the 56-65 years old category with 2 respondents making up 3%. The highest response came from respondents between 18-24years old category, with 31 respondents making up 52%.

3.1.2 Descriptive analysis:

The results are presented individually per protein type and type of test, and the most selected characteristics are shown.

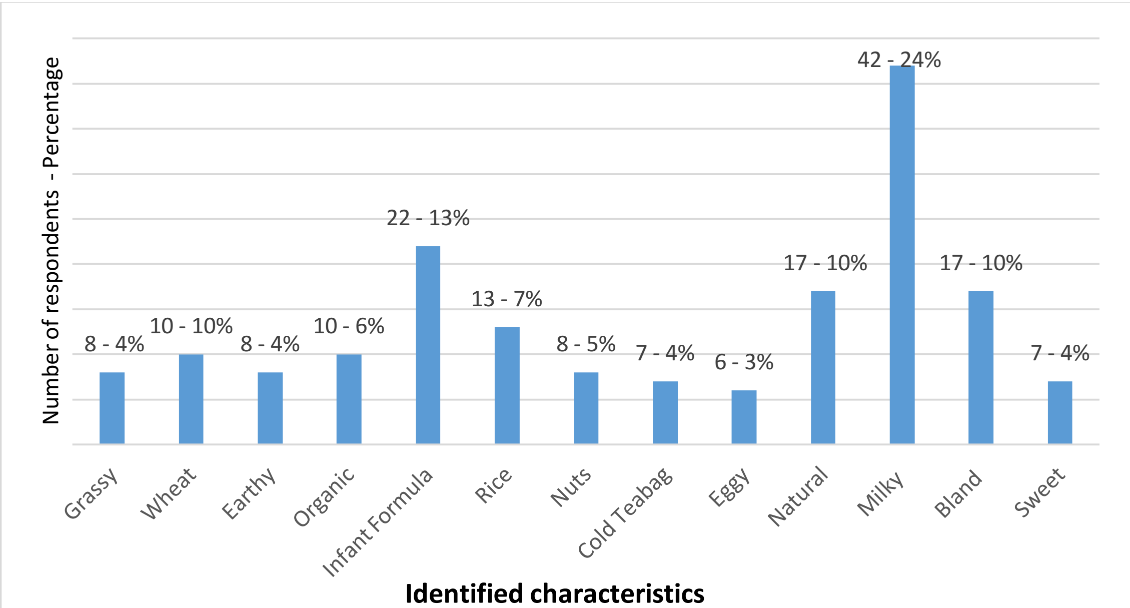


Fig 7 CATA ANALYSIS – Whey (043). Represents the number and percentage of characteristics identified by the respondents in whey protein sample.

n = 60

The characteristics mostly identified when respondents tasted whey protein sample are milky reporting 42 respondents and representing 24%, second characteristic mostly identified was infant formula representing 22 people (13%). The lowest characteristics identified were eggy (3%), sweet (4%), grassy (4%) and earthy (4%).

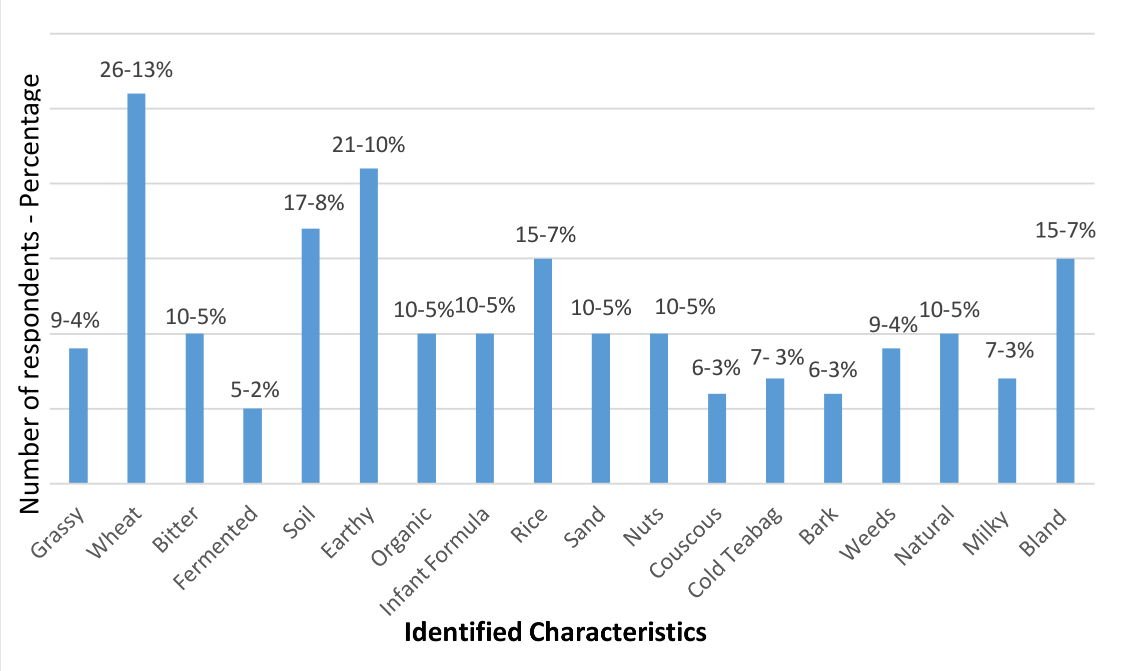


Fig 8 CATA Analysis – Pea (453). Represents the number and percentage of characteristics identified by the respondents in pea protein sample (%)

n = 60

The characteristics mostly identified when respondents tasted pea protein sample are wheat reporting 26 respondents and representing 13%, second characteristic mostly identified was earthy represented by 21 people (10%), and soil with 17 people identifying the characteristic, representing 8% out of the 60 people. The lowest characteristics identified were fermented (2%), milky (3%), couscous (3%), cold tea bag (3%), bark (3%) and grassy with 4%.

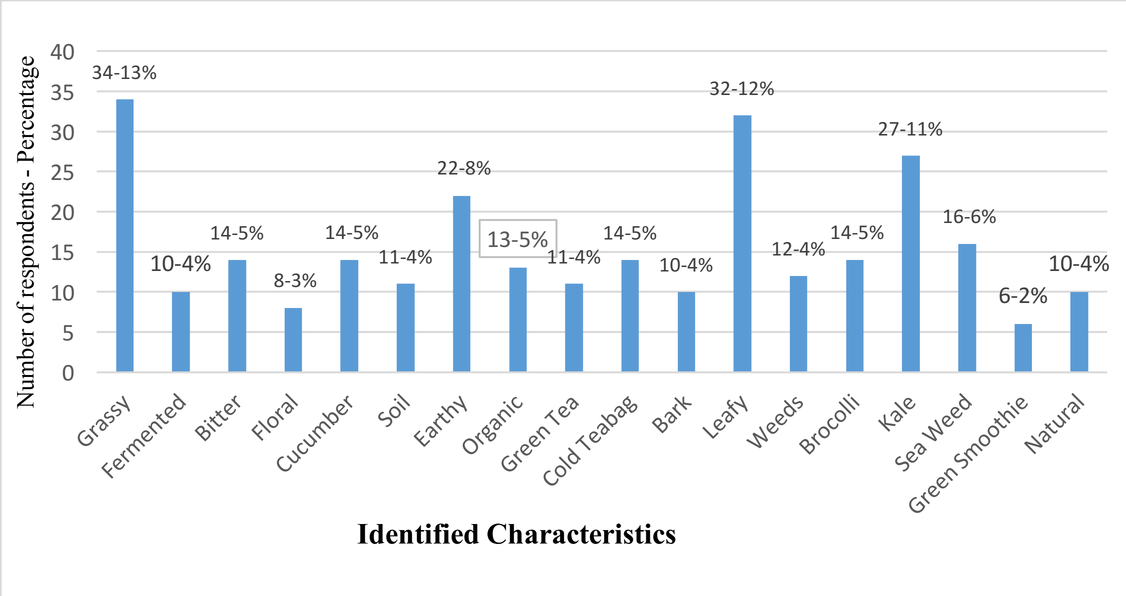


Fig 9 CATA Analysis – Chlorella (565). Represents the number and percentage of characteristics identified by the respondents in microalgae protein sample.

n = 60

The characteristics mostly identified when respondents tasted Chlorella protein sample are grassy reporting 34 respondents and representing 13%, second characteristic mostly identified was leafy represented by 32 people (12%), and kale taste with 27 people identifying the characteristic, representing 12% out of the 60 respondents. The least characteristics identified were green smoothie (2%), floral (3%), soil (4%), green tea (4%), bark (4%), weeds and natural with 4%.

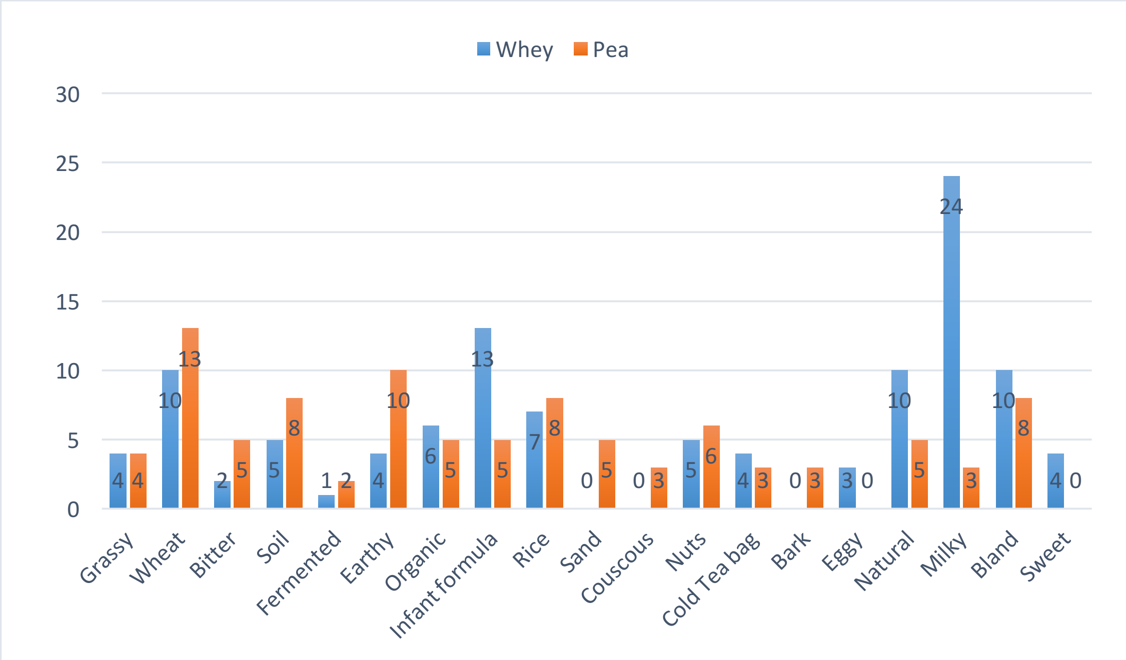


Fig 10 CATA Sensory results – Whey protein and Pea protein. Represents the percentage of characteristics identified by the respondents – A comparison between whey protein and pea protein sample.

n = 60

3.1.3 Ranking, preference and triangle test

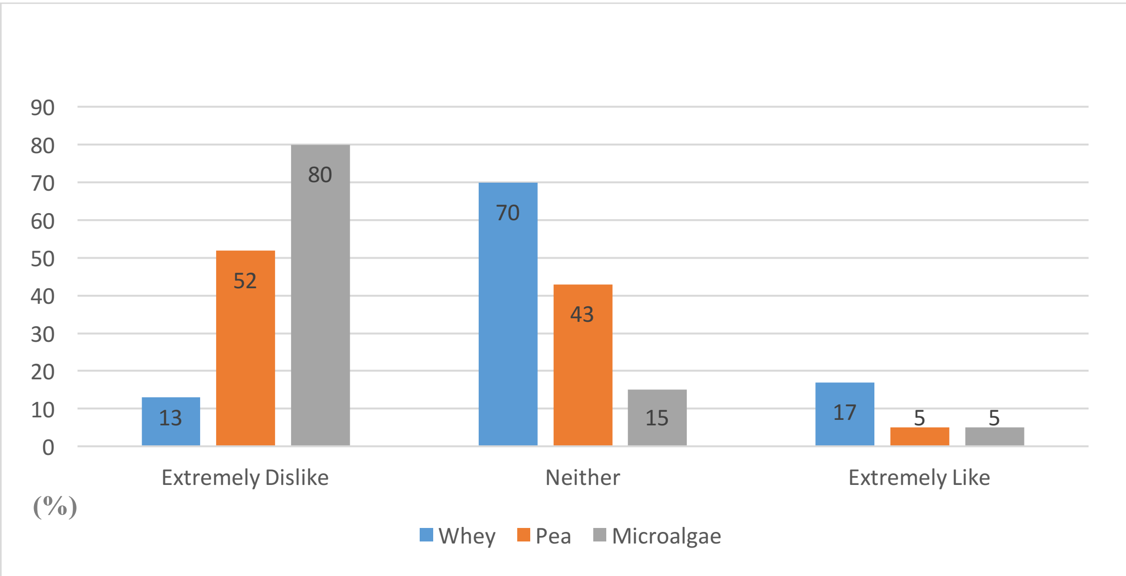


Fig 11 Ranking test. Represents the values for the taste preference, for each protein sample (%)

n = 60

Of the 60 respondents, 48 people ranked microalgae in the extremely dislike category, obtaining 80%, while whey protein was identified to obtain the highest level of extremely like category, reaching 10 people, 17% of the sample population, and on the intermediary category, “Neither”, representing 42 people (70%) of the respondents.

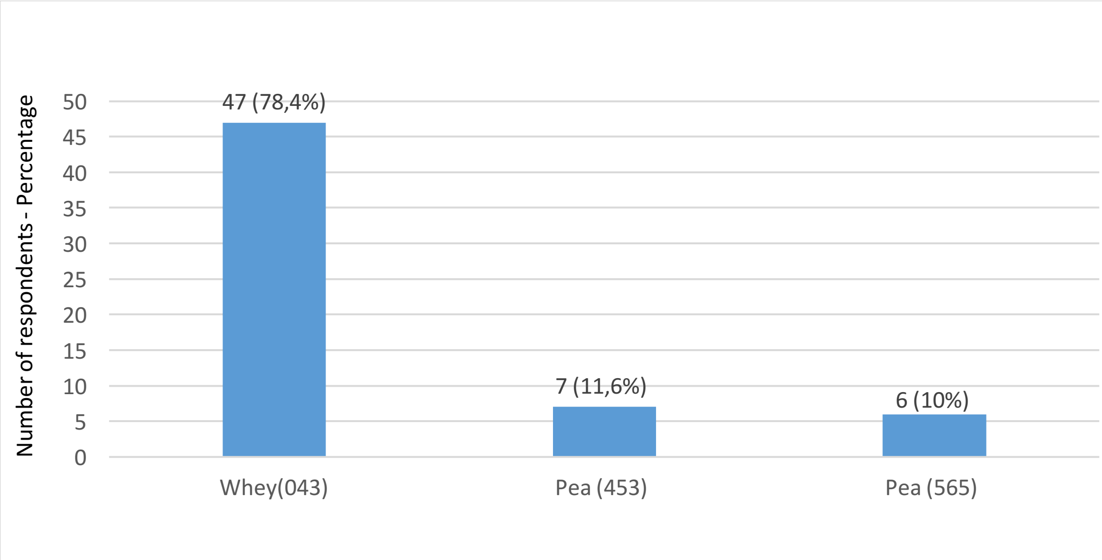


Fig 12 Triangle test. Represents which sample was identified as the most different taste (%)

n =60

Out of 60 responses, 78.4% of the respondents or 47 people reported whey protein as the different taste. Secondly, 11.6% or 7 people identified pea protein as the different taste. Finally, with the lowest response numbers, 6 people representing 10% of the participants, pea protein again was identified as the different taste.

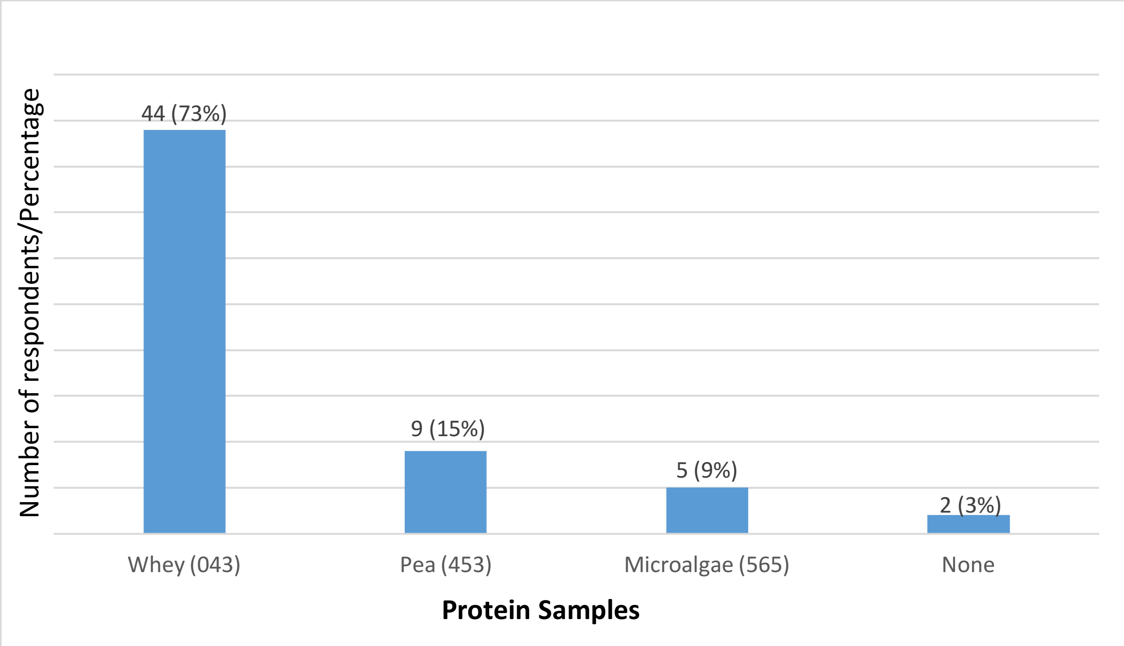


Fig 13 Preference test. Shows which sample was chosen as the favourite, regarding flavour (%)

n = 60

Out of 60 responses, 73% of the respondents or 44 people reported whey protein as their preferred taste. Secondly, 15% or 9 people identified pea protein as the favorited taste. Finally, with the lowest response numbers microalgae protein was identified as the less preferable taste. Two people (3%) did not choose any of the protein samples as favourite.

3.2 CUSTOMER ONLINE SURVEY:

Introduction:

Respondents were asked about their knowledge and purchase behaviour and opinion on plant based protein ingredients and its properties. A large scale of 221 respondents were randomly selected through Ireland's population. Furthermore, graphs are utilized for better comprehension. This questionnaire is composed of 8 multiple choice questions.

- Results per question:

Question one:

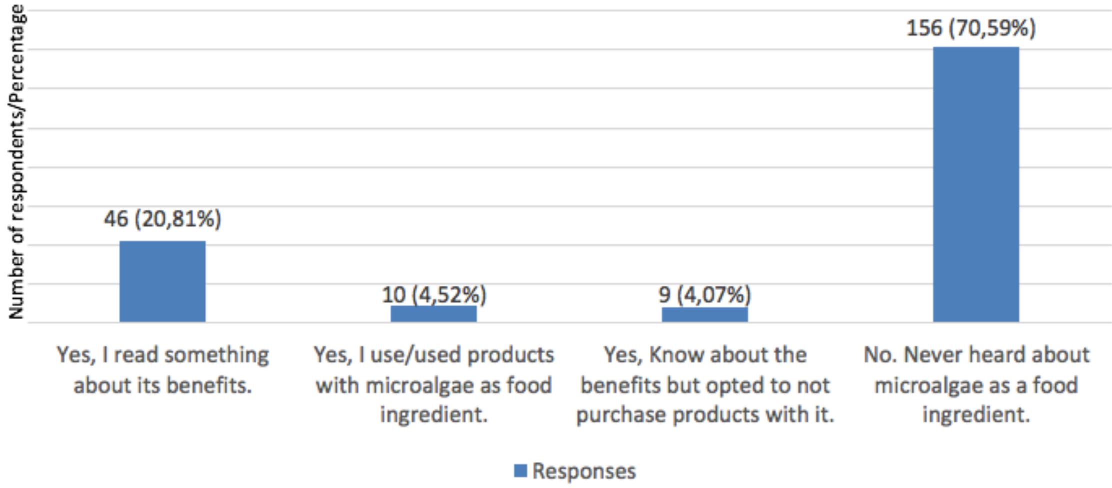


Fig 14 (Q.1 Are you aware of microalgae as a food ingredient). Represents the values for customer's knowledge about a specific plant protein used in the food industry.

n = 221

Four categories of answers were given, three are considered similar, with some specific details to differentiate and better classify the responses. Over half of the respondents (156) reported to not be aware of microalgae as a food ingredient, representing 70.59% of the total population. 20.81% of the respondents, totalling 46 people reported to be aware of microalgae as a food ingredient. 4.52% related to be aware of it and have used/use products with microalgae as a food ingredient, represented by a number of 10 people. 4.07% (9 people), related the awareness of microalgae, however opted to not purchase the product.

Question two:

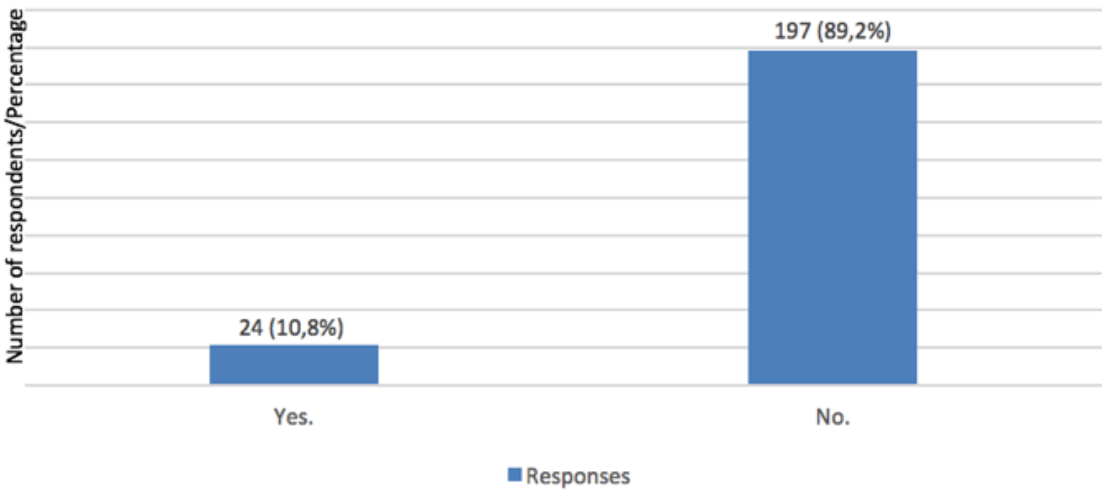


Fig 15 (Q2. Do you know any relevant nutrition benefit of microalgae?). Provide an insight about customer's awareness of microalgae and its benefits to human nutrition (%) n = 221

A total of 197 people, related to not know relevant nutrition benefit of microalgae, representing 89.2% of the population. 10.8% of the respondents (24 people), mentioned to be aware of their nutrition benefits.

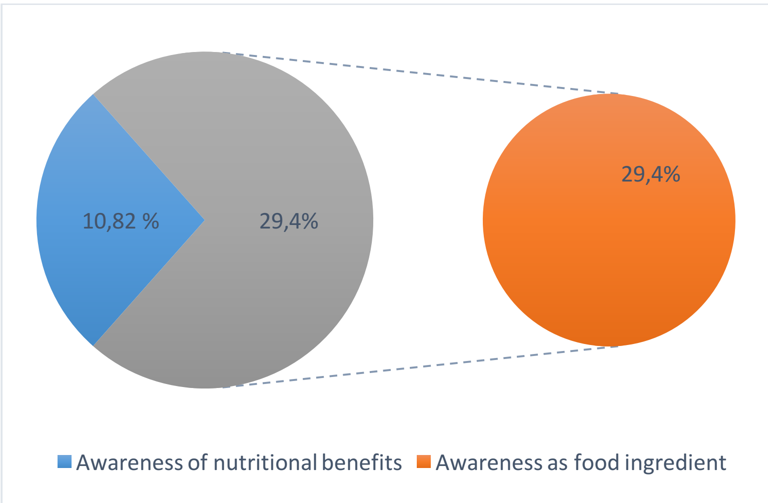


Fig 16 Represents the amount of respondents aware of microalgae as food ingredients (29.4%), and out of this value, 10.82% of the respondents confirmed to also be aware of microalgae nutritional benefits.

Question three:

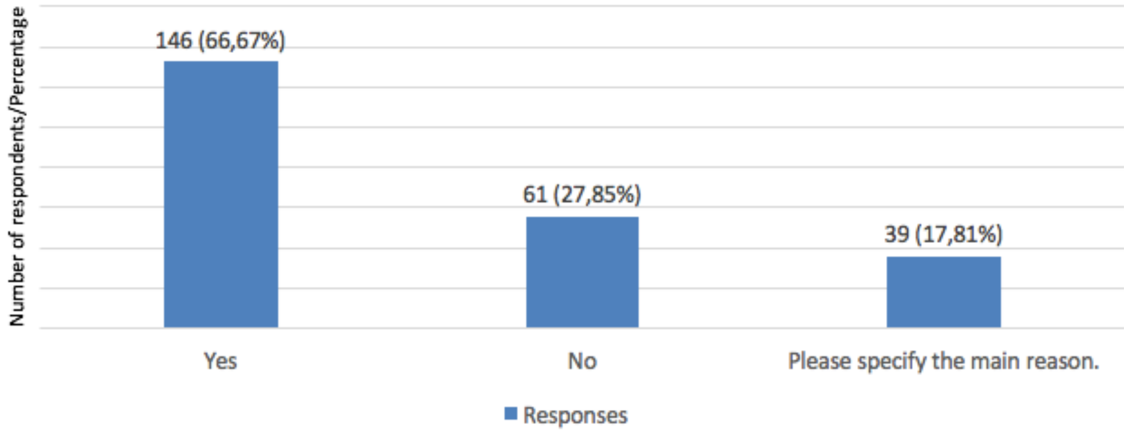


Fig 17 (Q3. Would you consider consuming microalgae as a source of high protein nutrition?). Represents customer's attitude on the likelihood of the consumption of microalgae because of its increased protein content. Additionally, specifications of the main reasons they would consume it or not, which is going to be presented on discussions section (%)

n = 221

A percentage of 66.67% people confirmed the possibility of the consumption of microalgae as a source of high protein nutrition. 27.85% of the respondents totalling 61 people reported to not consider microalgae consumption as a source of high protein nutrition. Moreover, 17.81% listed the main reasons they would consume or not the protein, specified on the discussion section.

Question four:

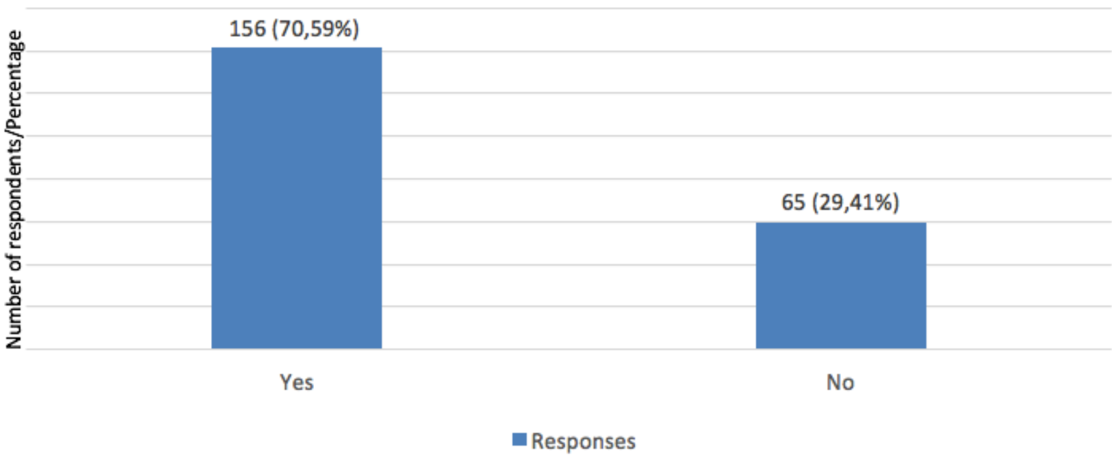


Fig 18 (Q4. Would you consider yourself familiar with the role of sustainable food products in the future health of the planet?). Represents the values for customer's awareness and worries on the use of sustainable food products in the future health of the planet (%)

n = 221

A percentage of 70.59% people confirmed to be familiar with the role of sustainable food products in the future health of the planet, totalling 156 of the respondents. 29.41% of the survey population confirmed to not be familiar with the importance of sustainable food products in the future health of the planet.

Question five:

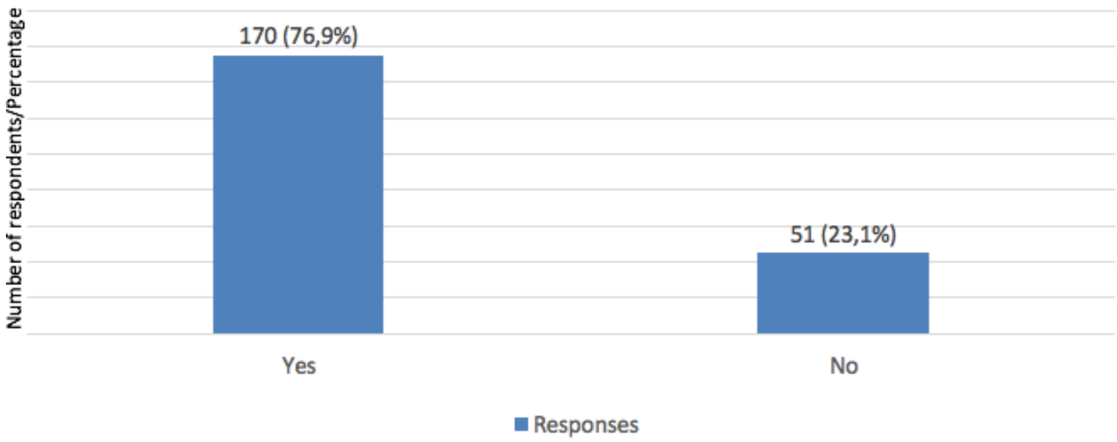


Fig 19 (Q5. Do you think a plant-based diet is important for the future sustainability of the food system?). Represents the importance customer's give to a plant-based diet relating to future sustainability of the food system (%)

n = 221

Of the 221 responses, 76,9% of the respondents or 170 people reported to consider the importance of a plant-based diet for the future sustainability of the food system. A number of 51 respondents, representing 23.1% of the total, did not consider a plant based diet important for the future sustainability of the food system.

Question six:

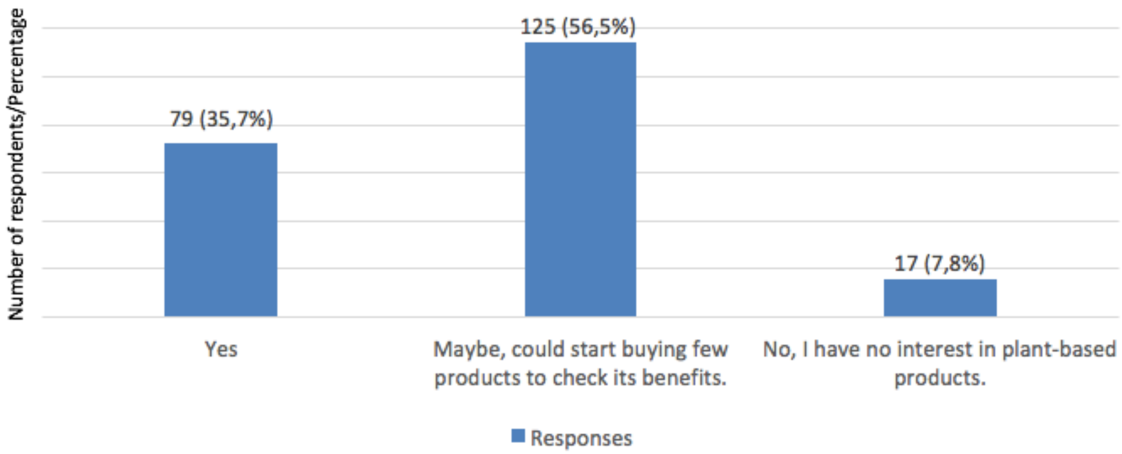


Fig 20 (Q6. Would you change to a plant-based diet if it could improve the future health of the planet?). Shows customer's opinion on changing to a plant-based died and the relationship with improvements it could bring to the future health of the planet (%)

n = 221

A total number of 125 people, representing 56.5% of the survey population reported that they maybe would move to a plant-based diet if it could improve the future of the planet, moreover, 79 people said yes for the change, representing 35.7%. Finally, 17 people (7.8%) reported to not have interest in plant-based products.

Question seven:

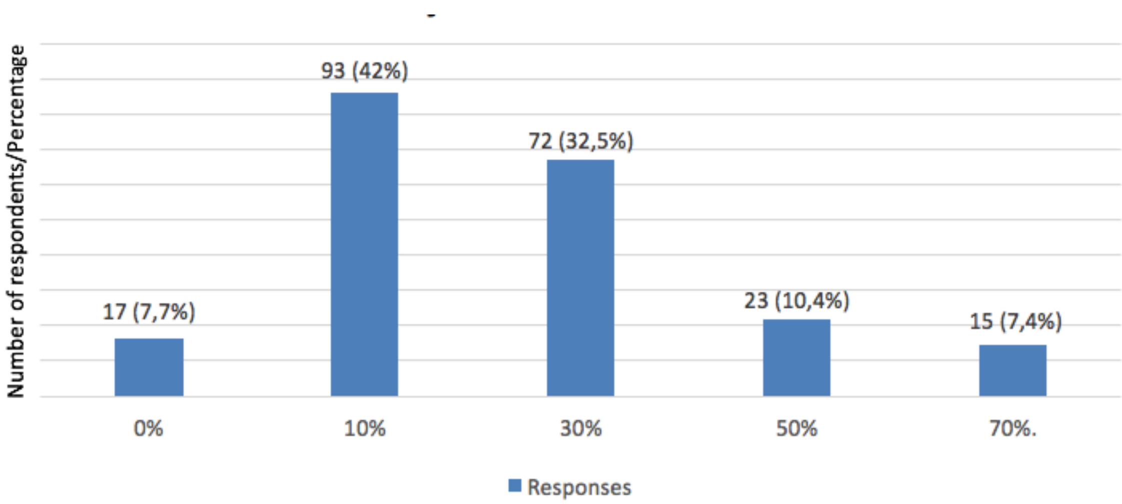


Fig 21 (Q7. Would you be prepared to increase your spending on food by the following amount, if it led to a fully sustainable food chain?). Refers to values customers would be prepared to increase their spending when purchasing plant-based products if that would lead to a sustainable food chain, in percentages (%)

n = 221

The vast majority of sample respondents, 42%, amounting to 93 people stated that they would be prepared to increase 10% of their spending on food if it led to fully sustainable food chain, followed by 32,5% of the survey population would be prepared to increase 30% on their spending. Thereafter, 10,4% of the population on a increase of 50% on spending, 7,7% stating a 0% of increase on their purchase spending, and finally, 7,4% (15 people) reported to be able to increase 70% of their spending of food, if it led to a fully sustainable food chain.

Question eight:

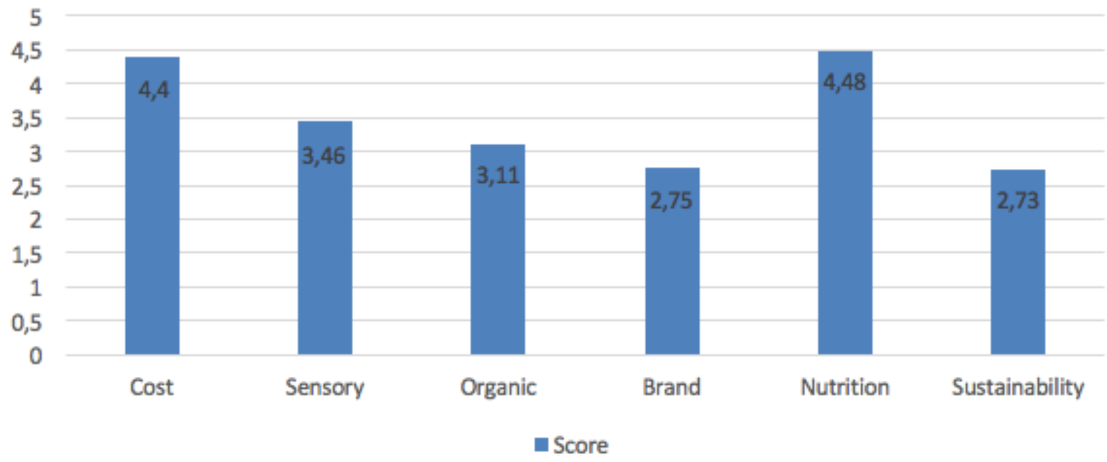


Fig 22 (Q8. When purchasing food products, place the following criteria in order of how you consider them). Represents what customers consider when purchasing a product, in order from the most important to least important.

n = 221

The criteria identified as the most important when purchasing food products is Nutrition, with a score of 4,48, followed by Cost with a score of 4,4 and Sensory with score 3,46. Organic is considered fourth criteria considered when purchasing a product, followed by the two last one Brand (score 2,75) and Sustainability (score 2,73).

SECTION FOUR

DISCUSSION

4.0 DISCUSSION

4.1 Introduction:

This chapter discusses, compares and contrasts the significant findings in this study, and concludes with the main features that needs to be considered in the future.

4.2 General analysis:

The first section of the sensory analysis questionnaire was focused on the Irish respondent's profile. This section was included to gain an insight into the general profile of the population sample, and with further investigation be useful to show if the study corresponds to the literature.

The majority of respondents to the sensory were female with 57% of the total, however not having that much difference to male respondents with 43%. The dominant age of respondents was between eighteen and twenty-four-year-old totalling 52% of the respondents, as figure 3.1 indicates, with the lowest response rate observed between fifty-six and sixty-five with only 3% of the respondents. According to Lang (2020), the results of the demographic analysis showed that the acceptance of the blending concept of plant based ingredients does not vary by age, gender, income or education.

4.3 Descriptive analysis:

Check-All-That-Apply (CATA) is used in this study with the aim to get an insight on how consumers perceive plant-based products against the commonly used animal based products used in the food industry, and also to identify the most relevant sensory attributes that affect adult's hedonic perception (Lungu & Muchenje, 2018).

A descriptive sensory analysis of liquid whey protein identified dairy and nondairy flavours. Nondairy flavours are typical of dairy products and include cardboard, animal or wet dog, cucumber, while for fresh sweet liquid whey contains sweet aromatic and buttery flavours. The difference in starter cultures and cheesemaking procedures, for example, between Mozzarella and Cheddar fluid way production, provide flavour differences (Carter & Drake, 2018).

Initial descriptive statistics on these behaviours reveal some interesting results. Firstly, for the check all that apply whey protein sensory analysis, the terms milky, infant formula, natural, bland, wheat, rice were positive drivers, the terms eggy, grassy and earthy were mentioned but showed less impact. The comparison of sensory characteristics between whey protein and pea protein is shown in Figure 10, one from animal origin and one from plant origin, with this analysis we could relate that similarities in were identified. Characteristics such as grassy (both 4%), organic (6-5%), rice (7-8%), nuts (5-6%), cold tea bag (4-3%) and bland (10-8%) were identified in both samples and in a very similar rate. On the other hand, characteristics such as infant formula (13-5%), milky (24-3%) and natural (10-5%) were mainly identified in whey protein samples, giving to them such a particular taste characteristic. Sweet (4-0%) and eggy (3-0%) taste was only identified in whey protein samples, not being a characteristic for pea protein samples. While characteristics, such as sand (0-5%), couscous (0-3%) and bark were only identified in pea protein samples, giving to them these particular features.

4.4 Ranking Test

This sensory test was implemented to assess taste acceptability of participants, a ranking scale for each protein sample was provided, and it has been found that most of the consumers indicated whey protein (17%) as the most likable sensory characteristics, and the least dislikeable (13%) between the other samples. On the contrary, microalgae sample is being identified as the most dislikeable (80%) taste preferences and considered at the same category of extremely liked as pea protein, achieving (5%). Unexpectedly, the results identified that whey protein in a high level (70%) of “neither” category, representing the scale neutral category. Pea protein also achieved a good percentage of the neutral scale category (43%). As you can see in Figure 11. To summarize, this results help us to understand that whey protein is still better accepted when compared to plant-based proteins, and that pea protein was had a lower dislikeable rate, and higher neutral point, when compared to microalgae sample, proving better acceptability.

An interesting study on consumer perception of plant-based proteins, a qualitative and quantitative survey, exploring ingredient perception. Aschemann-Witzel & Peschel (2019) results exposed that a clean label indication of the use of soy, pea or potato proteins as product ingredients provide a satisfaction in term of consumer perception, this generated a positive feedback from consumers. However, soy protein showed to have less positive effect on those knowledgeable consumers, probably because of its relation of consumption with breast cancer, allergic reactions and GMOs.

A study conducted by Wee & Forde (2019) analysed physical and sensory characterisation of noodles with added denatured pea protein isolate. The results revealed the addition of denatured pea protein did not change significantly textural or sensory qualities of noodles.

4.5 Triangle Test

Triangles tests are extensively used in food analysis to determine if differences between two products can be sensory detected. The participants are advised to select the different sample from the group of three, being two identical (Pea protein) and one different (Whey protein).

The majority of the respondents (78,4%) identified whey protein as the different taste, with a vast divergence with the other protein samples results, as seen in Figure 12. This results help us to understand that pea protein isn't considered an effective substitute in whey protein emulsions, considering sensory characteristics and not its functional properties.

By contrast, a study conducted by Aschemann-Witzel & Peschel (2019) with the need to evaluate consumer's perception of new alternative ingredients, such as proteins, and the aim to evaluate how these new ingredients would affect the overall product and subjective quality perception, conducted an experimental quantitative and qualitative survey exploring ingredient perception. The test results confirm a favourable view on plant protein as ingredient. When the manufactures highlighted the protein origin on labels, it led to a more favourable perception of the product, specially for consumers interested in plant-based food. Potato protein was identified more favourable in the quality dimension tests, also providing texture.

4.6 Preference Test

Preference tests provide information about whether people dislike or like a product. The aim is to determine if consumers like a product, if one product is preferred over another and if consumers intend to use a product.

This test's results provide an insight in what kind of products consumers choose, when they consume animal based products and plant-based. Figure 13 shows the evidence that over the 60 participants, 73% opted for whey protein sample as their favourite, a discrepant difference when compared to Pea protein (15%) and Microalgae accomplishing 9%. Relating to the literature review, customers are demanding products with plant-based ingredients, but still are linked to animal origin sensory characteristics.

A qualitative study focused on European consumer preferences and perceptions conducted by Banovic & Grunert (2018) a variety of participants could not differentiate between natural sources of protein and foods with increased protein content, no matter foods with animal or plant proteins. Researchs on consumer acceptance of foods with increased protein, and in particular on plant protein enrichment content is still scarce. Hence, the future use and acceptance of plant protein enriched foods rely on how these products get into people's reality.

A review on sustainable food proteins and its use in the food industry towards sustainability, health and innovation was studied by Fasolin & Vicente (2019), the results revealed that sustainable aspects of alternative proteins, used as ingredients, such as plant-based, insects and microbial are still little explored, mainly in micro and nanoscales.

5.0 Customer online survey

The online customer survey was developed with the aim to analyse participant's knowledge and usage of plant-based proteins and their attitude on purchasing products and its given importance to sustainability. In relation to question one and two, it has been found that mostly people are not aware about microalgae as a food ingredient (70,59%), neither know about their nutritional benefits (89,2%). Figures 14 and 15. In addition, is important to mention the relationship of respondent's awareness between microalgae as food ingredients and their knowledge about their nutritional benefits. Figure 16 shows that out of the 29,4% of respondents aware of microalgae as a food ingredient, only 10,82% confirmed to be aware of their nutritional benefits. The results provide us an insight that even though consumers are demanding for more 'clean label' products, with more sustainable ingredients, they are not really aware of some novel ingredients being introduced in the food industry, and not even into the nutritional benefits they can provide to human health.

Schunko & Vogl (2019) investigated organic consumers' knowledge and practices and they also concluded that organic consumers are unaware of wild ingredients used in organic products and this is related to poor labelling. This study also concluded that this category of consumers barely differentiates between organic and non-organic wild food, and there is a lack of awareness on market relevance from organic consumers. Cheung et al., (2016) related to the role of trust consumers give to the product itself, because they neither have sufficient knowledge about the ingredients nor would they notice whether ingredients are correctly displayed.

Most customers (66,67%) would consider the consumption of microalgae as a source of high protein nutrition, only 27,85% were not opened to this novel option of sustainable protein. The main reasons to consider the introduction of microalgae to their eating habits were mostly the nutritional benefits, such as being source of omega 3, rich in protein and antioxidants and provide immunity boost. Figure 17.

Accordingly, Lang (2020) investigated consumer acceptance of blending plant-based ingredients and the results exposed that there is a trend towards shifting from meat-centric to plant diets, and that their acceptance of blending plant-based ingredients into meat-based foods is increasing, and their acceptance is related to differences in food values and lifestyle.

When customers were asked if they are familiar with the role of the consumption of sustainable food products in the future health of the planet, the majority confirmed the awareness of the fact (70,59%), whereas, other customers' (29, 41%) confirmed not familiarity of it. Figure 18.

The understanding of consumers decision-making process and the use of sustainable food products for the future of the planet was investigated by Bangsa & Schlegelmilch (2020) and the insights revealed that the majority of the reviewed papers predominantly assume an increase in consumers worries and interest on sustainable products not only in food and drinks, but also in clothing, homes, mobility and footwear. In addition, the familiarity with the consumption of sustainable food products in the future health of the planet is related variables such as, socio-demographic, behavioural and lifestyle segmentation.

Following the questionnaire, customers were questioned about what they think about plant-based diet to the future of a sustainable food system. The majority of the respondents (76,9%) confirmed the importance they give to the reduce meat consumption and important changes that need to me done for a balanced and sustainable food system, whereas 23,1% people did not give the right importance for plant-based diet and its power of improvements. Figure 19.

Graça & Truninger (2019) analysed the current evidences and future directions that need to be taken in consideration for the reduction of meat consumption and for people to start following a plant-based diet. The results revealed that the transition to more plant-based diets is increasing, however it is still fragmented because of some barriers, such as lack of information for consumers, difficult to develop new cooking skills and positive taste expectations for plant-based meals are some of the variables stopping plant-based diet adoption.

Question six completes question five, when its asked if the respondents would change to a plant based diet if it could improve the future health of the planet. Evidences showed that they care about the subject and said yes (35,7%) to this changes, while other respondents would try to change (56,5%) starting with some few products to check the benefits it could bring to them. Figure 20. The amount of people that revealed to have no interest in plant-based products and its relationship with future health of the planet was very low (7,8%), compared to question five, when respondents said to not give importance (23,1%) to a plant-based diet for future sustainable food system. Figure 20.

Cleveland & Gee (2017) and Pérez-Martínez & Pérez-Jiménez (2019) has found a strong correlation between the importance of changes to plant-based diet and improvements in the future sustainability of the food system and health of the planet.

When the increase of spending on food products, if it led to a fully sustainable food chain was approached, customers had to answer in how much they were prepared to pay. Out of the 221 customers, 42% would be ready to pay an increase of 10% of the price, followed by 32,5% of the respondents would be ready to pay an increase of 30%. These results are surprisingly, because they show us that customers would pay more for sustainable products, only a small portion of the respondents (7,7%) would not be prepared to pay an increase of value. Figure 21.

The descriptive results of question eight reveals some interesting results. Respondents were asked to classify how they consider some attributes when purchasing products. Attributes such as cost, sensory, organic, brand, nutrition and sustainability, the results can provide an insight on the importance they give on each character, especially on nutrition and sustainability, and how cost is classified. The attributes of nutrition (4,48) and cost (4,4) were equated, nutrition is considered the most important aspect when purchasing food products by these 221 people. Aspects such as brand and sustainability were considered the least important to them. The results exposes to us that nutrition and cost are considered more important than sustainability and that sustainability is the last attribute customers take in consideration when purchasing food products. Sensory aspects and organic were classified just after cost and before brand. Seen figure 22.

The willingness to pay for a premium wine obtained with sustainable production method was analysed in Sicilian consumers by Lanfranchi et al., (2019). This study points out that the knowledge of sustainable production methods significantly influences the decision-making of consumers when the premium price increases, and also that they are more prone to buy sustainable wine only when the premium price is low. In addition, Ghyanidze et al., (2017) showed that price and nutritional information are considered more influential in consumers' food choices than information relating to the ecological impact of production or social responsibility of the producers.

A study conducted by Chege et al., (2019) analysed consumers' willingness to pay more for nutritious food, the sample population was focused on consumers at the base of the pyramid. This study finding revealed that poor urban consumers are willing to pay a premium for safe and nutritious porridge flour, in addition, when manufactures provide nutrition information on labels there is an increase on willingness to pay more for nutritious food.

On the other hand, Bangsa & Schlegelmilch (2020) related that in accordance to different purchasing realities and product categories, consumers do not consider sustainability as the most important attribute. Tebbe & Blanckenburg (2018) concluded that although consumers are willing to pay a small increase in price for sustainable-labelled plant-based products, consumers prefer to pay a premium similar animal-based product, which cost less. Indeed, sustainability might not always be an asset, thus, instead of sustainability attributes, many prioritize characteristics such as price, brand, taste and flavour, aesthetics or functionality.

SECTION FIVE

CONCLUSIONS & RECOMMENDATIONS

5.0 CONCLUSION

In conclusion, this study has highlighted several factors that influence customers behavioural when purchasing sustainable food products, their preferences and usage. The new generation of consumers are demanding more information on what they are consuming, the development of new technologies on the usage of novel plant-based products is observed, as well as, consumer's adoption of a plant based diet is increasing. Moreover, the findings identify that consumers would start to consume microalgae as a source of high protein nutrition mainly because of their nutritional benefits. However, the results provide an insight that the majority of the consumers are not aware of microalgae as a non-meat protein ingredient or neither its nutritional benefits. Seen this, we can mention the food industry role on educating customers, mainly providing more clear and complete label information about their ingredients and nutritional benefits and also implementing marketing strategies. Likewise, with the results of a sensory analysis test, was confirmed that whey protein taste acceptability is higher when compared to other plant-based proteins, such as pea protein and chlorella protein. This situation may change if some innovation on the ingredient side is done by the food industry, to improve their sensory profile, hence leading to a widespread uptake. This study also reveals that consumers are familiar with the usage of more plant-based products for the future health of the planet, their understanding affects their decision-making process, and there a significant number of people that worry and have an interest on sustainable products in general, not only in food. The relationship of the importance of the adoption of a plant-based diet with the future of a sustainable food system and progresses on the future health of the planet were investigated, and consumers revealed to care about it and they would definitely make these changes if it led to fully sustainability system. Furthermore, the findings on consumers' willingness to pay more for a food product in exchange of a sustainable food chain, the results exposed that the majority of the consumers are prepared to pay a small increase in price, however, the next questions approaching how they consider some attributes when purchasing food products, the least important to them was sustainability, the sample population was focused on nutritional benefits and cost. This finding means that in order to make sustainability more valuable to people, it must be made more attainable first. The findings observed in this study underscore the importance of attitudes, knowledge and behaviours of consumers on future changes in the increased usage of sustainable plant proteins, because when consumers demand, the food industry will be willing to achieve their requirements and it will lead to improvements not only for human health, but also for a sustainable food chain and healthy planet. It is evident no single individual or group can adequately undertake this problem alone, collaboration from an extensive range of actors along the supply chain towards sustainability is the key on creating long-term and sustainable results.

5.1 RECOMMENDATIONS

The reasons behind people's behaviour when purchasing food products are explained in this study, the relationship of extra cost and their willing to purchase and sustainable product instead of the usual one. Also the aspects respondents most take in consideration when purchasing food products, our results show dominant research interest in the cognitive consumer decision-making process, future research should focus more on affective and habitual decision-making, influenced more by emotional state or unconscious decisions.

One of the reasons identified behind consumer's not purchase more of plant-based products, is their not awareness of their benefits to human health, seen this, it is recommended that the food industry focus on consumer's knowledge about novel ingredients used by them which will bring improved health benefits to consumers and also benefits to the food industry, such as product stability and functionality. The role of educating customers, with clear and complete labels containing ingredients and nutritional information will bring benefits to both sides.

In terms of our review, was identified that the extant literature has made substantial contributions to our understanding of the nature of consumer decision-making in the sustainable-consumption context. However, with our surveys and sensory tests we also discovered a number of shortcomings, contradictions and research gaps, such as consumers are worried about purchasing sustainable food products if it helps the future health of the planet, they are prepared to pay a small increase of value on sustainable products and that consumers are focused on nutrition and cost when purchasing food products. These findings offer an abundance of future research opportunities in this knowledge domain.

Finally, although this research has provided more understanding of the attitudes, knowledge and behaviours of the population living in Ireland, towards plant-based proteins used in the food industry as ingredients and its properties, there are many still to be explored. In order to provide a complete framework on the usage of sustainable food proteins at national level in Ireland, further research should be conducted, a full country study is recommended. This should be mandatory study carried out by each county council in order to achieve accurate results per region.

SECTION SIX

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6.0 REFERENCES

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SECTION SEVEN

JOURNAL ARTICLE

7.0 JOURNAL ARTICLE:

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Food Proteins: Sustainable Sources of Proteins Responsible for Emulsions Stability in Food Products

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ABSTRACT

One of the major challenges the manufactures are facing is the fact that consumer's personal health standards are changing constantly, modern consumers are willing to know more about the ingredients used in their food. There is also the relationship with ethics and sustainability which is leading to changes in the food industry. Seen this, manufactures are opting for other sources of ingredients, such as plant-based alternatives. The use of sustainable plant proteins provides a variety of benefits to the product, such as added nutritional value, such as fiber, proteins and antioxidants, emulsion formation, product stability, extended shelf-life. There is a wide varied of plant-based proteins used in the food industry.

Due to the high number of theoretical approaches and research that have been done aiming to discover sustainable sources of plant proteins or even focused on improved methods to develop emulsifying properties of these proteins and to increase their usage in food industry, leading to satisfied consumers and the reduction of sustainability issues. This paper aim to identify consumer's behaviours, knowledge, attitudes, preferences, and awareness levels associated with sustainable sources of plant-based proteins used as ingredient in the food industry versus animal origin proteins of people living in Ireland.

The survey population was randomly selected to assess the extent of understanding and views from different customers, with the aim to obtain a wide variety. Moreover, the findings identified that consumers are demanding accurate information on labels, such as ingredients description and benefits. The food industry is developing new methods for improvements on the usage of plant-based proteins. Consumers are not fully aware of plant-based proteins used as product ingredient, neither are aware of their nutritional benefits. Most interestingly, the relationship of the use of more sustainable sources of food proteins and the development of a fully sustainable food chain with the future health of the plane is known by the respondents. This research offers an abundance of information for future research opportunity, and its recommended further research for a complete framework on the usage of sustainable food proteins usage at national level.

Keywords: Ireland, plant proteins, emulsion, sustainability, consumer behaviour, consumer attitude.

Type: Research paper

1. Introduction

Nowadays, changes in personal health standards, ethics and sustainability has led to the increased demand for plant-based food or ingredients in food products. Seen this, manufacturers are looking for other plant-based alternatives to use in the food industry to redesign these products formula and serve these new consumers (Chung, et al., 2019).

In the food industry there are many types of emulsifiers, they can be in a low molecular weight – synthetic (e.g. monoglycerides, sucrose esters, polyglycerol esters) (Lam & Nickerson, 2013) or natural (derived from milk proteins, polysaccharides, phospholipids and surfactants). The choice of an appropriate emulsifier is one of the most important aspect when formatting an emulsion-based product, a better understanding of the molecular basis of the emulsifier is of vital importance for the emulsions creation (Ozturk & McClements, 2016).

Soybean and wheat are historically considered to be the main sources of plant based protein ingredients, meanwhile, milk powder (casein, whey protein) are considered to be the major source of animal based protein used to emulsify mixtures. However, nowadays, all these protein emulsifiers are considered to cause allergic reactions in sensitive human being. The need for more researches on plant based emulsifiers are needed because of the increased concern to dietary restrictions linked to milk and egg protein. Allergic reactions, diseases such as bovine spongiform encephalitis and multidrug-resistant food-borne pathogens are some of the concerns mentioned (Burger & Zhang, 2019). A study carried out for Lam & Nickerson (2013) studied other natural sources of proteins and its possible emulsifying properties. It was analysed ingredients from legumes, such as faba bean, lentil, pea and chickpea, also oilseed and flaxseed crops emulsifying properties.

Proteins are formed by blocks, and twenty different types of amino acids are responsible to build these blocks structure. Nineteen of these amino acids have the same general structure $H_2N\text{-}C\alpha H(R)\text{-}CO_2H$, having only group R (side chain) as a divergence between each type of protein (Yada, 2017).

Whey protein its basically constituted by β-lactoglobulin, α-lactalbumin, bovine serum albumin and immunoglobulin. These amino acids form an amphiphilic protein, which in emulsions are responsible for emulsion stabilization and prevention against flocculation (Bai, et al., 2019).

Soybean is essentially composed 40% protein and 20% oil. Soy proteins are classified according to their sedimentation levels, known as globulins and albumins, which have a globular structure (Nishinari, et al., 2014).

Pea protein concentrates are normally composed by 3-10% carbohydrate, 0.5-3,5% lipid and 4-9% moisture. They contain approximately 20% - 30% proteins of different types, which mainly include globulins (65%-80%) and albumins (10%-20%) (Burger & Zhang, 2019).

Saponin's surface structure are considered active because it consists of both hydrophilic (such as rhamnose, xylose, arabinose, galactose, fucose, glucuronic acid) and hydrophobic chemical groups (such as quillaic acid and hypsogenic acid) on the same molecule (Yang, et al., 2013).

There are three main groups of potato proteins, representing up to 40% is the glycoproteins patatins, the second group representing up to 50% of the potato proteins is the protease inhibitors. The last group is formed by oxidative enzymes, such as lipoxygenase, polyphenol oxidase and enzymes associated with starch synthesis (Schmidt, et al., 2018).

Microalgae structure is composed by biopolymers, such as proteins and carbohydrates. These biopolymers might have potential role as texturizer, stabilizer or emulsifier in food products. Microalgae possess a range of 9-77% of proteins, 6-54% carbohydrates and 4-74% lipids (Bernaerts, et al., 2019).

Proteins are known in the food industry also because of its emulsifying properties, which are highlighted due to its amphiphilic nature. Having an amphiphilic structure, means that they contain hydrophobic and hydrophilic groups along the chemicals compounds chain. These groups are responsible for embracing the molecules that usually wouldn't format a stable emulsion. The understanding of the term "emulsion capacity" is defined as the ratio in grams of oil needed per grams of protein that can be help before a phase inversion (Lam & Nickerson, 2013) Emulsion stability (ES) is a measure of the amount of creaming over time (Burger & Zhang, 2019).

When determining protein's emulsifying characteristics, a main aspect that needs to be taken in consideration is their physiochemical properties. An important factor that influences proteins absorption in the oil side of the interface, is the surface hydrophobicity. This will lead to a better molecule integration, hence a higher emulsion stability. Surface charge of the protein is another factor that influences protein's solubility in the aqueous phase, because a high protein solubility is required for a better diffusion ratio to the interface (Lam & Nickerson, 2013).

Burger & Zhang (2019) refers to the large variety of methods used to measure emulsifications properties of proteins, such as turbidimetric techniques, conductivity and droplet size measurements, and relates to the reason why they were never comparable in studies. Turbidimetric techniques, which include Emulsifying Activity Index (EAI) and Emulsifying Stability Index (ESI) is responsible to measure the ability of the protein be absorbed to the interface, meanwhile EAI measures the absorbed layer's stability within a determined period of time.

Li et al., (2016) describes the protein absorption process of soy protein and specify three main steps, protein diffusion, formation of the protein monolayer and interfacial jellification. Protein diffusion is a process where the interfacial tension and complex viscosity does not go under changes for a period of time and can only be identified for a dilute protein solution. The following step is when the formations of the protein monolayer occurs, which include the processes of structural deformation, interfacial tension reduction and the rapid increase in complex viscosity. Third and last step of protein absorption, specially for globular proteins is the interfacial jellification. This step is when the multilayer is formed, with the aim to increase the complex viscosity a slight reduction in the surface tension is applied.

The nutritional and functional properties of the milk proteins are very important factors for many different processed foods. Their chemical structure, configuration, and aggregation state in milk protein food products has the capacity to stabilize the oil droplets in oil-in-water emulsions (A. Taneja, 2015).

Milk proteins are composed of polypeptide chains with amphiphilic nature, they contain hydrophilic and hydrophobic amino acids, for this reason they can be absorbed by oil-water emulsions and help the homogenization process encompassing oil droplets. Protein molecules are dense and it tend to be thick and diffuse in slower proportion if compared to other small molecular emulsifiers (Lam & Nickerson, 2013).

The emulsifying properties of these proteins derived from milk depend on their physiochemical properties, the hydrophobic group influences the oil side absorption, leading to a better interaction rate and higher emulsion ability. Whereas, surface charge with in the aqueous phase, is another important aspect that influences milk protein solubility and stability. Proteins derived from milk, soy and wheat have been studied, and also the partial hydrolysis properties of these ingredients. The main purpose to study the effects of partial hydrolysis is to evaluate if these proteins are providing better emulsifying properties and increasing emulsions solubility. The functionality improvement of hydrolysed protein increased the use in the food industry, this is because of reappearance of hidden hydrophobic groups which increases the surface hydrophobicity, reducing its molecular weight, enabling better oil-water interface adherence (Lam & Nickerson, 2013).

The food industry has a great interest on casein’s functional properties, however, casein’s micelle capacity to emulsify and stabilize oil-in-water emulsions is of most significance on the food industry interests. Casein’s micelles are known to be responsible, especially in dairy products, to contribute to their functional properties, an important role is the ability to form or to stabilize gels, foams and emulsions samples (Lazzaro, et al., 2017).

Chemical nature, conformation at the interface and aggregation state of caseins are directly linked with its emulsifying and stabilizing capacity. Caseins are known to be absorbed at the interface, in individual or aggregated form and are able to strongly perform emulsifying agent role. Sodium caseinate is an example of poorly aggregated casein system, which are less effective for the stabilization of emulsions than highly aggregated casein micelles (Lazzaro, et al., 2017).

The emulsifying characteristics of pea protein is one of their most important functional properties, and its need in the food industry as a plant-based emulsifier will broaden research. Many factors can influence pea protein emulsifying properties, such as heat treatment which can expose the hydrophobic groups in the protein molecules, hence leading to an increased protein aggregation, and better emulsifying properties of soy protein isolate. Protein molecule structure, chemical composition, pH, environmental conditions, ionic strength and the volume fraction of oil phase also contribute with the emulsions stability and absorption (Chen, et al., 2019). Thus, the commercial utilization of pea protein as emulsifier is still limited. The lack of knowledge on its structural and functional properties, and a large gap between commercially used pea proteins and the pea proteins prepared in laboratory (Burger & Zhang, 2019).

A study conducted in 2019 analysing the emulsifying and stability properties of pea proteins, highlighted some extraction methods to obtain less denatured proteins with better solubility, emulsifying and foaming properties. The most used methods to extract and produce pea proteins are alkali extraction, acid precipitation and spray drying, and to obtain less denatured protein methods such as salt extraction-dialysis and micellar precipitation are used (Chen, et al., 2019).

Tamanak et al., (2016) mentioned that pea proteins when compared to non-soy legume proteins (i.e. chickpea, faba bean), has poor solubility, limiting its emulsifying properties. It happens because of its low surface charge density, a more hydrophobic surface structure, leading to a relative poor solubility. Tamn et al., (2016) connects pea proteins poor solubility with their complex molecular structure, their globular form which may limit emulsification power and for having a compact tertiary structure, restricting the molecular flexibility.

Recently many studies have been interested in the use of saponins as a plant-based emulsifier. Saponins are extracted from the *Quillaja Saponaria Molina tree*, as a secondary metabolite. Saponins, when compared to other natural emulsifiers, can be used at low levels and be able to form fine oil droplets and keep the emulsion stable over different environmental changes. These metabolites are already available for the use in the food industry as some previous researches have reported quillaja saponin as an efficient emulsifier for forming and stabilizing oil-in-water (O/W) emulsions and nanoemulsions (Zhu, et al., 2019).

Natural surfactants, such as Quillaja saponin and Lecithin were used to evaluate the formation and stabilization of vitamin E based nanoemulsions. Ozturk et al., (2014) examined the effect of oil compositions (0-100% vitamin E acetate), surfactant type and concentration (0,0005-5%) on the particle diameter and vitamin loading capacity. The study showed that when increasing both surfactant concentration, the mean particle diameter decreased, with lower levels of quillaja saponin needed to form small droplets. Physical stability was also evaluated, Ozturk et al., (2014) took in consideration pH effects, ionic strength and temperature. Quillaja saponin samples were stable over a pH variation (3-8) and salt addition (concentration 0-300 mM NaCl), however showing flocculation at lower pH and higher salt concentrations. Both surfactants formed stable droplets over heating variation (30-90 °C for 30 minutes), in not salt addition conditions. This study proved that the use of quillaja saponin in the food industry will provide similar surfactants properties when to lecithin, which is known as an efficient surfactant.

In the past years, potato has been noticed as an innovative protein source for human consumption, and as novel plant based protein offering more sustainable and cost effective alternatives. Potato protein just as other natural sources of proteins possess high nutritional values, assuring food functional properties, forming structural networks such as gels, foams and emulsions. In food products where the formation of a final set gel is required, proteins are of great importance for gel building by binding water, as well as, for the textural properties of the final sensory perception of the food gel (Schmidt, et al., 2018).

Aside of the nutritional and functional properties, potato protein carries a particular feature, being gluten-free (GF). The development of new GF products is emerging because of changes in the dietary requirements for celiac disease, for people looking for non-allergenic ingredients, and because of the industry needs for product variation. These new requirements are leading to a new category of products in the food market, expanding the variety of products offered to this public (Calero, et al., 2013).

A study designed to analyse microwave radiation effects and protein addition to rice and potato starch was conducted by Villanueva et al., (2018), the supplement of protein added in the samples were 5% of calcium caseinate (CA) or soy protein isolate (SPI). To analyse the improvements that may have occurred in the samples, hydration, pasting properties and viscoelastic features were taking in consideration. The present study aimed to evaluate the physical modification of the systems and the possibility to develop ingredients with new functionalities. The results revealed that the microwave radiation treatment (MWT) was influenced by the botanical origin of the starch, the presence and the type of protein added. The key changes were notated when SPI was added, in addition, potato starch blends showed an increased water absorption capacity and decreased water solubility index. To sum-up, MWT on potato-protein blends supported gel stability, reduced pasting profile and improved viscoelastic moduli, seem this, Villanueva et al., (2018) could confirm that the combination of MWT with protein addition allows to develop new food products with tailored properties.

Soy proteins has been used in the food industry for many years, the reason for a wide use is its large possibilities in emulsifying properties of food products. Products such as beverages, infant formulas, ice cream and dairy products are examples of its usage. However, due to their large molecular weight and globular structure, improvements need to be made regarding soy protein poor surface activity, such as proteolysis which may improve their emulsifying properties (Li, et al., 2016).

A constant increasing interest on the development of food Pickering stabilizers has occurred in the food science environment, the development of novel non-thermal process to produce soy protein isolate (SPI) nanoparticles with the aim to perform as effective emulsions stabilizers. A study conducted by Ou et al., (2017) analysed the effects of the addition of Ca^{2+} would induce. The use of Ca^{2+} on a determined protein concentration (2%) induced aggregation and crosslinking, when the Ca^{2+} concentration was increased, a progressive turbidity and particle size was observed this indicates aggregation and formation of nano-sized particles. The results revealed that the addition of Ca^{2+} at SPI samples, seen the finding of larger particle sizes, lower surface charge, stronger internal integrity, lower emulsification performance and higher emulsion stability against coalescence, confirm that the fabricated SPI nanoparticles could perform as an effective pickering stabilizers.

A study conducted by Mozafarpour et al., (2019), analysed the structured of soy proteins and methods to improve interfacial activity and emulsifying property. Many different methods have been used in soy proteins such as chemical, thermo mechanical and enzymatic modifications with the aim to modify their physical properties. Also the use of hydrogen and disulphide bonds to stabilize their compact structure of globular soy proteins is mentioned as tactic to enhance their absorption. Partial denaturation under controlled heating and shear conditions followed by unfolding of their rigid globular structure was identified as responsible to improve protein surface activity, consequently improving absorption of soy proteins at air/water and oil-water interfaces. Heat treatment makes structural modifications, leading to a more flexible protein and the improvement of the molecule surface activity.

Soy lecithin is a relevant natural surfactant that has been used in the food industry as an effective emulsifier. It’s emulsifier properties are due to its amphiphilic molecular structure, which contain a lipophilic group formed of fatty acids and a hydrophilic group formed of phosphoric based esters, which are responsible for its great emulsification potential (Wang, et al., 2017).

A study conducted by Chung et al., (2019) investigated the properties of oil-in-water emulsions stabilized by sodium caseinate or soy lecithin in black coffee solutions. The aim was to verify the use of soy lecithin as a potential emulsifier in order to fully or partially substitute sodium caseinate in oil-in-water emulsions. The test identified that either caseinate or soy lecithin, in all model emulsions, had similar whitish appearances.

In addition, blends stabilized by both stabilizers at the same time were able to produce O/W emulsions with similar colours, particle sizes, and electrical properties, indeed, it was also able to whiten a black coffee slightly higher whitening power than those samples with only caseinate. Overall, was concluded that soy lecithin can be successfully used to replace sodium caseinate, fully or partially in O/W emulsions.

Microalgae is considered a novel functional ingredient, which provide scientifically prove of health benefits to human body and reduce the risk to develop disease. Aside to their multiple nutritional, health benefits and balanced composition, microalgae has also a structuring role acting as an effective texturizing ingredient in food. The specie Photoautotrophic microalgae is rich in proteins, storage polysaccharides and cell wall related polysaccharides, and these components are known as structural biopolymers. Nevertheless, even with the limited study on microalgae functionalities, food processing operations have demonstrated success in providing required microstructural and rheological properties (Bernaerts, et al., 2019).

Microalgae nutritional value is emphasized because of their similar quantities of protein, when compared to other protein sources like milk, soybean, egg and meat. Moreover, microalgae have naturally developed protective systems, due to extreme changes in the environmental conditions, this lead for the production of antioxidants and pigments, which are important for human consumptions as supplements, because it isn't naturally internally produced. In addition, in an environmental point of view, microalgae protein extraction is more efficient and requires less water sources, when compared to terrestrial crops production, such as wheat, pulse legumes and soybeans (Koyande, et al., 2019).

It's known that ruptured algae can clearly act as efficient surfactants and emulsifiers, however there is a gap about their real role when stabilizing emulsions, and to help to fill this gap a systematic study conducted by Law et al., (2018) was developed with the aim to understand emulsification and demulsification processes in the wet lipid extraction. Knowing the demulsification processes, will be easier to develop strategies to feasibly solvent recovery. The results revealed that proteins are great emulsifiers and the microalgae protein extracts have the same level of emulsification properties.

2. Materials and methods

2.1 Introduction:

Mixed research methods were implemented in this study, with the intention to collect, analyse and mix both qualitative and quantitative data, within a single study at some stage of the research process, aiming to understand and evaluate the current trends evolving plant-based proteins. The main reason to put the survey results together is to capture the details of the situation, because neither quantitative not qualitative method is sufficient by themselves.

To measure respondent's attitudes, beliefs and opinion a "Likert Scale" method was used in some questions.

2.2 Sample selection:

A total number of 221 people were selected to complete the customer's online questionnaire, and 60 participants for the sensory analysis study. The selection of the respondents was random in order to assess the extent of understanding and views from different customers and to obtain a wide variety, while the sensory analysis was applied in a face-to-face method. The diversity of people and different age range and reality, give better inference as to a customer behaviour knowledge, and attitude toward plant-based proteins used in the food industry and its nutritional properties.

2.3 Questionnaire Design:

An online questionnaire was developed comprising only one section and containing 8 quick questions to be answered. These questions were developed to gather information regarding costumer's knowledge about plant-based proteins used in the food industry as an ingredient, its functional properties and correlating with costumer's worries on sustainability. Each question was carefully developed in order to comprise the study objectives.

The sensory analysis study consisted of three protein samples and four different tests. The protein samples were whey protein, pea proteins and chlorella protein (representing microalgae). It consisted of section 1, asking about their gender and age range, followed by Check-All-That-Apply (CATA) analysis, where the participants were asked to consume a portion of the solution and review all the sensory characteristics they thought did apply for each sample, separately. The test was designed to allow multi answers options from respondents. This test is a simple and fast sensory profiling tool, which allows researchers to analyse sensory similarities and differences between samples.

The second test was the liking test, where respondents were advised to choose their level of taste liking for each sample. Three main points were stablished on the scale, Extremely Dislike, Neither and Extremely Like. There was also the triangle test, aiming to determine whether there is a sensory difference between two samples, two of the same (pea protein) and one different (whey protein). They were asked to identify the sample which they felt was different. The last test was the preference test, designed to evaluate proteins' acceptability. Participants were asked to identify their favourite sample between three different samples (Whey, Pea and Chlorella).

Once the questionnaire was constructed and corrected, a pilot test was carried out to the first 20 random participants for the online customer survey and 3 for the sensory analysis. After ensuring the results of the pilot survey were accurate, and the questions were fully understood, it was sent for the next 201 respondents for the online survey and 60 respondents for the sensory analysis, increasing the likelihood of the survey.

2.4 Data preparation and collection:

All questionnaire responses were inputted into Survey Monkey. The survey was distributed randomly to a total of 221 anonymous Irish participants, while the sensory analysis study was conducted face-to-face in a particular population of 60 anonymous people living in Ireland. The online questionnaire was distributed through social networking site, such as, E-mail, Facebook, LinkedIn, etc.

The entire survey took 48 days, from October 31 to December 18 of 2019, to be completed the results were collected directly through Survey Monkey. The data collection for sensory analysis was initially done with college students and lately with close friends. The results were put together in MS Excel table to be analysed and created results.

3. Results and discussion

The sample size of the research was 221people for the online customer's survey and 60 for the sensory analysis study, which was made up 34 females and 26 male respondent's, giving a 57:43 females to males' ratio. With relation to age range on the sensory analysis results, the age category of most of the respondents were between 18 to 24 years old, representing 52%, or 31 respondents. The second category of age mostly identified was 25 to 35 years old, comprising 28% of the respondents, the smallest group was 56 to 65 years old respondents, with only 3%.

In regard to the descriptive analysis, the Check-All-That-Apply (CATA) indicated the differences and similarities of characteristics in pea protein samples and chlorella samples. In regard to cross tabulation, the main difference was Chlorella with 13% of grassy characteristics compared to only 4% for pea proteins. For pea proteins, the characteristics that mainly distinguish from Chlorella proteins were soil (8-4%), bland (8-1%) and rice (8-1%). While, infant formula (5-0%), sand (5-0%), couscous (3-0%), nuts (6-0%) and milky (3-0%) were identified in a good percentage for pea proteins, but not appeared in Chlorella sensory characteristics. This study is used with the aim to identify relevant sensory attributes that affect adult's hedonic perception, also used the know how consumers perceive plant-based products against commonly used animal based (Lungu & Muchenje, 2018).

The ranking test was implemented to assess taste acceptability of participants, a ranking scale for each protein sample was provided, and it has been found that most of the consumers indicated whey protein (17%) as the most likable sensory characteristics, and the least dislikeable (13%) between the other samples. On the contrary, microalgae sample is being identified as the most dislikeable (80%) taste preferences and considered at the same category of extremely liked as pea protein, achieving (5%). Unexpectedly, the results identified that whey protein in a high level (70%) of “neither” category, representing the scale neutral category. Pea protein also achieved a good percentage of the neutral scale category (43%). This results help us to understand that whey protein is still better accepted when compared to plant-based proteins, and that pea protein was had a lower dislikeable rate, and higher neutral point, when compared to microalgae sample, proving better acceptability.

A study conducted by Wee & Forde (2019) analysed physical and sensory characterisation of noodles with added denatured pea protein isolate. The results revealed the addition of denatured pea protein did not change significantly textural or sensory qualities of noodles.

Triangles tests are extensively used in food analysis to determine if differences between two products can be sensory detected. The majority of the respondents (78,4%) identified whey protein as the different taste, with a vast divergence with the other protein samples results. This results help us to understand that pea protein isn’t considered an effective substitute in whey protein emulsions, considering sensory characteristics and not its functional properties.

By contrast, a study conducted by Aschemann-Witzel & Peschel (2019) with the need to evaluate consumer’s perception of new alternative ingredients, such as proteins, revealed that when the manufactures highlighted the protein origin on labels, it led to a more favourable perception of the product, specially for consumers interested in plant-based food. Potato protein was identified more favourable in the quality dimension tests, also providing texture.

Preference tests provide information about whether people dislike or like a product. The evidences show that 73% opted for whey protein sample as their favourite, a discrepant difference when compared to Pea protein (15%) and Microalgae accomplishing 9%. Relating to the literature review, customers are demanding products with plant-based ingredients, but still are linked to animal origin sensory characteristics.

A review on sustainable food proteins and its use in the food industry towards sustainability, health and innovation was studied by Fasolin & Vicente (2019), the results revealed that sustainable aspects of alternative proteins, used as ingredients, such as plant-based, insects and microbial are still little explored, mainly in micro and nanoscales.

The online customer survey was developed with the aim to analyse participant's knowledge and usage of plant-based proteins and their attitude on purchasing products and its given importance to sustainability. In relation to question one and two, it has been found that mostly people are not aware about microalgae as a food ingredient (70,59%), neither know about their nutritional benefits (89,2%). In addition, is important to mention the relationship of respondent's awareness between microalgae as food ingredients and their knowledge about their nutritional benefits. The results expose that out of the 29,4% of respondents aware of microalgae as a food ingredient, only 10,82% confirmed to be aware of their nutritional benefits. The results provide us an insight that even though consumers are demanding for more 'clean label' products, with more sustainable ingredients, they are not really aware of some novel ingredients being introduced in the food industry, and not even into the nutritional benefits they can provide to human health.

Schunko & Vogl (2019) investigated organic consumers' knowledge and practices and they also concluded that organic consumers are unaware of wild ingredients used in organic products and this is related to poor labelling. This study also concluded that this category of consumers barely differentiates between organic and non-organic wild food, and there is a lack of awareness on market relevance from organic consumers.

For most customers (66,67%) would consider the consumption of microalgae as a source of high protein nutrition, only 27,85% were not opened to this novel option of sustainable protein. The main reasons to consider the introduction of microalgae to their eating habits were mostly the nutritional benefits, such as being source of omega 3, rich in protein and antioxidants and provide immunity boost.

Accordingly, Lang (2020) investigated consumer acceptance of blending plant-based ingredients and the results exposed that there is a trend towards shifting from meat-centric to plant diets, and that their acceptance of blending plant-based ingredients into meat-based foods is increasing.

When customers were asked if they are familiar with the role of the consumption of sustainable food products in the future health of the planet, the majority confirmed the awareness of the fact (70,59%), whereas, other customers' (41%) confirmed not familiarity with it.

Bangsa & Schlegelmilch (2020) research insights revealed an increase in consumers worries and interest on sustainable products not only in food and drinks, but also in clothing, homes, mobility and footwear. In addition, the familiarity with the consumption of sustainable food products in the future health of the planet is related variables such as, socio-demographic, behavioural and lifestyle segmentation.

Consumers opinion about plant-based diet to the future of a sustainable food system. The majority of the respondents (76,9%) confirmed the importance they give to the reduce meat consumption and important changes that need to me done for a balanced and sustainable food system, whereas 23,1% people did not give the right importance for plant-based diet and its power of improvements.

Graça & Truninger (2019) research findings reveals that the transition to more plant-based diets is increasing, however it is still fragmented because of some barriers, such as lack of information for consumers, difficult to develop new cooking skills and positive taste expectations for plant-based meals are some of the variables stopping plant-based diet adoption.

Question six completes question five, when its asked if the respondents would change to a plant based diet if it could improve the future health of the planet. Evidences showed that they care about the subject and said yes (35,7%) to this changes, while other respondents would try to change (56,5%) starting with some few products to check the benefits it could bring to them. The amount of people that revealed to have no interest in plant-based products and its relationship with future health of the planet was very low (7,8%), compared to question five, when respondents said to not give importance (23,1%) to a plant-based diet for future sustainable food system.

Cleveland & Gee (2017) and Pérez-Martínez & Pérez-Jiménez (2019) has found a strong correlation between the importance of changes to plant-based diet and improvements in the future sustainability of the food system and health of the planet.

When the increase of spending on food products, if it led to a fully sustainable food chain was approached, customers had to answer in how much they were prepared to pay. Out of the 221 customers, 42% would be ready to pay an increase of 10% of the price, followed by 32,5% of the respondents would be ready to pay an increase of 30%. These results are surprisingly, because they show us that customers would pay more for sustainable products, only a small portion of the respondents (7,7%) would not be prepared to pay an increase of value.

The descriptive results of question eight reveals some interesting results. Respondents were asked to classify how they consider some attributes when purchasing products. Attributes such as cost, sensory, organic, brand, nutrition and sustainability, the results can provide an insight on the importance they give on each character, especially on nutrition and sustainability, and how cost is classified. The attributes of nutrition (4,48) and cost (4,4) were equated, nutrition is considered the most important aspect when purchasing food products by these 221 people. Aspects such as brand and sustainability were considered the least important to them. The results exposes to us that nutrition and cost are considered more important than sustainability and that sustainability is the last attribute customers take in consideration when purchasing food products. Sensory aspects and organic were classified just after cost and before brand.

Lanfranchi et al., (2019) study points out that the knowledge of sustainable production methods significantly influences the decision-making of consumers when the premium price increases, and also they are more prone to buy sustainable wine only when the premium price is low. In addition, Ghyanidze et al., (2017) showed that price and nutritional information are considered more influential in consumers' food choices than information relating to the ecological impact of production or social responsibility of the producers.

4. Conclusions

In this study, consumer's behavioural factors when purchasing sustainable products has been highlighted, factors such as preferences, usage and knowledge. The demand for more information coming from the new consumer's generation is increasing, the food industry is developing new technologies to reach this demand, and developing novel plant-based products.

Results obtained, revealed that consumers would consume sustainable plant proteins because of its nutritional benefits. However, the data presented expose that they are not aware of for example, microalgae as a non-meat protein ingredient or neither their nutritional benefits. The sensory analysis results revealed whey protein, when compared to pea protein and chlorella protein, is still preferred by the consumers. The relationship of the importance of the adoption of a plant-based diet with the future health of the planet, and also with the development of a fully sustainable food chain was investigated and the majority of consumers exposed to significantly care about it, be aware and even be prepared to pay a small increase of value when purchasing sustainable food products.

These findings underline the food industry need to educate customers and invest in marketing strategies, to provide more clear and complete label information about their ingredients and nutritional benefits. If innovations on the ingredients side is done, aiming to improve plant based proteins sensory profile, their usage and acceptability will widespread uptake. To conclude, in order to make sustainability more valuable to people, it must be made more attainable first.

Conflict of interest

The authors declare no conflict of interests.

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Highlights:

Novel Consumer's health standards are enforcing sustainable changes in the food industry

Plant-based proteins are capable of provide health benefits to humans

Sustainable plant proteins are being used as efficient emulsifier in food products

Consumers are willing to pay a small increase of price if it leads to sustainability

SECTION EIGHT

APPENDICS

8.0 Appendics

Appendics 1 – Customer online survey

Plant based products (protein emulsifiers) - Customer

FOOD PROTEINS: DIFFERENT SOURCES OF PROTEINS RESPONSIBLE FOR EMULSION STABILITIY IN FOOD PRODUCTS

This survey is designed to identify consumer knowledge and preferences of food protein as an ingredient, and the relationship of purchase power and sustainability. This questionnaire can help researches develop common practices or misconceptions that should be addressed. This survey is part of the Masters in Food Safety Management program at Technological University of Dublin, Ireland. If you decide to take part of this study, all the information provided will be confidential and the respondent can not be identified. I appreciate your help and participation.

1. Are you aware of microalgae as a food ingredient?

- [ ] Yes, I read something about its benefits.
- [ ] Yes, I use/used products with microalgae as food ingredient.
- [ ] Yes, Know about the benefits but opted to not purchase products with it.
- [ ] No. Never heard about microalgae as a food ingredient.

Other (please specify)

2. Do you know any relevant nutrition benefit of microalgae?

- [ ] Yes.
- [ ] No.

If yes, (please specify one benefit)

3. Would you consider consuming microalgae as a source of high protein nutrition?

☐ Yes

☐ No

☐ Please specify the main reason.

4. Would you consider yourself familiar with the role of sustainable food products in the future health of the planet?

☐ Yes

☐ No

5. Do you think a plant-based diet is important for the future sustainability of the food system?

☐ Yes

☐ No

6. Would you change to a plant-based diet if it could improve the future health of the planet?

☐ Yes

☐ Maybe, could start buying few products to check its benefits.

☐ No, I have no interest in plant-based products.

7. Would you be prepared to increase your spending on food by the following amount, if it led to a fully sustainable food chain?

- ☐ 0%
- ☐ 10%
- ☐ 30%
- ☐ 50%
- ☐ 70%.

8. When purchasing food products, place the following criteria in order of how you consider them.

- Cost
- Sensory
- Organic
- Brand
- Nutrition
- Sustainability

Appendics 2 – Sensory analysis study questionnaire

Check All That Apply Sensory analysis

Gender:

Age range:

☐ 18-24 ☐ 25-35 ☐ 36-45 ☐ 46-55 ☐ 56-65 years

The following solutions contain several allergens. **If you suffer from any food allergy, please do not partake in this study.**

You will be presented with a sample solution. Please consume a portion of the solution, allowing it to wash over your tongue. Once you have consumed the solution, please review the sensory characteristics and tick those that you think are present in the sample. You may select as many as you think are appropriate.

☐ Grassy	☐ Weeds	☐ Infant Formula
☐ Wheat	☐ Broccoli	☐ Rice
☐ Bitter	☐ Kale	☐ Sand
☐ Floral	☐ Fruit	☐ Seeds
☐ Astringent	☐ Sea Weed	☐ Nuts
☐ Cucumber	☐ Grapes	☐ Couscous
☐ Fermented	☐ Green Smoothie	☐ Bitter
☐ Soil	☐ Natural	☐ Cold Tea bag
☐ Earthy	☐ Milky	☐ Bark
☐ Organic	☐ Bland	☐ Leafy
☐ Green tea	☐ Sweet	☐ Eggy

Liking Test.
Panelist Instructions;

- You will be given three samples, taste each sample beginning with the sample on the left.
- Mark with an x the point on the scale which correlates to your liking of the sample.
- Rinse and swallow before moving on to the next sample.

043

|---|---|

Extremely Dislike	Neither	Extremely like

453

|---|---|

Extremely Dislike	Neither	Extremely like

565

|---|---|

Extremely Dislike	Neither	Extremely like

Triangle test.

- You will be given 3 samples.
- Two samples are the same and one is different.
- Taste each sample from left to right.
- Rinse and swallow before moving on to the next sample.
- Circle the sample which you feel is different.

043 453 565

Preference test.

- You will be given three samples.
- Taste each sample from left to right.
- Rinse and swallow between each sample.
- Circle the sample which you prefer.

043 453 565