FIPDes

Food Innovation & Product Design

FIPDes Day 2020
International talents in Food Innovation and Product Design

Students' Book of Executive Summaries

Congratulations to the FIPDes Cohort 8 !

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We shall strive for infallibility without pretending to reach it

"We shall strive for infallibility without pretending to reach it": this was the motto of the great French chemist Michel Eugène Chevreul, who discovered the chemistry of fat, the same person that introduced the word "organoleptic", so widely used in our food science and technology circles, the same that... Stop! The list of his achievements is much too long, in particular because Chevreul would live for hundred and two years... And he said, during an interview at the age of 100 years old, before the state celebration of his birthday, that he was "the dean of the students".

Illustration 1: Michel-Eugène Chevreul (1786-1889), who discovered the chemistry of fat, said of himself, when aged 100 years old, that he was the dean of the students.

Now, we are ready for the discussion, because we have the two poles between which I want to build this introduction to the yearly book of our wonderful Erasmus Plus master programme: improvement, on one hand, and students, on the other.

For sure, our Erasmus Master programme is excellent, because all participants are wonderful.

Let us begin by looking at our programme, because it was created about ten years ago, and during all these years, it was improved by the professors and administration It is analyzed regularly by the Consortium in charge of running it, it was guided by the Advisory Board, and evaluated by committed students.

But the order of my enumeration is not right, and students are first. By the way, this makes me think that perhaps it would have been a better option to ask this foreword to be done by one student, one alumnus... or to a trio including student, professor, and administration? This will be for the next year. Here, let us begin the discussion by savoring the fact that our master program attracts students from all over the world, in huge numbers: as a consequence, only those who demonstrated the highest commitment, as well as the strong desire to cross the world, leave families and friends, with the view of improving themselves. And, as it is well known when selection occurs, the happiness, as well as the confidence of the happy few that we receive each year creates a very special environment, which drives our young friends even further. Contrary to what is said, the mathematical theorem of regression towards the mean, here excellence generates even more excellence.

By the way, imagine that you are yourself one of these students: would you sacrifice your time for giving so much effort to join the FIPDes community? This sense for responsibility, along with a personal pleasure to study that was responsible for their previous university results, is a key asset for their success.

And finally, I am happy to testify that many of our young friends come from countries where there is a lot to do, to develop a useful food industry. Here also, we contribute to build the knowledge and skills on firm grounds.

You will find an examination of all this context in the following pages of this book.

About professors, now, our consortium links four of the best European universities: good universities means good scientists and professors. Of course, there are many debates, within the science, technology and education communities about what does it mean to be a good scientist, or a good professor, but could we not usefully make the following analysis:

1. The (scientific) knowledge was increased regularly since the beginning of modern science, in the Renaissance, with remarkable individuals such as Galileo Galilei, Francis Bacon, François Viete and then many others.

2. Century after century, layers of knowledge were deposited, until the current "mountain of knowledge" was established.

3. At each time, the new knowledge was transferred to technique through "technology" and, of course, the most efficient technology transfer was from the tip of the mountain; at each time, one would be probably late in exploring the possibilities of transfer from older knowledge.

4. As a consequence, if we want the students to be really innovative, we have to help them to be at the tip of the mountain, this tip is where the best scientists are.

5. And who knows better the more modern science than the best scientists?

All this is to say that the professors in the environment of our FIPDes master programme are wonderful as well. Of course, I would be more easy at writing this if I were not myself one of the professors, but anyway, writing it is also a commitment, a promise to deserve the honor of being part of the team that tries to contribute to support our students in their studies.

You will hopefully find an emanation of all this context in the following pages of this book.

Finally, even if it is frequently said, we have to repeat it: the junction between student and professor would hardly be possible without an efficient administration. Imagine the efforts to get visas, when so many countries are concerned. Imagine finding lodgings, when you are moving from one country to another, in the course of your curriculum... For sure, today, the Earth is a village... but borders of so many kinds remain! National borders, health borders (remember the covid!), cultural borders...

Our colleagues in the administration do a wonderful job. In chemistry laboratories, our keywords are "Safety, quality, traceability"... but these are universal keywords. And to ensure it, we need friends that focus less on science and technology and more on "real life"!

By the way, what does "administration" mean? For sure, it means individuals in the foreign affairs of the universities, it means people responsible for students lodgings, etc. but it means also the direction of the universities, because their impulse is so much needed, and it also means the wonderful support of the European Commission, that gave us the honor to consider that our program was of excellent quality. Building the FIPDes (and others) master programme is certainly not for the faint hearted, because, for sure, Europe as well is a small village... but with different national rules, different cultures... The European vision is the key basis for our master program.

But all human enterprises are imperfect, and we have to make huge efforts to improve them

All this said, you guess that I am more at ease at trying to look for (and propose) improvements. And indeed, this book showcasing achievements of the FIPDes students is the right place, because it is a demonstration of their skills.

For sure, there are imperfections, as any human enterprise, but don't worry, and consider instead that we can strive toward infallibility without pretending to reach it. Improvement! How can we do it? I am happy to share a new vision, a new paradigm. Indeed, the idea is simple, but it includes many points, and it is based on the right use of words: after all "communication" is key in this book, and the precision of the language is tremendously important, is it not?

About this, one should always remember the introduction of the *Elementary Treatise of Chemistry*, by the great Antoine-Laurent de Lavoisier, the founder of modern chemistry, who wrote very wisely (following an underestimated genius, the philosopher and economist Etienne Bonnot de Condillac):

"We think only through the medium of words. --Languages are true analytical methods. -- Algebra, which is adapted to its purpose in every species of expression, in the most simple, most exact, and best manner possible, is at the same time a language and an analytical method. -- The art of reasoning is nothing more than a language well arranged."

Thus, while I thought myself employed only in forming a Nomenclature, and while I proposed to myself nothing more than to improve the chemical language, my work transformed itself by degrees, without my being able to prevent it, into a treatise upon the Elements of Chemistry.

The impossibility of separating the nomenclature of a science from the science itself, is owing to this, that every branch of physical science must consist of three things; the series of facts which are the objects of the science, the ideas which represent these facts, and the words by which these ideas are expressed. Like three impressions of the same seal, the word ought to produce the idea, and the idea to be a picture of the fact. And, as ideas are preserved and communicated by means of words, it necessarily follows that we cannot improve the language of any science without at the same time improving the science itself; neither can we, on the other hand, improve a science, without improving the language or nomenclature which belongs to it. However certain the facts of any science may be, and, however just the ideas we may have formed of these facts, we can only communicate false impressions to others, while we want words by which these may be properly expressed."

Illustration 2: Antoine Laurent de Lavoisier (1743-1794), the father of modern chemistry, was also a firm believer in the importance of the precision of words.

But enough, let us put this in action regarding our FIPDes master programme in particular.

1. The question is not "education", as it is so often said, but rather "instruction". Education really means politeness, manners, etc. but our FIPDes students are adults, well-educated individuals, sometimes even already having jobs. For sure, our program tries to give information on do's and don'ts in the industry, on ethics, on values, but the FIPDes students, with their remarkable cultural diversity, have as much to tell to the professors and administration as they get from them. No, the question is not education; it is to study.

2. I do not like the word "teaching", because I have the feeling that teaching is an impossible task. Learning is an active process, performed by a responsible person, and nobody can teach us, really, but on the contrary we can learn, if we decide to. Moreover, the question of "teaching to a class" is worse: in a simplistic view, let us imagine a professor explaining a specific scientific idea at a certain velocity, with a certain level of pre-requisites, with an assumed level of knowledge and skill; on the other side, the students are all different, have all their particular backgrounds, so that the sole probability (remember: the number of favorable cases divided by the number of possibilities) of a matching between a « teacher » and the students is zero.

On the contrary, professors can "profess", i.e., they can speak in front of an audience, trying to light on the Great Fire of the Pursuit of Knowledge and Skills, a burning desire to Study: again, the question is to study. Not to teach, but to study.

3. And now we can see that the students are at the core of our training institute. Not the administration, not the professors: the students are first. Of course, this does not mean that all the instructional environment counts for nothing, but it means that when goals are discussed, always the question is to study. To study in the best conditions but to study. And this puts a heavy load of responsibility on students: the question is to study!

4. So now the search for improving our program is perhaps easier to make, because the question is clear: how can we improve all details of our organization so that studies by the FIPDes students are improved? Even if the content of this book is of good quality, how can we ensure that next year will be even better? As we are what we do, and judged from our results (at work, we recognize the craftsman), this book is an evaluation of our common organization... and the next book will be -I hope- an evaluation of the efficiency of this new paradigm if, as I hope, but I have few doubts, my colleagues, older or younger, share it.

5. Of course, the question of technology and innovation is of key importance, in the deeper analysis that we made, because, as well said in the title of our master, we focus on technology, *i.e.*, the transfer from science toward technique, and to production. How do we improve with regard to this?

Here you remember that I already discussed it above, but this is the end of the analysis, because any goal should be based on values, on ethics. The question of helping future engineers to work in full conscience of their responsibility is exceedingly difficult, and even if the works that you will find in this book are explicitly tackling the issue, we always need to improve the methodology for answering it.

Yes, we need a methodology so that the future engineers can participate efficiently in the social dialogue between the public (I prefer to speak of citizens rather than of consumers), the industry, the NGO's, the social networks... Today, too often, the food industry is accused of too many things, and wrong ideas about food products from industry are used in public discussions... because the public does not have the real understanding of what they buy and eat. Such words as flavorings, additives, and transformation, generate fears, often -but not only- because some political forces rely on them to fight organizations that they dislike. Of course, all those who have the feeling to work for the good of others (let's remember that we could not live today without

the food industry) find this unfair, but this is not a positive attitude: we have to fight, we have to explain, we have to demonstrate, and the more actively the better. For decades, the French Academy of Agriculture is explicitly active in this explanatory enterprise, but it is the responsibility of the food industry, and of all engineers, to contribute as well to the public debate.

Illustration 3: We have to say it to our younger friends: for sure, they are right if they intend to work for the industry, the industry that makes all these "daily miracles".

And now, because the works shown in this book include much scientific research, along with technological research -and mainly because science is my absolute passion-, it is my duty and pleasure to end on this.

First, let me tell you that, when I was myself freshly graduated, I was complaining that the older people had already discovered everything: quantum mechanics, relativity, supramolecular chemistry, molecular biology, etc. This was naive, because, science and technology had already done a lot, for sure scientific and technology journals are announcing every day new achievements about flavor release, nano-encapsulation, in-depth analysis of complex chemical systems such as melanoidins, new analytical "omics" methods, and insights on the biological effects of food components... But so much remains unknown!

Can you imagine, for example, that we do not understand why an apricot tart is perceived to be sour, after cooking, even when made with very sweet apricots? Or can you imagine that we ignore how the compounds that are released by carrots or onions are indeed released when a simple carrot stock is made? Or can you imagine that we still ignore what is going one when wine is cooked (remember: cooked wine is included in about 50 % of the sauces in French cuisine)? Or can you imagine...

Illustration 4: Here, a raspberry in a gel: after some hours, the colour and flavour of the fruits distribute in the gel. How could you prevent this to happen, in the yogurt industry, with products that are stored for days?

Indeed, we have mostly questions, not answers, and the American physicist Richard Feynman said it well: "there is plenty of room at the bottom". This observation is especially pertinent in food science and technology: the microscopic, nanoscopic and molecular worlds are full of mysteries that young and brilliant minds can study.

But wait... Who should study this? I want to finish this discussion of the relationship between science and technology, as well as this introduction to a book by future masters by a paradox: you see, people like me find science so wonderful that our passion can be contagious, and this is unfair! It is unfair, because we have no right to change the way of our FIPDes friends, who decided to come on our program because they had the wonderful political vision of contributing to the world through their work in the food industry. Let us remember: innovation, design!

For sure, science can appear wonderful to some of us, but we have a higher responsibility: keep our young friends on the road that they decided, so they keep on wanting to know the most advanced science but only with the view of usefully transferring it to the food of humankind.

What a beautiful goal theirs is!

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Role of Pea Protein and Pea Starch on Functionality and Formation of Volatile Organic Compounds in Bakery Applications

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Introduction

The growing interest in pulses like peas is due to their rich nutritional profile, low environmental impact and as a promising crop to help tackle malnutrition. This has led to its incorporation in several food systems like bakery. The promotion of pea ingredients in foods does not only introduce variety but also provide a healthy plant-based alternative to ingredients derived from cereals and animals (Amin *et al.,* 2016; Thakur *et al.,* 2019).

However, the application of several emerging pea ingredients (e.g. flour, starch or protein fraction) in bakery foods may significantly influence the final product quality like the physicochemical and sensorial characteristics. This is because of the very peculiar chemical composition very different from that of cereals like wheat (Reineccius, 2006). In addition, several extrinsic factors like processing conditions, may also have an impact on the formation of volatile organic compounds (VOC's) via the biochemical pathways of lipid oxidation, mainly impacting odour quality (green and grassy odours) (Azarnia *et al.,* 2011) which likely undergo further chemical interactions during thermal treatments like baking.

Research objectives

- To investigate the physicochemical properties of pea ingredients (flour, starch, and protein fractions) in comparison to wheat
- To evaluate the impact of formulations with these pea ingredients on the physicochemical properties and the generation of VOC's within a sponge cake with a wheat-based reference

Methodology

Physicochemical characterization of ingredients

Determination of moisture, pH, colour (CIEL*a*b*) and particle size distribution were done on the wheat flour [WF], yellow pea flour [YPF], pea starch [PS] and pea protein isolate [PPI]. Furthermore, the level of starch granule damage and pasting properties were analysed on the flours and pea starch only.

Batter and cake development process

Batters were developed using a kitchen aid equipment (5KSM150, St. Joseph, Michigan, USA) which involved the forming of a liquid foam by introducing air into a continuous liquid phase of egg (45%) and sugar (25%) at maximum speed. After, flour (25%) and sunflower oil (5%) were folded in to form batter at minimum speed in order to optimize air incorporation and prevent batter splattering.

Batters obtained per formulation were poured into 21 aluminium molds each (8.0cmx 4.5cmx3.5cm) and baked at 170˚C in an instrumented oven (Bongard, Wolfisheim, France) specially designed to ensure thermal homogeneity (Fehaili *et al.,* 2010).

For developed batters, density, pH and colour measurements were done together with microstructure observation by confocal laser scanning microscopy (CLSM). Moisture, volume, pH, colour and texture measurements were also done on cakes developed.

In addition, the volatile profile of formulated cakes from wheat flour and yellow pea flour were determined using a GC-MS equipment with an automatic Head Space – Solid Phase Micro Extraction (Trace GC Ultra/Tri Plus, Thermo Scientific Model, USA).

Results and discussions

Flour and fractions physicochemical properties

All samples were low in moisture but had significant differences ($p<0.05$). The very low moisture content in PPI may be due to the additional drying step (usually spray drying) after the extraction process (Boye *et al.,* 2010).

Table 1: Physicochemical characterization of ingredients

Parameter	WF	YPF	PPI	PS
MC [%]	12.15±0.00^{a}	8.04±0.12^{d}	4.19±0.13^{e}	9.07±0.07^{c}
pH	6.26±0.03^{d}	6.39±0.01^{c}	7.07±0.01^{b}	7.95±0.04^{a}
PSD (D50) [µm]	65.84±0.59^{a}	28.88±0.18^{c}	31.79±0.23^{b}	24.32±0.27^{d}
SD [%]	5.66±0.06^{a}	2.11±0.02^{d}	31.79±0.23^{b}	24.32±0.27^{d}
PV [mPa.s]	11.30±1.41^{b}	6.88±0.40^{c}	-	13.40±0.50^{b}
FV [mPa.s]	8.16±0.76^{a}	5.64±0.09^{b}	-	5.23±0.09^{b}
SV [mPa.s]	3.14±0..66^{c}	1.24±0.41^{c}	-	8.17±0.42^{b}
Color				

MC– moisture content, PSD– particle size distribution, SD– starch damage, PV– peak viscosity, FV– final viscosity, SV– setback viscosity

Different letters within the same row have significant differences ($p<0.05$)

PSD and SD content have been studied to have a major impact on flour quality because they influence the behaviour of flour during hydration in processing (Monnet *et al.,* 2019). These two properties are closely linked to the fine milling process applied due to the high degree of particle size reduction required to liberate the starch granules from protein matrix that breaks into smaller matrix. As a result, it technically leads to some degree of starch damage in the final flour obtained (Maskus *et al.,* 2016; Pelgrom *et al.,* 2013, 2015) which may impact its functionality.

For starch pasting properties, the peak viscosity of PS was about twice the value of YPF, which could have been due to the interference of co-existing nutrients like proteins, lipids, and fibres in the YPF unlike PS. Hence, this may have to some extent, restricted the swelling and dispersion of starch granules, which therefore affected its viscosity during heating

(Wang *et al.,* 1993). On the other hand, the peak viscosity of WF was also relatively higher than YPF but not significantly different from that of PS.

The final and setback viscosity depicts the point where pasted starch experience retrogradation when cooled (Shafie *et al.,* 2016). These two properties can impact the volume of a sponge cake. It is postulated that, released amylose during baking rapidly retrograde during cooling, which provides a structural support to cake crumb, producing a large volume of cake (Choi and Baik, 2014). With regards to this, PS having a higher setback viscosity will not only have a supported crumb structure without sinking at the centre of the cake, but also a high cake volume in its formulations compared with the flour formulations.

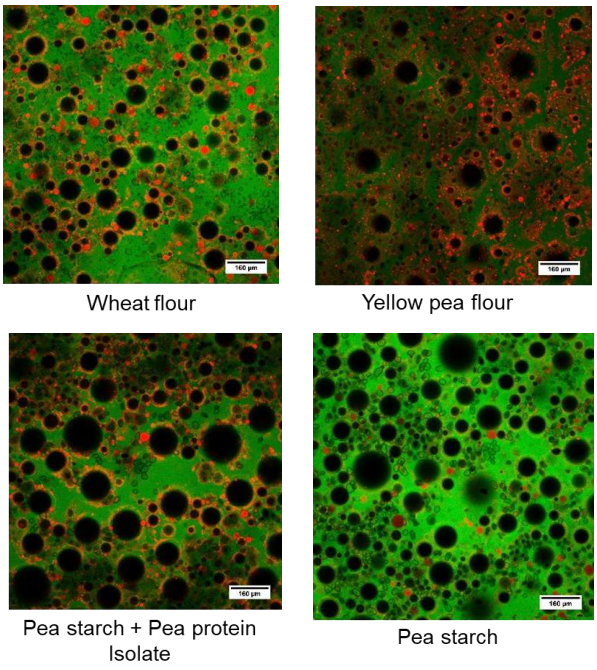

Figure 1 : Confocal superimposed images of different batter formulations at x10 magnification, green— protein structure, red – lipid structure, dark round circles –air bubbles

Effect of ingredients on different batter formulations for sponge cake development

On batter structure

The properties of the developed batters were coherent with the final sponge cake properties. With respect to the different batter formulations, the flour formulations had higher batter densities (YPF = 0.63±0.06 g/cm^3, WF = 0.55±0.06 g/cm^3), than the formulations made from starch and protein fractions (PS+PPI = 0.42±0.00 g/cm^3 and PS = 0.43±0.00 g/cm^3)

Although the batter densities of WF and YPF were significantly different ($p<0.05$), there were no significant differences among the fraction formulations. This depicts how different the functionality of the starch and protein components within the pea flour are from when separated into individual fractions. It can be postulated that, the possible absence of minor flour constituents like fibre within pea starch and pea protein isolate could have significantly enhanced their functionality in volume expansion during baking hence their lower batter densities.

From **Figure 1**, it can be seen that the batter microstructures of wheat (reference) and yellow pea are quite similar, except that the proteins and lipids in yellow pea batter seem to be prominently situated around the air bubbles than that of wheat batter. This may be due to the high protein content in yellow pea flour which tend to stabilize more of the air bubbles as compared to wheat.

However, comparing the batter structures of yellow pea and PS + PPI, the latter has a high distribution of large-sized air bubbles (160—170µm) than the former, which corresponds to its lower batter density. The same can be said of the pea starch batter.

On cake structure

Evidently, the differences observed in the batter structures (**Figure 1**) impacted different characteristics in their final cake products. Batters with lower densities resulted in lower cake densities, meaning increasing in cake volume due to the entrapment of more large-sized air bubbles as found in the batters of the starch and protein fractions (**Figure 1**).

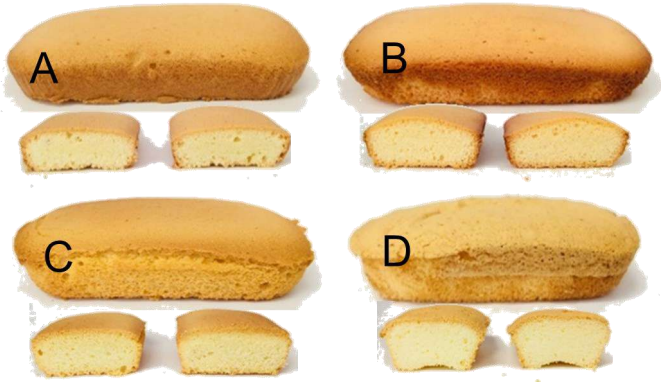

Figure 2: *Appearance of the 6 different cake formulations ; Key:* ***A****– Wheat,* ***B****– Yellow pea,* ***C****– PS+PPI,* ***D****– Pea starch*

Wheat cakes had the lowest volume (48.10 ±0.35 cm^3) , followed by the yellow pea cake (58.10 ± 0.36 cm^3) , which corresponds to their higher batter densities. PS+PPI cake on the other hand, serving as a reconstituted pea flour formulation, appeared not to be only significantly different from pea flour in its batter properties but also in the final sponge cake product by having a higher cake volume (62.63 ± 0.32 cm^3). Furthermore, cakes developed with only PS had the highest volume, 72.17 ± 1.37 cm^3.

Apart from cake volume and density, batter density also influenced another important property of cake such as texture. In this study, the texture of the developed cakes was determined by a measure of apparent Young's modulus (E*), which gives an idea of the stiffness of a viscoelastic material. Lassoued *et al.* (2018) suggested that, high values of E* in baked products are undesirable since they are associated with perceived firmness of the crumb which may in some cases, negatively impact the consumer's acceptability.

Table 2: Physical properties of formulated cakes

Cakes	Moisture[%]	pH	Density [g/cm^3]	Texture [kPa]
A	14.37±0.04^{e}	7.63±0.01^{d}	0.36±0.00^{a}	36.99 ± 1.32^{bc}
B	14.92±0.02^{d}	6.98±0.01^{e}	0.32±0.00^{b}	81.13 ± 4.91^{a}
C	14.45±0.05^{e}	8.01±0.04^{b}	0.27±0.01^{c}	41.36 ± 0.53^{b}
D	16.26±0.01^{b}	8.52±0.05^{a}	0.23±0.00^{e}	30.78 ± 2.41^{c}

Different letters within the same column have significant differences (p<0.05)

VOC profile of wheat and yellow pea cakes

Besides changes in cake structural properties, yellow pea flour was also very reactive during baking in generating the highest concentrations of volatile organic compounds. Among them, hexanal and 1-hexanol were the most abundant resulting from lipid oxidation of reactive precursors present in pea. Secondly, Strecker's aldehydes and pyrazines were formed from Maillard reaction mainly occurring during baking.

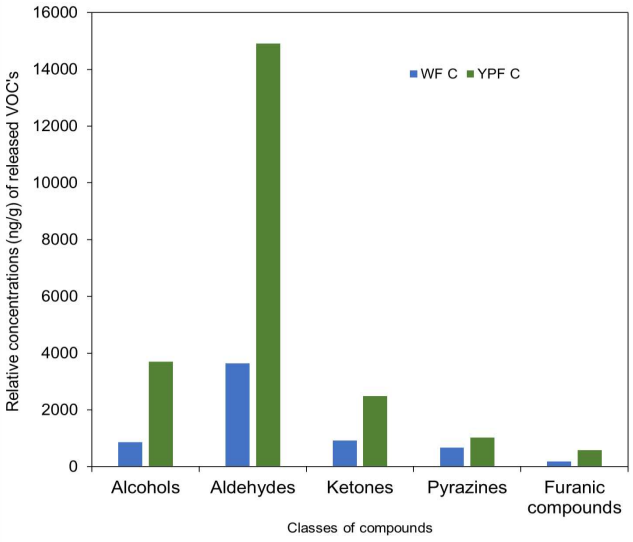

Figure 3: *Overview of concentrations of released classes of VOC's from wheat and yellow pea cakes*

Conclusion

The incorporation of yellow pea ingredients (flour, protein and starch fractions) significantly impacted the structuration and chemical reactivity of the formulated batters and cakes. Individual fractions possessed enhanced functionality compared to the complete pea flour as mere ingredient. These findings does not only advance the knowledge in research, but also provide the food industry with other alternatives when it comes to some functional ingredients, which to an extent adds value to pea and pulses.

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Evaluation of bagged lettuce quality stored in different packaging and fridge conditions

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Introduction

The convenience that bagged lettuce offers has often coupled with consequence of rapid quality deterioration, especially at home after the consumers open the package. The quality issues begin to emerge and to worsen during post-purchase of bagged lettuce at consumer's household. As soon as consumer opens the lettuce bag, the barriers (atmospheric and physical) that have been protecting the salad quality will be broken. This results in the acceleration of salad quality degradation, shortening the shelf life of product. This is undesirable from a consumers' perspective as they expect longer freshness quality, especially when the products are stored in the fridge. This study aims to provide thorough understanding of the behavior change of fresh cut products at end-consumers with different possible scenarios on how consumers store the product in their fridge.

Research objectives

1. Evaluate the effect of different fridge conditions (emphasis on temperature and RH) on salad freshness quality

2. Observe the effect of different packaging from end consumer's perspectives

3. Study consumer behaviors on salad consumption and storage in household fridge

Methodology

The materials used in this project are categorized into several main groups: various types of lettuces, packaging materials, cooling systems (fridges), and analysis equipment.

This study was conducted as the initial approach of understanding packaged food behavior, focusing on lettuce, in different fridges at Electrolux. It consisted of several stages: preliminary, first, second, and third stage. The preliminary stage was focused on selecting the type of lettuce to be further observed. Observing the effect of temperature on salad quality, analyzing the impact of storage in fridges based on consumer perspectives, and conducting online survey to better understand consumer's behaviors in storing salad are the first, second, and third stage of research, respectively.

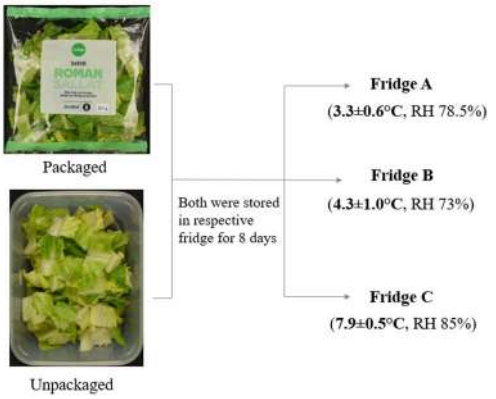

Fig 1. Set-up for evaluating the effect of temperature

Lettuce was stored in different temperatures (2°C, 5°C, 8°C), different relative humidity (RH) (21%, 56.5%, 82.5%), and different packaging types (sealed bag, open bag, folded bag, unpackaged) for 8 to 14 days. The following quality parameters were assessed throughout the storage period: weight loss, texture, color change, vitamin C, and condensation build-up in the packages.

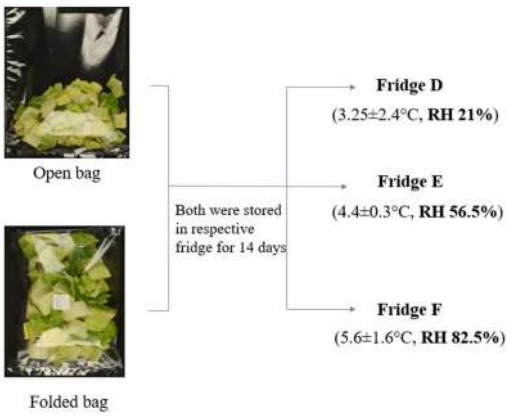

Fig 2. Set-up for evaluating the effect of RH

Results and discussions

The preliminary stage of this research revealed the importance of initial quality of lettuce cuts in determining the deterioration level during storage. Lettuce that has more damaged tissues will undergo more severe and more rapid deterioration process than that with less damage.

It was observed that weight loss was significantly affected by relative humidity. Meanwhile, texture was found to not be significantly affected by temperature and relative humidity in this study. However, the firmness decreased over storage time in all conditions. Condensation was noticed to be significantly affected by temperature and relative humidity. The result implied that temperature, its fluctuation, and relative humidity are the combination of storage conditions that affect the condensation phenomenon in bagged salad. As for color, salad stored in higher RH can better preserve the color and that exposed to less oxygen showed less browning formation.

Different types of packaging provided different level of protection on salad quality, with sealed bag provided the highest protection and unpackaged provided the least. Higher exposure to oxygen resulted in higher deterioration rate of salad quality

Fig 3. Comparison of salad image stored in different packaging

The questions related to visual assessment and condensation acceptance were asked to understand the consumers perception about salad quality. The visual assessment question revealed the importance of color on salad quality perception as consumers link browning to bad quality.

Conclusions

- Higher RH was more favorable for salad storage with better retention of the following quality parameters: color change and weight loss
- Lettuces stored in the folded bag had three times less weight loss, better color retention, and lower browning formation as compared to open bag
- The perception of salad quality varied between individuals, but the majority have similar perception on bad visual quality linked to color change of lettuce

Future use of the research

1. It was clear how different salad preparation methods can give different impact to the quality of fresh-cut products. It is important for the salad producer to carefully design the production line to minimize the damage to plants tissues as much as possible.
2. The packaging company may consider redesigning the packaging to give better protection for salad during storage after the bag is opened. Making the packaging to be easily resealable, such as adding zipper or removable tape, is an alternative of preserving salad quality during storage at home.
3. For the fridge company, thorough studies in this topic can help the company in assessing whether current cooling system and fridge design are sufficient in preserving the freshness quality of fresh cut products.

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How to pack better ? A study on meat batter

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Introduction

Increase in the trend of green consumerism has been observed as more consumers are becoming more concerned with the environmental impact of the products that they are purchasing. Thus, food manufacturers, specifically for canned products, are making the shift from metal packaging to a more sustainable material such as Tetra Recart®. The latter has been proven to have less environmental impact as compared to metal cans but still having the same properties needed to provide safe food products.

The Tetra Pak R2 Machine with the piston filler has been used to fill different types of food product for the Tetra Recart package®. The focus of the study is meat batter as it could represent viscoelastic products. To predict the fillability of the meat batter in TPR2 machine with standard product tank and when to recommend Pressurized product tank for products that exceeded the limit of the former, the study aims to achieve the following objectives.

Objectives

1.) To understand the rheological characteristics of meat batter with different formulations
2.) To investigate the product's rheological properties which can be correlated to its fillability in the TPR2 machine with standard product tank

3.) To understand the pressure drop in the filling pipe

4.) To correlate the meat batter's rheological properties and their corresponding pressure profile Hypothesis The assumption is that the rheological parameters of the meat batter can be used to correlate with the pressure drop

during the filling process.

The specific hypothesis of the study includes:

1.) The use of rheometer can produce reproducible results to quantify and make a rheological model for the meat batter

2.) The rheological model established can be used to predict the pressure profile of the batter inside the filling machine

3.) The rheological characteristics of the different meat batter can predict the fillability of the product in the machine

Materials and Methodology

The materials used for this study were pork belly class 3 (HKScan), pork shoulder class 2 (Martin&Servera), modified starch, salt, and water. The meat was cured, grinded, and mixed with other ingredients using different equipment for laboratory and pilot plant scale. The resulting products were meat batter samples with varying percentage of added water which had different rheological properties and fillability. These were analyzed in duplicates using rotational rheometer with the serrated cup and four-blade vane attachment.

The flow curves of the meat batter were analyzed, and a Non-Newtonian fluid flow model could be fitted. Also, the corresponding rheological parameters, and the thixotropy property were studied. Build-up and breakdown tests were performed to study the thixotropy property of the meat batter. Amplitude sweep test was also performed to analyze the viscoelastic property of the meat batter.

The K and n values were derived from the flow curves which were used to estimate the apparent viscosity. The estimated pressure drop was also calculated in order to compare with the experimental pressure drop.

The different meat batter samples were tested in the TPR2 machine with standard tank to gather data on the pressure profile and pressure drop curves. The experimental pressure drop values were then compared to the calculated pressure drop.

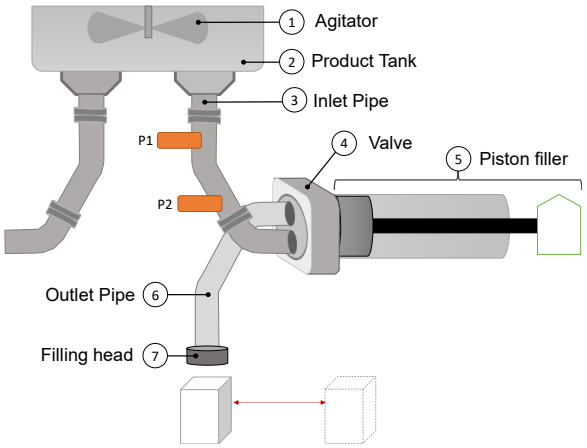

Figure 1. The simplified diagram of filling system of Tetra Pak R2 filling Machine with the additional installation of pressure meters

Conclusions

The conclusions obtained from this study were:

1. The rheological characteristics of the meat batter could be studied using the vane geometry in the rotational rheometer which showed that the meat batter could be described by the Power Law Model. The rheological measurements provide K and n values of which the former decreased as the % added water was increased, while the latter was not significantly affected. Shear thinning behaviour was observed among all the samples. The calculated apparent viscosity and pressure drop estimated from the rheological measurements showed decreasing trend as the % added water was increased. The calculated K value and amplitude sweep test can be used to predict the meat batter's fillability in the filling machine with a standard tank and when to recommend the filling machine with pressurized tank. The values obtained from products produced in laboratory and pilot plant scale were not significantly different from each other.

2. The analysis of the flow curve could be used to study the thixotropy property of the meat batter which showed decreasing trend with the additional water. The filling duration is shorter than the time needed for the meat batter with lower %added water to build-up. The amplitude sweep test showed that the sample with lower water had more elastic property.

3. The pressure measurements during the filling in the machine showed the difference in the typical pressure profile curves for products with different fillability. The meat batters which were able to be filled in the TPR2 machine with standard product tank were 20% and 30% added water meat batter that followed the viscous fluid behavior. This showed the limit of the machine without pressurized product tank as no less than 20% added water. However, samples of 15% and 10% added water meat batter were not able to be filled exhibited more elastic property during the filling process.

4. The Rabinowitz-Mooney derived from the Power Law model used to predict the pressure drop of the meat batter samples was only applicable to 30% AWMB.

5. The fillability of the meat batter was significantly affected by its elastic property. The calculated pressure inside the piston for lower added water samples was found to be at the pressure in which water molecules could boil at 10˚C which could create steam inside the piston. In addition, the mixture of meat batter and air in the pipe might have affected the filling behavior of the samples.

Figure 2. Filled packaged with pilot scale meat batter a) 10% AWMB, b) 15% AWMB, c) 20% AWMB and d) 30% AWMB

Recommendations

The following are recommendations based on the study:

1. Meat batter products can be further investigated in terms of viscoelastic properties and adhesiveness/stickiness by Texture Profile Analysis. This is to further understand its behaviour and to study the possibilities to correlate to its behavior during filling.

2. The fat, protein and quantitative analysis of the meat particle size can be done to determine the effect of the meat composition and microstructure on its rheological properties. The effect of varying the fat content of the meat batter can also be studied and be correlated to its fillability in the machine, to know the applicability of the prediction model in different conditions.

3. More parameter of machine response could be studied to understand how the machine works and responds to the product. This may include the motor response, synchronization to the piston movement, and the pressure measurement in the piston.

4. Study of other fluid flow models including the elastic properties could be done to determine the best fit prediction model for the meat batter with lower added water.

5. To provide a substitute for meat batter in the machine test, further study in the proxy product could be performed such as build-up, breakdown and amplitude sweep in order to compare to the rheological properties of meat batter. Machine test of the proxy product could be made in order to gather pressure data measurements and to increase the understanding of machine fillability in a systematic way.

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3D printing to produce model food structures to study food oral processing

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Introduction

Food Oral Processing (FOP) is a dynamic process that changes food structure and conditions the evolution of the food perception with time (Wilkinson, et al., 2000). Understanding the relationship between food structure, food oral processing and food texture in mouth is important to develop novel food products addressing the specific demands and needs of senior consumers.

To develop novel food structures, food research should assess and optimize new manufacturing processes, such as 3D Food Printing (3DFP). This process consists of a 3-axis stage, an extrusion unit and a user interface (Sun, et al., 2015).

3DFP is characterized by a high degree of control enabling the design of novel food structures (Campbell, et al., 2017) and can become an important research tool to study the relationship between food structure and food properties in mouth.

To optimize this process, the following factors should be considered: the deposition process of the printed ink, the capability of the material to build stable structures and the strength acquired by the printed object to resist mechanical treatments (Godoi, et al., 2016).

Reaserch objectives

- Evaluate the feasibility of 3D printing to produce model food structures.

- Study the effect of the structure design on the mechanical and dissolution properties, assessed in conditions inspired by food oral processing.

Methodology

The project involved:

-A rheological characterization of the starch pastes used for 3D printing to evaluate its flow during printing and yield stress;

- A geometrical analysis to measure and compare the 3D Printed Structures (3DPS) with their target dimensions;

- An *in vitro* texture analysis to assess the mechanical properties of the 3DPS in conditions inspired by tongue-palate compression and involving also a progressive wetting and dissolution.

Structure Design

Two different families of cubic 3D structures were specifically designed to evaluate the effect of size and porosity on the mechanical and dissolution properties:

-Group 1 contains four structures having the same internal pattern and number of holes, but different size (Figure 1);

-Group 2 contains three structures with the same size but different internal patterns and number of holes (Figure 2).

The structures were named as follows: LXX_PYY, where XX is the side of the cube in mm and YY is the number of vertical pores in the structure.

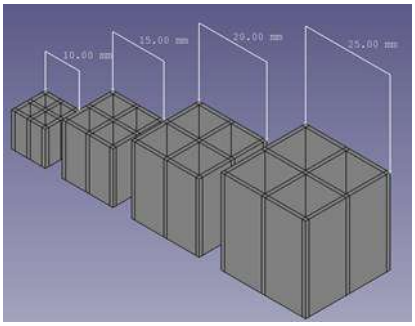

Figure 1. Group 1. From left to right; L10_P4, L15_P4, L20_P4 and L25_P4

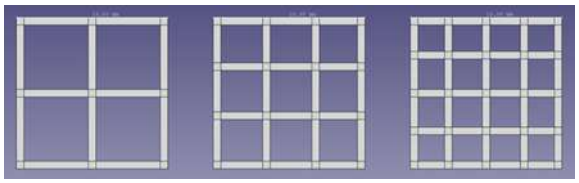

Figure 2. Group 2. From left to right; L20_P4, L20_P9 and L20_P16.

3D Printing

A Foodini 3D printer by Natural Machines (Spain) was used for this project.

All prints are produced in a closed environment where the ink is pushed down from a capsule through the nozzle by a piston kept at constant temperature (46 ± 2 ˚C). The printer includes a carriage that transports the syringe at the desired coordinates The printing parameters used in this study are illustrated in Table 1.

Parameters	Foodini
Nozzle diameter	0.8 mm
Nozzle temperature	46 ± 2 ˚C
Layer thickness	0.9 ± 0.2 mm

Table 1. Printing parameters

Wet compression & Dissolution tests

In order to evaluate food oral processing, a dissolution test coupled with a five-count compression test was performed in a customized set-up (Figure 3).

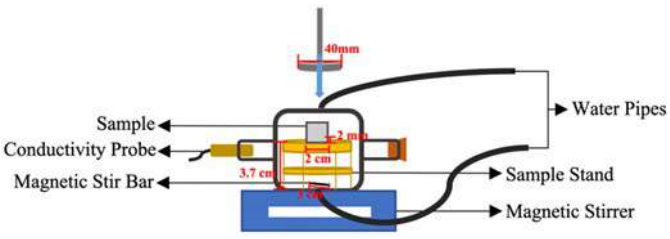

Figure 3. Scheme of the compression and dissolution tests

The objective of this test was to evaluate the mechanical behavior of the different geometries, simulating the compression of a soft food between the tongue and the palate, while the food structures are wetted. The evolution of the compression forces (N) and water conductivity (mS) were recorded, while the vertical displacement and temperature were controlled.

The 3DPS were partially immersed into deionized water, to mimic wetting caused by saliva in mouth.

Results and discussion

Rheological properties

One important mechanical property conditioning the extrusion process is the material yield stress. It influences the minimum pressure needed to start the flow and the stability of the prints (Liu et al., 2019).

The paste yield stress was 182 ± 2 Pa (Figure 4), which enables producing tall stable food structures.

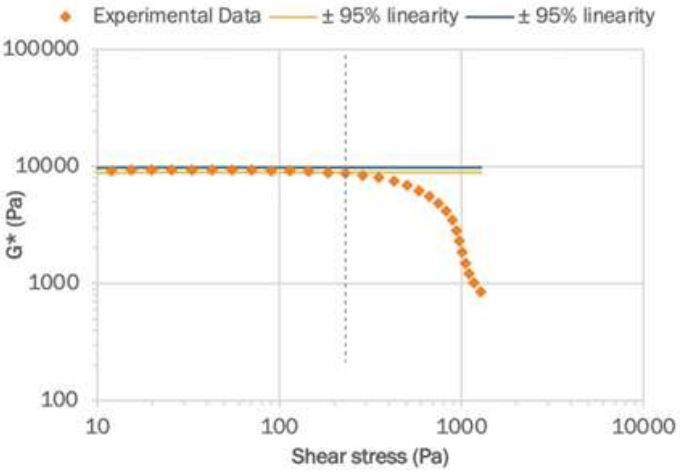

Figure 4. Yield stress determination

Figure 5 shows the shear-thinning behavior of the starch paste used in this project. In 3DFP inks require to have shear-thinning behavior and shear recovery properties due to the high shear applied at the nozzle (Liu et al., 2019a).

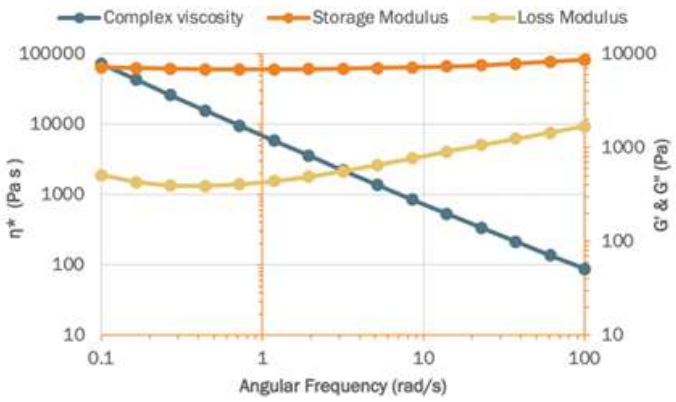

Figure 5. Complex viscosity, Sorage (G') and Loss (G'') Modulus

Besides, the elastic modulus (*G'*) relates elastic solid-like behavior such as resistance to deform elastically, reflecting the mechanical strength of materials (Yang et al., 2018).

In contrast, the loss modulus (*G''*) represents the viscous response of the material. Figure 8 shows that G' > G'' suggesting an elastic behavior for our paste.

Dimensional Characteristics

The structures were successfully printed by following the design pattern, dimensions and number of pores. However, variations were visually recognizable between the target, and the printed structures (Figure 6).

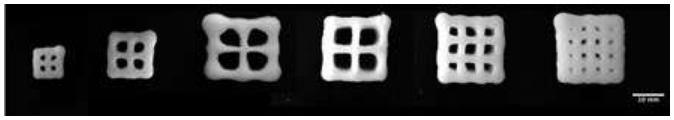

Figure 6. From left to right structure L10_P4, L15_P4, L25_P4, L20_P4, L20_P9 and L20_P16.

Quantitatively, a small difference was observed between the printed height and the target height (<6%). Besides, the difference with the target length and width was <15%, but as a result, structures L10_P4 and L20_P16 showed a significant difference in pore width (40% and 46%, respectively).

"Yield Point" in Dry vs. Wet compression

The yielding of the printed structures was observed for most structures during compression. Wet conditions reduce by 50% the "yield point", i.e. the stress needed to yield a structure when compared to compression performed without wetting (Figure 7).

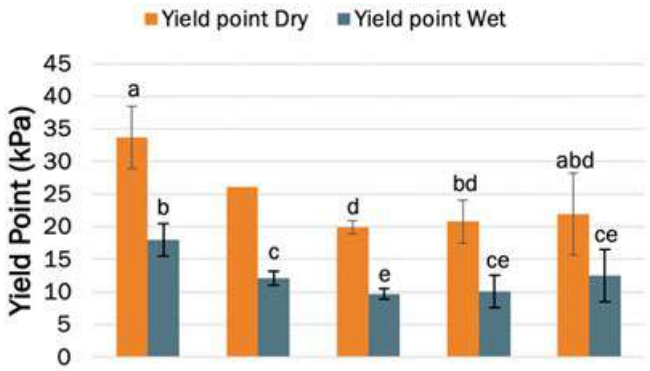

Figure 7. From left to right structure L10_P4, L15_P4, L20_P4, L20_P9 and L20_P16.

Figure 8 illustrates the influence of structure porosity for Group 1 structures (different dimensions with same number of pores) and Group 2 structures (same external dimensions but different number of pores) under wet conditions. When porosity decreased the yielding point of the structures in Group 1 clearly increased. However, the results from Group 2 are less conclusive on the role of porosity.

Results reported in Figure 7 and 8 suggest that the external dimensions (which are maintained constant in Group 2) have a dominant effect on the structure yielding behaviour and that the Yield Points increase when the structure dimensions decrease.

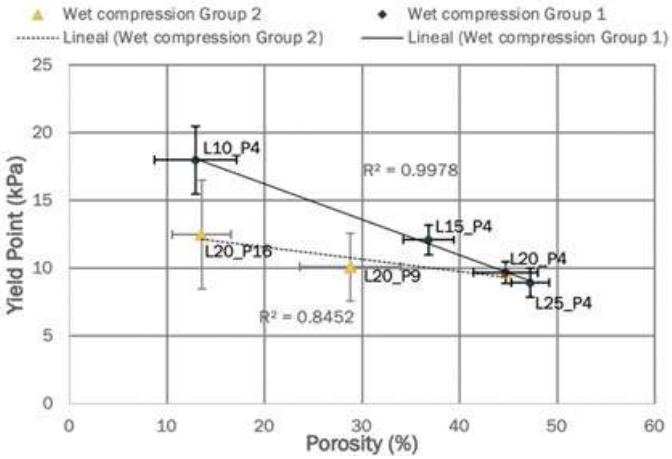

Figure 8. Relation between yielding points and porosity from Group 1 and 2 under wet compressions

Conclusions

In this study, it was demonstrated that 3D printing can be used to create controlled product structures made of starch. The limitations in dimensional accuracy were assessed.

The Yield Points of the 3DPS were strongly affected by the change in external dimensions and porosity.

About 50% reduction in the pressure needed to yield the structures was observed, when wetting of the 3DPS was performed simultaneously to compression, in conditions inspired by tongue-palate compressions.

In conclusion, the development of model food structures has a great potential as a research tool to understand the link between food product structure and perceived texture and to foster the development of novel food products to satisfy the specific consumer needs.

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Fermentation as a tool to improve immunity: design of a postbiotic drink

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Specialized Nutrition at Danone constantly innovates based on consumer needs. Over the last years, healthy ageing is the center of attention in the food industry. Knowing that arthritis is the third biggest disease affecting adults, the development of a product targeting the market niche was at scope.

The aim of this project was to design a stable postbiotic dairy drink prototype which is able to prevent and decrease inflammation response in arthritis patients. Trial for optimizing pH decrease rate on yoghurt starter culture and increase fermentation yield on Bifidobacterium were done prior to Pilot plant trial. The use of pectin as a stabilizer to prevent phase separation was studied on pilot scale. Pasteurization is essential to the production of postbiotic molecules and helps the adsorbance of casein micelles with pectin. The heat treatment extends the product shelf life in comparison with natural yoghurts present on the market.

Results of lab trials presented the adaptability of yoghurt starter culture fermentation to the designed process. Media supplementation has a non-significant impact on CFU count of Bifidobacterium. Pilot plant trial showed the influence of pectin in stabilization of prototype.

Experiments demonstrated the technical feasibility of the process in pilot scale due to the reproducibility of both microbial fermentations and use of pectin. This study can be used as a starting point on further prototype development.

Confidential topic

Transforming the food system for nutritional and planetary health

Surfacing innovations and building transition pathways using an adapted Delphi methodology

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Introduction

According to estimates, the 2019 world population was 7,7 billion and **more than 820 million people were still undernourished** (1). This last number has been on the rise since 2015 (2). **Food systems are failing to meet the worlds dietary requirements**. Additionally, undernutrition currently increases alongside overweight and obesity (3). This **double burden of malnutrition** is evolving at unacceptably high levels. Global malnutrition is expected to worsen due to a **further increase in global food demand** as population growth is projected to reach about 10 billion by 2050 (4). A large proportion of people suffering from undernutrition **simply cannot access or afford a safe and healthy diet**. For many, the availability of nutrient-rich food is too low.

Current malnutrition trends, the affordability/ availability challenges combined with the projected demographic developments, will **dramatically worsen health outcomes if no large-scale action is taken.**

Besides malnutrition outcomes, the food system is also amongst the major drivers of global environmental degradation. The **way we produce, use, and dispose of our food contributes to climate change, land and soil degradation, biodiversity loss, and interferes with the global nitrogen and phosphorus cycles,** amongst others (5).

Our food system drives and contributes to the transgression of the planetary boundaries (6). On the other hand, **the current global environmental changes and transgression of planetary boundaries equally impede and challenge the world's capacity to meet food security and nutritional needs** through in-

creased water scarcity and temperatures, changing rainfall patterns, extreme weather events, increased atmospheric CO2 levels, etc.

Climate changes and environmental degradation directly influences what food is available and at what price. **The impact is expected to be negative, reducing food supplies and raising food prices** (7). Thereby further threatening global malnutrition (8).

This is projected to worsen in the future and is expected to be the hardest on populations in the Global South, especially **Sub-Saharan Africa and South and South Asia** (7).

The **current climate emergency combined to the malnutrition challenge thus make it even more crucial to rethink food systems.** Food systems should thus feed the global population and cease land conversion and degradation, safeguard biodiversity, reduce wasteful freshwater water use, substantially decrease nitrogen and phosphorus pollution, produce zero carbon dioxide emissions, and cause no additional increase in methane emissions.

Innovative interventions, technological changes and novel mitigation measures are urgently needed to make this possible.

Research objectives

Our immediate challenge is to **ensure that healthy and sustainably produced food is the most accessible, affordable, and desirable choice for all.** Disruptive innovation is essential to achieve this goal as incremental innovation will not ensure global food security while dramatically reducing the negative impacts of the food system on our planetary system. Planetary diets have been established and the need for a sustainable transition of the food system acknowledged. However, **innovative ideas need to be generated and large-scale dissemination investigated collaboratively**. These interventions need to be tailored to the specific geographic and cultural context of different developing countries. The urgent need for jointly disrupting climate and nutrition issues called **for this study aiming at surfacing win-win innovations with a 10-year horizon rooted in the concrete challenges of specific geographical settings.**

This will be done through an **iterative Delphi Process which involves a diverse panel of experts responding from three different geographic settings : Urban Bangladesh, Semi-arid Rural Ethiopia, Tropical Coastal Mozambique.**

Ideas are generated, prioritized, and then discussed in depth to identify innovative pathways for successful dissemination of the identified innovations.

Methodology & results

The Delphi Method is a recognised qualitative tool used to make predictions and support decision-making. This tool helps **reaching consensus of opinions by subjecting a group of experts to a successive series of questionnaires**. The Delphi technique thus allows for structured communication between a large group of experts, whereby all provide relevant contributions to tackle a complex challenge (8).

The Delphi method was adapted to surface and investigate dietary and planetary health innovations. The lead question of the conducted Delphi Study was the following: **“Which innovations can be game-changers in emerging markets to make affordable, safe & nutritious foods available in an environmentally sustainable way by 2030?”**

This lead question was rooted in three different food system settings: **the semi-arid rural setting of Ethiopia, the tropical coastal setting of Mozambique and the tropical urban setting of Bangladesh.** These are high priority contexts where nutrition and environmental challenges are particular barriers for development.

As a first step, a diverse **panel of 52 global experts** and experts based or with experience in one of these three defined settings were enlisted. The experts where then subdivided amongst the three contexts to further answer the different questionnaires from that specific perspective.

Three rounds of questionnaires were elaborated, answered, and analysed between February and June 2020. Panellists were invited by email to actively participate in the 3 rounds, each including a virtual discussion session followed by an online anonymized survey to capture the experts' input and insights. The 3 rounds were ordered following a funnel approach: **divergence, convergence and detailing** (Figure 1). A final webinar was facilitated to summarizing project results and reflections on the process and participants were invited to provide any additional thoughts and feedback in a final reflection survey.

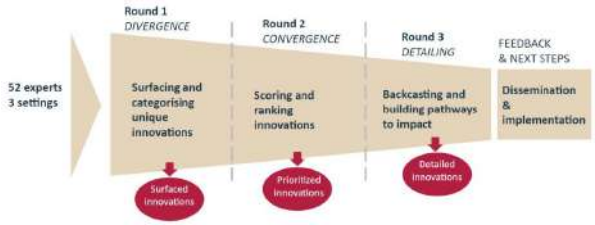


Figure 1. *Delphi study design*

Round 1: methods & results

Methods

The first round of questionnaire consisted of a **disruptive innovation scan**. It was designed to be an open, outside-of-the-box, solution-focused brainstorming on innovations rooted in the real-life challenges of the three selected settings. The goal was to identify innovation that could really leapfrog the current situation. Experts were asked to **propose and describe three to five innovations** that can be game-changers in making affordable, safe & nutritious foods available in an environmentally sustainable way by 2030 for the setting that they were assigned to.

All responses were analysed. First, similar innovations were merged and the descriptions were streamlined. The innovations were categorised on the supply chain, on their maturity and in six distinctive clusters. **The six clusters are Crops and animal agriculture, Food science and technology, Logistics and distribution, Digital and Agtech 4.0, Education and outreach, Public/private institutions**.

A catalogue was elaborated containing all this information and distributed to the experts for round 2.

Results

In the first round, a total of 48 experts provided a total of 151 innovations. After merging, 71 innovations remained. 14 extra innovations were added from a previous literature search, totalling up **to 85 innovations.**

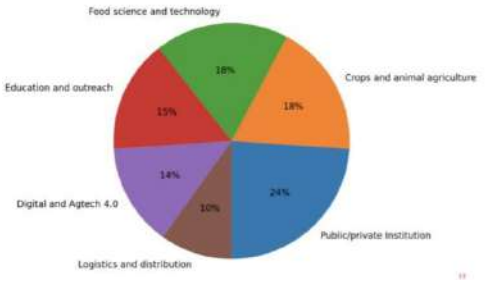


Figure 2. *Distribution of the innovations amongst the 6 clusters.*

Round 2: methods & results

Methods

The second round of questionnaire aimed at **evaluating the innovations on different dietary and planetary health criteria, building consensus and prioritizing innovations that have a combined high dietary, planetary and leapfrogging impact**

Experts were asked to select **their "Top 10" favourite innovations from the innovations catalogue** for their specific setting. This Top 10 had to include 1 innovation from each of the 6 clusters (Table 1) as well as an additional "favorite 4" from any of the clusters.

Additionally, experts were asked **to score the potential impact of each of 10 innovations on 14 different criteria. The 14 criteria consist of 5 criteria related to dietary health and 7 criteria related to planetary health as well as a leapfrogging and equity.** The criteria related to dietary health are the following: food quality, food safety, availability, affordability, and desirability. While the 7 criteria related to planetary health are climate mitigation, climate adaptation, water use, soil health, reducing biodiversity loss, increasing biodiversity, reducing pollution.

All responses were analysed. The frequency of selection of the different innovations was investigated across all settings as well as per setting. Experts scored each innovation on 14 criteria. For each criteria, the "Top 5" innovations with the highest score were identified. This was also done for the overal planetary and dietary score (Table 1).

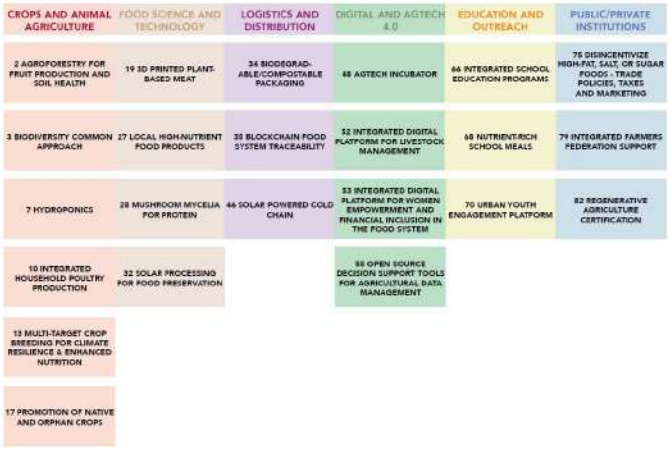
Secondly, a decision tree was established to select the priority innovations for each setting. These priority innovations were further characterised using expert's input and redistributed to the experts for round 3.

Results

A total of 48 experts responded to the second round. Excitingly, innovations that were provided for one specific context were chosen by experts answering from another context too. This shows that there can be interesting **cross pollination of ideas between different contexts. 83 out of the 85 innovations were selected at least once.** The two innovation that were not selected were "gas-based fermentation to produce proteins" and "reusable crates/ backhauling". As much as 49 innovations were selected at least 5 times, while 3 innovations were selected at least 15 times. The Top-5 ranking innovations for the dietary and planetary criteria are displayed in table 1.

Top 5 Dietary health	Top 5 Planetary health
Street food innovations	Integrated farmers federation support
Blockchain food system traceability	Biodiversity common approach
High pressure processing	Regenerative agriculture certification
High tech greenhouses	High tech greenhouses
Mushroom mycelia for protein	Mushroom mycelia for protein

Table 1. *Top 5 for dietary and planetary health criteria*



Round 3: methods & results

Methods

In this final round of the Delphi process, experts were asked to **identify what was necessary for these priority innovations to foster a major step-change towards having significant positive impact on both human and planetary health. Experts were asked to unleash their imagination.**

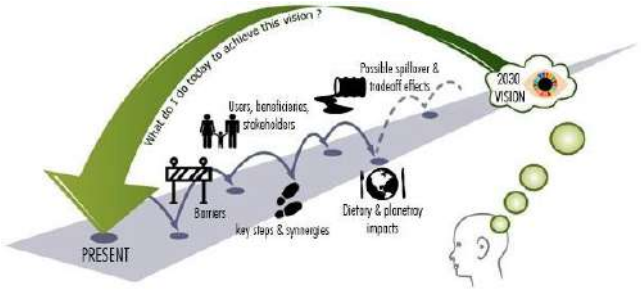
A **backcasting approach** was used for this purpose. Each expert was asked to review two innovations through the backcasting approach. This is a technique that **starts by defining an ideal vision and then works backwards to identify the missing steps to achieving this ideal vision** (9).

Experts were asked to imagine and describe the ideal scenario where the innovation has

reached its maximal dietary and planetary potential impact by 2030 in their specific setting.

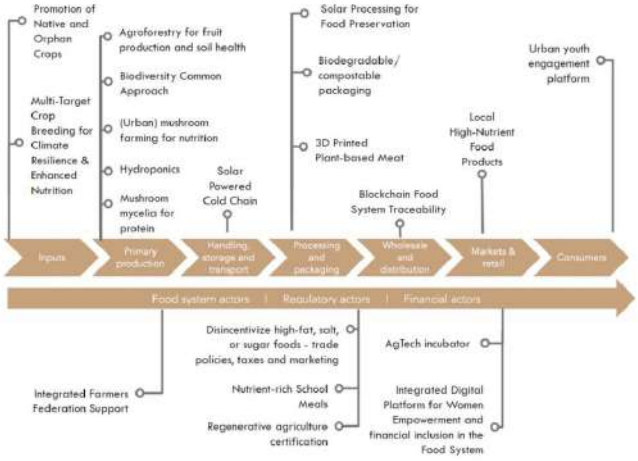
Figure 4. Adapted backcasting approach

Results



37 experts responded in the third round and 19 innovations of the 23 were selected.

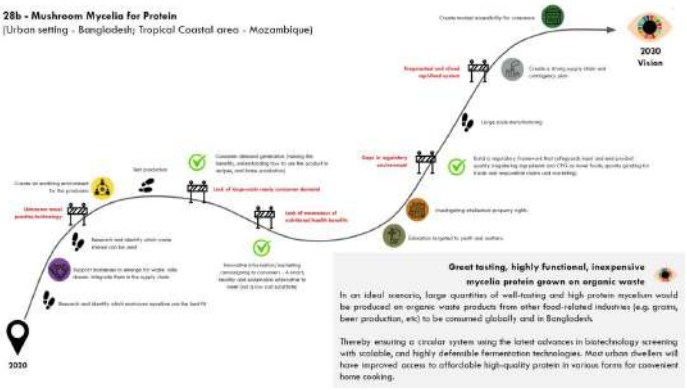
Figure 5. Priority innovations mapped on the supply chain



The selected innovations cover the different segments of the supply chain. This is very relevant as a diversity of solutions spreading across the supply chain will be needed to sustainably improve food systems in an holistic manner.

The expert's input from the backcasting and pathway building exercise were processed and merged. **Ultimately, pathways towards the ideal 2030 uptake vision were structured and assembled for each of the 20 innovations.**

Figure 6. Pathway to impact of one of the 20 final innovations



Conclusion

Thanks to the insightful contributions of 52 multidisciplinary experts, 85 innovations surfaced and detailing for a diverse set of 20 selected focus innovations that include technological, nature-based and policy/institutional solutions, was possible. Essential actions for transition pathways have been identified and will inform action strategies. The Delphi consensus-building methodology did not lead to unimaginable futures, rather it emphasized that existing technologies, when contextualized in place, and analysed across environmental, health, and social impact criteria have significant potential for positive transformation. Cross-pollination between experts, innovations, and context settings was a motivating benefit of the process and resulted in a constructive exchange of solutions. The process revealed that game-changing is also about portfolio-building, creating enabling environments, facilitating flexibility, and supporting across value chain interactions.

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Development of texturized vegetable protein by application of low moisture extrusion

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Introduction

Nowadays, there is a trend towards the protein transition. One of its main drivers is the growth of the world population, as by 2050, it will reach 10 billion, and by this year, the protein demand will increase up to 50% (WUR, 2020). To prepare for this, it is necessary to start broadening the plant-based products in the market. Actually, this transition has been accelerated during the pandemic of coronavirus. One of these plant-based products is texturized vegetable protein or TVP, which is mainly produced through the extrusion of soy ingredients. It is important to remark that TVP requires hydration before its consumption (Riaz, 2006).

Research objectives

The aim of the thesis was to determine the effect of pre-processing and extrusion conditions of soybean ingredients on TVP produced in a low moisture extruder. And later on, compare the extruded products with similar commercial products available in the Austrian market.

Methodology

Materials and equipment: The raw materials used to produce the TVP were soybean grit (SG), treated with solvent to reduce its fat content; inactive soybean flour (I), previously heat treated to inactivate enzymes; and active soybean flour (A), without any of these treatments. The benchmarks were Vega Vita, Veggy Star and Top OP. To produce the TVP, a low-moisture extruder (Cincinnati CM45, conical, counter rotating twin screw extruder, motor power 19.6 kW, 1986) was used.

Experimental Procedure: The experimental procedure consisted on the characterization of the raw materials (SG, I, A), and extrusion try outs (T1, T2, T3).

Characterization of raw materials:

The most relevant analysis was the determination of protein solubility (PS), which is based on the measurement of the percentage of total protein that is soluble in water under controlled conditions. For this, 100 mg of the sample were weighed, and 10 ml of the water at different pH (from 2 to 9) was added. The dispersions were continuously homogenized for 30 minutes, an then they were centrifugated at 4000xg for 30 minutes. The supernatants were frozen and their protein content (SP) was determined by Kjeldahl method. PS was calculated dividing the SP by the total protein content of the raw material (d.b.).

Extrusion try outs:

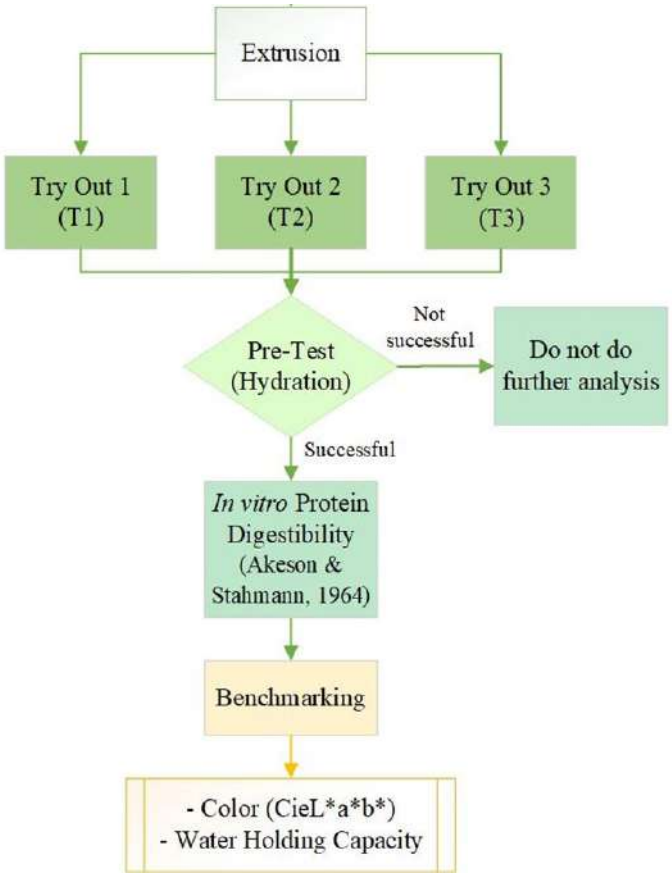

Figure 1.- Experimental procedure of the extrusion try outs

Try Out 3 (T3)		
Extrudates	Temperature Profile (°C)	Moisture $_{Feed}$ (%)
T3A1	80 – 110 – 140 – 160 – 80	30
T3A2	90 – 130 – 160 – 180 – 100	30
T3A3	90 – 140 – 170 – 180 – 120	30
T3A4	90 – 140 – 170 – 170 – 140	35

Table 1.- Processing conditions of extrusion Try Out 3

During T3, the nozzle diameter was reduced from 4mm to 2.5 mm, to increase the retention time and allow a better melting and cooking of the food matrix.

Specific Mechanical Energy (SME): The SME was calculated with the following equation:

$$\mathrm{SME} = \frac{P_{max} \cdot \left(\frac{\tau_f - \tau_i}{100}\right) \cdot \left(\frac{v_A}{v_E}\right)}{\dot{m}_T}$$

Where,

SME = Specific Mechanical Energy [kJ/kg]

Pmax = Maximum power of the extruder [kW]

= Running torque [%]

= Running torque with empty extruder [%]

= Actual speed of the extruder [rpm]

= Engine speed [rpm]

= Total mass flow [kg/s]

Pre test: The extrudates were exposed to a hydration pre-test (50 g of product in 125 ml of cold water for 1h), to determine if they can absorb water and preserve their structure when being in water.

In vitro protein digestibility: It was carried out according to the pepsin-pancreatin method of Akeson & Stahmann (1964).

Benchmarking: The color was determined through the CIELAB color space method.

For the water holding capacity (WHC), the samples were hydrated (50 g of product in 125 ml of cold water for 1h) and then sieved. The WHC was calculated as it follows: mass of water absorbed by the sample (g) / mass of the dry sample (g).

Statistical analysis: The data obtained from the characterization of the raw materials and from the analysis of extrudates and commercial products were analysed through Statgraphics 18 Software. The results of the mean, standard deviation, one-way ANOVA and Tukey test were obtained.

Results and discussions

Pre-test: Hydration: The extrudates from the first and second try out did not pass the hydration pre-test, as the samples got dispersed in water and formed an agglomerated paste. From the extrudates of Try Out 3 (T3A1, T3A2, T3A3, T3A4), extrudate T3A1 got completely dispersed in water after hydration. However, extrudates T3A2, T3A3, T3A4 were successfully hydrated (see Figure 2).

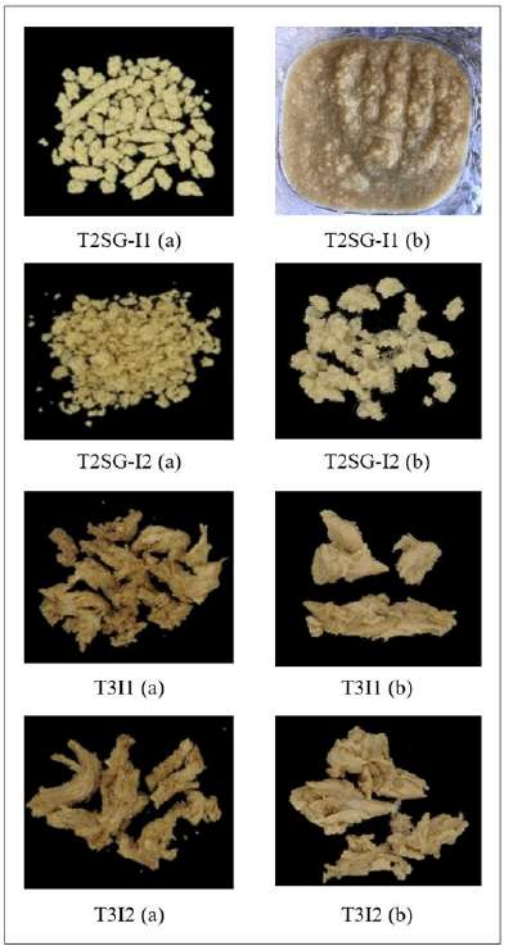

Figure 2.-Try Out 3 extrudates before (a) and after (b) hydration

Protein solubility:

The positive results on the pre-test of extrudates T3A2, T3A3 and T3A4 were strongly influenced by the higher solubility of active soybean flour, as protein solubility is important for the formation of a more stable structure of TVP. The difference on the solubility of the three raw materials might be attributed to previous pre-treatments, as heat and solvents may change the original structure of the protein, reducing its solubility in water. Figure 3 presents the protein solubility of the raw materials.

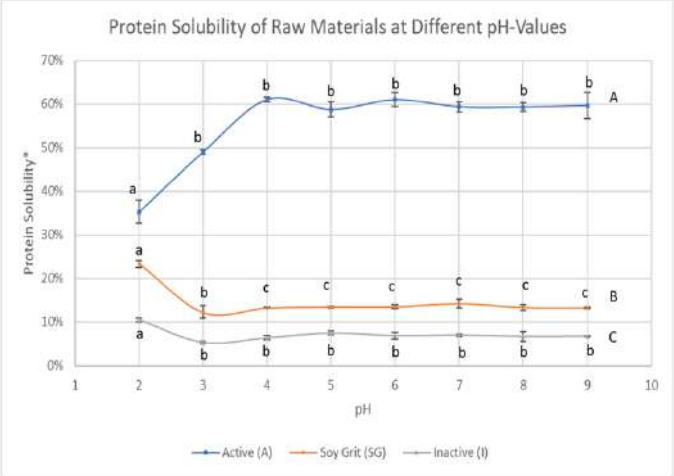

*Figure 3.- Protein solubility of raw materials. * The values are the average of two repetitions and error bars represent the standard deviation. A, B, C Different letters show significant difference ($p < 0.05$) between the groups (raw materials). a, b, c Different letters show significant difference ($p < 0.05$) between the groups (pH of each raw material)*

Protein digestibility in vitro and Specific Mechanical Energy (SME):

In general, protein digestibility decreased after extrusion process. This might be due to the formation of cross-linked complexes, that reduce the protein digestibility (Lin, Lu, Kelly, Zhang, & Zheng, 2017). Moreover, T3A4 showed a higher digestibility than T3A3. This might be due to the changes on heat distribution and water content during the process of these extrudates.

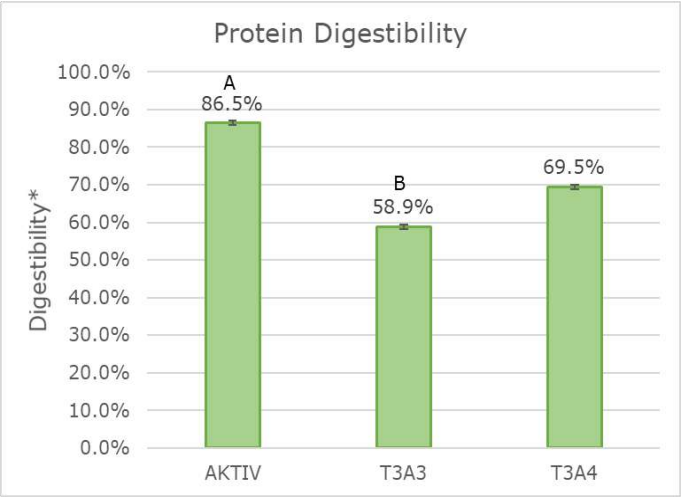

*Figure 4.- Protein digestibility in vitro. * The values are the average of two repetitions and error bars represent the standard deviation (SD = 0.6)* [A, B, C] *Different letters show significant difference (p < 0.05) between the groups.*

Water has a lubricant effect during extrusion. When the feed moisture increased, viscosity of the food matrix might decrease, and the food mass moved towards the outlet easily, so a lower input of mechanical energy was required. With lower mechanical energy, the shear and friction between the food matrix and the barrel of the extruder decreased, so the cross linking was also lower, leading to a better digestibility (Guerrero, et.al, 2012). As it can be observed in Figure 5, at a lower Specific Mechanical Energy, the digestibility of the extrudates was higher.

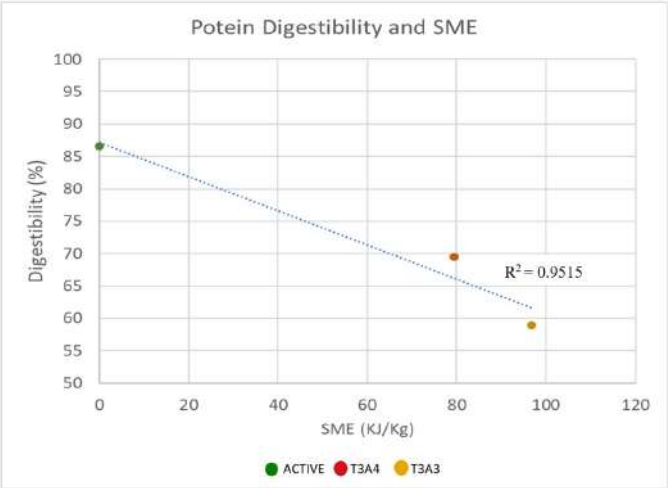

Figure 5.- Protein digestibility and Specific Mechanical Energy (SME) of extrudates.

Benchmarcking:

The results of the l*a*b* analysis showed that in general, the extrudates were darker and redder than the commercial products. This might be attributed to higher temperature used during the extrusion process.

Product	L*	a*	b*	
Vega Vita	57.62 ± 0.56 [A]	9.97 ± 0.34 [A]	28.85 ± 0.07 [A]	
Top OP	55.88 ± 0.40 [B]	9.13 ± 0.29 [A, B]	27.24 ± 0.30 [B]	
Veggy Star	60.77 ± 0.66 [C]	6.04 ± 0.50 [C]	26.63 ± 0.55 [C]	
T3A2	55.74 ± 0.20 [C]	8.89 ± 0.49 [B]	32.88 ± 0.33 [D]	
T3A3	41.50 ± 0.83 [D]	15.22 ± 0.29 [D]	29.00 ± 0.66 [A, E]	
T3A4	43.52 ± 2.07 [D]	14.81 ± 1.10 [D]	29.54 ± 0.19 [E]	

*Table 2.- * The values correspond to the average of four repetitions ± standard deviation. A, B, C, D Different letters show significant difference (p < 0.05) between the groups in the same column.*

After hydration, the main observation was that the intensity of the color values reduced. This might be due to the effect of water on the reflection of light. Hydrated TVP can present pink color that resemble cured meat, tuna or salmon (Riaz, 2006).

Figure 6.- Texturized vegetable protein (T3A3): dry (left) and hydrated (right).

Regarding the water holding capacity (WHC), all the samples were significantly different. The extrudates produced in the pilot plant showed lower WHC than the benchmarks. Nevertheless, the WHC values of the extrudates matched with values reported in literature, which are normally between 1 and 3 g of water / g of product (Riaz, 2006).

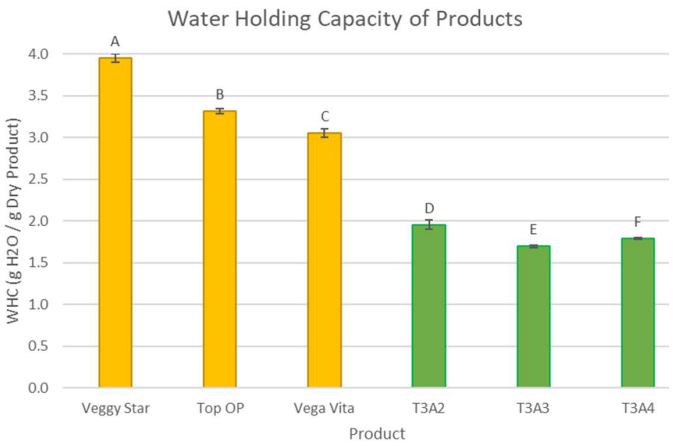

Figure 7.- Water Holding Capacity of commercial products and extrudates. A, B, C, D, E, F Diffrent letters show significant difference ($p < 0.05$) between the groups.

Conclusion

- It was possible to produce texturized vegetable protein with low-moisture extrusion.
- The pre-treatment of the raw materials influenced the quality of the final product.
- Higher water content during extrusion process led to lower specific mechanical energy and higher digestibility of the final product.

Finally, regarding the benchmarking, the extrudates presented a darker color and a lower WHC than the benchmarks. Nevertheless, the WHC of the extrudates matched values reported in the literature.

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Evaluation of food quality and safety for packaged ham using innovative packaging material by analyzing its physicochemical properties

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Introduction

Incomparable characteristics of petrochemical plastics made it difficult to find alternatives for them and reduce the huge adverse impact of petrochemical plastics on the environment (MacArthur et al., 2016), among these unique chracteristics, is high barrier properties. PLA (Poly Lactic Acid) is a commercial polymer made of conversion of corn starch into lactic acid, which is then polymerized (Dugan, 2001). This material is one of the biopolymers which receives considerable interest by researchers as a potential alternative for petrochemical plastics (Neethirajan & Jayas, 2011; Weiss et al., 2006) . GASP project with the objective of PLA reinforcement with nanocellulose crystalline (CNC) (Perez et al. 2017) aims in improving barrier properties of this bio-degradable and bio-compostable alternative of plastics (Trifol et al. 2016b). However, the application of such material in food packaging sector is not possible before evaluation of its safety and quality level and its stability in preserving food products during their desired shelf life (Gremli, 1996; Nielsen & Jägerstad, 1994).

Research objectives

- Chemical safety evaluation following the Regulation EU n°10/2011
- Hygienic properties using microbial adhesion and cross-contamination tests
- Evaluation of the performance of a bio-based and bio-degradable packaging in application to real food.

Methodology

1. Overall Migration Test based on the protocol described in Regulation EU n˚10/20:

2 groups of special migration cells (8 ml) filled with different food simulants: water, isooctane. One-side contact between plastic material and food simulant (2.2 cm diameter) was done. Evaporation until complete remove of food simulant and weight measurement of the residue was done after 10 days of storage at 4 ºC and at 40 ºC. Maximum acceptable amount by regulation:60 mg per kg food. (samples: PLA, GASP: multilayered (confidential) ,PLA+CNC).

2.Non Intentionally Added Substance (NIAS) Test:

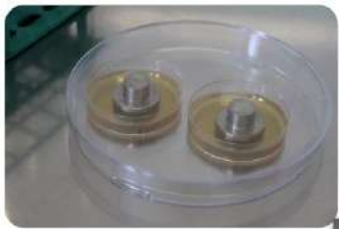
Addition of 1g of plastic material to the solvent (20 ml methanol + 5 ml chloroform), and GC-MS analysis of the solvent after 48 hours storage at room temperature + stirring at 150 rpm(Samples: PLA+CNC1, PLA+CNC2, PLA+CNC3, GASP). Confidential percentages

3. Bacterial Adhesion Test

Material in 11×14 mm size was immersed in bacterial suspension (107 CFU/ml) for 2h at 8ºC. After ultrasonic detachment for 2min, serial dilutions was done, incubation on BHI agar, at 37ºC for 24h and enumeration of colonies.

4. Bacterial Cross Contamination Test

The same protocol as adhesion test was performed and after 2h of adhesion, the material was placed on BHI agar for 24h. The medium and the material underwent ultrasonic detachment separately. Serial dilution, incubation and enumeration was done as adhesion test. (samples for adhesion and cross contamination test: PET, PLA, PLA+CNC. Bacterial strains: *Hafnia Alvei, Listeria Monocytogenes*).



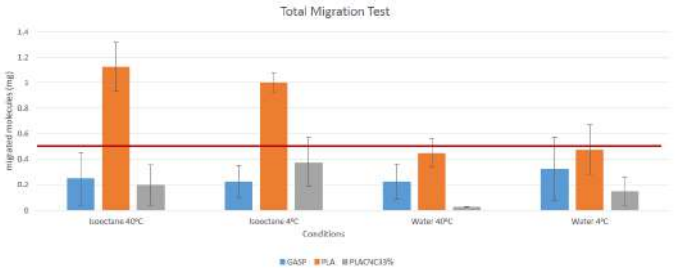
5. Shelf life Tests

Tests consist of pH, color, total microbial flora, and gas composition measurements. The tests were done on day 1, 3, 7, 14, 21 after packaging of 2slices of ham (11cm×16cm, thickness: 3mm) in trays and lids under modified atmosphere condition (80% N2, 20%CO2). In each point 6 trays, (1 empty tray with oxygen sensor, 1 with ham and sensor, 4 with ham and without sensor) for 2 groups separately: PLA trays+ PLA lids, PET trays and PET lids. Each measurement was done in triplicate.

pH: 1g of ham+20 ml of distilled water

Color: For each slice of ham, 5 points (4 on the margins, 1 in the middle) were measured

Gas composition: for all trays, at each point was measured (destructive method)



Oxygen content: Everyday measurement for samples with sensor. (Non-destructive).

Total flora: 10g of ham + 90ml of physiological water→ in stomacher for 90sec. Serial dilution, incubation on PCA for 72h in 30˚C and enumeration.

Results and discussions

1. Total Migration Test: All samples were under the threshold, except pure PLA in isooctane at 4˚C and 40˚C.

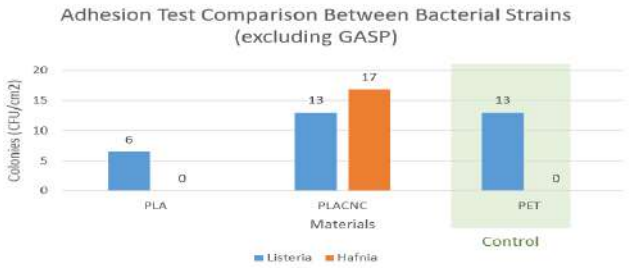
2.

2. NIAS: Lactic acid was found in all samples with PLA. Internal standard was not identified→ the test was not reliable. Samples did not show any non-authorized compounds.

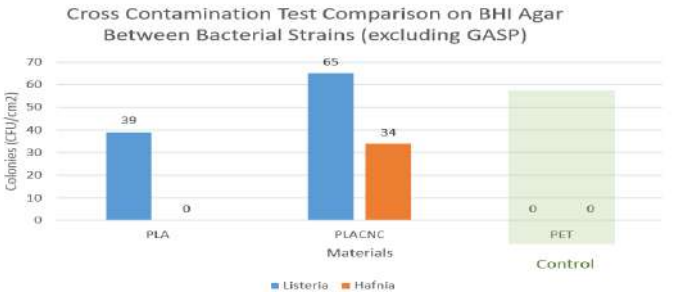
Compounds found in GASP (PET/CNC/PE) GC-MS analysis	
1-Methyl-3-(3,4-dimethoxyphenyl)-6,7-dimethoxyisochromene	13.348
Silane	14.664
Pyridine	15.796
3H-Isobenzofuran-1-one, 6,7-dimethoxy-3-p-tolylamino	15.853
Anthracene	16.595
Cyclononasiloxane	16.779
5-(4-amino-6-chloropyrimidin-5-yl)-6-nitrobenzimidazole	17.356
METHYL ESTER OF DI-O-METHYLISOPHOMAZARIN	19.193

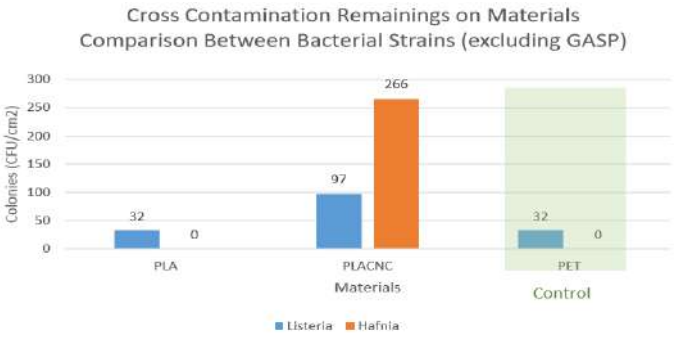
Methodology needs to be adapted for the materials due to the lack of internal standard.

3. Adhesion Test: High adhesion level for PET/CNC/PE (too high to be displayed in the graph): due to material delamination. Higher adhesion level of PLA+CNC comparing to PLA for both strains→ perhaps due to the positive correlation between the roughness of cellulose particles and adhered bacteria.



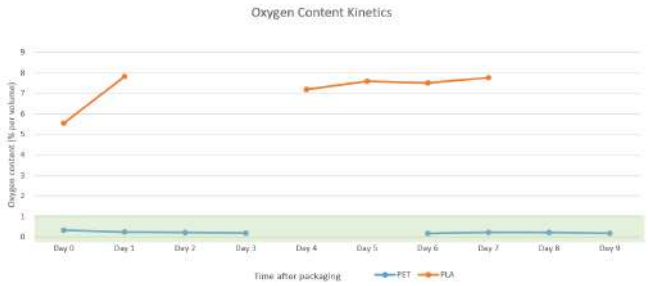
4. Cross Contamination Test: According to the Mats test, Listeria was hydrophobic and Hafnia was hydrophilic. A hypothesis is that CNC makes PLA more hydrophilic and more favourable for Hafnia. GASP material was again too contaminated to be compared.





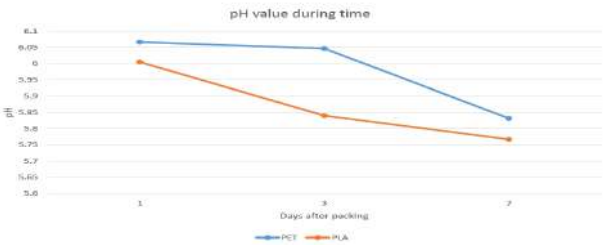
5.Shelf life Tests:

A) A quick increase and further equilibrium in oxygen content of PLA trays comparing to PET trays.



B) More stability in gas composition of PET trays and equilibrating gas composition of PLA trays with atmosphere.

D) PLA has faster decrease in pH. Initial difference on pH value is due to a delay of two days in packaging. pH value can be interpreted as an indicator for microbial activity.



Conclusion

The GASP project showed promising achievements in fabrication of an alternative for plastic packaging materials. Two different prototypes were tested during the internship: i) a multilayer material with a novel gas barrier layer(CNC), sandwiched between PE and PET and ii) new nanocomposites composed of PLA and CNC. Chemical safety tests gave a proof of both types regarding migration of molecules and presence of harmful compounds. However the specifications of NIAS test needs to be improved. The microbiological tests showed higher adhesion and cross contamination for PLA+CNC. Delamination of multilayered material made the results of this test unreliable and the lamination properties of this material needs to be improve for this experiment.

Shelf life test was not done with any of the GASP material due to Covid-19 crisis and the difficulty in receiving the materials from our suppliers. Moreover, the tests were not complete for the same reason. To conclude, GASP materials do not exhibit supplementary risks as food contact materials in comparison to other polymers.

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Effect of plant proteins on starch digestibility: an *in vitro* study

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Profile in a nutshell:

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Introduction

Diet represents one of the multiple factors determining metabolic diseases such as obesity and type 2 diabetes mellitus (Augustin *et al.,* 2015). Low glycaemic load diets including low Glycaemic Index (GI) foods may be a valuable nutritional strategy to reduce the risk of type 2 diabetes and cardiovascular diseases (Livesey *et al.,* 2019).

While developing new products with a low glycaemic index it is crucial to recognize an effective method to slow down the starch digestion. Starch digestibility is affected by the starch structure, food processing conditions and individual components present in the food matrix, i.e. lipids, dietary fiber, anti-nutrients and proteins. Specially, proteins are known to affect the rate of starch hydrolysis in cereals (Ezeogu *et al.* 2008).

Several *in vitro* and *in vivo* studies have shown that proteins play an essential role in reducing the digestibility of starch, eventually contributing to lower postprandial glycemia. The findings could help to optimize the protein content, cooking method and dietary source of starch during the formulation of products with a low GI.

Research objective

The key aim of this research was to evaluate the effect of hydrolyzed pea protein on the digestibility of wheat starch in a bread model *in vitro*.

Methodology

Conventional bread (traditional recipe) and two breads enriched with two different concentrations of Pea Protein Isolate (PPI) (5% and 20% w:w) were formulated and produced. The prototypes were subjected to enzymatic digestion by *Infogest method (*Minekus *et al.*, 2014) and the release of glucose (as a marker of the starch digestion) (Goni *et al.,* 1997) and of free amino groups (as a marker of the protein digestion) (Nielsen, 2002) over gastric and intestinal simulated digestion were assessed.

Results and discussions

Effect of pea protein isolate incorporation on dough rising

The nutritional composition of the three breads is reported in Table 1. The samples were varied in the content of Whole Wheat Flour and Protein Isolates. At 5% PPI concentration increased water absorption was observed compared to the control. This might be due to increased water absorption capacity due to protein increase. Low water absorption increase (1%) by 5% addition of Pea protein was observed in a study by Dabija and colleagues (2017). Further increase in concentration did not result in more uptake of water and similar findings were reported by Sissons and colleagues (2005) by a 4% increase of gluten content.

Table 1: Nutritional composition of the three breads

Samples	Calories (kcal)	Carbohydrate (g)	Dietary Fiber (g)	Protein (g)	Fat (g)	Sodium (mg)
Control Bread	335	72	13.2	9.97	1.96	440
5 % PPI Bread	334	69	12.6	13.9	1.89	438
20% PPI Bread	333	61	11	25.71	1.68	445

Figure 1 shows the effect of formulation on dough rising. Control and bread with 5% PPI show no difference in rising when assessed visually while in bread with 20% PPI rising was lower.

Figure 1: After leavening for 2 hours the dough volume for Control, 5% and 20%.

Free amino groups release during in vitro digestion

The amount of Free Amino Groups (FAG) released during the in vitro digestion is shown in Figure 2. As expected, the amount of the FAG at the beginning and over the in vitro digestion was increased as the protein concentration was increased. Although there was no significant difference in the release of the FAG during the Gastric Phase of all the three breads, there is gradual progression visible in control and 5% PPI bread.

The release of very low amounts of FAG during the gastric phase is in agreement with another study where a similarly slow release in static and dynamic in vitro digestion models was found (Egger *et al.,* 2019). This finding may be due to the fact that during gastric digestion pepsin enzyme was used and this enzyme is an endopeptidase that breaks the peptide bonds inside the proteins and large peptides to produce more peptides, while in the intestinal phase pancreatin enzyme contain other enzymes which continue the peptide digestion and also produce FAG (Berg *et al.,* 2002). A sudden spike can be noticed from the graph during the transition from gastric to intestinal phase in Control and 5% PPI bread. The significant increase of the FAG concentrations at all the time points of the intestinal phase in the Control and 5% PPI

bread can be due to the effect of Pancreatin during the intestinal phase (Egger *et al.*, 2019).

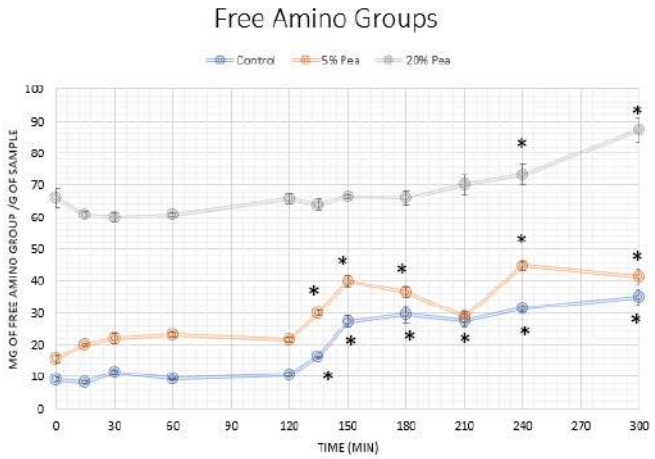

*Figure 2: Free Amino Groups released during Gastric Phase (0-120 min) & Intestinal Phase (120-300 min). ANOVA Single Factor Based on estimated marginal means (n=3) with Tukey Test. * indicates p<0.05 in comparison with respective baseline conc.*

Effect of Plant Protein Hydrolysis on Starch Hydrolysis

Figure 3 shows the concomitant glucose and FAG release at monitored time points during the in vitro digestion. There was a significant difference in the glucose release within different time points for all the three samples ($p<0.05$). AUC of the Starch Digested was also not significantly different among the samples (Control, 5% and 20% PPI enriched bread).

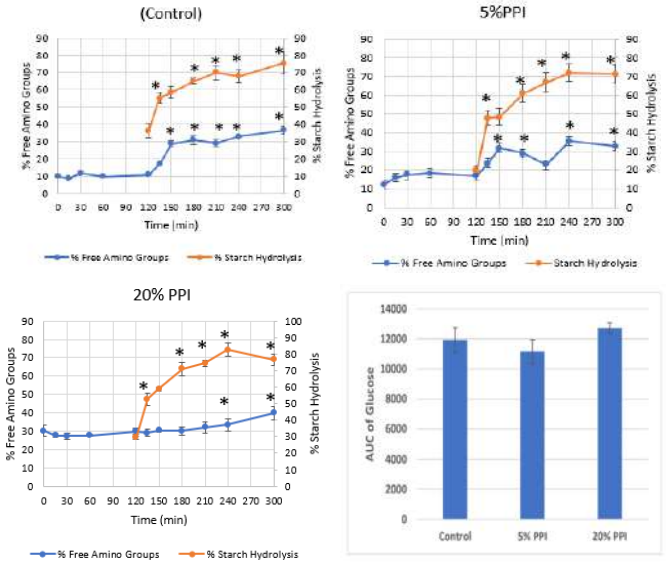

*Figure 3: A. Free Amino Groups (FAG) release Vs Starch Hydrolysis in Control, 5% PPI and 20% PPI Bread Samples. * when p<0.05 for %FAG and % Starch hydrolysis by ANOVA and Tukey post hoc test. B. Total glucose released during the intestinal phase (120 to 300 min) assessed as AUC.*

These results are contrary to previously reported studies in which a negative correlation was found between protein and starch digestibility (Barón et al., 2017; Singh, Dartois & Kaur, 2010). However, neither of those studies involved leavening process and hence this gives rise to a need of deep understanding of a combined effect of processing and food matrix on the starch and protein digestibility. Time and type of processing can affect starch-protein interactions and, in turn, glucose release (Berti et al., 2004; Barbiroli et al. 2013; Wolter et al., 2013).

Effect of Protein concentration on Glycaemic Index

Table 2 shows the GI of the three breads. There is a significant difference between the GI of control bread and 5% PPI enriched bread. While no significant difference between control and 20% PPI bread was recorded. Noteworthy there was no significant difference between the starch hydrolyzed (over total starch of each sample) in the three breads but GI was found to be different. Normalizing of the starch hydrolysis results with total starch might be the reason for this difference.

Table 2: AUC Mean, Glycemic Index (GI) and Hydrolysis Index (HI)

Sample	Index	1	2	3	Mean AUC	Mean GI	Std Dev
Control	AUC	11073.54	12681.21	12029.82	11928.19		ST.DEV
	HI	100.00	100.00	100.00		100	0
	GI	100.00	100.00	100.00		100	0
5% PPI	AUC	11406.65	10256.02	11788.72	11150.47		
	HI	103.01	80.88	98.00		93.96	11.60
	GI	96.26	84.11	93.51		91.29	6.37
20% PPI	AUC	12795.36	13024.49	12313.18	12711.01	MEAN	ST.DEV
	HI	115.55	102.71	102.36		106.87	7.52
	GI	103.15	96.10	95.90		98.38	4.12

In addition, another interesting observation noticed was that to normalize the Gastric pH (3) to intestinal pH (7) during the in vitro digestion, the amount of NaOH added, varied significantly for the three samples. Indeed, NaOH was added in a volume of 200 µL, 400 µL and 900 µL to maintain the pH of 7 in Control, 5% and 20% PPI bread samples, respectively. The reason for the increasing requirement of NaOH in 20% PPI bread is the higher protein content. Mennah Govela and colleagues (2019) reported that higher protein concentration and smaller surface area result in higher buffering capacity during in vitro digestion. This may be a proof that in 20% PPI bread a consistent proteins' aggregation might have occurred that reduced the starch-protein linkages ameliorating starch hydrolysis. This is in agreement with the fact that baking at high temperature can increase proteins aggregation due to disulfide linkages between added proteins (Cabra, Arreguin, Vasquez and Farres, 2006). Therefore, processing can also affect starch digestibility and have a major role when high concentrations of proteins are used in a leavened baked product.

Conclusion

In the present study, the effect of the PPI at two different concentration (5% and 20%) on the starch digestibility of bread was evaluated. Increased water absorption was observed at 5% concentration but not at 20%. The FAG formed during in vitro digestion increased as the protein concentration was increased. There was no significant difference in the AUC of FAG during gastric phase while the level of digested proteins from 10 to 35%, 10 to 34% and 30 to 80% (by weight of bread) for control, 5% PPI and 20% PPI bread respectively from the start of the gastric digestion to the end of intestinal phase. AUC of glucose was similar between the breads. However, the 5% PPI bread had a significantly lower glycemic index than the control bread while no significant difference between control and 20% PPI bread was recorded. This finding was explained as a result of a higher protein aggregation in 20% PPI bread.

The findings of this study shed light on the effect of PPI on GI of bread, offered some indications to produce PPI enriched bread with a lower GI and highlighted the importance of the protein type and processing in using protein-starch interactions to reduce starch digestion. In vivo studies are necessary to determine whether 5% PPI enriched bread significantly affect post-prandial glycaemia.

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Purification of commercial plant protein ingredients

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Introduction

Plant proteins can contain up to 200 non-protein compounds. Although these components offer health benefits, some are not suitable for specific individuals. Purification of plant proteins is the prerequisite for producing a commercial ingredient compliant with health, safety & quality, and nutritional recommendations.

Research objectives

1) Remove or reduce unwanted compounds in plant proteins to comply with safety, quality and nutritional standards.

2) Minimize protein loss and retain amino acid quality.

3) Maintain protein functionality.

Methodology

The plant proteins (PP) underwent two different purification treatments (T1 & T2). PP were solubilised in warm water (X % Total Solids) before undergoing treatment. For T1, the goal was to remove unwanted compounds (1, 2, 3 and 4) as much as possible to validate a one-off feasibility trial carried out previously and provide data to estimate removal rate over time and function of water used. For T2 the PP was dissolved in warm water (X % TS) and pH adjusted and fed into equipment for treatment. The goal was to explore the feasibility of reducing compound 2 from PP as much as possible. Finally, the protein yield, as well as the content of the compounds 2 and residual minerals, within

the resulting PP, were examined as well as ingredient functionality characteristics.

Results and discussions

Commercially available PP contains unwanted compounds in concentrations greater than recommended in guidelines. Compound 1 was significantly (96%) reduced from PP with T1, validating the first feasibility trial. Compounds 2 was reduced by 78%, ensuring improved mineral bioavailability. Compounds 3 was almost fully removed (99.9%) and compound 4 was partially removed (18%).

T2 suggested it was feasible to reduce compound 2 from PP (33%), although T2 was less effective than T1. This was thought be due to the treatment conditions which can be optimised.

For T1 the protein and amino acid content increased in the ingredient and for T2 there was no difference. The colour of T1 PP was lighter and less yellow than the starting material. The PSD was similar, although viscosity increased.

Conclusion

Compounds 1 removal permits PP application in Specialised Nutrition products. Food safety guidelines are met for compound 3, although further purification steps are required for compound 4 to ensure food safety. further optimisation and validation is required for both T1 & T1. Recommendation have been provided for treatment conditioning and scaling up the process.

Confidential topic

Quantifying enteral amino acid absorption in the human colon: pilot study

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Master Thesis tutor :
Prof. Paola VITAGLIONE and PhD. Suzanne HODGKINSON

Introduction

While, on one hand, the demand for high-protein foods by consumers keeps increasing, on the other hand, the problem of supplying the right amount of protein to the growing population worldwide is one the main challenges nowadays. Proteins are fundamental nutrients for body maintenance and growth. However, not all the proteins contain the essential amino acids required by the body for protein synthesis. The gold standard method used to evaluate protein quality has been defined by the Food and Agriculture Organization (FAO) and is represented by the Digestible Indispensable Amino Acids Score (DIAAS) which is based on ileal true digestibility. The latter one relies on the assumption that absorption of amino acids in the colon can be neglected, as previously reported in animal studies. However, that has not been proved in humans yet.

Research objectives

To provide information useful for the main study aimed to quantify amino acids absorption in the human colon, while being infused during a colonoscopy exam.

Our body can synthesize all the proteins needed for the organism only when all the essential amino acids are present in a specific ratio and quantity. When one essential amino acid is missing, the other amino acids provided cannot be utilized by our body and subsequently get converted into urea. Therefore, an increase in the urea production in the liver due to the excess of the other amino acids would be expected. Urea to creatinine ratio has been used as a uri-

nary biomarker to detect differences in high- and low-quality protein administration. Urinary creatinine is considered as an index of muscle mass and assumed to be constant over time.

A pilot study has been used to determine:

1. the level of lysine – one of the essential amino acids – which has to be served in a meal ("degree of deficiency") in order to provide a sufficient contrast in the nitrogen: creatinine (N:C) ratio in the urine so that if lysine is absorbed in the colon the N:C ratio in the urine will significantly decrease;

2. the duration of urine collection time postprandially

3. the intersubjective variability of the urinary N:C ratio to calculate the sample size required for the colonoscopy trial.

Methodology

Twelve participants were recruited and received, in two separate occasions, two test meals, one providing the complete amino acid pattern suggested by FAO 2013, and the other one 60% lysine deficient. Urinary samples were collected at time 2,4 and 6 hours after the test meal and they were analyzed for urea and nitrogen to creatinine ratio.

Results and discussions

Urea: creatinine ratio showed no statistical difference between the two test meals administered among the twelve participants as we can see in the graph below.

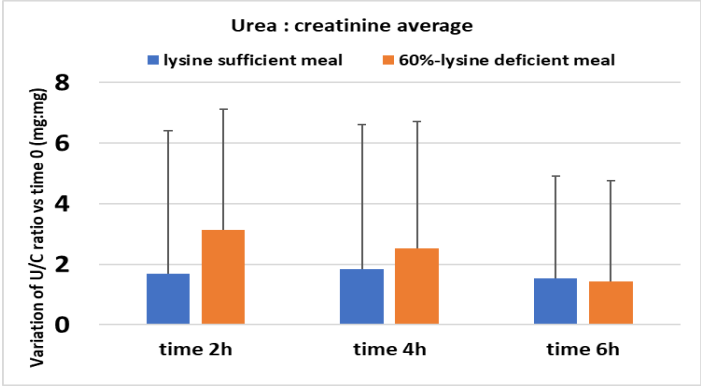

Figure 1. Urea to creatinine ratio average among the participants during the two test meals.

From these results, it can be deducted that 60% of lysine deficiency did not create a sufficient contrast in the biomarker chosen. Also, the definition of an optimal timing was not possible. Lastly, given the high inter-individual variability, a too great sample size was calculated for the colonoscopy study.

For all these reasons, a new protocol has been designed aimed at establishing the ideal conditions to perform the study reducing the impact of some limitations. The scheme of the new protocol is presented in Figure 2.

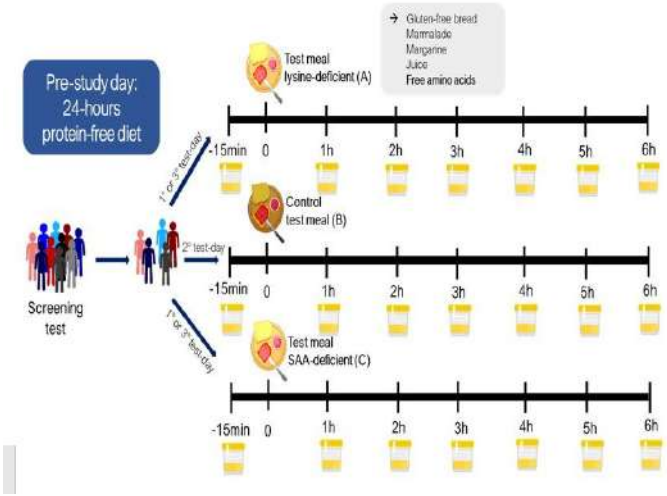

Figure 2. New protocol study design

The major changes concerned:

- Greater lysine deficiency will be used;
- A protein-free diet will be provided in the pre-study day, in order to allow a standardization of the participants baseline;
- Free amino acids will be part of the test meal instead of the protein used before, namely zein, in order to allow higher precision and lower excess of other amino acids;
- A sulfur amino acid-deficient test meal will be added to the trial since sulfate has been shown to be a potential urinary biomarker useful to quantify its absorption;
- Urine samples will be collected hourly instead of on a 2-hours basis;
- Baseline urine sample will be collected after discarding the first urine of the day.

Conclusion

In order to quantify amino acids absorption in the colon, a pilot study was carried out to define the parameters to be used for the study, envisaging infusion of specific essential amino acids directly into the human colon, in participants undergoing colonoscopy. Being urea the product of amino acids metabolism in the liver, an evaluation of urinary urea: creatinine ratio would allow to notice whether a difference is present when high-quality protein, providing all the essential amino acids, versus a low-quality one, missing in some of them, is administered. However, inconclusive results were reached. A new protocol has been designed for a future pilot study aimed at reducing the impact of some limitations of the previous study on the outcomes.

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Evaluation of Alternative Packaging for Solid Oral Dosage Forms

An assessment from Elanco's perspective

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The animal health industry is a highly competitive and innovation-driven market that has undergone many changes in recent years. In 2016, around 9.9 billion US dollars were spent on animal health pharmaceutical products. One of the main players in the animal health business is Elanco Animal Health Inc., a global animal health company with headquarters in Greenfield, Indiana (USA), is one of the main players in the animal health business with a product range that covers companion animals (dog, cats), farm animals (cow, pig, chicken) and aquatic animals (fish). Elanco, like other animal health companies, is under constant pressure to innovate, hence this project in assessing innovative packaging of animal health products. The packages currently in use at Elanco include bottles, dispenser systems, tubes, syringes, vials, bags, flexible intermediate bulk containers and blisters.

This project intended to innovate in the companion animal product packaging space, specifically, the aluminum blisters (see Figure 1) currently used at Elanco Animal Health were challenged and compared to alternatives. Elanco's experience with the aluminum blister has highlighted many advantages to this type of packaging: excellent product protection and good consumer acceptance. However, even as the blister appears well suited to Elanco's purposes, there are disadvantages to its use, such as difficulties for elderly or disabled customers, child resistance and environmental concerns.

Figure 1: Typical aluminum blister

This project aims to overcome these shortcomings by assessing 22 possible solutions for the improvement of blister packaging and its alternatives. Ten of the alternatives are attempts to improve the current blister, while the others change the packaging system completely.

The alternatives have been found in a mixture of already existing ideas from the packaging development team, ideas that were inspired by the benchmarking process, ideas that have come from different stakeholders and the consumer behavior study. These alternatives have been selected from three sources: novel ideas already in the minds of Elanco's packaging team, the results of the benchmarking study that was carried out to identify innovations in pharmaceutical packaging, and a consumer behavior study.

The alternatives were assessed using a packaging scorecard with the following attributes: product protection, consumer acceptance, marketing, environmental impact, regulatory, investment and material costs, technical feasibility, and novelty.

The aspects were assessed during expert interviews from experts within Elanco and throughout the industry. It includes experts from tooling companies, foil suppliers and contract manufacturers. From Elanco, experts from regulatory, marketing, procurement, and research and development were consulted. A consumer study and a benchmark study were conducted to better define the intended area of innovation.

The benchmark study indicated that blisters and bottles are the main packaging systems used in the animal and human health industry for tablets. The consumer study showed rising concerns in environmental impact, convenience, and ease of opening.

The alternative solutions vary from minor improvements in the visual appearance of the blister to flexible, multi-dose systems. Of the 22 assessed alternatives (see Figure 2), ten have scored higher than or equal to the current aluminum blisters. Overall, the flexible, single-dose solution (Solution 7) seemed to be the most promising alternative. This alternative enjoys the functional aspects of blisters while decreasing its lack in convenience and environmental impact. In addition, the solution shows potential further improvements in environmental friendliness.

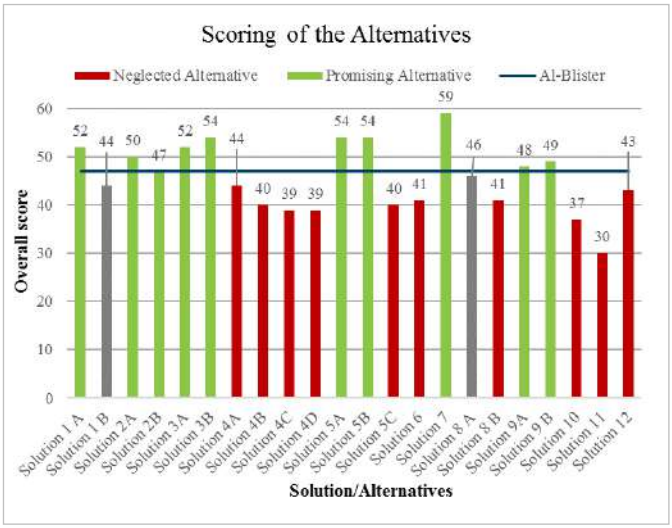

Figure 2: Overall scoring of the alternative packaging alternatives

Solutions, which improved upon the currently used blister, also appeared promising. A trend was noticed in which alternatives that achieved greater environmental friendliness often lacked in sufficient product protection to be able to replace the current blister packaging. No significant relation between the scores of a particular aspect and the overall score were found.

Confidential topic

Development of an aromatic matrix to be added in beer before refermentation step

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Beer can be traced back around 5000 years ago and, nowadays, its consumption around the world is estimated to be over 100 billion liters per year. Stimulated by craft brewers and by consumer preference for hop aroma over bitterness in beers, hop aroma became focus of researches. Bioflavoring is one possibility that has been studied for improving organoleptic quality of beverages, and that can be relevant for hopped beers. Although hop aroma is quite complex, the main subjects being pursued are the biotransformation of monoterpene alcohols and polyfunctional thiols and its precursors, present in some hop varieties. Fermentation with brewer's yeast presents relevant results to obtain hoppy aroma, like citrusy, through the combination of linalool, geraniol and citronellol. Recent studies also bring the importance of the presence of some thiols – i.e. 4MMP, which characterizes some hops' fruity aroma – and that is possible to enhance this characteristic in the presence of microorganisms' enzymes.

Through the selection of ingredients, formulation and process parameters, this study aims to develop a new aromatic product that will be used in beer refermentation, using hop in presence of yeast to obtain a fruity profile. Different conditions were evaluated through sensory and volatiles analysis, and application tests were performed to understand the product viability. The resulting product will bring new opportunities to brewers with an innovative concept.

Confidential topic

Food product development:
An innovative jelly dessert with satiating properties

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The aim of this study was to develop a jelly dessert with enhanced satiating properties provided by a combination of specific macro nutrients and ingredients with scientific studies in reference to satiety, weight loss and/or weight management. Along different development stages a base formulation was developed, optimized and further evaluated for potential consumers in a clinical trial test to corroborate the efficacy in terms of satiety. Finally, with the inclusion of other ingredients to potentiate the effect founded in the previous study, different incompatibilities were founded in between the food matrix and the new ingredients, making non viable the inclusion of most of them without a modification that affect the obtained results with consumers.

Confidential Topic

Durum and Common Wheat in Breadmaking: Biochemical and Rheological Differences

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Introduction

This research looks into the origins of quality differences between durum wheat flour (DWF) and common wheat flour (CWF) in breadmaking. Common wheat is typically used for bread, biscuits, and other baked goods, while durum wheat is usually reserved for pasta. This distinction arises from both quality concerns and legal restrictions in some countries, including France (Boyacioglu & D'Appolonia, 1994 ; Décret n°55-1175, 1955; Décret n°93-1074, 1993). DWF is used in regional breads around the Mediterranean, but attempts to replace CWF with DWF in standard bread types yield a lower-quality product that is smaller and denser (Sissons, et al., 2012).

This project investigates the biochemical differences between DWF and CWF, and then it explores the rheology and biochemistry of doughs and breads made from mixtures of the two flours. Further work will draw correlations to understand how the biochemistry leads to differences in the final products.

Research objectives

- Biochemical characterization of DWF and CWF, including lipids, proteins, sugars, and enzymes
- Evaluation of the rheology of doughs made with mixed flours
- Quality assessment of breads made with DWF and CWF, as well as mixed flours

Methodology

Flour. Lipid cartography and carotenoid analysis was performed on the flours (Castello, et al., 1998). A Folch extraction with 20 mL each of methanol, chloroform, and salt water was

performed on both flours, the mixture was centrifuged, and the lower chloroform phase was recovered. Thin-layer chromatography was used to separate the lipid fractions. The fatty acids were hydrolyzed and methylated using BF_3 in methanol (14%, m/v). The sample was extracted in pentane and then redissolved in heptane for analysis by gas chromatography using a flame ionization detector (Trace 1310, Thermo-Fischer Scientific, Waltham, MA, USA). The lipid cartography was performed in triplicate.

The Kjeldahl method was used to determine the protein content of both flours (Organisation internationale de normalisation, 2006). The mineralization step was performed with a Digest Automat K-438 (Büchi, Flawil, Switzerland), then the distillation step was performed automatically with a Distillation Unit B-324 (Büchi, Flawil, Switzerland) using 80 mL 30% NaOH, 50 mL water, and 30 mL boric acid. Titration was performed using a Titroline 500 pH meter (SI Analytics, Mainz, Germany) using a 0.1 M sulfuric acid solution and with a target pH of 4.6. Sucrose was used as a negative control and the titration value for sucrose was subtracted from the sample values. The analysis was performed four times per flour.

Soluble and insoluble fractions of gluten proteins were quantified by SE-HPLC according to Morel, et al. (2000). Flour (160 mg) was dissolved in 20 mL of sodium phosphate buffer (0.1 M, pH 6.9) with 1% SDS and agitated, then centrifuged and the supernatant was filtered and analyzed as soluble proteins. The pellet was dissolved again in 20 mL of buffer, agitated, sonicated at 7 W for 90 seconds, and centrifuged as before. The supernatant was recovered, filtered, and analyzed as insoluble proteins.

Both solutions were separated on a size exclusion column (TSK gel G4000SW, 300 mm x 7.5 mm, Tosoh Bioscience, Tokyo, Japan) using sodium phosphate buffer (0.1 M, pH 6.9) with 0.1% SDS at a flow rate of 0.7 mL/min. The HPLC set-up (Varian, Les Ulis, France) uses a photodiode array detector at 214 nm. Each flour was analyzed six times.

Dough. Model doughs were mixed using a consistograph (Chopin Technologies, Villeneuve-la-Garenne, France) using only flour and water. Eleven bread doughs with varying ratios of DWF and CWF were made using 10% increments (100%, 90/10, 80/20, etc.). Hydration was set at 56% based on preliminary trials with the consistograph targeting a maximum pressure between 2000 and 2500 mb for 100% DWF and 100% CWF doughs. Doughs were mixed for 480 seconds at 60 rpm using a double arm mixer and maintaining the temperature of the mixing compartment at 25 °C.

Bread. Breads were prepared using the same 11 ratios of DWF and CWF as above. The standard BIPEA French bread-making test protocol was followed by a trained baker, who scored each dough and bread based on the color, quality, hydration, and fermentation of the dough as well as the volume, density, color, and appearance of the bread.

Slices from BIPEA breads were scanned and analyzed using ImageJ software (NIH, Bethesda, MD, USA). Regions of 1000 x 600 pixels were extracted from the center of the image, converted to 8-bit grayscale, and threshold analysis was performed according to the method of Otsu (Scheueur, et al., 2015). The threshold was considered the division between air cells (dark areas) and cell walls (light areas), and particle analysis was undertaken to analyze the size and number of air cells in each slice. The analysis was performed on 3 images per type of bread.

Results and discussion

Flour. Total lipids were found to be higher in DWF than in CWF, and the distribution of lipid types and polyunsaturated fatty acids (PUFA) was similar between the two. Table I shows the total lipid concentration from lipid cartography, which was calculated by adding all of the lipid fractions.

Table 1. Lipid content and fatty acid ratio in DWF and CWF.

	Lipid content (µmol.g^{-1})	SMUFA	PUFA	
		µmol.g^{-1}	µmol.g^{-1}	%
DWF	37.5 ± 2.6	13.8 ± 0.5	23.8 ± 2.1	63
CWF	31.2 ± 1.4	10.8 ± 0.4	20.4 ± 0.9	65

The concentration of total fatty acids was significantly higher in DWF than in CWF ($p<0.001$), but the ratio of saturated and monounsaturated fatty acids (SMUFA) to PUFA is consistent between the two types of flour. The amount of PUFA is important because these fatty acids are substrates of lipoxygenase, an enzyme that can impact characteristics such as dough color and strength.

The type of lipids, particularly the distribution between nonpolar and polar lipids, can impact the bread-making quality of the flour. Figure 1 shows that the distribution of lipid types is similar between the two kinds of flour, with the majority found in the form of TAG, followed by TPL. Most of the additional lipids found in DWF are in the form of TAG, which is more than 40% higher in DWF. A higher level of TAG in DWF could play a role in decreased bread volume, as it has been shown that increasing nonpolar lipids in bread has a negative impact on volume (MacRitchie & Gras, 1973; Sroan & MacRitchie, 2009).

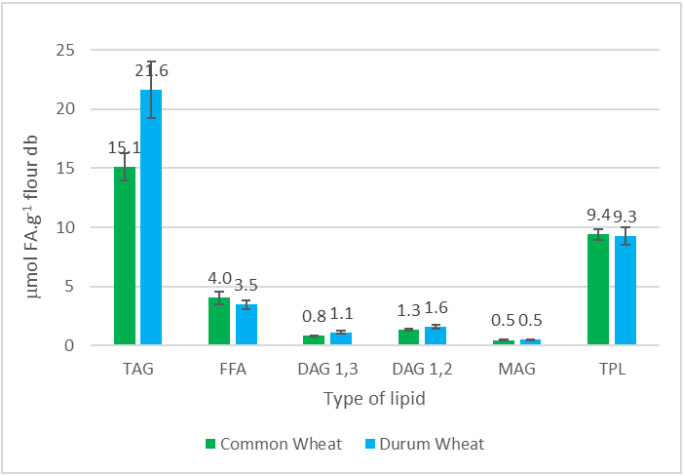

Fig. 1. Concentrations of each lipid type in DWF and CWF based on lipid cartography, n=3. TAG: triacylglycerols, FFA: free fatty acids, DAG: diacylglycerols, MAG: monoacylglycerols, TPL: total polar lipids.

The total protein content was found to be higher in DWF (12.2 ± 0.4%) than in CWF (11.5 ± 0.1%), as was expected (Boyacioglu & D'Appolonia, 1994). Figure 2 shows a comparison of both the total protein and the relative amounts of insoluble and soluble protein in both types of flour.

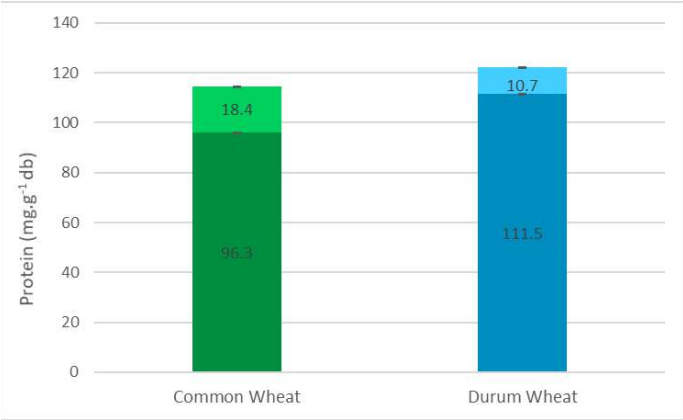

Fig. 2. Comparison of insoluble (light area) and soluble (dark area) protein as a portion of total protein in DWF and CWF, n=6.

Analysis of wheat proteins by SE-HPLC identified significant differences in the distribution of types of proteins. The amount of insoluble protein was much higher in CWF, accounting for 16.0% of total protein in CWF and only 8.7% in DWF (Fig. 2). Despite its higher protein content, the reduction in insoluble protein in DWF may contribute to the lower volume of its bread because the insoluble protein fraction, made up of large polymers of glutenins, is strongly correlated to dough strength and bread-making quality (Khan & Shewry, 2009; Gupta, et al., 1993).

Dough. The 100% formulations were repeated six times each and show a significantly higher maximum pressure (PrMax) value for 100% DWF (2279.3 ± 74.4 mb) than for 100% CWF (2026.0 ± 122.1 mb) ($p<0.001$). The pressure toward the end of the mixing period also reflects a change in the dough with more DWF (Fig. 7). The pressure at 450 s increases from 975 mb with 100% CWF to 1184 mb with 100% DWF and follows an approximately linear increase. Higher values for both maximum

pressure and pressure at the end of mixing shows that increasing the proportion of DWF creates a stiffer dough.

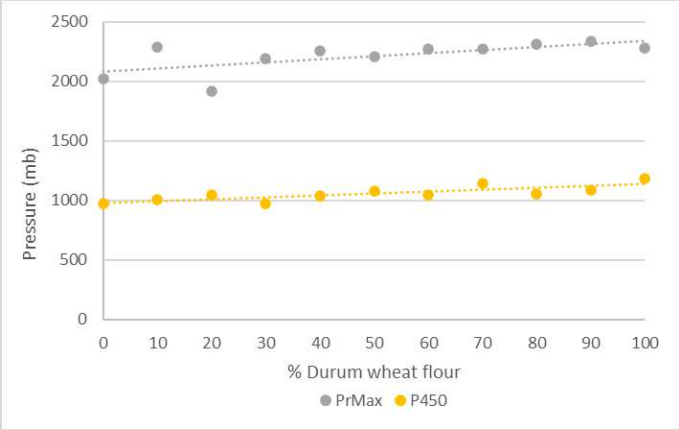

Fig. 3. PrMax and pressure at 450 s in consistograph for doughs with varying amounts of DWF, n=6 for 0 and 100% DWF, n=1 for all others.

Bread. The volume of 100% CWF bread was higher than that of 100% DWF bread (1701 ± 16 cm^3 vs. 1433 ± 17 cm^3 for loaves of 280-290 g, p=0.060). The maximum volume was found at 40% DWF (Fig. 4), indicating a possible synergistic effect between the two flours. The volume remained similar to or greater than control (100% CWF) until 70% durum wheat flour, at which point the volume decreased with each further addition of DWF.

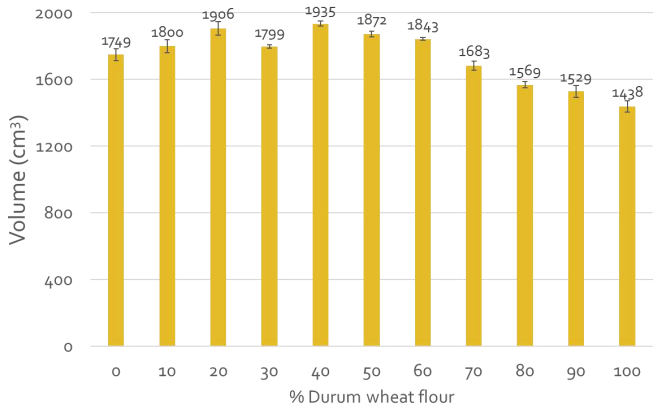

Fig. 4. Volume of breads made using the standard BIPEA method with varying amounts of DWF, n=4 loaves.

Volume is a quality indicator for bread and demonstrates the lower quality of DWF bread, as the literature predicts (Boyacioglu & D'Appolonia, 1994).

Digital image analysis was used to compare the texture of the crumb of breads made with different formulations. Using false color application, a qualitative difference was observed between the two kinds of breads (Fig. 5) with 100% CWF bread having a finer crumb.

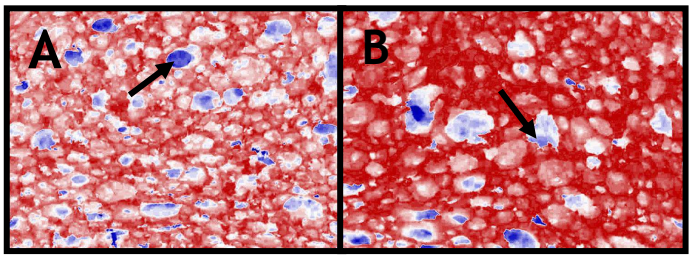

Fig. 5. False color images of 100% CWF (A) and 100% DWF (B) bread slices. Dark areas in the original image are shown in blue (indicated by arrows), while light colors are shown in red.

Threshold and particle analysis led to quantitative results that supported this observation, with a greater number and smaller size of air bubbles in 100% CWF bread.

Table 2. Number and size of air bubbles in DWF and CWF bread.

	# Bubbles	Avg. size (pixels)
CWF	817.0 ± 14.5	256.5 ± 3.2
DWF	517.3 ± 10.7	319.5 ± 41.2

Larger air bubbles in the high-DWF breads are a sign of low quality and likely indicate that smaller bubbles coalesced because of instability. This may be linked to the higher levels of nonpolar lipids in DWF: air bubbles in bread dough are stabilized by a liquid lamella, and higher levels of non-surface-active TAGs could interfere with bubble stabilization (Sroan & MacRitchie, 2009).

Conclusion

The present study found significant biochemical and rheological differences between DWF and CWF, as well as quality differences in breads made from the two flours and their mixtures. In the analysis of the flours, the significant differences in the concentrations of insoluble protein and nonpolar lipids between

DWF and CWF stand out as noteworthy. Bread doughs made with high proportions of DWF were found to be stiffer in terms of consistograph pressure. Higher proportions of DWF in breads were also associated with reductions in loaf volume and with loss of crumb fineness as determined by digital image analysis.

Further research is needed to draw more definitive links between the biochemical differences between the flours, the behavior of the doughs, and the quality of the breads. The next step will be full biochemical characterization of the doughs made with varying ratios of DWF and CWF, including protein, lipids, and carbohydrates. Once all of these data are collected, further statistical analysis will be useful to make connections between the different phases. Correlations and principal component analysis will help determine which differences have the greatest impact on the quality of the final product, which will explain the observed differences in breads made from the two different flours.

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Transcriptomic analysis of *Listeria monocytogenes* grown at low temperature with unsaturated fatty acids

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Introduction

Nutritional recommendations for optimizing fatty acid balance in foods are very popular among the food industries and consumers (PNNS: 2006-2008). Generally, there is a severe decline of fats placed on the market through the reformulation of existing food products, aiming to increase the amount of polyunsaturated fatty acids consumed. On the other hand, recent scientific data showed that some foodborne pathogens including *Listeria monocytogenes* are able to grow at low temperature in the presence of exogenous unsaturated fatty acids (UFA). Bacteria can use these fatty acids to fluidify their membranes when temperature gets lower. In these conditions, bacteria can grow and reach infectious doses much quicker than it had been predicted. To date, few studies examine how these unsaturated lipids can impact pathogen development and none have identified the genetic regulation of lipid metabolism of *L. monocytogenes* in this context. In order to identify which genes are differentially expressed among the conditions studying, a transcriptomic analysis will be conducted. However, the RNA should be extracted first. The RNA extraction is a very common molecular biology practice and accurate gene expression data rely on the proper extraction of purified and high-quality RNA (Toni et al., 2018).

Research objectives

The objective of this project is to decipher the physiological mechanisms which allow L. *monocytogenes* to grow faster in presence of UFA at low temperature. The increase of bacterial growth rate was evaluated in presence of oleic

acid (C18:1). Transcriptomic analysis would be performed on cultures grown with and without oleic acid (C18:1) at low (5˚C) and optimum (37˚C) temperatures. Before sending the samples for transcriptomic analysis, by using RNA sequencing, the RNA extraction method was optimized.

Methodology

Cultures of *Listeria monocytogenes were* grown at low (5˚C) and optimum (37˚C) temperature with and without the addition of oleic acid (C18:1).

For the experiment of studying the growth rate, the sampling was conducted at specific times, every 1 hour and twice per day from the moment of inoculation, for the cultures of *Listeria monocytogenes* grown at 37˚C and 5˚C respectively, by measuring the OD_{600} until the culture reached the stationary phase. For the calculation of the maximum specific growth rate (μ_{max}), the modified Gompertz model was used (Zwietering et al., 1990) and the data of OD directly fitted to the model (Dalgaard and Koutsoumanis, 2001).

The optimization of the RNA extraction method was essential for achieving the ideal purity and quality before the RNA sequencing. The goal was to collect the samples at the middle exponential growth phase. From the growth curves of *Listeria monocytogenes* grown at the different conditions, the middle exponential phase was at the level of OD_{600} around 0,1 ± 0,05. The cells were harvested and the pellets were submerged into liquid nitrogen for flash freezing, aiming to stop all the transcriptional processes. The storage of pellets at -80˚C for stabilization of RNA was following until the extraction. The total RNA extraction was conducted by using a modified Trizol reagent (Invitrogen, USA) protocol (Chomczynski, 1993). After the extraction the quantity and purity of the RNA was assessed by using a microvolume UV-Vis spectrophotometer (NanoDrop™). Then, the RNA samples were treated with DNase in order to remove the contaminating genomic DNA and an RNA Cleanup Kit (NEB) was used for removal of DNase and purification of RNA.

The quantity and purity of RNA were measured again by using a microvolume UV-Vis spectrophotometer (NanoDrop™). The RNA is has acceptable purity when the ratios $A_{260/280} \geq 1,8$ and $A_{260/230} \geq 1,2$. Next step was the determination of quality using the BioAnalyzer (Agilent 2100 Bioanalyzer System). Intact RNA samples must have a RNA Integrity Number (RIN) ≥ 7,5 in order to be used in downstream applications.

After successfully optimizing the total RNA extraction method, the samples collected will be sent for RNA sequencing.

Results and discussions

First of all, from the graph illustrating the OD_{600} measurements at logarithmic scale during time at 37˚C (Fig. 1), it could be observed that the cultures of *Listeria monocytogenes f*ollowed the same pattern of growth irrespective of the addition of oleic acid solution as a source of exogenous unsaturated fatty acid. Moreover, by comparing the maximum specific growth rates (μ_{max}), it was not observed any significant difference ($p>0,05$) between the samples.

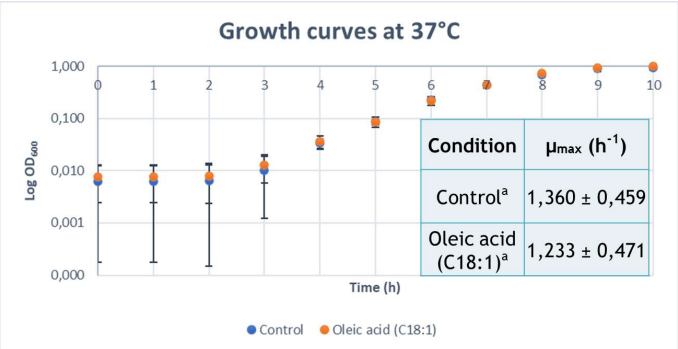


Fig. 1. Growth curves and maximum specific growth rates (μ_{max}) of Listeria monocytogenes at 37˚C (Mean values of 4 biological replicates).

At 5˚C, there was difference in the growth of the cultures, because of the presence of oleic

acid. The parameters of growth differ between the culture with presence of oleic acid and the other without (Fig. 2). The lag phase in the case of the cultures with the addition of oleic acid was shorter and there was also difference in the final OD_{600} reached, with the culture without the presence of oleic acid being incapable to reach the same level of OD_{600}.

Moreover, by comparing the maximum specific growth rates at 5˚C, it can be observed a significant increase ($p<0,05$) of it between the two cases tested in this study. The presence of oleic acid influenced the growth of *Listeria monocytogenes* by increasing its growth rate from 0,034 h-1 to 0,050 h-1. Hence, the results of previous studies conducted at the team were confirmed. In the presence of exogenous UFA, *Listeria monocytogenes* grown at low temperatures reaches infectious doses faster than expected, posing a threat to humans' health.

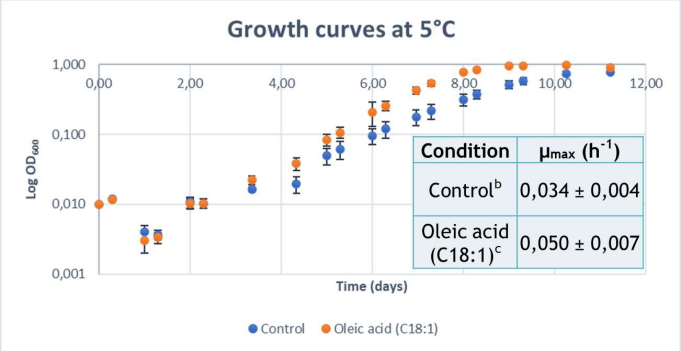


Fig. 2. Growth curves (Mean values of 3 biological replicates) and maximum specific growth rates (μ_{max}) (Mean values of 8 replicates) of Listeria monocytogenes at 5˚C.

Of course, due to the originality of this project, there are no available data in the literature about growth of *L. monocytogenes* at low temperatures in the presence of exogenous UFA in order to compare the results of this study. Of course, it has been studied the effect of exogenous unsaturated fatty acids on other bacteria and the positive effects of them in terms of growth. For instance, De Sarrau et al. (2013) observed that UFAs from spinach incorporated into the membrane of Bacillus cereus to help the adaptation of bacteria grown under anaerobiosis and at low temperature. The results of it was the increase of the maximum specific growth rate 2 to 3 times compared with the control samples, reaching the growth at low temperatures with presence of oxygen.

Last but not least, a significant difference ($p<0.05$) was observed at the maximum specific growth rates (μ_{max}) between the different temperature tested irrespective of the presence of oleic acid in the medium. Other studies (Szczawiński et al., 2017) had estimated the maximum specific growth rate at several low and high temperatures, confirming the big differences in the growth of *Listeria monocytogenes* at optimal and suboptimal conditions.

In terms of RNA extraction, the method used may influence RNA quality and purity. So, the total RNA extraction protocol should be optimized depending on bacterial species but also growth conditions. Many modifications of the initial protocol were implemented in order to find the best method for obtaining RNA of high quality and purity.

The first point that needed modification was the step of harvesting the cells of Listeria monocytogenes. The objective of this step was the collection of the cells for the following total RNA extraction. The initial protocol involved two centrifugations at low temperature, low speed and for long time (20 minutes in total) and with a step of washing the cells with TE buffer in between them before the pellets were submerged in liquid nitrogen. At the final protocol, there was only one centrifugation at low temperature, high speed of 12000g and for very short time of 30 seconds.

The half-lives of the majority of bacterial mRNAs vary from 30 seconds to 50 minutes, with average time of 3 minutes, whilst the half-lives of eukaryotic mRNAs can last for many days (Belasco and Brawerman, 2012).

The short half-lives are a crucial part of strategy to respond rapidly to the environmental changes. Hence, with the initial protocol, part of mRNA that had been produced as a result of the exposure at the experimental conditions studying would have been degraded and the results of the downstream applications would have given erroneous and not representative results.

The next modification was about the RNA precipitation. In this step, the incubation time, the temperature during incubation, the temperature of isopropanol and the addition of sodium acetate were tested in order to achieve to obtain a combination of high RNA yield, purity and quality. At the final protocol, 15 minutes incubation time at room temperature was chosen by adding cold isopropanol. At the end, very good yields were achieved without incubating at low temperature. Last but not least, according to the results from the BioAnalyzer (Fig. 3), DNA precipitation also occurred at the samples incubated more than 15 minutes irrespective of temperature. Of course, the DNase treatment was the next step, after the extraction at the final protocol, however, the least DNA contamination was wanted. Any peaks that are typically around or larger than 4000 nt, as in the electropherogram of the Figure 3, could be attributed to DNA.

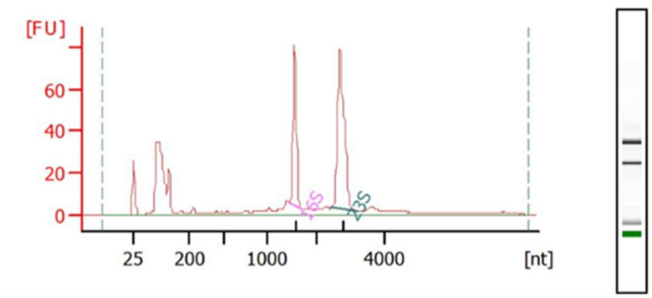
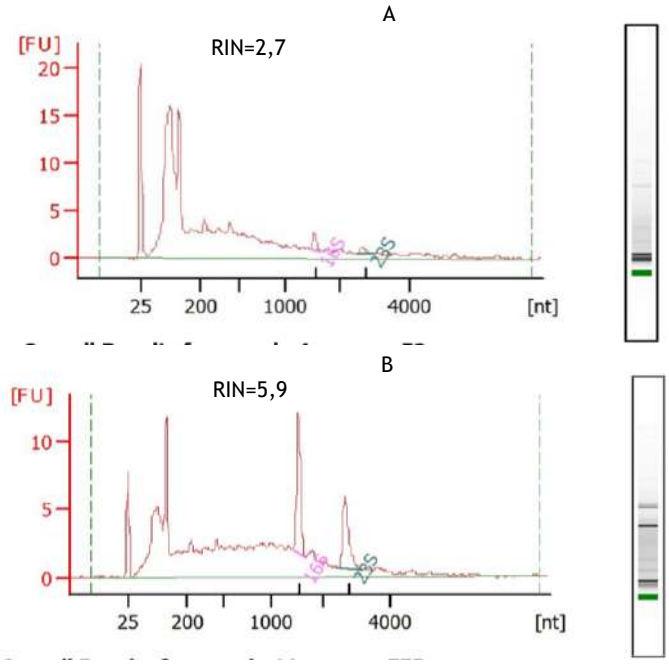


Fig 3. Electropherogram of total RNA extraction: RNA with possible DNA contamination, obtained from samples grown at 37°C without the presence of oleic acid.

In some samples the RNA was degraded (Fig. 4). Generally, an elevated threshold baseline that was observed in some samples, such as the samples illustrated in the Figures 4.a and 4.b, and small peaks of 23S and 16S, both are indicative of degradation (Mueller et al., 2004). Moreover, as it was illustrated in the Figure 4.b, the degraded RNA shifted the RNA size distribution into several smaller fragments, that could be observed in the electropherogram, but also in the gel image that is presented next to the graph. On top of that, a significant reduction in fluorescence signal was observed in degraded RNA samples. This total RNA, presented at the Figures 4.a and 4.b could not be used in any downstream applications, because the mRNA used for analyses, such as RNA sequencing and RT-qPCR, must be intact and have high quality, because the use of low-quality RNA endangers the gene expression results (Imbeaud et al., 2005). On the other hand, the total RNA illustrated in the Figure 4.c was an example of RNA with the highest quality and no observed degradation (RIN=9,9), ideal for any downstream application. No smaller fragment of RNA and no elevated threshold of baseline were observed. In addition, the fluorescence signal was high and the peaks of 23S and 16S RNA were clear, with good ratio.



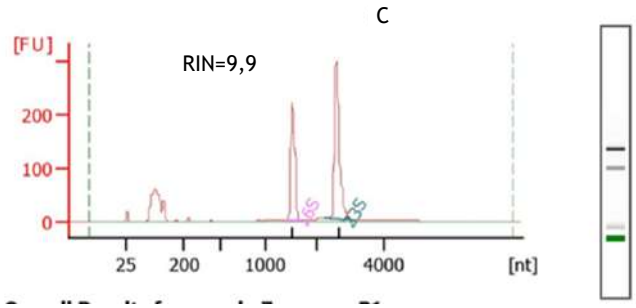


Fig. 4. Electropherogram of total RNA extractions. (A), (B): Different levels of RNA degradation; (C): Intact RNA with the highest quality.

Conclusion

The objective of this study was to identify the mechanisms which allow *L. monocytogenes* to grow faster in presence of UFA at low temperature, by conducting a transcriptomic analysis. Before the transcriptomic analysis, by using RNA sequencing, the RNA extraction method needed to be optimized. And the biggest part of this study focused on the optimization process. At the end, the total RNA extraction protocol was optimized and RNA samples of high quality (RIN>7,5) and purity ($A_{260/280}$>1,8 and $A_{260/230}$>1,7) will be sent for RNA sequencing. Moreover, the increase of maximum specific growth rate (μ_{max}) was also evaluated in presence of oleic acid (C18:1) at low temperature (5°C), confirming the fact that *Listeria monocytogenes* grows faster than expected at low temperatures and in presence of exogenous UFA.

This study was the first step for the identification of the mechanisms which allow *L. monocytogenes* to grow faster at low temperatures using the UFA from the food product, leading to higher risk of listeriosis in chilled product rich in unsaturated fatty acids.

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Mathematical Model of Ohmic Heating

Application of computational fluid dynamics analysis for optimal design

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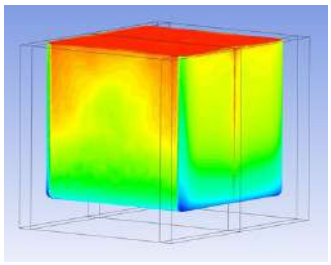


$$\frac{\delta\rho}{\delta t} + \nabla \cdot (\rho\vec{v}) = 0$$

$$\nabla \cdot (\sigma(T)\nabla U) = 0$$

$$\frac{\delta(\rho E)}{\delta t} + \nabla \cdot \left(\vec{v}(\rho E + p)\right) = \nabla \cdot (k\nabla T) + \sigma(T)|\Delta U|$$

$$\frac{\delta(\rho\vec{v})}{\delta t} + \nabla \cdot \left(\rho\vec{v}^2\right) = -\nabla \mathrm{p} + \nabla \cdot (\bar{\bar{\tau}}) + \rho\vec{g}$$

Introduction

Ohmic heating (OH) is a promising technology which comprehends the use of electric current passing through food with the primary purpose of heating it. Various studies have probed potential advantages of this technology for the food processing, such as fast volumetric heating rates. However, this technology needs to be optimized as the formation of cold spots limits its safety assurance (Salengke & Sastry, 2007).

The development of safety protocols is a challenging task, as the complexity of the process, intricated arrangement of the equipment and the short treatment periods result in a low availability of data and information during experimental studies (Shynkaryk & Sastry, 2012). Moreover, food itself can be portrayed as a complex matrix of heterogenous phases and multiple biochemical and microbial interactions. (Erdogdu et al., 2017). This implies a high level of complexity for the experimental characterization of food processing technologies, such as OH.

One crucial component in understanding and improving OH-technologies and applications lies on development of mathematical models, which can then be used to simulate various typical as well as worst-case scenarios (Salengke & Sastry, 2007).

Research objectives

The present research project is focused on the development and validation of an accurate mathematical model that can serve as a powerful tool for characterization and design optimization of OH Systems, using salted water as a model for liquid food, through the assistance of computer software (ANSYS Fluent). Thanks to the model it is possible to characterize the temperature homogeneity during the treatment and to identify cold spots formed during the OH process. The validation of the model was performed with real data from actual the OH-treatment cell.

Methodology

Development of the Mathematical Model

Geometrical model

By taking into consideration the volumetric heating feature of OH and based on the preliminary models of the system, it was acceptable to assume a symmetry plane (see Fig. 1) in order to reduce the computational expenses of the model and consequently the time for its calculation.

Fig. 1 Geometrical Model of the ohmic treatment chamber.

Governing Equations

To determine and simulate the system's behaviour through the OH process it is necessary to stablish the governing equations that correspond to the main physical phenomena that are taking place during the OH procedure, these are:

Mass Balance:

$$\frac{\delta\rho}{\delta t} + \nabla \cdot (\rho\vec{v}) = 0 \quad \text{(Eq. 1)}$$

Convergence of the Electric Potential:

$$\nabla \cdot (\sigma(T)\nabla U) = 0 \quad \text{(Eq. 2)}$$

Heat Balance:

$$\frac{\delta(\rho E)}{\delta t} + \nabla \cdot (\vec{v}(\rho E + p)) = \nabla \cdot (k\nabla T) + \sigma(T)|\Delta U| \quad \text{(Eq. 3)}$$

Momentum Conservation :

$$\frac{\delta(\rho\vec{v})}{\delta t} + \nabla \cdot (\rho\vec{v}^2) = -\nabla \mathrm{p} + \nabla \cdot (\bar{\bar{\tau}}) + \rho\vec{g} \quad \text{(Eq. 4)}$$

Discretization of the geometric domains

The discretization of the geometrical model and generation of the computational field was carried out through the meshing application integrated in software package of ANSYS STUDENT R1, which is based on the application ANSYS Mechanical.

The mesh of the geometrical model (see Fig. 1) was discretized to obtain the mesh for the simulation. The fines mesh was generated for the fluid domain, the grey volume in Fig. 2, as in this particular domain of the most relevant physical phenomena takes place. Additionally, the curvatures of the contact surfaces of the electrodes (green domains in Fig. 2) to the fluid domain were also refined as the heat transfer and heat losses needed to be calculated with a high accuracy. The total amount of elements for the final mesh was 479 790 elements, in which the governing equations were solved simultaneously.

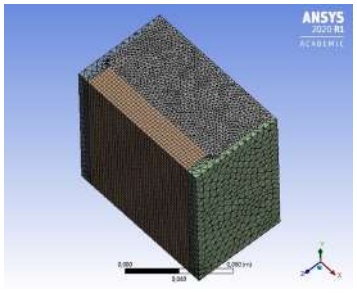


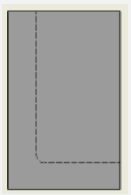
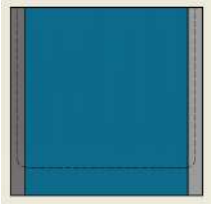
Fig. 2 Discretization Mesh of the Geomatrical Model of the ohmic treatment chamber.

Boundary Conditions

At the boundary of the domains it is necessary to define the conditions that the differential equations need to fulfill so that the model will represent with accuracy the reality (Erdogdu et al., 2017).

The boundary conditions (see Table 1) set at the different domains, permitted the heat transfer from the heated fluid to the solid domains and to the surroundings as the system was defined as closed one.

Table 1 Boundary Conditions of the Ohmic Heating Cuboid Treatment Cell model.

Boundary	Conditions
High Voltage Electrode (E) 	No slip. Heat exchange is allowed. Electric and thermal properties of SS were set. Electric potential defined through cuboid_voltage_vol UDF.
Ground Electrode (G) 	No Slip. Heat exchange is allowed. Electric and thermal properties of SS were set. Electric potential was set to 0.001V (as 0 is understood as isolation by Fluent (I. ANSYS, 2020a)
Air Layer (AL) 	No slip . Heat exchange is allowed. Electric and thermal properties of Air were set. Electric potential was set to 0 V.
Insulator (I) 	No slip. Heat exchange is allowed. Electric and thermal properties of POM were set. Electric potential was set to 0 V.

Calculations Residuals

The residuals of the model were set to a low order, with the goal to reduce drastically the linearization error of the model. The tolerance of the residuals for each governing equation is shown in Table 2.

Table 2 Tolerance criteria for the governing equations of the Ohmic Heating Cuboid Treatment Cell (OH-CTC) Model.

Governing Equation	Tolerance Criteria
Mass Continuity (Eq. 1)	1×10^{-6}
x-velocity (Eq. 4)	1×10^{-6}
y-velocity (Eq. 4)	1×10^{-6}
z-velocity (Eq. 4)	1×10^{-6}
Energy (Eq. 3)	1×10^{-6}
Potential (Eq. 2)	1×10^{-9}

Determination of the electrical conductivity of the model fluid

For the determination of the electrical conductivity of the model food an aliquot of 250 mL was taken from a mother solution. The initial electrical conductivity was determined with conductometer (Mettler Toledo EL3, Mettler Toledo Inc, Columbus, Ohio) with a 0 % temperature compensation set-up. Afterwards, the same aliquot was microwaved for five seconds at a power of 400 W, stirred to homogenize the temperature distribution and immediately the electrical conductivity and temperature were determined, to prevent heat loses to the surroundings.

Experimental Set- Up for the validation of the Model

The temperature profiles at the three different locations were measured and compared for an OH treatment with a power generator (Deutsches Institut für Lebensmitteltechnik e.V., Quakenbrück, Germany) delivering 1500 W to the chamber with a frequency of 12 kHz and a electric voltage of 500 V.

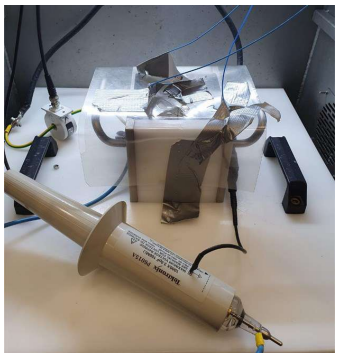


Fig. 3 Experimental Set-Up for Validation of the Ohmic Heating Cuboid Treatment Cell Model (Part I).

The time, temperature, electrical current and voltage were recorded (see Fig. 3 and Fig. 4) until the temperature at geometrical centre reached the value of 358.15 K.

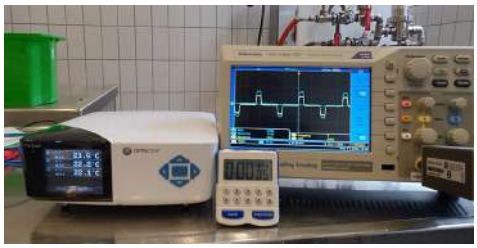


Fig. 4 Experimental Set-Up for Validation of the Ohmic Heating Cuboid Treatment Cell Model (Part II).

Positioning of Temperature Probes

The temperature probes were positioned in three specific locations: at the cold-zone (T1), hot-zone (T3) and in the geometrical center (T2) of the treatment chamber, based on previous simulations of the model (see Figure 5).

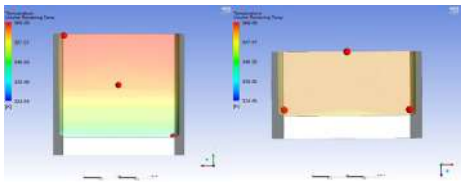


Fig. 5 Positioning of the temperature probes.

Statistical Analysis

The validation of the model was carried out through the comparison of the measured values and the numerical solution of the model through the calculation of the Relative Rood Mean Square Error (RRMSE).

Results and discussions

Validation

Two validation measurements where carried out. By comparing the power delivered by the generator during both measurements, it is possible to observe a difference of 1.59 % between the two validation measurements. These difference represents and experimental error which is not contemplated during the numerical calculations.

The maximal RRMSE of the model comprehended 4,59 % as shown in Fig. 6, Fig. 7 and Fig. 8:

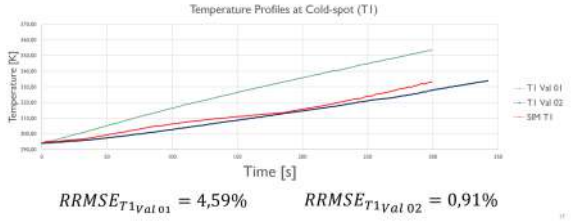


Fig. 6 Validation curves at cold-spot (T1) and their respective Relative Root Mean Square Error (RRMSE).

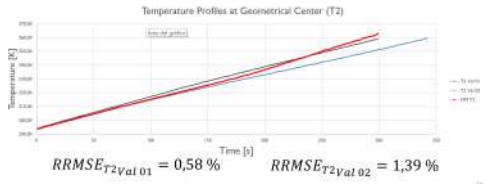


Fig. 7 Validation curves at the geometrical centre of the fluid domain (T2) and their respective Relative Root Mean Square Error (RRMSE).

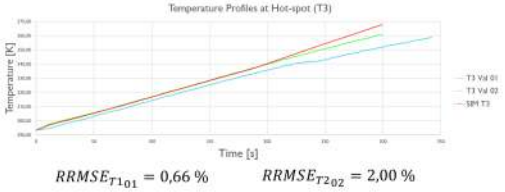
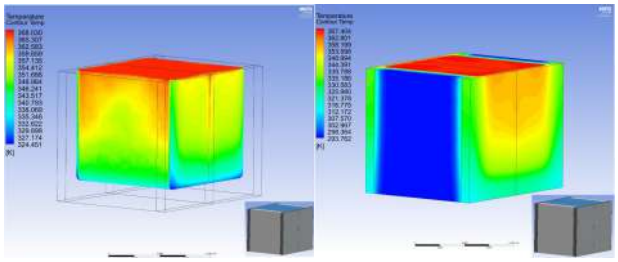


Fig. 8 Validation curves at hot spot (T3) and their respective Relative Root Mean Square Error (RRMSE).

Based on the validated temperature profiles it is possible to conclude that the predictions of the model are valid for the OH procedure of salted from 293.15 K to 358.15 K. This means that the temperature distribution at the end of the treatment in Fig. 9 accurately describe the final state of the modelled system.



(a) (b)

Fig. 9 Temperature distribution at the end of the OH treatment according to the simulated model for (a) the fluid domain and (b) the whole system

Mechanistic characterization of the cold-spot's formation

As stainless steel is not only a good electrical conductor but also a thermal one, part of the heat generated through OH is lost to the environment, as the temperature fields of Fig. 9 show.

Additionally, thanks to the simulations it was possible to determine that the heat distribution through the system is also being affected by the natural convection of the fluid (See Fig. 10). This leads to an uneven distribution of temperature in the fluid being heated, which causes an irregular electrical conductivity over the fluid.

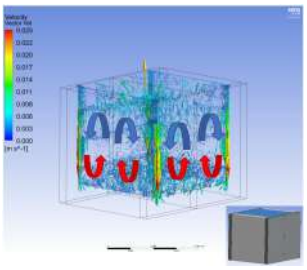


Fig. 10 Natural convection velocity vectors in the fluid domain.

Based on these two phenomena, the cold-spots are generated at the bottom corners of the chamber, as the intensity of the convection and conduction of heat to the exterior are higher in this zone.

Conclusions

As the RRMSE<5%, the model can predict and describe the OH treatment with a good accuracy.

Based on the simulation it was possible characterize the generation of cold-spots in the system, whereas they are due to the heat losses to the environment and the buoyancy effect of the natural convection.

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Healthy diet and planetary health: Food waste estimation upon a Mediterranean diet and a Western diet

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Introduction

Mediterranean diet

Mediterranean diet is a dietary pattern, which was traditionally consumed in Mediterranean region. It is rich in plant-based foods: vegetables, fruits, legumes, tree nuts, seeds, olives, and cereals, preferably whole grain, with olive oil as a main source of added fat, along with high to moderate consumption of fish and seafood, moderate intake of eggs, poultry, and dairy products, and low consumption of red meat, including moderate intakes of alcohol which is mainly wine during meals. In addition to food intake, cultural and lifestyle aspects are key elements for Mediterranean diet such as moderation, socialization, culinary activity, physical activity and rest with respecting local and seasonal products consumption (Bach-Faig et al., 2011). Several epidemiological studies show that the adherence to Mediterranean diet is inversely associated with non-communicable diseases such as cardiovascular disease, hypertension, obesity, diabetes, cognitive decline, and some types of cancers (Sánchez-Sánchez et al., 2020). The focus on sustainable aspects of Mediterranean diet has increased, and its plant-based dietary pattern is recognized as eco-friendly (Dernini et al. 2016).

Food waste

Roughly one-third of edible part of food produced for human consumption is lost or wasted, and food loss and waste issue needs to be addressed (Gustavsson et al., 2011). It is set as one of the targets of the Sustainable Development Goals to halve per capita global food waste at the retail and consumer levels

and to reduce food losses along production and supply chains including post-harvest losses by 2030 (UN, 2015). Food is lost and wasted at any stages of food supply chain from production to consumption. In developing countries, loss occurs mainly at production to process stage, whereas, in industrialized countries, food is mainly wasted at consumption level (Gustavsson et al., 2011). This consumer-level waste is the results from complex attitudinal and behavioral factors of consumers toward wasting food (Secondi et al., 2015). It is important to understand the food waste situation accurately in order to define the focus of waste reduction approach, and food waste measurement has been carried out on various scales. Direct measuring methods such as garbage collection and self-reporting by participants are more accurate, on the other hands, indirect measuring methods such as calculation using second-hand data as used in the present study are easy to be applied to large scale and suitable to grasp overall pictures (Xue et al., 2016). Fruit and vegetables tend to be wasted the most in mass (WRAP, 2012; Kranert et al., 2012), however, animal-derived products, especially red meat, can cause larger environmental burden. Thus, it is suggested that reducing meat waste has environmental benefits due to its environmental intensity, while less wasting vegetables, fruit, and cereals is beneficial because of their quantities (Usubiaga et al., 2018), and to change dietary patterns may have impacts on food waste situation.

Research objectives

The final goal of this study is to help realizing sustainable life through the reduction of food waste along with enhancing human health. In order to take actions for this, it is important to understand the impact of dietary patterns on food waste and decide an effective focus. Although Mediterranean diet has been mentioned as healthy and sustainable, the impacts on food waste are underinvestigated. Hence, the aim of this study is to characterize Mediterranean diet from the perspective of food waste. To estimate food waste quantity and to evaluate its environmental impacts of Mediterranean diet through comparing with Western diet were set as objectives.

Methodology

In the present study, waste from only edible part of food was considered and inedible waste such as bone of chicken was excluded.

Dietary data

Firstly, dietary data were extracted from a nutritional intervention study with Mediterranean diet in overweight and obese subjects conducted at University of Naples Federico II (Meslier et al., 2020) During 8-week intervention, participants in Mediterranean diet group consumed individual tailored Mediterranean diet without changing their habitual energy intake, and Western group kept their habitual diet as control. Participants completed food frequency questionnaire of 142 food items on a weekly basis at baseline and every 4 weeks. In the present study, food consumption data of 80 subjects, 42 subjects in Mediterranean diet group and 38 subjects in Western group, at baseline and after 4-week intervention were obtained.

Food waste estimation

Edible waste quantity was calculated from the intake data and percentage value of edible (%EW) and inedible waste (%IW) to purchase quantity per food groups (Vanham et al., 2015) as the following formula.

$$\text{Edible waste (g)} = \frac{\text{Intake (g)} \times \%\text{EW}}{1 - \%\text{EW} - \%\text{IW}}$$

Environmental impact estimation

Three indicators of carbon, water, and ecological footprint were used in the present study to assess environmental impacts of food waste. Carbon footprint represents the quantity of greenhouse gas emission as CO_2 equivalent, water footprint measures volume of water resources consumed and/or polluted. Ecological footprint indicates land and aquatic area required to provide food and resource for production and to absorb the emissions related to production system using biologically productive areal unit of global hectare (gha). The footprint data of per quantity food commodities or food groups were accessed at Double Pyramid Study (BCFN, 2015) and multiplied by waste quantity, then, footprints of edible food waste were estimated.

Results and discussion

Food waste quantity

The quantity of edible food was significantly increased after 4-week following Mediterranean diet, and it was larger than Western group (Fig.1).

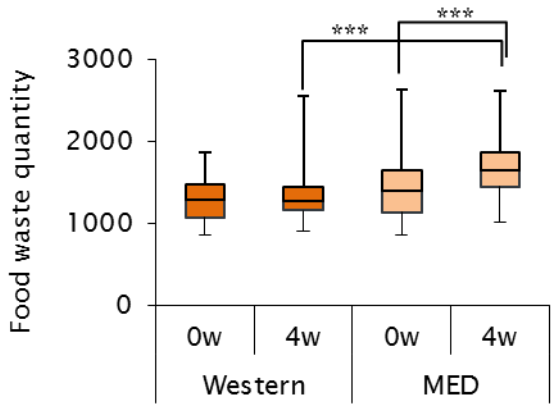

*Fig. 1 Edible food waste quantity over intervention. Western: Western diet (n=38). MED: Mediterranean diet (n=42). 0w: baseline. 4w: the 4th week of intervention. ***p<0.001 0w vs 4w (Wilcoxon signed-rank test) and Western vs MED (Mann-Whitney test).*

The variation of total food waste quantity from baseline to the 4th week of intervention in Mediterranean group was significantly larger than that of Western group, and the increases of waste from fruit and vegetables had large impacts on the changes of total waste in Mediterranean diet. (Fig.2).

Environmental impact of food waste

Carbon footprint of wasted food significantly decreased after 4-week following Mediterranean diet, and it was significantly smaller than Western group (Fig.3). Water footprint of wasted food had same trend as carbon footprint, but ecological footprint did not show significant difference between diets.

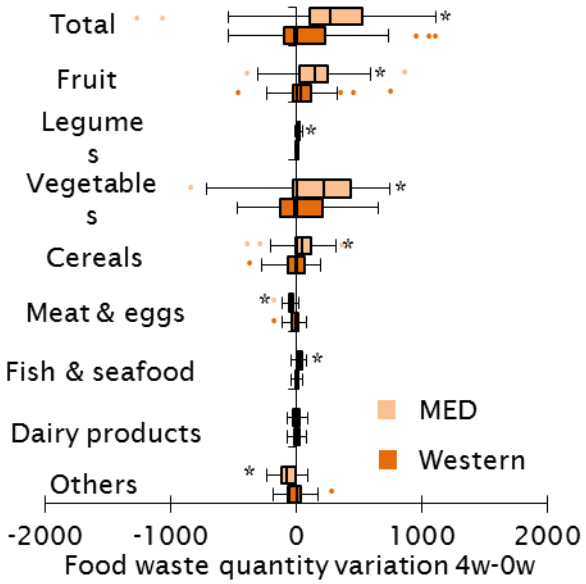

*Fig. 2 Edible waste quantity variation between 4-week intervention. Western: Western diet (n=38). MED: Mediterranean diet (n=42). *p<0.05 vs Western (Mann-Whitney test)*

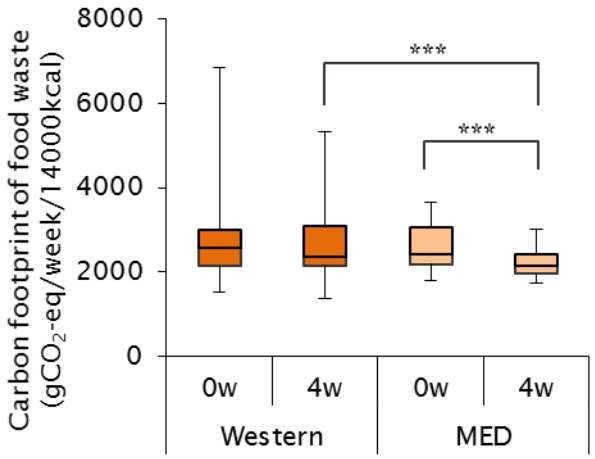

*Fig. 3 Carbon footprint of food waste. Western: Western diet (n=38). MED: Mediterranean diet (n=42). 0w: baseline. 4w: the 4th week of intervention. ***p<0.001 0w vs 4w (Wilcoxon signed-rank test) and Western vs MED (Mann-Whitney test).*

Possible recommendation

This study suggests the potential of dietary pattern to impact on the food waste quantity and its environmental impacts. In particular, shifting toward plant-based Mediterranean diet from animal and fat rich Western style diet can increase the amount but reduce environmental burden of food waste. Hence, raising the adherence to Mediterranean diet can be recommended as one of the choices for sustainability in terms of food waste and its impacts in addition to health benefits. However, larger waste generation can also means the larger room in Mediterranean diet for reducing food waste. Therefore, it can be another recommendation to enhance abilities and opportunities of consumers for efficient use of food products with particular focus on managing plant-based products of vegetables, fruits, that are main contributor to waste in Mediterranean diet. Examples for keeping these perishable foods longer include improvement of knowledge to prolong shelf life and equipment for proper storage (van Geffen et al., 2019). Other concepts of Mediterranean diet might have a potential to help preventing food waste. For example, social norms, which are important factors to motivate consumers (van Geffen et al., 2019), may be possible to be pushed toward waste preventive direction with utilizing socialization culture of Mediterranean diet, and over-purchasing and over-cooking could be prevented with concept of moderation.

Conclusion

The quantity of edible food waste can be larger in Mediterranean diet than Western diet. However, despite of the quantity, environmental impacts of food waste can be smaller in Mediterranean diet than Western diet. The findings in the present study suggest that following Mediterranean diet including its cultural aspects has potential to realize healthy and less wasteful lives, and promoting particularly perishable food management can be recommended as one of the effective focuses for waste reduction in Mediterranean diet.

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Fate of probiotic *Bacillus clausii* spores through simulated Gastrointestinal Tract (GIT)

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Master Thesis Tutor:
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Introduction

The demand of probiotic functional foods is growing rapidly due to increased awareness of consumers about their impact on health (Tripathi & Giri, 2014). However, preserving the efficacy of probiotic bacteria is a paramount challenge to exert proposed health claims (Terpou et al., 2019). As, probiotic microorganism's survival is critical during the shelf life of product as well as during the passage through gastro-intestinal tract (GIT) (Soares et al., 2019). *Bacilli* have been traditionally proposed as probiotics for functional foods due to their spore's smooth survival to the harsh conditions occurring along food production (Terpou *et al.,* 2019). *B. clausii* is one of the mostly used probiotic *Bacillus* in clinical studies due to its functional and therapeutic properties. From a probiotic perspective, vegetative cells and spores have separate, but complementary functions. Some properties like immunomodulation and adsorption can be promoted by both vegetative cells and spores, while others, like antimicrobial activity, are a specific trait of vegetative cells (Lopetuso *et al.*, 2016). Probiotic *B. clausii* are highly resilient, but they need to regain their metabolic activity after entering the host to provide benefits (Cutting, 2011). Thus, it is of interest to understand the dynamics of *Bacillus* spp. germination in the gut including the fate of ingested *Bacillus* spores and the ratio of vegetative cells and spores in the different gut sections across different hosts, the length of time that the *Bacillus* persist in the gut after their withdrawal from the diet, and the influence of GIT physiology and of *Bacillus* strain (Bernardeau *et al.*, 2017).

Aims of the Project

a) To evaluate the fate of *B. clausii* spore's germination upon in-vitro GIT simulation

b) To understand the impact of different GIT conditions on *B. clausii* spores germination

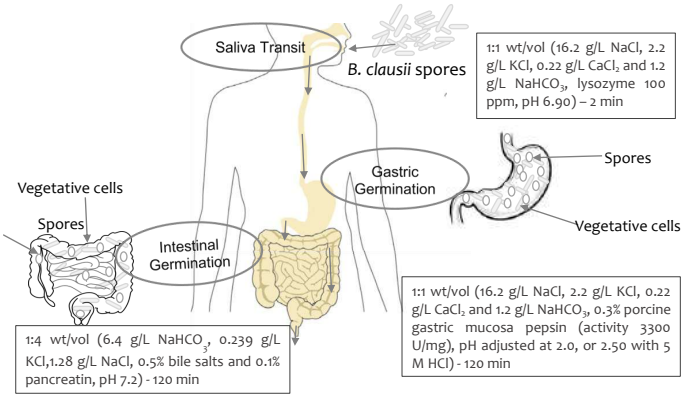
c) To assess the spores' and vegetative cells number during GIT.

d) To isolate the four strains of Enterogermina

Materials and Methods

In-Vitro GIT Simulation of *B. clausii* Spores

(Ricciardi *et al*. 2014)

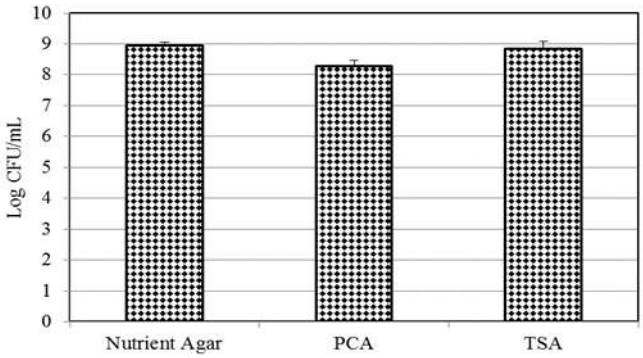


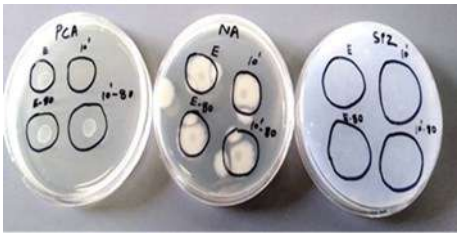
*After each step, vegetative cells were assessed by CFU drop-counts and spore by means of Petroff-housen chamber.

Results and Discussions

1. Assessment of Optimal Conditions for *B. clausii* Growth and Sporulation

- Nutrient Agar (NA) and TSA growth media's population level was almost one Log CFU/mL higher than that of PCA. This was likely due to presence of growth stimulators (Fig.1).
- Spizizen medium (SM) approached to minimize spore's germination from nutrient medium induction. Surprisingly, there was no growth at all on SM medium





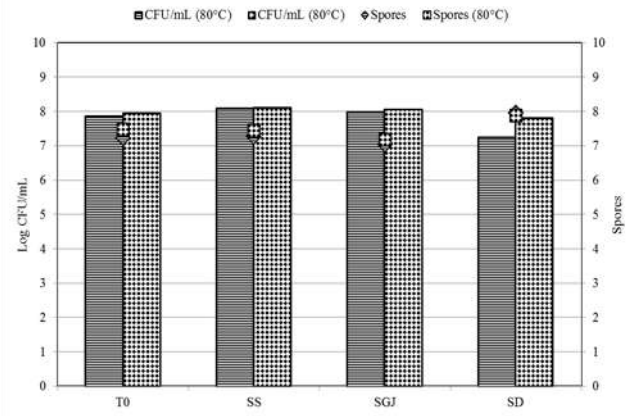
2. Behaviour of *B. clausii* spores during simulated GIT

Gastric pH: 2.5, Intestine pH: 7.20

Nutrient agar

Thermal treatment exerted no significant difference on both spores and cells counts.

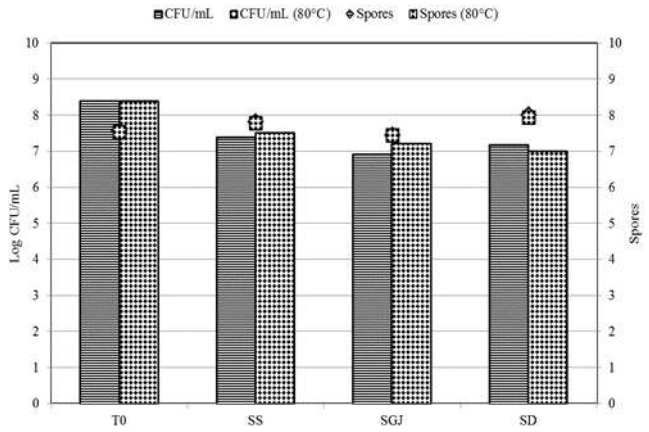
All spores counted at the microscope were able to germinate on the nutrient agar independently by the thermal induction



Gastric pH: 2.5, Intestine pH: 7.20

PCA

On PCA, a low germination rate was observed, likely due to medium composition

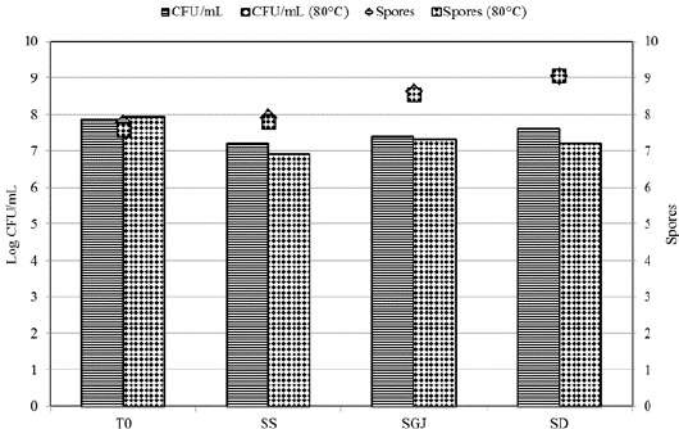


Gastric pH: 2.0, Intestine pH: 7.20

PCA

Vegetative cells recovery after gastric juice was a little bit lower than that recorded at pH 2.5.

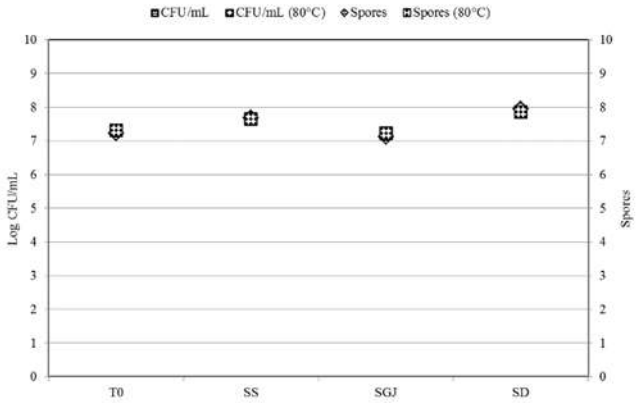
Higher spores germination observed during the Intestinal transit is probably related to duplication due to long time transit (2 hours).



Gastric pH: 2.0, Intestine pH: 7.20

Spizizen medium

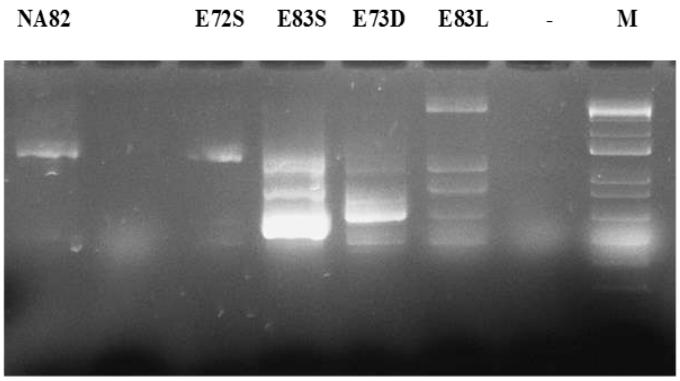
On SP medium, no vegetative cells growth found that didn't provide any concrete result towards germination.



3. Isolation of *B. clausii* Strains

DNA fingerprinting by RAPD-PCR

Primer M13-R2 provided the best results allowing the four different genotypes hosted by the Enterogermina vials to be discriminated



Conclusion

B. clausii spores have the unique capacity to germinate in unfavourable environment especially human GIT where low acidic pH, bile salts, low oxygen availability, and high enzymatic activity are the main limiting factors for almost all probiotics. In this study, the fate of *B. clausii* spores was investigated through simulated human GIT conditions. *B. clausii* spores survive transit through the human gastrointestinal tract. They can undergo germination, outgrowth and multiplication as vegetative forms. Low acidic pH 2 at the gastric transit is maximal threshold level to survive for spores. *B. clausii* spores have more capacity to duplicate in the colon at pH 7.2 even after low pH gastric transit. Moreover, growth media directly affect on the count of vegeta-

tive cells as nutrient rich media support spores germination. Thermal treatment to facilitate spores activation and sporulation didn't affect final results significantly. Overall, *B. clausii* spores well survived throughout the GIT conditions with noticeable spores germination.

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Recyclability by Design for Multi-layer Plastic Flexible Packaging

A comparative study of recyclability by design guidelines and alignment among various stakeholders

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Profile in a nutshell:

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Introduction

The year 2018 marked an important paradigm shift in plastic packaging industry. The ban on plastic waste import for recycling by China, forced western world to find plastic recycling solutions in a short time. PET, glass and paper industries are relatively developed packaging recycling industries when compared to multilayer flexible plastics. Multilayer plastic flexible packaging (MPFP) type has proven to be one of the most challenging for recycling due to lack of structural standardization, centralized data and clear regulations. The study evaluates the current state of MPFP packaging recyclability by assessing most trusted recyclability by design (RBD) guidelines by comparing them against the sorting and recycling ground realities in EU. 'Recyclable by Design' can be interpreted as design of a packaging that fits the sorting and recycling stream in a region. Hence, RBD guidelines are set of instructions for a packaging design that enables it recyclability as per current state of recycling or make it future recycle ready. Various stakeholders like packaging developers, suppliers, recyclers and consumers play important role in MPFP value chain (especially at end of life stage) and hence their views on the topic are important to assess. Current sorting and recycling technologies cannot process MPFP due to non-standardized design parameters like additives, multi-layers materials, barrier layers, etc., which might not be compatible with existing recycling streams and may pollute them on entering. The study explores current state of MPFP recyclability by assessing different guidelines, views of stakeholders and state of sorting and recycling of MPFPs in Da-

none Nutricia's major EU market countries. Conclusions are then drawn based on the analysis, about the ideal MPFP structure, brand and producer responsibility as well as recommendations for recyclable MPFP design and future research.

Research objectives

Research Objective 1: To compare recyclability by design guidelines and sorting and recycling operations with the aim of contributing to Danone's D4R guideline.

Research Objective 2: To compare alignment of thought with respect to current RBD guidelines for MPFP among stakeholders using qualitative and quantitative data with the aim of exposing the factors that hinders effective recyclable MPFP development.

Methodology

The data collection was divided into two parts based on research objectives. RO1 corresponds to secondary data collection i.e. data collected via online research of reports, studies and research journals. RO2 corresponds to primary data collection i.e. data collected directly by author via interviews and online consumer survey. Each RO corresponds further to two data collection steps, which are analyzed separately and eventually devises four analysis. Finally, the result is derived in the form of a recommended RBD guideline, recommendations for improvements in Danone's internal design guideline (D4R) as well as suggestions for future research.

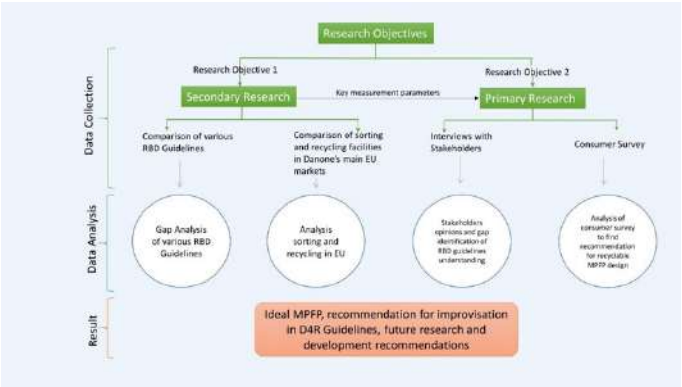

Figure 1. Study approach split into three parts – data collection, data analysis and result

Results and discussions

The result of the study suggests the scope of improvement in Danone's D4R guideline for MPFP design could be introduction of sub-parameters and specifications for coatings and barriers, additives and labels. A need for consolidated data for MPFP recycling and sorting at EU level was identified. Integration of life cycle assessment tools in D4R guideline (or vice versa) was also stated as one of the scopes. Finally, a need for categorization of all the design data corresponding to each EU market country was identified as one of the major scopes of improvement for D4R. A comparison of various RBD guidelines revealed lack of harmony and missing information in suggested design parameters. This is true for more specific parameters whose percentage use are smaller in MPFP design. Since brands generally refer to these guidelines for developing in-house design guideline, a non-unanimous behavior can cause confusion for a packaging designer/developer and may not present a clear data. All the guidelines that were assessed showed incomplete information for one or other parameter and sub-parameters. An analysis of sorting and recycling data in Danone Nutricia's major EU markets reveled that poly-ethylene (PE) and poly-propylene (PP) are major developing plastic recycling streams. This information also gives a course about the material MPFP design should aim for, as per the plastic stream in a country. Among the countries that were compared for recycling efficiency, France, though has developing PE recycling stream, is found to be the least recycling efficient, whereas The Netherlands is the most recycling efficient country. The qualitative research (stakeholder interview and consumer online survey) revealed a lack of information exchange among brands, packaging suppliers and recyclers as a major factor for non-

recyclable design of current MPFP, which is multi-material layers packaging and generally have a metal barrier for high performance and food safety assurance. 16 stakeholders were interviewed, out of which 70% agreed that metallized layer and a non-uniform material structure should be changed to a non-metallized version with mono-material structure.

One recycler have tested mono-material (PE based) MPFP to be 100% recyclable. However, currently available mono-material (mostly PE and PP based) MPFPs have lower barrier and machine performance than their metallized counterparts. Another differentiating factor is the price. Mono-material based MPFP has a higher raw material cost than conventional MPFP as the technological development of barriers is still at nascent stage. Some stakeholders have argued that the primary purpose of a packaging is to ensure quality and safety of food which is questionable with mono-material based MPFP. Corresponding to this, two chemical based solvent recycling technologies (categorization as chemical recycling is arguable) have successfully recycled post-consumer MPFP with food grade quality and without deteriorating the structural integrity of the polymer. These recycling technologies fulfills the criteria for plastic circular economy but weather it fits the definition can be argued. The online consumer survey revealed that consumers do their part in segregating the MPFP plastic, however, majority thinks that the conventional MPFP is recyclable. Some readers may argue that the ignorance on consumers part does not impact MPFP recyclability, but the consumer awareness have ability to change plastic policies and brand profitability.

Conclusion

A MPFP design guideline with sub-categories for the percentage (amount per gram), recommended value and to avoid values have potential to add clarity for a packaging developer/ designer. A strong need of collaboration with recyclers has been identified as a major factor for future recyclable MPFP since recyclers are involved in the ground work. This could be in the form of recycling tests for the MPFP at concept stage. Mono-material packaging based on PE or PP has been identified to be a focus material that has potential to be 100% recyclable in Danone's major EU market. Hence, it is safe to assume that the MPFP packaging portfolio in Early Life Nutrition can focus on PE or PP material to meet their 2025 goal of 100% recyclable packaging.

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How to pack better ? A study on meat batter

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Introduction

Increase in the trend of green consumerism has been observed as more consumers are becoming more concerned with the environmental impact of the products that they are purchasing. Thus, food manufacturers, specifically for canned products, are making the shift from metal packaging to a more sustainable material such as Tetra Recart®. The latter has been proven to have less environmental impact as compared to metal cans but still having the same properties needed to provide safe food products.

The Tetra Pak R2 Machine with the piston filler has been used to fill different types of food product for the Tetra Recart package®. The focus of the study is meat batter as it could represent viscoelastic products. To predict the fillability of the meat batter in TPR2 machine with standard product tank and when to recommend Pressurized product tank for products that exceeded the limit of the former, the study aims to achieve the following objectives.

Objectives

1) To understand the rheological characteristics of meat batter with different formulations
2) To investigate the product's rheological properties which can be correlated to its fillability in the TPR2 machine with standard product tank

3) To understand the pressure drop in the filling pipe

4) To correlate the meat batter's rheological properties and their corresponding pressure profile Hypothesis The assumption is that the rheological parameters of the meat batter can

be used to correlate with the pressure drop during the filling process.

The specific hypothesis of the study includes:

1) The use of rheometer can produce reproducible results to quantify and make a rheological model for the meat batter

2) The rheological model established can be used to predict the pressure profile of the batter inside the filling machine

3) The rheological characteristics of the different meat batter can predict the fillability of the product in the machine

Materials and Methodology

The materials used for this study were pork belly class 3 (HKScan), pork shoulder class 2 (Martin&Servera), modified starch, salt, and water. The meat was cured, grinded, and mixed with other ingredients using different equipment for laboratory and pilot plant scale. The resulting products were meat batter samples with varying percentage of added water which had different rheological properties and fillability. These were analyzed in duplicates using rotational rheometer with the serrated cup and four-blade vane attachment.

The flow curves of the meat batter were analyzed, and a Non-Newtonian fluid flow model could be fitted. Also, the corresponding rheological parameters, and the thixotropy property were studied. Build-up and breakdown tests were performed to study the thixotropy property of the meat batter. Amplitude sweep test was also performed to analyze the viscoelastic property of the meat batter.

The K and n values were derived from the flow curves which were used to estimate the apparent viscosity. The estimated pressure drop was also calculated in order to compare with the experimental pressure drop.

The different meat batter samples were tested in the TPR2 machine with standard tank to gather data on the pressure profile and pressure drop curves. The experimental pressure drop values were then compared to the calculated pressure drop.

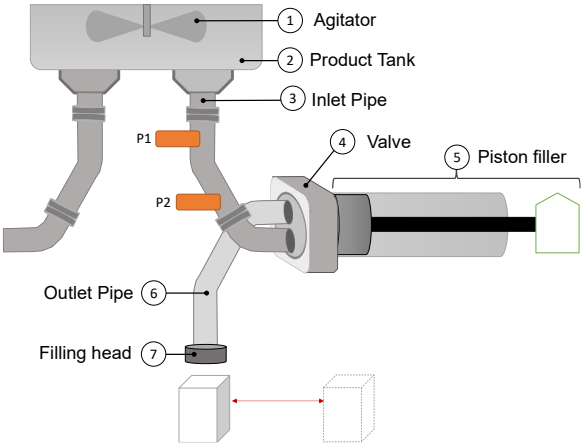

Figure 1. The simplified diagram of filling system of Tetra Pak R2 filling Machine with the additional installation of pressure meters

Conclusions

The conclusions obtained from this study were:

1) The rheological characteristics of the meat batter could be studied using the vane geometry in the rotational rheometer which showed that the meat batter could be described by the Power Law Model. The rheological measurements provide K and n values of which the former decreased as the % added water was increased, while the latter was not significantly affected. Shear thinning behaviour was observed among all the samples. The calculated apparent viscosity and pressure drop estimated from the rheological measurements showed decreasing trend as the % added water was increased. The calculated K value and amplitude sweep test can be used to predict the meat batter's fillability in the filling machine with a standard tank and when to recommend the filling machine with pressurized tank. The values obtained from products produced in laboratory and pilot plant scale were not significantly different from each other.

2) The analysis of the flow curve could be used to study the thixotropy property of the

meat batter which showed decreasing trend with the additional water. The filling duration is shorter than the time needed for the meat batter with lower %added water to build-up. The amplitude sweep test showed that the sample with lower water had more elastic property.

3) The pressure measurements during the filling in the machine showed the difference in the typical pressure profile curves for products with different fillability. The meat batters which were able to be filled in the TPR2 machine with standard product tank were 20% and 30% added water meat batter that followed the viscous fluid behavior. This showed the limit of the machine without pressurized product tank as no less than 20% added water. However, samples of 15% and 10% added water meat batter were not able to be filled exhibited more elastic property during the filling process.

4) The Rabinowitz-Mooney derived from the Power Law model used to predict the pressure drop of the meat batter samples was only applicable to 30% AWMB.

5) The fillability of the meat batter was significantly affected by its elastic property. The calculated pressure inside the piston for lower added water samples was found to be at the pressure in which water molecules could boil at 10˚C which could create steam inside the piston. In addition, the mixture of meat batter and air in the pipe might have affected the filling behavior of the samples.

Figure 2. Filled packaged with pilot scale meat batter a) 10% AWMB, b) 15% AWMB, c) 20% AWMB and d) 30% AWMB

Recommendations

The following are recommendations based on the study:

1) Meat batter products can be further investigated in terms of viscoelastic properties and adhesiveness/stickiness by Texture Profile Analysis. This is to further understand its behaviour and to study the possibilities to correlate to its behavior during filling.

2) The fat, protein and quantitative analysis of the meat particle size can be done to determine the effect of the meat composition and microstructure on its rheological properties. The effect of varying the fat content of the meat batter can also be studied and be correlated to its fillability in the machine, to know the applicability of the prediction model in different conditions.

3) More parameter of machine response could be studied to understand how the machine works and responds to the product. This may include the motor response, synchronization to the piston movement, and the pressure measurement in the piston.

4) Study of other fluid flow models including

the elastic properties could be done to determine the best fit prediction model for the meat batter with lower added water.

5) To provide a substitute for meat batter in the machine test, further study in the proxy product could be performed such as build-up, breakdown and amplitude sweep in order to compare to the rheological properties of meat batter. Machine test of the proxy product could be made in order to gather pressure data measurements and to increase the understanding of machine fillability in a systematic way.

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Development and Evaluation of a Sustainable Paper Straw Wrap: A Packaging Design Project for Tetra Pak

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Introduction

In Europe, annual plastic waste accumulation is reached up to 25.8 million tonnes, and globally up to 70% of the plastic waste ends up as litter, thereby creating plastic pollution without being included in the circular economy (MacArthur, D. E., Waughray, D., & Stuchtey, M. R., 2016). The impacts of plastic waste are dramatic as more plastic waste accumulates in the environment. In May 2018, The Single-Use Plastic (SUP) Directive proposed phasing out single-use plastics. The increasing quantity of disposable single-use items is one of the major causes of waste accumulation in the environment due to becoming litter versus being recycled. Today, packaging waste litter has severe effects on the environment. Herewith, as the EU has reported, curbing packaging littering is a complex issue that must be tackled. Thus, packaging design is one of the keys to accelerating circular solutions while solving this complex issue (European Union, 2018).

Research objectives

The project is carried out with Tetra Pak, which has developed a U-shaped paper straw as a sustainable alternative to the plastic straw. As compatible with the sustainability approach, the plastic wrap of the paper straw should be replaced by a more suitable alternative. The new solution of the wrap should help to prevent littering by keeping the parts attached to the package and ensure the wrap is recycled along with the rest of the package in the same waste stream. Moreover, the solution should also facilitate easy opening functions for the consumer. As the aim of this project, a sustainable solution for the Tetra

Pak paper straw wrap should be developed and evaluated, while considering accessibility and littering prevention aspects, in particular.

Methodology

In this study the Delft packaging design method (ten Klooster, 2002) has been practised. The Delft packaging design method provided substantial assistance in choosing and understanding the right perspective and supported to the researcher for controlling the process fluently and iterating purposefully (Van Boeijen, Daalhuizen, van der Schoor, & Zijlstra, 2014). The development of the design solution was conducted in three different modules: design concepts, wrap material trials, pattern configurations for the ladder band. Figure I represents the fundamentals of the development process of this research on the Delft packaging design cycle model. The guidelines for preventing littering by packaging design (Wever, Gutter, & Silvester, 2006) and the guidelines on opening feature of packaging design for accessibility (Fain, n.d.) were used as an approach in the development process.

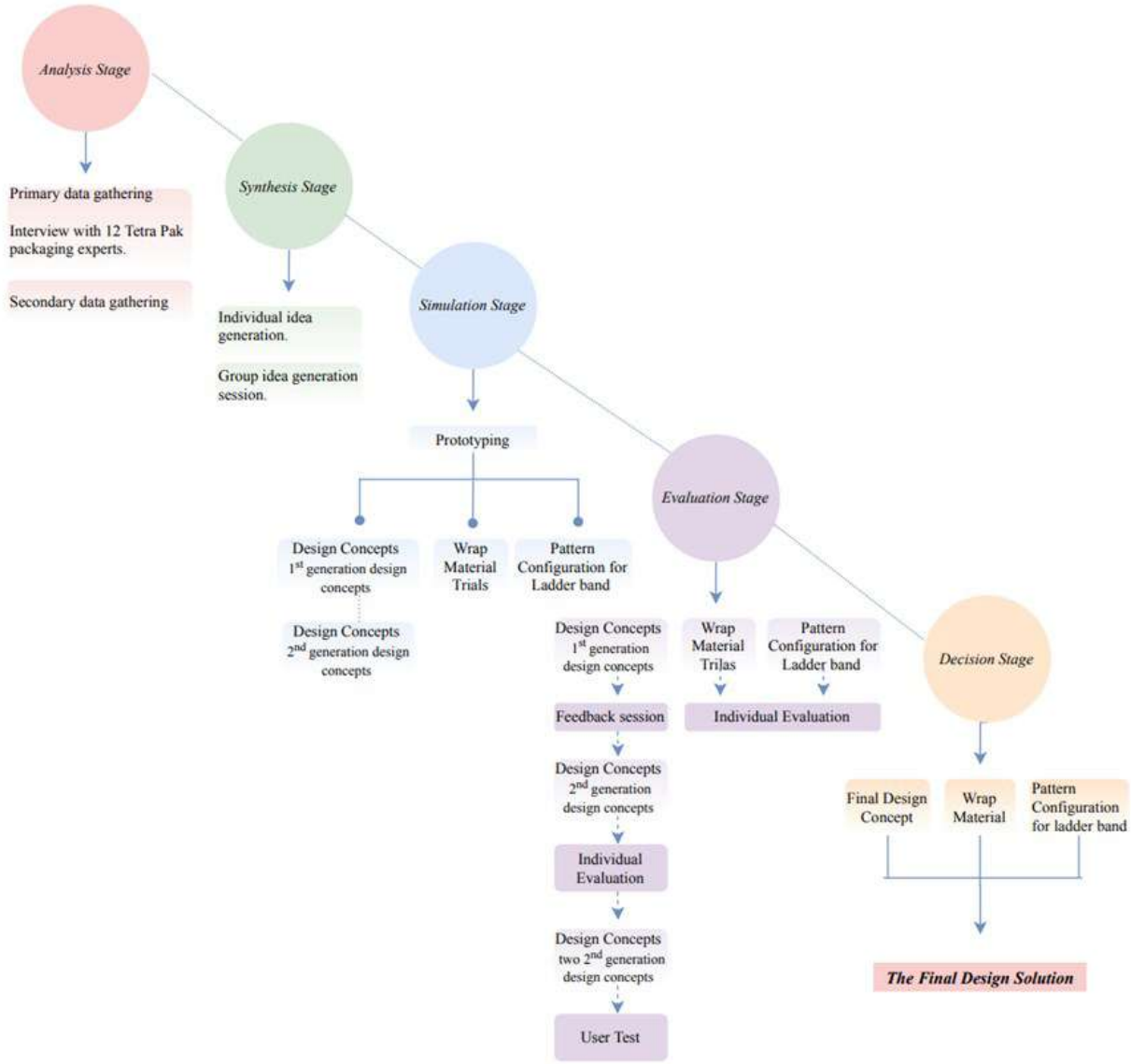

Fig. 1—Layout of the methodology

Results and discussion

The final design solution of this study was concluded as 'Pull-Strip' wrap design. The pull strip wrap has the mechanism of pulling the strip and taking the straw out by smooth sliding from the slipcase to facilitate easy opening and restrains the littering behaviour. The pull-strip model comprises an attached strip at the interior surface of the wrap. The folded layers are placed behind the straw's neck to form the mechanism of taking the straw up when the strip is pulled. The design mechanism facilitates an easy opening function for the U-shape straw wrap (see figure II). The design solution requires a loose sealing at the wrap opening that does not break off the paper when the strip is pulled, where embossed seam promotes easy opening function. Furthermore, the embossed seam sealing is a more sustainable option compared to the other sealing methods since it does not require any sealants or an energy-intensive application.

Fig. 2—The final design solution of the paper straw wrap.

As a natural renewable source and the U-shaped straw is made from paper, paper is chosen as the main substrate of the wrap material. The required functionalities of the wrap are provided with polyethylene extrusion coating. Polyethylene coating also assists in keeping the material variation as similar to the product system that increases the overall collection rates per materials for the specific variants.

The way the straw is attached to the package helps to curb littering and eliminate fossil-based adhesive, reducing the plastic content of the unit package. The design solution's material, sealing and functionalities promote enhancing the recycling activities in the waste management stream. The new pattern configuration of straws in its multipack facilitates 16% more efficient material and volume utilization in bulk loading (see Figure III). The final design solution of the wrap can be manufactured in the existing wrapping line with minimum adjustment in the machinery.

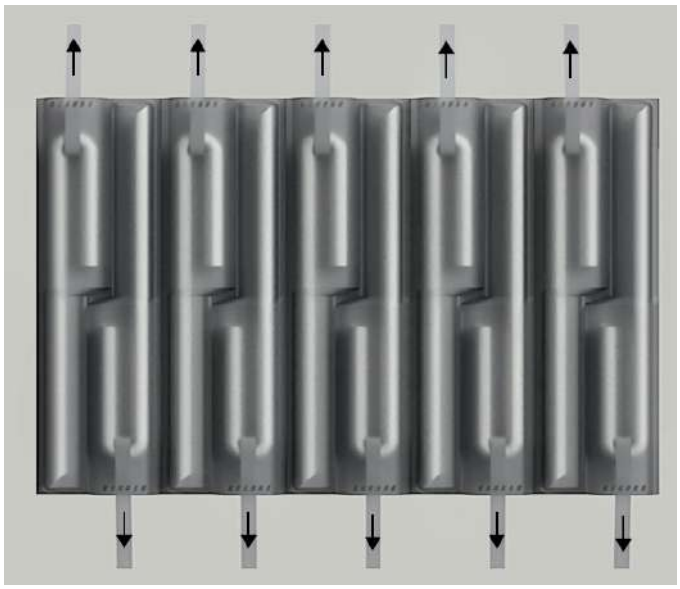

Fig. 3— The final design solution of the pattern configuration of the ladder band.

Conclusion

In the developing process of the design concepts, many design suggestions were created. However, most of those suggestions could not fulfil the requirement of littering prevention aspect or easy opening function of this project at the same time either because of their design or functionality. For those reasons, the

design concepts were developed in two stages and were evaluated in many sessions. In the early stages of the development process, a single particular aspect at a time was focused on the design concepts to fulfil. However, this created certain difficulties when the design was wanted to be improved, such as the design concepts that had an easy opening function did not fully meet the requirements of littering prevention aspects. The important outcomes were obtained in the second stage when the design concepts were developed more in a holistic perspective such as, how they are interacting with the other elements of the product system and in which level they meet the requirements of this study etc.

One of the concerns during the development of the material trial was the amount of fossil-based plastic used for coating the paper samples. The polyethylene layer of the paper sample could be coated as a thinner line. Thus, that would decrease the fossil-based plastic content of the wrap, consequently, that would be a more sustainable option compare to the final design solution. However, a certain strength and elasticity of the wrap material were needed to prevent the possible break off the material and to overcome the force that is applied when the opening grasp is pulled. In the sustainability perspective, a non-fossil-based plastic could be applied as the coating layer of the wrap material. Although, that could decrease the compatibility of the product system's materials in the waste stream while increasing the cost of the unit product due to the non-fossil based plastic price.

In conclusion the design solution that was the result of the thesis work conducted is able to address these objectives through the following:

- Littering behaviour is curbed by the design and the sealing method modification on the wrap to package attachment.
- Easy opening function is facilitated by Pull-Strip wrap design with embossed seam sealing at the wrap opening.
- The design solution is theoretically manufacturable in the existing straw wrapping line with small adjustment in the machinery.
- The material suggestion for the wrap enhances material collection and compatibility rates with the rest of the packaging system in the waste management stream. Improved waste management of the materials will help placing them in a circular economy and recreating value of the material as resources, thus leading to more circular and sustainable systems.
- The wrap to package attachment with heat-sealing eliminates fossil-based adhesive on the product system. The plastic content of the unit package is decreased and the risk of contamination from the hotmelt in the paper waste stream is eliminated.
- The rearrangement of the pattern configuration of the ladder band provides efficient utilization of the wrap material. The new pattern configuration facilitates 16% more efficient material utilization. This also positively influences the volume utilization in bulk loading.

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The Ecology of *Salmonella spp.* in pork meat processing industry - comparative genotyping and phenotyping.

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Introduction

Pork products are among the most frequent foodstuffs implicated in human salmonellosis in the EU, right after poultry. Considering that, pork is the third most frequently contaminated meat after fresh chicken and turkey (EFSA, 2019). Therefore, monitoring and surveillance activities have been implemented along the food chain to assess the risk caused by pork and products thereof as a source of *Salmonella* spp. to prevent outbreaks. Additionally, pig and pork meat are reported worldwide as sources of *Salmonella* spp. resistant to clinically important antibiotics, representing a major threat to the treatment of invasive infections in humans. According to the EFSA 2019 summary report on zoonoses, zoonotic agents and food-borne outbreaks, *Salmonella* spp. was responsible for 24.4% (91,662) of food-borne outbreaks in the European Union (EU) in 2017. EFSA (2019) estimates that 4.5% of outbreaks are associated with pig meat and products thereof. The common practice of antibiotic use in intensive food-animal production has been considered the main driver for the selection and transmission of antibiotic-resistant foodborne bacteria, including *Salmonella* spp., to humans (EFSA, 2019).

Research objectives

In this study we investigate a set of 95 strains, used for wet lab research and 96 genome data for dry lab research of *Salmonella enterica* subsp. *enterica* that have been isolated during routine sampling of pork carcasses from various meat processing environments in the Netherlands.

We use a combined approach of comparative laboratory phenotyping and genotypic analysis to establish the diversity within this set of Salmonella isolates.

Moreover, we assessed possible clustering within this set, and draw a comparison to a broader set of 95 *Salmonella enterica* subsp. *enterica* genomes in order to elucidate patterns that may explain the presence in the meat processing facilities.

Methodology

- Bacterial strains, culture conditions and pre-culturing

Ninety-five *Salmonella enterica* subsp. *enterica* strains were used in this study, from which 92 were isolated from pig and 3 from bovine carcasses in meat processing facilities in the Netherlands (SWBR strain collection). All strains were stored at -80˚C in glycerol (Sigma, 25% v/v final concentration) until further use. Collection involved also whole genome sequencing data of all 95 isolates and one extra sequence, complying in total of 96 genome data.

Cells from -80˚C stocks were streaked on BHI agar plates and incubated at 37˚C for 24h. Single colonies were transferred into 5 mL of BHI broth, and incubated statically overnight (~ 18h) at 37˚C. After incubation, this procedure was repeated in order to the obtain second overnight cultures, which were used for analysis.

- *In silico* serotyping

In silico serotyping and antigenic profiles were determined using SeqSero. An external, second group of ninety-five publicly available whole *Salmonella enterica*, subsp. *enterica* genomes was obtained from NCBI as an external reference group. This collection mainly includes isolates of human clinical origin (n=61; 63,2%) and also environmental swabs (n=20; 21,0%) and plant or sea food-based sources (n=15; 15,8%).

- Phenotypic characterisation

Growth performance:

A fast screening method using 100 well (Honeycomb) plates in the Bioscreener C system was to estimate the maximum specific growth rate (μ_{max}) of many strains in parallel. OD600 was measured every 15 min, for 4 – 7 days depending on the temperature settings. Raw data was processed using the statistical programming language R (R Core Team (2019), version 3.6.2). The growth rates package (Kahm et al. (2010), version 0.8.1) was used to log transform the OD data and fit a spline through the steepest part of the slope. The angle of this slope was taken as a proxy for maximal growth rate (Proxy_mumax (h-1)).

Biofilm formation on solid media:

Luria-Bertani (LB) plates without salt, supplemented with 40 mg/L Congo red (CR) and 20 mg/L Coomassie brilliant blue were used to determine the Congo red-binding property of the cells. LB agar plates supplemented with 200 mg/L Calcofluor (C) were used simultaneously to determine the cellulose production by comparing the fluorescence of the test strains under UV light.

Antimicrobial susceptibility testing:

Antibiotic resistance of strains was measured according to the Kirby-Bauer agar disk diffusion method (Perilla, 2003), with some modifications. Equally sized colonies from BHI agar were transferred to PPS and turbidity was adjusted to match a 0.5 McFarland standard. The suspension was inoculated onto Mueller-Hinton agar using a sterile swab, followed by antibiotic disk application. The following antibiotic disks were used: Apramycin (15µg), Neomycin (10µg), Ampicillin (10µg), Amoxicilline (30µg), Colistin (10µg), Flumequine (30µg), and Trimethoprim (25µg).

- Genotyping characterisation

Distribution of antimicrobial resistance genes

FASTA files from 96 strains of the SWBR collection and 96 strains of the external collec-

tion of S. enterica were screened for antimicrobial resistance (AMR) genes using the ResFinder 3.0 database

Prophage diversity:

Prophage sequences within the assembled contigs of each genome were identified with the PHAge Search Tool Enhanced Release (PHASTER), with recommended settings.

Typing of isolates by core genome multilocus sequence typing:

For cgMLST analysis, the whole-genome sequences of both the SWBR and external strain collection were uploaded to public databases for molecular typing and microbial genome diversity (PubMLST) and schema of total 3003 loci was retrieved using cgMLST v2. The minimum spanning tree was generated by PHYLOViZ Online (N locus variant = 25), using cgMLST and related epidemiological data.

Results and discussions

A cgMLST scheme was created using 3003 core loci shared within all Salmonella in the SWBR isolates and a minimum spanning tree (Figure 1.) based on this scheme, divided collection into 2 clusters, that correlate well with serovar *S.* Typhimurium (blue) and *S.* Derby (orange). No clustering was observed for location or sampling time.

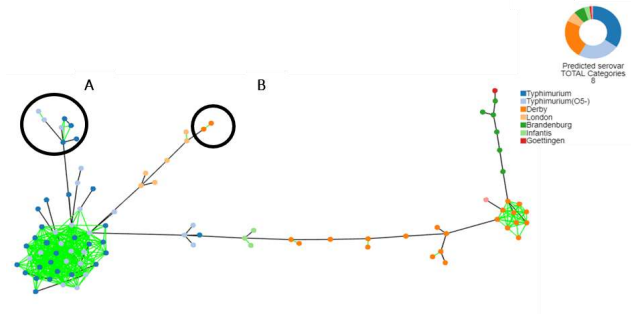

Figure 1. Minimum spanning tree of Salmonella enterica genomes from the SWBR strain collection. The circles are colour coded per serovar. Clusters connected by green lines show isolates that differ in less than 25 alleles.

Comparative phenotypic and genotypic analysis showed that growth performance was significantly influenced by temperature, but it was stable over serovars, even though outliers were identified. On figure Figure 1. Group A: The slowest growers at 15, 20, 30 and 37˚C among *S.* Typhimurium serovars; Group B: The fastest growers at 15, 20, 30 and 37˚C among *S.* Derby serovars.

Expression of both curli and cellulose and therefore biofilm formation capacity was found to be strain and temperature dependent.

Protein sequences of SWBR isolate collection were considerably more conserved when compared to the external collection, with strain serovar specific allelic variants of virulence factors FimH and SipD (Figure 2.).

SWBR collection External collection

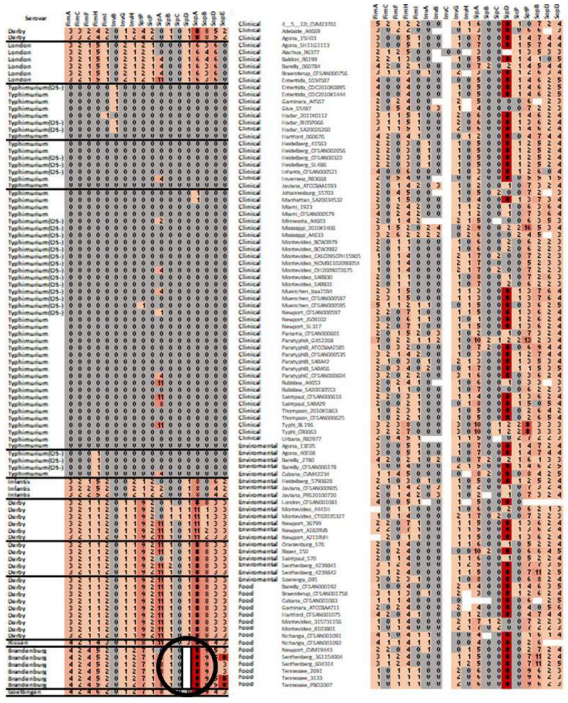

Figure 2. Collection of virulence factors and protein alignments identified allelic protein variants among the SWBR and external strain collection genomes.
(Legend: grey – low allelic change (≤6 changes), red – high allelic change (≥7 changes); white – absence; black circle – a loss of SipD gene from S. Brandenburg in SWBR collection)

Even though, more than 90% of isolates of the SWBR collection showed multidrug resistance, the number of isolates that carried putative resistance genes was higher than the number of resistant phenotypes (Figure 3.).

SWBR Collection External Collection

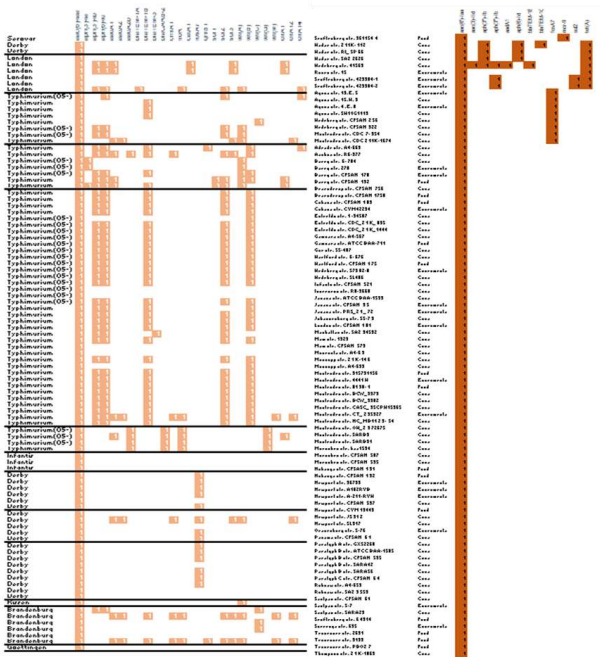

Figure 3. Collection of multidrug resistance genes among the SWBR and external strain collection genomes. (Legend: orange – ≤2 genes, red – ≥3 genes; white – absence of gene)

Variability in prophage types illustrated the correlation between prophage repertoires and the genome diversity of different Salmonella serovars (Figure 4.).

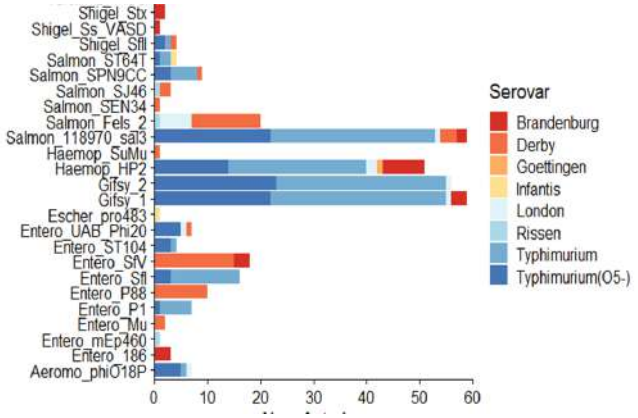

Figure 4. Pattern of intact prophages per predicted serovars indicated in the SWBR collection strain serovars.

Conclusion

Taken together, assessment of parameters was highly relevant in order to make better predictions on prevalence, virulence potential and persistence of *Salmonella enterica* subsp. *enterica* in environment. Further research is needed to determine the factors explaining the overall persistence of the SWBR isolates in industry and its apparent adaptation to swine. For better understanding of microevolution in environment (Tassinari et al., 2019), we proposed looking closer at virulence factors, especially at diversity indices and genetic diversity, to have greater insight in level of allelic variation and multilocus linkage disequilibrium (LD) for diversity at each locus and horizontally transferred sequences for better understanding of multidrug resistance, such as plasmids and prophage sequences. As a further study we also suggest an integration of microbial genome-wide association studies (mGWAS), since use of this research can help improve antibiotic use as well as the discovery and development of more effective antimicrobials (San et al., 2020). Obtained data, could be also further used for application of machine learning (ML) models for prediction of phenotype from genomic data, which could reduce high cost of biological studies and take the advantage and challenge of highly complex, dimensional and high resolution of omics data (Xu and Jackson, 2019).

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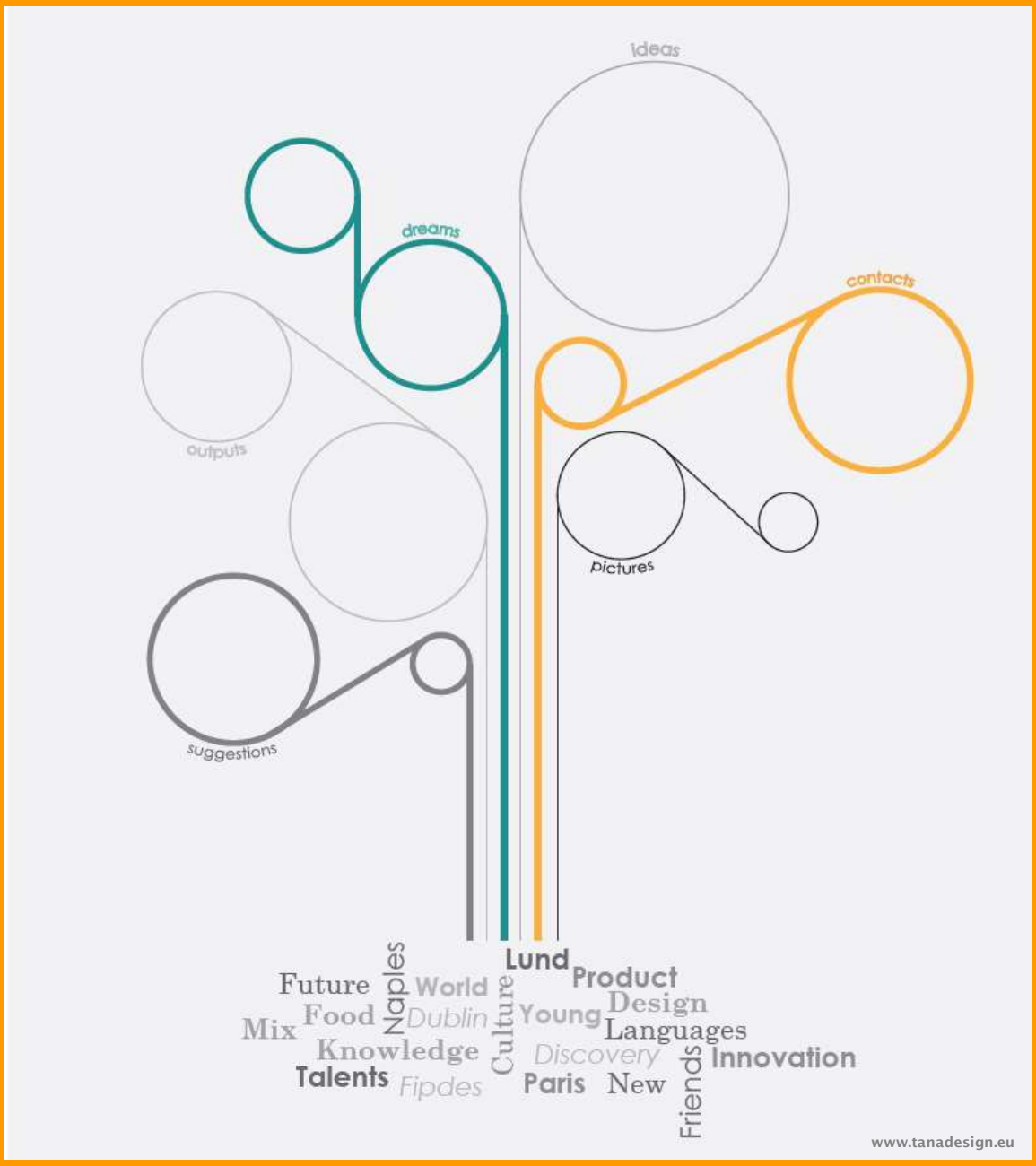

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