

# Food Innovation & Product Design





**Congratulation to the FIPDes Cohort 5 !**



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## Introduction

By **Erik ANDERSSON**, Research Coordinator (Lund University, Sweden)  
FIPDes Consortium Committee Member 7

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### **Sarai ARCE ESPINOSA**

Study of stability of Bifidobacterium in infant formula during processing and storage 12

---

### **Volkan BAGDADLI**

US consumers and sparkling wine: a generational and behavioral segmentation analysis 13

---

### **Elena BAIXAULI MARIN**

Towards a bottle weight reduction - Evaluation in a selected milk plant in the United Kingdom 18

---

### **Nancy CALDERON RAMIREZ**

Salivary N-acyl ethanolamines upon food mastication in humans: influence of individual nutritional status 21

---

### **Izza Patricia CORTEZ**

Effects of process variations on the particle size and rheological properties of ice cream mix as measured on-line and at-line 25

---

### **Brendan CROSSE**

An Explorative study into growth strategies used for developing a large-scale niche dairy Industry in Ireland 26

---

### **Yamile Sayaret DOMINGUEZ HERNANDEZ**

Competitive intelligence analysis of the dairy Light category to drive nutrition superiority in Product Design 29

---

### **Wally E. GARCIA CASTILLO**

Folding carton and internal printing: A technical approach to consumer differentiation and food safety 30

---

### **Julian David GONZALES ROMERO**

Cottage Cheese Optimization 34

---

### **Ziwei GUO**

Enzymatic Processes to Create Added Value Ingredients in Early Life Nutrition Product 35

---

### **Natalija KOZAREVSKA**

Comprehension of the pH effect on the degradation kinetics of Vitamin C during the heating of a liquid model solution 36

---



<b>Júlia KRAMMER</b>	41
Exploring the last phases of product development: From kitchen to plant production	
<b>I-Chin (Elizabeth) LEE</b>	45
Formula Optimization of New Cricket Flour Based Cookies Using Sensory Evaluation	
<b>Nan MA</b>	48
Study to examine iron absorption in a matrix of young child formula	
<b>Elizabeth Ama MENSAH</b>	49
Effect of antimicrobial activation of a compostable package on the shelf life of selected food products	
<b>Divya MOHAN</b>	53
Evaluating the behaviour of probiotic Lactobacillus plantarum 299v in non-dairy oat based yogurt using two different packaging materials	
<b>Laura MURILLO GONZALEZ</b>	58
Potential functionality of protein hydrolysate-enriched biscuits for glycaemia control	
<b>Yoga PUTRANDA</b>	66
Impact of biobased packaging materials on quality of fully-baked frozen bread	
<b>Laura RUIZ MORFIN</b>	69
The Effect of Added Multicomponent Antioxidant Formulations in Sunflower Oil based Model and Food Systems	
<b>Siddharth SHARAN</b>	76
Future of Fermentation - Idea Generation and Development of Prototypes	
<b>Muhamad Syahir SUHAIMI</b>	78
Evaluation of the Corrugated Box Strength Performance in Supply Chains : A Case Study of Duni AB	
<b>Juan Camilo VARELA TABARES</b>	82
Effect of the variation of the formulation ingredients and process parameters on the stability and sensory properties of aerated desserts	
<b>Ling WANG</b>	83
Comparative Study of Different Characterization Methods of the Cooking Level in Cereal-based Products	



## Join forces across borders - feed the world through collaborative innovation



**Joint food innovation, represented by international students from the Erasmus Mundus Joint Master Degree FIPDes, Food Innovation and Product Design.**

**The students are engaged in small-scale production of yogurt and fresh cheese in Grignon, France.**

Photo by Divya Mohan/FIPDes, 5 November 2015.



*"The pullover marked size 48 has the width of a size 54 and the length of a size 38. I don't know how the employees of Milena come to these measurements. In the capital, there are no people who are so small and square. If it's our fault that we cannot accommodate the central plan with our sizes, we would like to apologise for that. In this case we shall endeavour to become, in the future, smaller and squarer."*

This amusingly subtle criticism of the GDR party by the character Christine Kerner, played by the actress Katrin Saß in the movie *Good Bye, Lenin!*, is a good example of how enterprises, state-owned or private, can clearly benefit from collaboration with their customers. In this film, the example was about pullovers, obviously being too wide and too short to fit the general East Berlin citizen. But in general terms, this approach – to work together through collaborative innovation – could relate to just about anything out there: healthcare, education, household goods, consumer electronics – and not least – food. Why? Well, it would be fair to say that the current global challenges in providing humans with safe and healthy food are nothing short of enormous.

With an ever-growing population, we quickly need to find innovative solutions to these challenges: to fight under- and overnutrition, to reduce food waste and to have sustainable and sound food production. However, in order to succeed, we need to re-think our common view on innovation, and simultaneously focus on the needs of individuals, take into account the needs of societies, ensure the availability of natural resources and re-

<sup>1</sup> *Good Bye, Lenin!* (2003), Quote by actress Katrin Saß (playing the character Christine Kerner), Directed by Wolfgang Becker, X Filme Creative Pool, Germany





duce the environmental impact. This will require a new generation of talented people capable of working across borders and sectors. Many of these young students, researchers and entrepreneurs now meet across the world, in new educational programmes and constellations, in networks, competitions and other initiatives, in order to find joint solutions. Let us look at three examples.

#### Food powder – the future for ugly food

Why throw away enormous amounts of nearly expiring fruits and vegetables, when they still are perfectly fine to eat? A few years ago, this fact triggered an idea in an international group of young, bright students, who met at the university. Why not gently convert the precious food items into powder? In doing so, the fruits and vegetables would keep their high nutritional value, while extending their shelf life up to two years. After spending some time refining their idea, the students organised themselves under the name FoPo Food Powder and submitted a video pitch to the world-known Thought for Food Challenge. The result was a huge success – to their surprise they won the runner-up prize and had some seed money for their continued work. After a successful fund-raising campaign, they started with a trial in the Philippines and small-scale production of their first food powders. Now these powders are for sale on-line, and the innovative idea, once born and bred in the minds of a group of young, talented people, has flourished into an investor-backed business with a clear sustainable development approach.

In this example, two important circumstances were crucial for the success:

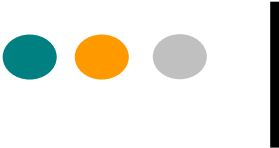

The diverse and complementary background of the students. Combined, the founding team had extensive experience in research and development, engineering and design, business management and development, technical sales, and operations and consumer insight, a knowledge that can hardly be found in one single person.

The very valuable training and network gained during the Thought for Food Challenge. Thought for Food is a non-profit innovation platform dedicated to tackling global food challenges. It is obvious that the students in FoPo Food Powder could highly benefit from the genuine collaborative and sharing attitude that exists within this network.

#### Eat more insects!

Would you consider having crisp bread, cereals, pasta or falafels made of cricket flour? No? The group behind Eat:em would like to change that. To overcome peoples' reluctance to insect food, this international group of students has come up with a three-step plan to normalise these novel ingredients for consumers. The idea is to use food product development as a tool and: 1) introduce people to insects as food by offering cricket





flour crisp bread and cereals, 2) make insects a part of the everyday meal by promoting cricket flour pasta, and 3) replace meat with insects in the form of cricket flour falafel. By focusing on simple ingredients, low investments and feasible technology, this approach could definitely help in the breakthrough of insect-based food in the areas of the world where this is not currently embraced. The use of storytelling by fictitious characters that people could identify themselves with will further help in the transition from being sceptic to being curious and interested consumers, who would actually consider trying this unusual but nutritious food.

Once again, we can see that food innovation is best performed jointly, in multidisciplinary teams. However, for this to happen, we need a place or a platform where these interested and talented people can meet. In this specific case, the enabler was the Food Hackathon, a competition concept that can be found in various places around the globe. It works like this: For an extended weekend, all people interested – farmers, engineers, scientists, artists, linguists, teachers, journalists, policymakers, and others – are invited to share their ideas and form interdisciplinary teams. The teams highlight and generate ideas to solve specific food-related issues. The focus can vary from preventing obesity among children and youth by making healthy eating more popular, to the connectivity of food – linking food with logistics, art or other areas. As with the Thought for Food initiative, the ambition is to generate new, viable business ideas to help build a sustainable food system for the future.

#### Edible straws – making a big impact with something that appears to be insignificant

Sometimes it is overwhelming to hear the alarming reports about what we humans do to the environment. An example is the enormous amounts of plastic waste we produce. Did you ever think about a small, insignificant thing that contributes to this – the straw? There is a rising concern about it, but one of the most innovative solutions is probably the edible straw. Three international students, tired of hearing about the devastating state of the oceans and how landfills are overflowing with disposable packaging, came up with the idea. Thanks to university awards and EU climate innovation investments, they are now developing this novel approach into commercial straws that will be edible, with zero waste and fortified with nutrients. The goal is to replace plastic straws globally, which would have a big impact and inspire future entrepreneurs.

In this third and last example of key enablers for joint food innovation, I want to emphasise the role of higher education. All students in this case are enrolled in FIPDes, Food Innovation and Product Design, which is one of several Erasmus Mundus Joint Master Degrees on food. These are highly competitive educational programmes acknowledged and supported by the European Commission. Students come from all over the world to

share experiences, acquire knowledge and learn new tools in a European setting. Each programme has its unique profile and mobility plan. The students visit several countries during their studies, but what they share in common is an intercultural exchange, collaborative approach and life-learning experience. Subjects range from aquaculture, food science and technology, sustainable cropping systems, grape cultivation and winemaking, to food innovation and product design.

These were just a few cases of how interdisciplinary teams of talented, young people can meet and come up with new, innovative ideas on how to tackle some of the food challenges of today. However, one should remember that in addition to groups like these and their supportive platforms, competitions and higher educational programmes, established food businesses are also taking on joint collaboration. Cluster organisations and university incubators further help in the transition from in-house to open and collaborative innovation practices. To sum up, the cases and examples presented clearly show that the days are over when a single, uplifted innovator solely handles innovation. Tomorrow's sustainable food innovation process is transversal, with the overarching principles of collaboration, mutual trust and a vibrant exchange of ideas.

*To the graduating students in FIPDes cohort 5*

More than most of us, you know what it means to be part of interdisciplinary teams, to experience an international exchange and to pursue joint collaboration projects. During these two years of studies, you have proven to have the talent, strength and joyful engagement to join forces across borders and face tomorrow's challenges. It has been a sheer privilege to work with you, and I wish you all the best!

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## Study of stability of Bifidobacterium in infant formula during processing and storage

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In the present study, data was obtained to contribute to the expertise on Bifidobacterium stability in infant formula by evaluating the impact of several factors on its viability during processing and storage. Results showed a strong influence of water activity and temperature, whereas oxygen was found to be less detrimental at specific conditions. A methodology to build a prediction model based on Arrhenius plot and linear regression equations was developed to conceivably shorten future shelf life studies and set boundaries during storage. On the other hand, a potential pre-blend preparation was found to be promising for specific carriers and a new methodology was found to be useful for evaluating matrix effect on bacterial viability. The insights obtained during this project will be of great value for the development and launch of new products containing Bifidobacterium.

*Confidential topic*



## US consumers and sparkling wine: a generational and behavioral segmentation analysis

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### Introduction

Wine industry is regarded as highly dynamic and fragmented, a fast-paced industry which makes it currently one of the most complex and competitive agri-food businesses globally (Thach & Olsen, 2006). Growing shift in consumer behaviour in wine enhances the rivalry in ever-competitive landscape; thus keeping the pulse of the market and understanding its components have become keys to corporate survival (Orth *et al.*, 2007; Spawton, 1991).

Although global wine production was stable around 270 million hl (OIV, 2015) during last two decades, the value has doubled and reached 300 billion USD by 2015 (Euromonitor, 2016). United States have become the largest national wine market by 2012 surpassing long-time leader France. Besides its massive market magnitude corresponding to 14% of global consumption and 20% of value, United States stand in the heart of wine marketing research as yet the market is far from saturation and possesses vast opportunities for global producers.

Despite promising trends in total wine consumption in U.S., sparkling wine receives special attention by marketing professionals given that it has been and forecasted to be the fastest growing segment; comprising 9% of total wine sales and demonstrating 10% of growth in the last year (Euromonitor, 2016). Whilst sparkling wine in U.S. is dominated by imports from Italy and France in-

cluding Champagne; Prosecco from North Italy has attracted most of the attention over the past several years by consumers and demonstrated 23% growth in 2016 (Nielsen, 2017). Prosecco is a vital player in the market due to not only being driver of growth in sparkling wine segment in U.S. but also assisting growth in total as it mediates adaptation of wine consumption behaviour in younger consumers. It is vital for companies and producers to understand behaviour of younger generations in U.S. (Fountain & Fish, 2010) towards sparkling wine and Prosecco as they are already considered to be major reason of upward trend in consumption (Mueller et al., 2011; Heeger, 2006) and will be future consumers and opinion leaders (De Magistris *et al.*, 2011; Lockshin & Corsi, 2012).

### Research objectives

The main aim of the study is to discover sparkling wine and Prosecco consumption behaviour and preferences of a sample drawn from the U.S. population. Through profiling consumers by their preferences and revealing possible competitors; investigate clear target groups and market opportunities for Prosecco growth.

### Methodology

Questionnaire data is processed in SPSS 22.0. Exploratory factorial analysis (EFA) is used to analyse underlying constructs within a large set of purchase influencing statements and preferences when consuming sparkling wine. Principal component analysis (PCA) and principal axis factoring (PAF) are applied as factor extraction methods in EFA so to achieve a higher reliability on factor loadings. For similar purposes, two rotation methods of direct oblimin and varimax are used. The reduction of the constructs into factors have formed the basis for subsequent

partitioned cluster analysis. Descriptors for each cluster have been identified after examining factors and factor loadings. Multinomial logistic regression (MLR) is used to explain levels of Prosecco consumption by characteristic consumption behaviour and preferences of generations and identified clusters as well as to reveal possible competitors of Prosecco in relation to purchase patterns in other types of sparkling wines across various price segments. Observed differences between generations and identified clusters on purchase and consumption behaviour have analysed with statistical significance tests of ANOVA, Mann-Whitney U and Kruskal-Wallis H depending on the nature of the assessment in the questionnaire and the statistical analyses.

### Results and discussions

Exploratory factorial analysis and subsequent clustering resulted in four segments based on sets of purchase influencing statements and preferences when consuming sparkling wine; enjoyment-oriented (24%), origin-oriented, information seekers and reassurance seekers. Enjoyment-oriented seeks pleasure, were characterized with high appreciation to socializing attribute of sparkling wine consumption were more interested in ideas to serve, mix or food-pair. They were mostly Millennials generation who preferred wine and sparkling wine of lower price segments while demonstrating lowest frequency in wine consumption and highest increase in Prosecco consumption. Origin-oriented (27%) had distinct interest and willingness to pay (WTP) in extrinsic attributes, mainly region and origin of production which to them addresses certain levels of quality. They were of higher age with highest con-



sumption frequency both in wine and sparkling wine and were highest in familiarity with types of sparkling wine. Information seekers (30%) were highly enthusiastic in enlarging their knowledge about intrinsic and extrinsic attributes of wine as well as production methods and appellations. They were mostly males and Baby Boomers, consuming the least in volume but had highest price paid for a bottle. Despite the cluster members had lowest consumption of Prosecco, they demonstrated highest familiarity towards types of Prosecco. Reassurance seekers (19%) who look for indications of better quality and ways to decrease perceived risk in their choices such as medals, recommendations. Members were mostly Generation X, highly

Generational differences has observed within the sample as expected. Older generations, more specifically Baby Boomers wine significantly higher than younger generations; while younger generations, more precisely Millennials consume more sparkling wine and Prosecco. These results were in parallel with literature (Agnoli et al., 2011; Atkin & Thach, 2012; Thach & Olsen, 2006, Mueller et al, 2011; Bruwer et al., 2011; Olsen et al., 2007; Onofri et al., 2015; Fountain & Fish, 2010; Charters et al., 2011). Another set of differences are observed in purchase patterns across price points and familiarity towards various sparkling wine types. Millennials prove a higher tendency to consume wine and sparkling wine of lower price points although higher price points were dominated by Generation X (Atkin & Thach, 2012; Olsen et al. 2015). Millennials are interested and open to more consume wines of import more than other generations; while older generations were similar in behavior towards domestic types. Champagne receives a special treat from all generations, penetrating into all

age groups (Thach & Olsen, 2015; Charters, 2005; Charters, 2009; Beverland, 2006; Ritchie et al., 2011; Fountain & Lamb, 2011; Nielsen, 2017). Millennials were the generation with highest interest in Prosecco.

Logistic models revealed being male and/or resident in state of California had decreased loglikelihood of Prosecco consumption. These results are attributed to higher tendency drinking bubbles of higher price tags for males which indicated 'prestige' and domestic bubbles in California. The model also revealed Champagne, Cava, Moscat from Piedmont, sparkling wines from France and California were significant competitors of Prosecco varying among generations.

## Conclusion

This study provides insight into U.S. sparkling wine consumer behavior. Consumer characteristics in selection and consumption of wines and sparklings wines are found highly heterogenous. Generational and preferential segmentation analyses are valuable tools in grouping and better understanding similarities in behavior that can be utilized to form clear target groups. This study allows reader to determine specific market gaps which can be then manipulated and evolved into opportunities for further growth of Prosecco.

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# Towards a bottle weight reduction - Evaluation in a selected milk plant in the United Kingdom

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## Introduction

The dairy industry represents a significant role in most of the economies worldwide, due to the high consumption of dairy products and its relatively low prices. Goods for private consumption are being packaged increasingly in plastic. Pasteurized milk in the United Kingdom is mainly packed in HDPE (Figure 1), the most popular packaging material, accounting for a 71% volume share in 2016 (Euromonitor International, 2016). Over the years, the industry has developed ever lighter and thinner plastic packaging, while at the same time providing identical or improved functionality – a benefit for the environment, the industry and the consumer (Hanser Velarg, 2004).



**Figure 1. Examples of HDPE milk bottles.**

## Research objectives

The primary purpose was to study the possibility of **light weighting HDPE bottles** used for pasteurised milk in a selected milk production plant.

The second purpose is to study **alternative materials and technologies** for a future implementation that can potentially reduce costs and supply the high production de-

mand, **evaluating the designs used by competitors** and researching other possibilities.

### Methodology

A combination of different methods was used in order to gather all the information needed:

- i. Semi-structured interviews
  - a. understanding the major problems encountered in the line production
  - b. comprehending the effects of light weighting
  - c. collecting information about alternative materials and technologies
  - d. understanding the designs used in the industry
- ii. Observations - evaluation of the current packaging in the processing line
- iii. Literature review
- iv. Weight measurement
- v. Thickness measurement
- vi. Drop test - evaluation of the ability to withstand free-fall impact forces
- vii. Vacuum chamber - evaluation of leakages or closure problems
- viii. Compression test

### Results and discussions

#### Designs available in the market

The main findings were:

- i. Milk packaging in the UK suffers a lack of innovation. Most companies use HDPE bottles with ECO or Infini designs for pasteurized milk.
- ii. Other designs found in the market are mainly for Extended Shelf Life products or enriched milk, in either HDPE or PET. Different agents of decontamination are used for a more thorough cleaning (hydrogen peroxide or peracetic acid). In this case, an appropriate design is necessary for

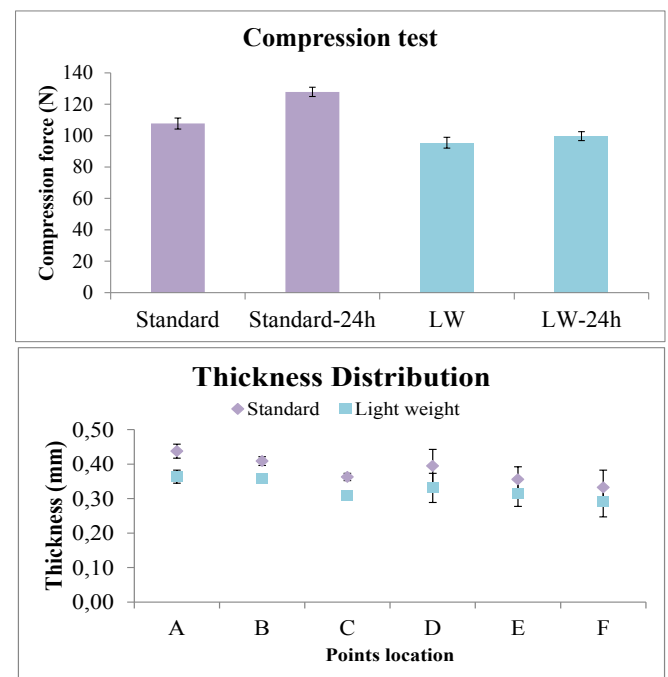
allowing an easy-flow of the solution in the packaging.

- iii. Titanium dioxide a photo-responsive white compound that protects the milk from oxidation is included in the packaging, due to its ability to scatter light and absorb UV light energy (Johnson, et al., 2015).
- iv. Clear PET is also used for enhancing the visibility of the product. A clean presentation gives an impression of transparency between brand and consumer, as well as a feeling of authenticity of the product itself (Bordbia, 2016).

#### Light weight HDPE pasteurized milk bottles

In the line study, only 0.08% of the bottles were damaged in the filling and cap station, due to a side-to-side deformation at the entrance of the equipment.

The results from the 10% weight reduction showed a diminution in the top load strength (Figure 2) and positive results for drop and vacuum tests. Thickness values presented an



**Figure 2. Standard and light weight bottle compression force values (N) and minimum thickness values (mm) comparison.**

even reduction (Figure 2). However, the top part sections had a lower value than the established in the specifications.

#### Alternative technologies and materials

PET prices are around 22% inferior to HDPE. Injection blow molding is the technology that produces PET bottles that unlike extrusion blow molding cannot produce a handle.

However, different packaging suppliers have developed technologies that can include an external or integrated handle (Sidel, B&R Industries, Plastipak, etc.) in an injection blow molding system.

Other similar resins such as PETG or EPET are compatible with extrusion blow molding.

Besides, there are alternative technologies to extrusion blow molding that can produce HDPE bottles and potentially reduce the cost.

- i. Compression blow forming and compression stretch blow forming are recent processes developed by Sacmi.
- ii. The use of foam blow molding HDPE (or PET) allows a reduction of material used due to its structure.
- iii. LiquiForm™ a recent technology for HDPE and PET can reduce the production time.

#### **Conclusions and further work**

The market analysis of the milk products pointed out the lack of innovation in the sector. All the different packaging found were Extended shelf life products or with additional ingredients that need extra packaging protection due to its composition. Other products look for brand differentiation among competitors. A suggestion is to continue exploring the products available in the market that can be used as a source of inspiration for future innovations in milk packaging.

The light weight trial presented controlled re-

sults for the weight values and successful results in the drop, compression and vacuum tests. Only thickness values in the top part section were below specification that can cause problems mainly in the packing and transport.

Further research is needed in the production line for ensuring the feasibility of the bottle. Moreover, the study of the bottle storage and transportation would be necessary for the complete assessment of the line. Environmental implications of the material reduction could be evaluated.

In regards to alternatives technologies and materials, there are possible solutions that can supply the high demand and can potentially reduce the costs. An extensive analysis is necessary for comprehending the total impact of substituting the line production and its economic and environmental repercussion.

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## Salivary N-acylethanolamines upon food mastication in humans: influence of individual nutritional status

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The aim of this study was to evaluate the effect of individual nutritional status on the salivary N-acylethanolamines (NAEs) upon mastication (before swallowing) of a solid model food with a different fat composition. To this purpose three model biscuits with different types of fat such as extra virgin olive oil and palm oil or without fats (control) were developed and a cross-over modified sham feeding protocol was carried out. Saliva samples collected were analyzed by LC/MS/MS for the content of LEA, PEA and OEA.

Data showed that the concentration of NAEs in both unstimulated and stimulated saliva was significantly higher in obese than normal weight subjects. Mastication of food caused an increase of salivary NAEs. During food mastication salivary NAEs was independent from the types of biscuits in normal weight subjects, while in obese subjects the biscuits contributed to the NAEs salivary content in the following order: extra virgin olive oil>palm>control biscuit.

### Introduction

Endocannabinoids (ECs) and N-acylethanolamines (NAEs) are lipid mediators present in human saliva in concentrations that vary depending on the nutritional status. Regarding NAEs, these include mostly Oleoylethanolamide (OEA), Palmitoylethanolamide (PEA) and Linoleoylethanolamide (LEA). These compounds are known to



provide anorectic and anti-inflammatory effects.

The mechanism by which NAEs can regulate food intake is by activating different receptors related with the modulation of food intake. These receptors are G protein-coupled receptor 119 (GPR119), which is activated by OEA and PEA, and peroxisome proliferator-activated receptors (PPAR $\alpha$ ) that is activated by AEA and other NAEs (Hensen, 2010). OEA is one of the most studied NAEs due to the important role it plays in regards to the regulation of food intake. Several studies have shown the anorectic effects of OEA. In the study of Oveisi *et al.* (2004), oral administration of OEA in rats caused a persistent inhibition of food intake. It was reported that OEA induces satiety by activating the receptor PPAR $\alpha$ , regulating feeding and body weight in mice (Fu *et al.*, 2003). Moreover, OEA lowers lipid levels in liver and blood by enhancing lipid oxidation (Fu *et al.*, 2005). Likewise, in the study of Mennella *et al.* (2015), it was demonstrated that consumption of a meal with a high content of oleic acid can increase plasma OEA levels and reduce energy intake at subsequent meals. This finding was in line with the evidence that specific fatty acids can determine the circulating levels of specific NAEs thus being precursors for their formation in the enterocytes (Joosten *et al.*, 2010). Moreover, it was recently demonstrated in normal weight subjects that NAEs can increase in saliva upon food mastication and can persist at a different extent (depending from the food matrix) in the oral cavity after eating thus being possibly involved in gustatory processes underpinning food reward and appetite (Kong *et al.*, 2016).

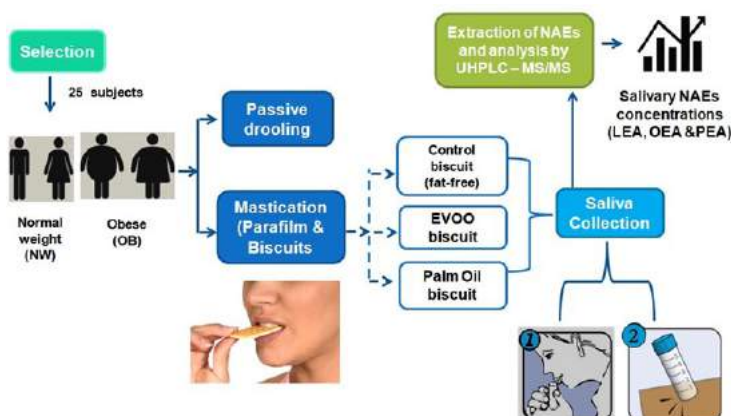
## Objectives

- To evaluate whether salivary NAEs during mastication are influenced by the individual nutritional status.
- To evaluate the effect of food on the release of salivary NAEs during mastication.
- To verify the contribution of different types of fats on the release of LEA, PEA and OEA

## Methods

Three biscuits differing for the types of fat, extra virgin olive oil (EVOO), palm oil and paraffin oil (fat-free control biscuit) were developed.

The design of the human study is shown in **figure 1**. Twenty-five healthy subjects were selected and invited to the lab in three different days in order to perform modified sham feeding experiments. During each experimental session subjects collected unstimulated saliva (by passive drooling), stimulated saliva (by parafilm mastication) and food boluses obtained by 3 min. mastication of one of the 3 biscuits developed in the study. Saliva samples were analyzed by UHPLC in order measure the concentrations of salivary OEA, LEA and PEA.



**Figure 1. Study design**



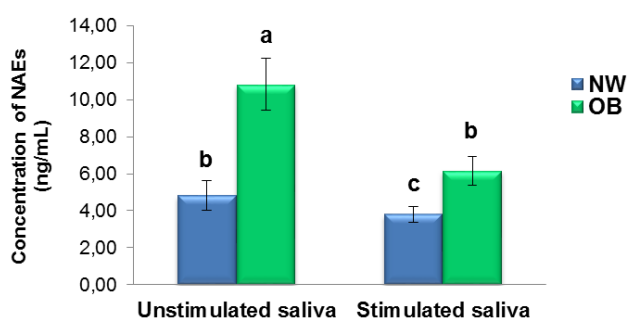
## Results and Discussions

### Subjects

25 healthy subjects, 12 normal weight (NW) and 13 obese (OB) were selected and participated to the study.

### Salivary NAEs in unstimulated and stimulated saliva

Total salivary NAEs (LEA, PEA and OEA) levels were significantly higher in obese subjects in comparison to normal weight subjects (Figure 2) for unstimulated saliva and stimulated saliva. These results are partially in agreement with the study of Matias *et al.* (2012), where it was demonstrated that NAEs are present in human saliva and that their concentration was dependant from the individual nutritional status, higher the BMI, higher the NAEs concentration. This may be due to a decreased FAAH expression and/or activity as a result of obesity or high fat intake (Engeli, 2008). Moreover, the results show in general higher concentrations of NAEs in unstimulated saliva than in stimulated saliva (parafilm mastication). Such difference may be due to the different glands activated during the collection. In fact, unstimulated saliva is secreted mainly by the submandibular and sublingual glands, while the stimulated saliva is secreted by parotid glands. The type of stimulation is an extrinsic factor that has a great impact on saliva flow, composition and rheology (Mosca and Chen, 2017).



**Figure 2.** Concentration of total salivary NAEs in Unstimulated and Stimulated saliva in NW and OB subjects. Different letters on the bars indicate significant difference between types of saliva and individual nutritional status.

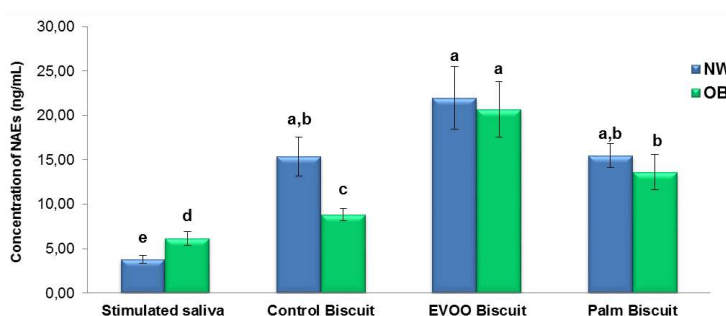
### Salivary NAEs upon food mastication

In all subjects, salivary concentrations of total NAEs increased significantly upon mastication of the biscuits with EVOO or palm oil in comparison with no-food condition (stimulated saliva) and the control biscuits (Figure 3).

In NW subjects no significant effect of biscuit type was found. Conversely, in OB the mastication of EVOO biscuits provided the highest increase of salivary NAEs, followed by palm oil and control biscuit.

These findings were likely linked to the different salivary enzymatic activity as well as mastication behavior of OB vs. NW subjects. It was hypothesized that a higher  $\alpha$ -amylase activity in OB could determine a higher release of NAEs or NAPEs from the biscuit matrix; thus, facilitating the degradation by FAAH. As a result, an overall reduction of NAEs in obese in comparison with normal weight subjects could occur. Regarding to the mastication it was hypothesized a lower mastication rate in obese subjects that could results in a lower extraction of NAEs from the food.

Finally, even though there is no significant difference between NW and OB for salivary NAEs during mastication of EVOO biscuit, a higher concentration of NAEs in comparison with palm and control biscuits was observed. It was hypothesized a role of dietary oleic acid to activate NAPE-PLD and inhibit FAAH, since oleic acid is the most abundant fatty acid present in EVOO (Schwartz *et al.*, 2008).



**Figure 3.** Concentration of total salivary NAEs for Stimulated saliva, Control, EVOO and Palm biscuit in NW and OB subjects. Different letters on the bars indicate significant difference among the samples.

## Conclusions

- OB subjects showed a higher release of NAEs in Unstimulated and Stimulated saliva than NW subjects
- During food mastication higher release of NAEs were found in NW and OB, compared to no-food condition.
- In OB the types of biscuit contributed to total salivary NAEs. While in NW the increase of salivary NAEs was independent from the biscuit types.
- Further studies should be carried out to elucidate the mechanisms underpinning these findings.

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## Effects of process variations on the particle size and rheological properties of ice cream mix as measured on-line and at-line

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The ice cream mix greatly influences final product quality after freezing thus, it is important to understand the mix behavior during its processing. This study evaluated the effects of mix process variations on particle size and rheological properties using conventional and novel analytical equipment. On-line and at-line measurements were employed to characterize the mix at different process stages. Process parameters to be used for ice cream evaluation were generated from the results of this study.

*Confidential topic*

# An Explorative study into growth strategies used for developing a large-scale niche dairy Industry in Ireland.

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## Introduction

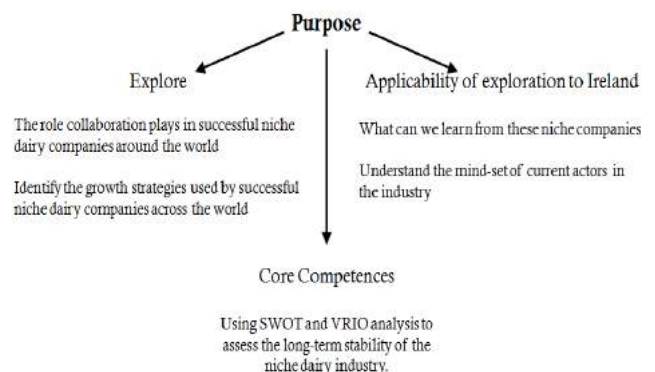
The Irish dairy industry has seen significant growth in recent times. It is also seen as one of key drivers in the Irish government's plans to grow food and drink exports. However, there is an environmental cost to this. The niche dairy industry has been stagnant for years. This project looks at the potential for the niche dairy industry to grow and resulting in higher economic impact but lower environmental impact compared to the conventional dairy industry. Niche milk in Ireland refers to organic cow, sheep and goat milk.

## Research objectives

**Research question: How can the Irish niche dairy industry be developed and grown?**

What core competencies are necessary for its long term success?

What learnings can we get from successful niche dairy companies around the world?



## Methodology

**Theoretical Framework:** Help the researcher and reader develop their own knowledge and understand the fundamentals behind this paper from theories surrounding different growth strategies to business analysis techniques.

**Literature Review:** Familiarisation with previous work done in the area, gather data on nutritional, environmental information to niche milk production and information surrounding the topic of product and packaging selection.

**Document Analysis:** Gathering relevant information on the companies studied in this thesis in order to decipher the different business strategies used and the level of collaboration seen in their business.

**Market Research:** Analysis of consumer trends and needs in order to understand if there is a need for these niche products in the market. Identify potential markets and products for the industry to develop.

**Interviews:** Conducted in person, over the phone and via email in order to obtain insights from relevant stakeholders and experts in the industry.

## Results and discussions

Summary table of the document analysis is shown below. There are a number of different companies analysed during the research. This provided a broad view on how niche dairy companies achieved their growth.

Market penetration does not play a large role in the niche dairy industry as it would lead to profit margin reduction. It is much more favourable to diversify the product offering or develop new markets for the products.

There are a number of different external growth strategies used in the niche dairy industry. Due to main benefits such as shared risk, in-

creased flexibility and market reach, access to new resources such as staff, technology and finance. Working with external growth strategies in a lot of cases, provides the framework that makes the niche dairy industry viable and a realistic alternative to conventional dairy.

Company		External Growth Strategy	Internal Growth Strategy
Dairy Co-Op	Goat	Merger	Market Development
Spring Milk Co.	Sheep	Joint Venture	Market Penetration/ Market
Goat Powder B.V.	Milk	Joint Venture	N/A
Advanced Food Concepts	Con-	Joint Venture	N/A
Epicom		Strategic Alliance	N/A
Little Milk Company		Strategic Alliance	Market Development
Blue River		Acquisition	Diversification
Alimenta S.R.L.		Acquisition	Diversification
Emmi Group		Acquisition	N/A

The SWOT and VRIO analysis proved that a large scale, niche dairy industry in Ireland has the capabilities to enjoy a sustained competitive advantage over its international counterparts. This was mainly due to the low-cost pasture agricultural practices in Ireland, the rarity and difficulty to imitate the products and the skills and expertise in Ireland are world class in the dairy industry.

There are numerous consumer trends and needs to which the niche milk products could align with this enhances their value and improves the chances of consumer acceptability. The main trends and needs spotted were"

sportification", trustworthiness of brands, on-the-go packaging and dairy intolerances.

The interviews highlighted that there is an appetite for a large niche dairy industry at all levels of the value chain. The main hurdles will be seen at the secondary processing level where a significant investment needs to occur so that the proper products can be developed that cater for export markets and meet customer requirements.

### Conclusion & Future research

It is undeniable in the researcher's opinion that Ireland is missing out from a purely economic perspective the potential to grow a significant niche dairy industry. It would provide another viable farming enterprise for small landowners throughout the country who are struggling with the lack of profitability of other farming enterprises such as beef and sheep meat farming.

Collaboration plays a huge role in many ways of life but especially in the niche dairy industry as it allows the actors to benefit from the economies of scale that most of the conventional dairy companies profit from

The research conducted on the various growth strategies deployed by various companies in the industry provided a value insight into how the Irish niche industry could grow. Especially, the New Zealand companies who are operating in a similar environment.

The environmental sustainability of the niche dairy industry was not as good as previously thought. The impact may be negated in the future once the farming systems are optimised to Ireland's farming landscapes.

Any interested parties should lobby for government support for this project; there is significant industry ready for Ireland to enter. The government support should allow the early

adopters to fast-track their way to creating a viable, sustainable business.

A niche dairy association needs to be developed for each of the Goat, Sheep and Organic cows in Ireland and these associations could collaboratively work together.

Further research needs to be completed with regards to the uniqueness of specifically Irish produced niche milks and the consumer benefits that go with these products and on the environment sustainability of these niche industries in an Irish agricultural setting and how to improve their rating by optimising farming and feeding practices.





## Competitive intelligence analysis of the dairy Light category to drive nutrition superiority in Product Design

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Unilever Germany GmbH

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PhD. Alexis KLEIN

PhD. Marine MASSON



The Diet and Light markets across food category are under dramatic challenge and transformation driven by the wish of the millennial generation for more natural and healthy propositions. Among dairy light category, products using artificial ingredients, whether it is sweeteners, or colorants are left aside. Keeping a product low in calories, while using only natural ingredients, is a challenge for innovation teams and product development in particular.

The aimed of the study was to develop an efficient methodology that will allow the R&D teams to get support during the New Product Design phase by identifying the big trends in the light categories on claims, ingredients and proposition, by selecting relevant database and sources on that matter; Unveil gaps in the existing proposition among dairy category or trendy ingredients not used today that will allow maintaining the low caloric range in the product. The insights obtained during this project will be of great value to Identify New Product Design opportunity that can fit with the most promising tracks.

***Confidential topic***





## Folding carton and internal printing A technical approach to consumer differentiation and food safety

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### Introduction

Packaging can be used to offer to consumers a complete brand experience. The emotional level enhanced during opening moment make consumers engage with the product and therefore, with the brand. Brands can firstly use printing to stand out at the point of sale and can use internal printing to enhance consumer experience at home.

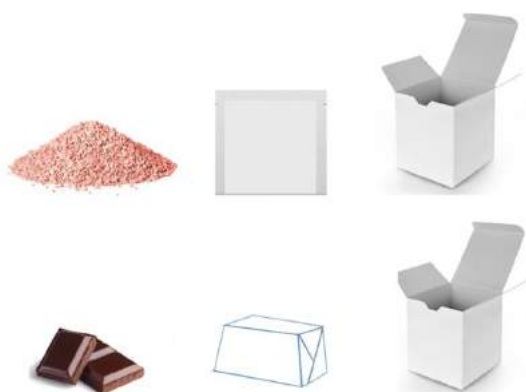
Internal printing allows to design a new experience for the consumer based on innovative decorating solutions printed on the internal side of the product. In the FMCG Industry, brands can firstly use printing to stand out at the point of sale and can use internal printing to enhance consumer experience at home. The development of internal printing on a packaging must be evaluated considering all the external and internal aspects involved in the topic. It is compulsory to consider from the beginning the intended end use for the consumer unit, to identify and control the opportunities and risks. This includes, for instance, the material specifications, the food product and the utilized printing inks.

Internal printing needs to be analyzed through the lenses of three pillars for a right execution: technical feasibility, consumer perception and safety. This thesis evaluates the necessary work to achieve internal printing, asks what do consumers think about internal printing and evaluates how to do it in a safe way. Based on the findings of this thesis, Unilever will have a better understanding of opportunities and constraints on internal printing.

## Research objectives

The purpose of this master thesis is to offer recommendations of the most technically feasible alternatives to present an attractive food packaging with internal printing in a safe way. The research objective was achieved considering a three-pillar perspective: feasibility, safety and perception.

The thesis evaluates the necessary work to achieve internal printing, using offset technology, on a board based consumer unit pack containing multiple individually sealed aluminum based sachets or unsealed aluminum based wrappers. The formulation of the food product to be considered is not strictly mentioned, therefore, the packaging solution proposed applies for a range that goes from a dry powder product to a fatty paste product.

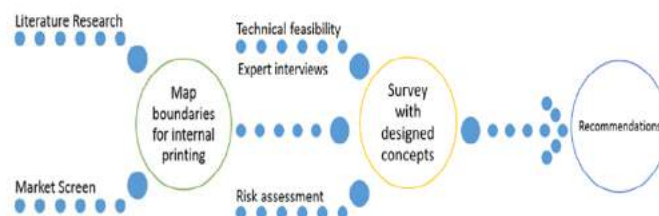


**Figure 1. Packaging systems considered for the study.**

## Methodology

The research process carried out in this thesis is designed by secondary and primary researches. The secondary research considered an extent literature research on the topics regarding the three pillars with a special focus on regulation frameworks. On the other hand, the primary research consisted on an initial market screening and interviews with an ink supplier, convertor, risk assessment expert and a consumer insight expert. Additionally, data was collected through one risk assessment and three feasibility assessments and then translated into insights to be used on a final consumer

perception assessment, where innovation tools helped to understand consumers' thoughts about different internal printing technical concepts through an online survey



**Figure 2. The research process**

## Results and discussions

### Results from Safety assessment

For the individual sealed aluminum based sachet scenario, it can be considered that a well-sealed sachet can guarantee the protection against migration. Regarding the unsealed aluminum based wrapper scenario, gas migration may occur if fractures on the wrapper film happen. For this thesis, corner fractures were analyzed through microscopy on 23 preselected samples to assess the effect of packaging process on the material and consequently, a possible negative impact on its functional barrier in the folded corners. Fractures were found in some of the corners, with aluminum ruptures from 520  $\mu\text{m}$  to 2250  $\mu\text{m}$ .

From an overall risk management perspective, it is recommended to run an overall migration test to determine the impact of fractures with the potential migrant substances of the ink. Even though both inks claim low migration properties and a mineral oil-free formulation, the safety of the food product needs to be assessed. The food product formulation is as relevant as the packaging materials used, so only by having full visibility of all production steps and the intended end use, a relevant assessment can be done. This must be assessed individually case by case considering its specific situation and the

Regulation needs, for instance, Regulation 1935/2004 in the European Union.

### Results from Feasibility assessment

A comparative technical short run test of two offset inks was run to get an initial feasibility insight. A potential direct food contact ink and a low migration not-intended food contact ink were printed on both side of the board in the same machine, the test proved that both inks show a similar machine behavior, the potential direct food contact ink achieved technically feasibility. The uncoated internal side of the board and the ink ingredients properties impact the final appearance on the substrate. A color space assessment showed differences between the potential direct food contact ink application on the inside and the low migration ink application on the outside. A final Robinson test showed a non-perceptible transfer of odor from the inks.

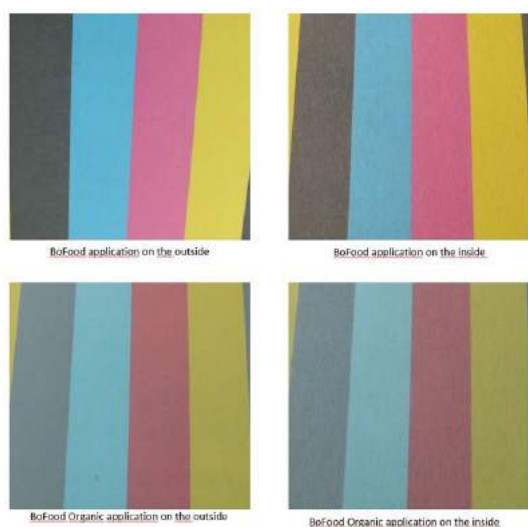


Figure 3. Inks applied on both sides of the boards

### Results from Perception assessment

The collected technical feasibility insights from the market screen and the feasibility assessment were used to develop a morphological chart to create technical artwork concepts. These concepts were then used on an online survey through a prototype. After collecting 123 surveys, it has been shown that internal printing has a positive impact on consumer perception. Additionally, an insight showing a pref-

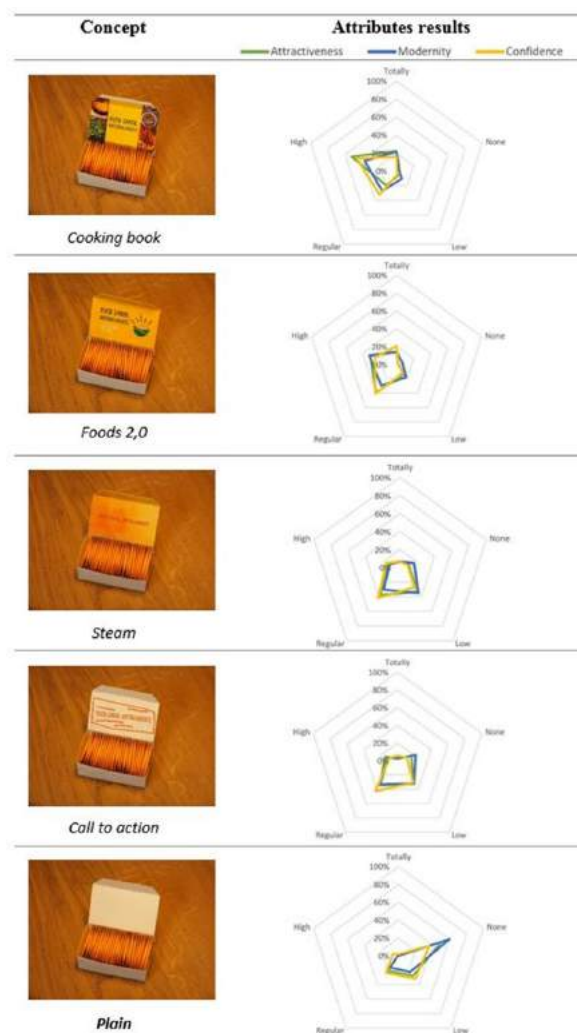


Figure 4. Attributes results from survey

		Excellent	Great	Good	Number of answers
	Cooking Book	64	23	9	96
	Foods 2,0	42	59	13	114
	Steam	13	27	64	104
	Call to Action	2	9	24	35
	Plain	2	5	13	20

1st place  
 2nd place  
 3rd place

Figure 5. Preference results

erence for a completely printed surface on the internal printing was obtained.

## Conclusion

To ensure consumer safety of the product, each execution needs to have an in-depth assessment and an overall migration test is recommended. The integrity of the primary pack needs to be ensured in case a direct food contact has been identified as a consumer safety risk. Regarding regulations, it could be shown that there are specific regulatory details which varies depending on the region and country where the final good is to be sold, however, the basis to achieve safe food products for consumption is the same all around the world.

An initial market screening showed that internal printing already exists in different food products with different artwork designs. It has been observed that most of the products were multi-pack: several consumer unit per selling unit. The initial feasibility assessments during this thesis are quite promising: internal printing is achievable under the current specifications of the packaging materials. This thesis only considered the offset printing technology and a technical assessment for a direct food contact intended ink and a low migration ink for food products, both printed on the same board. The technical insights regarding final appearance need to be considered by Unilever for their further research.

Thanks to the innovation tools used for the artworks concepts creation, the survey was useful to achieve the purpose of the thesis and even more, a robust set of recommendations were offered to Unilever for further discussions. The technical developed concepts pointed out that consumers are more likely to have an internally printed folding carton, specially a fully multicolored printing, increasing the consumer positive perception. In this way, Unilever can offer differentiation on their products.

This thesis only considered a specific series of conditions over the vast world of possibilities and solutions the Packaging Industry offers.

Five years ago, internal printing was limited to some technologies (e.g. inkjet) on some substrate and was not available for every food category, for instance, it could only be found on cereal boxes containing a serial number. Nowadays, internal printing is frequently used in the food industry, this allows a conclusion: there is value of creating an enhanced experience through inside printing which is relevant to the printing ink industry, who is working on safe solutions, to brand owners, who invest into internal printing while ensuring consumer safety and the consumer, valuing the enhanced experience by repeatedly purchasing those products and/or willing to pay a higher price.

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## Cottage Cheese Optimization

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This study is divided in two parts; in the first part, it was desired to see the effect on some coagulations parameters like; coagulation time, firmness and time to reach the cutting pH, when milk protein content is standardized versus non-standardized milk. The objective with this first part was to study the reproducibility of the process.

The second part of the study was the adaptation of the cottage cheese recipe, in order to achieve a successful downscale of the process. Trials were done focusing in the cutting, cooking and agitation parts, being those the most automatized steps of the cheese making process.

***Confidential topic***





## Enzymatic Processes to Create Added Value Ingredients in Early Life Nutrition Product

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Cow's milk-based infant formula is the first alternative when breast milk is not available. Cow's milk, however, hardly contains an essential component of human milk — oligosaccharides, which are thought to make a special contribution to the development and natural protection of an infant's health. Therefore, oligosaccharides are added to infant formula to mimic the beneficial effect of human milk oligosaccharides.

Oligosaccharides are carbohydrates, containing a small number of monosaccharides linked by glycosidic bonds, widely used in food industries. Mixtures of oligosaccharides with different degrees of polymerization and linkages are usually formed in the enzymatic processes.

The aim of this project was to gain insight into the enzymatic synthesis of oligosaccharides. Prior to the enzyme-catalyzed bioconversion, various methods of extracting enzyme from bacteria were investigated and enzyme harvested after mechanical or chemical treatment was characterized with respect to their response towards temperature and pH changes. Further on, a better understanding of the mechanism behind the oligosaccharide production using the enzyme was obtained through analyzing different factors that could possibly influence the reaction. By adjusting reaction conditions and altering source of enzyme, the yield of oligosaccharides can be significantly affected.

***Confidential topic***



## Comprehension of the pH effect on the degradation kinetics of Vitamin C during the heating of a liquid model solution

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The essay endeavors to investigate the kinetics behavior of ascorbic acid and its oxidized form dehydroascorbic acid due to the pH effect in liquid model solution during heating. Validation of analytical technique for real-time simultaneous quantification of AA and DHA by UHPLC-CAD was the first objective followed by data acquisition in order to test the previously set hypothesis concerning the possible degradation pathways of AA and DHA and their link to pH variation. Finally, identification of kinetic parameters and building of further predictive model was performed.

Degradation kinetics were tested at 70 °C for all the experiments using pH 2; 3.8; 5 and 8 buffer solutions containing 1g/L of ascorbic acid. The behavior of AA in lower pH such as 2 and 3.8 showed higher stability than when kinetic experiments were performed at pH 5 and 8.

First rate kinetic model fitted very well and the rate constants  $k_{app}$  and  $k_2$  were identified. As firstly observed the  $k_{app}$  was higher for pH 8 and 5 being  $1,70 \times 10^{-4}$  and  $1,47 \times 10^{-4}$  respectively, while for pH 3.8 and 2, values of  $9,11 \times 10^{-5}$  and  $8,33 \times 10^{-5}$  were obtained. Regarding the  $k_2$  for DHA, value of  $2,62 \times 10^{-4}$  was calculated only for pH 3.8 as for the rest of the pH the concentration detected was very low.

### Introduction

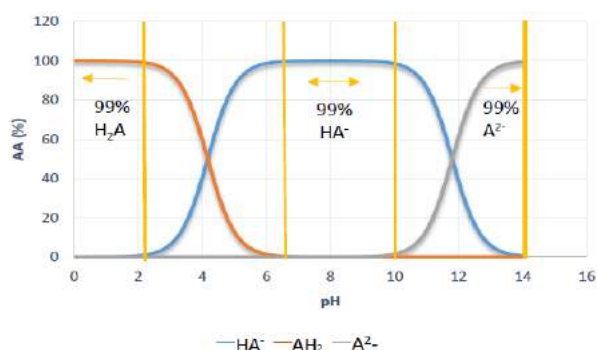
Vitamin C, with all its nutritional and functional properties is presented by L-



Ascorbic acid (AA) including its oxidized form L-dehydroascorbic acid (DHA) represented by 80% bioequivalency (Barrett & Lloyd, 2012). It is classified as one of the most important nutritional factors and known as global quality marker for processed food. It is used as additive to prolong shelf-life of products due to its anti-oxidant capacity. Effect on the stability of AA and DHA means effect on the quality of food products such as fruits and vegetables and juices causing color and flavor deterioration during processing and storage.

Two main degradation pathways of AA are classified into oxidative and non-oxidative (Yu *et al.*, 2013; Yuan & Chen, 1998). Oxygen, temperature, metal ions, light and pH can influence the degradation. The stability of AA in food matrix has been found to be different than the one in simple media due to the presence of other factors that enhance its degradation.

Due to the pH effect, different forms of AA are formed (Fig 1). The different reactivity of these forms leads to different proposals found in the literature regarding the degradation pathways and the formation of DHA. Bradshaw *et al.* (2011) proposed that the only reactive form is the ascorbate ion which is the one involved in the formation of DHA.



**Fig.1. Formation of different forms of AA due to pH effect**

In order to test this hypothesis, good method of quantification is required for AA and DHA in liquid model solution with different pH. Recently in literature, charged aerosol detection (CAD) paired with liquid chromatography has been tested due to its high sensitivity for small polar molecules such as AA and DHA (Novakova *et al.*, 2009). This method is characterized by fast analysis unlike other detection methods such as UV-spectrophotometry that involves reduction of DHA to AA prior to analysis due to its inability to detect DHA in UV above 210 nm, in which AA is detected.

**The aim of this study is to answer to hypothesis made by Bradshaw: the ascorbate ion is the most reactive and the only one from which DHA can be formed.**

### Research objectives:

1. Develop and validate a new method for short time analysis by UHPLC-CAD for combined detection of AA and DHA.
2. Acquire data for the degradation of AA and formation of DHA.
3. Build model of degradation for the chosen conditions.

### Methodology:

#### 1. Kinetics experiment

Degradation kinetics in laboratory reactor system with buffer solutions of pH 2; 3.8; 5 and 8 at 70°C and [AA]<sub>0</sub> = 1 g/L during 300min, sampling every 30.

#### 2. AA and DHA determination by HPLC-UV

Sample preparation: dilution in 1/50 MPA for stability and reduction of AA to DHA prior to analysis.

#### 3. Modelling tools: Microsoft Excel®.

## Results and discussions:

### 1. Validation of a new method UHPLC-CAD for detection of AA and DHA in buffer solution

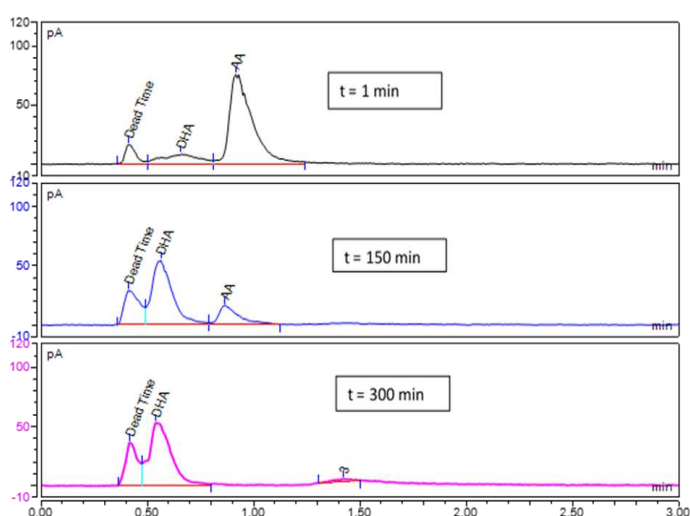
- Preliminary experiments:

Test of different parameters such as flow rate, organic solvents in mobile phase, different time of analysis, integration methods, analytical column and change of pH adjusting agents for the buffer solutions in order to increase detectability and quality of peaks.

- Calibration curves for AA and DHA for pH2 and pH 6

Calibration curves were made in triplicate to prove its repeatability. The nature of the curves was found to be quadratic and validation of the curves was performed successfully obtaining a good recovery of the concentration when the measured one was compared to the theoretical concentration. Further on. LOD and LOQ were calculated for both AA and DHA.

- Application of the method for detection of AA and DHA degradation kinetics in malate buffer



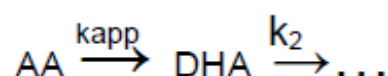
**Fig 2. Chromatograms of degradation kinetics of AA solution (1 g/L) in 20 mM malate buffer pH 3.8 adjusted with  $\text{NH}_4\text{OH}$ .**

The results obtained for  $t = 1$  min show AA peak with high intensity as expected,  $t = 150$  min the intensity of DHA peak is increasing and decrease of AA peak, as expected. However at 300 min, DHA peak was still present, while increasing the intensity of the dead time peak was as well observed, no AA was detected as expected.

It was concluded that it was not possible to use this method for the kinetics purpose, data acquisition for degradation kinetics was performed using HPLC-UV.

### 2. Degradation kinetics of AA and DHA in simple model medium.

AA degradation pathway is presented as following:



where  $k_{app}$  is apparent degradation constant influenced by constant oxygen and temperature while  $k_2$  is the degradation rate of DHA.

First order reaction was observed, as usually described in the literature for the degradation of AA for all the pH environments.

The rates of AA and DHA regarding the simple apparent model are presented as following:

$$\frac{d[\text{AA}]_t}{dt} = -k_{app} \cdot \text{AA}$$

$$\frac{d[\text{DHA}]_t}{dt} = k_{app} \cdot \text{AA} - k_2 \cdot \text{DHA}$$

Due to the variability in  $[\text{AA}]_0$  identification of standard  $[\text{AA}]_0$  by fitting the experimental data to first order kinetics equation.

## 2.1. Simple apparent model for ascorbic acid and dehydroascorbic acid degradation in pH 2; 3.8; 5 and 8.

After the standardization step, modeling of the data of AA and DHA for each of the experimental conditions was performed and the kinetic rate constants were calculated (Table 1) by minimizing the difference between the standardized data and the model

**Table 1 . Determination of  $k_{app}$  and  $k_2$  for first order model of AA degradation.**

pH	$k_{app}$	$k_2$
2	8,33E-05	Not identified
3.8	9,11E-05	2,62E-04
5	1,47E-04	Not identified
8	1,70E-04	Not identified

From the observations it could be noticed that the degradation is higher at pH such as 8 and 5 while for pH 2 and 3.8 lower degradation was detected. Higher stability on lower pH can be explained by the predominance of the fully protonated form same reported by Herbig & Renard (2016). The highest degradation rate for pH 8, in which  $AH^-$  is the only present form shows that the hypothesis by Bradshaw regarding the highest reactivity of this form is correct and also complies with the findings in the literature reporting highest instability of AA on this pH. (Rogers & Yacomini, 1971; Wilson et al., 1995; Rojas & Gerschenson, 1997; Yamauchi et al., 1993). However, different results were observed for the formation of DHA. Low, almost no DHA was formed at pH 8, which is opposite of Bradshaw's hypothesis where the highest formation of DHA was expected to be formed.

The same was observed for pH 2 and 5 while for pH 3.8 different behavior of DHA was ob-

tained where DHA concentration was observed to be higher or more stable than in the rest of the pH, therefore  $k_2$  was identified only for this pH.

Two possible explanation can be that no DHA is formed in the other pH or that the stability of DHA at those pH is very low and its degradation rate is as fast as its formation rate.

## Conclusion and Future Perspectives:

**Successfully completed the three objectives.**

Regarding the first objective it can be concluded that the new UHPLC-CAD method can be used for the purpose of detection of AA and DHA as pure compounds in liquid model solution as it was validated for its repeatability and accuracy. However, the method cannot be applied for the kinetics purpose due to retention of other AA degradation compounds at the same time as DHA. Due to the inability of the column to better separate the small compounds.

As further work to be performed could be testing the retention and detectability using another column.

Concerning the pH effect on the degradation kinetics it could be concluded that the experimental data fitted perfectly for first order kinetic model, where higher stability was observed at lower pH (2 and 3.8) than in higher (pH 5 and 8) when exposed on temperatures as high as 70 ° C.

Identification of  $k_{app}$  was successful for each pH while for  $k_2$  was only identified for pH 3.8.

The difficulty to conclude its behaviour in the other pH environments is due to its low levels of concentration. As possible explanation can be the high instability of

DHA in these pH environments or the fast degradation rate similar to the formation one.

As future recommendation can be considered performing kinetic analysis starting with DHA as initial compound. This could enable the chance to better observe DHA behaviour in different pH. Besides, anaerobic degradation of AA where direct cleavage of the ring( decarboxylation) and no formation of DHA was reported in the literature from Yuan & Chen (1998). It could be interesting to check if the kinetic rate of this cleavage is affected by the pH.

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## Exploring the last phases of product development: From kitchen to plant production

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### Introduction

An increasing demand for artisanal and craft products has been observed in recent years, this trend also reaching the alcoholic beverage market. This led to an undeniable growth in the popularity of craft and specialty beers, with an explosion of demand for variety in the brewing sector. Consumers show an increased interest in exploring new and exciting flavors, taste and aroma being the main driving factors when trying a new brew ("Scarlet Lane Brewing Company: Carving Out a Niche in the Craft Beer Market," 2016). The popularity of craft brewing is largely linked to the diversity and uniqueness of these beers. Consumers are stepping away from mass-produced, mainstream beers and are in search of new options that are exciting and can express their taste, style and individuality ("Craft Beers in the US & UK Case Study: How Mainstream Brewers can Benefit from the Trend Towards Craft Beers," 2011). Besides the popularity of craft brews, ginger and ginger flavored products have also been received with growing interest and excitement both in the form of beverages and other food products, expert even going as far as calling it "the ubiquitous flavor of the moment" (Black, 2011). Thus, the emergence and quick growth of the alcoholic ginger beer market does not come as a surprise.

However, the production of alcoholic ginger beer has been very little researched, thus

the literature available regarding this topic is very limited. This thesis aims to fill this gap by exploring the final stages of the development process of a new alcoholic ginger beer, identify the difficulties encountered during production and evaluate various solutions to overcome them. It is often disregarded that production upscaling can lead to unexpected challenges, an adaptation of the processing method to the new processing conditions being necessary.

## Objectives

The main objectives of this study were:

- To identify the challenges encountered during the upscaling of ginger beer production from kitchen scale to microbrewery level.
- To find and propose alternative processing methods in order to overcome said challenges, while maintaining a feasible production.

## Methodology

An Action Research strategy was used in this study which is a solution-driven process meant to give a deeper understanding of a specific situation where the researcher is given the freedom to intervene, carrying out different actions (Hart, 1996). In the first stage of this process, the problem is identified and explored for a deeper understanding, followed by an action-intervention from the researcher. The effects of this intervention are then observed and evaluated (Mertler, 2009). Following the Action Research strategy led to the elaboration of a process composed of eight different cycles and within each cycle the four stages of: plan, act, observe and reflect were methodically fulfilled. The eight cycles tackled in this thesis were: the optimization of ginger aroma and flavor extraction, the optimization of fermentation, the selection of fermenting yeast (wine/cider/beer),

the selection of beer yeast strain, selection of yeast Inactivation method, the analysis of the product's sensory characteristics by a trained panelist, a plant trial and finally, the analysis of acceptance by consumers. All these cycles were chosen in order to overcome the challenges of production upscaling.

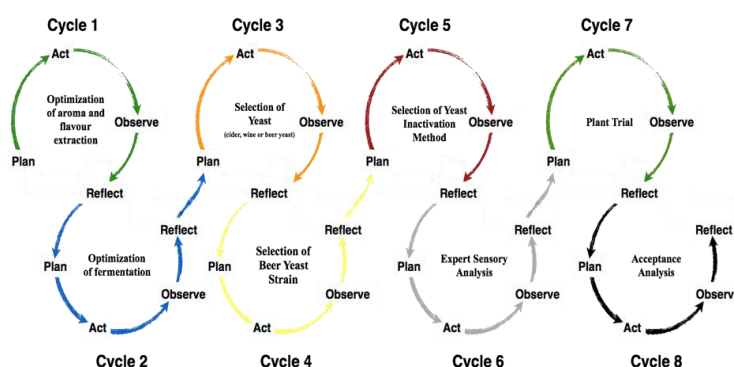


Figure 1. Cycles of the action research used in the context of this thesis

## Results and discussions

First, the main challenges brought on by the upscaling of ginger beer production have been identified as:

- the high risk of clogging due to frequent transfers of the ginger beer wort, containing ginger and lemon solids;
- the reduction of boiling time in order to minimize the usage of the kettle, which is one of the bottlenecks at the microbrewery and largely influences the flexibility of production;
- the difficulties of yeast inactivation for the production of a safe and stable product with a long shelf life;
- the selection of the yeast strain, which had to be readily available for purchase in larger quantities and it had to develop a balanced flavor profile while ensuring a short fermentation time.





**Figure 2.**  
**Solids captured**  
**during filtration**

After the challenges of upscaling have been identified, various measures were recommended to be carried out to overcome them, such as reducing the risk of clogging by eliminating the necessity of transferring the ginger beer wort from the kettle to the fermentation tank. This was achieved by implementing a different extraction method, where the extraction of flavor and aroma compounds from the ginger and lemon solids took place directly in the fermentation vessel. Furthermore, the time spent in the kettle was also significantly reduced and the cleaning process was faster and easier, thus making production more flexible. For the inactivation of yeast, bulk pasteurization was chosen as the only feasible and viable option despite a slight change in flavor quality and, finally, various yeasts were tested and a lager yeast was ultimately chosen for the production of a well-balanced ginger beer and an optimal fermentation period.

## Conclusion



**Figure 3. The Final Product:**  
**Sysskonhem Alcoholic Gin-**  
**ger Beer**

By exploring the last phases of the development process of an alcoholic ginger beer through implementing an action research strategy, valuable insights have been gained and a feasible ginger beer production method was elaborated. Furthermore, the advantages and disadvantages of implementing a cyclical development process compared to a linear one were also discussed. This work chronicles the development stages of a feasible production process in an attempt to act as an example and contribute to a better understanding of the process.

## Recommendations for future research and development

The further tweaking of Sysskonhem Alcoholic Ginger Beer recipe by testing of other lager yeasts is recommended in order to improve the flavor profile of the final product, reduce acidity and eliminate the strong yeast flavour. Furthermore, a shelf-life and stability test is strongly recommended to be carried out before the full launch of the product to accurately determine the period of time where the product is sure to maintain its quality and remains safe for consumption.

Besides the recommendations given for the manufacturing of ginger beer, the following topics are suggested to be further studied, thus contributing to the limited literature available on the development of ginger beer: the influence of ginger and lemon particle size on the extraction rate and efficiency; the impact of pasteurization on the flavor profile of ginger beer and identification of flavor compounds; the evaluation of product stability during storage; the exploration of the initial stages of the new product development in the case of ginger beer; and evaluation of the effects of the starter culture on the for-





mation of flavor compounds.

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## Formula Optimization of New Cricket Flour Based Cookies Using Sensory Evaluation

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### Introduction

"Insect food", this term has become familiar but also creepy for the Europeans, since the Europeans don't have a history of eating worms.

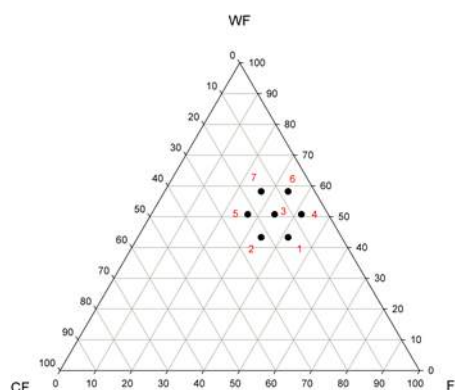
To create an ideal cricket-based cookie product, formula optimization is a common method to evaluate a food product one looks at simultaneously many attributes such as flavor, texture, color, and possibly cost (Gacula Jr., 2008). This is a way to consider different attributes at once and show the weight for each attribute in order to make an ideal product of its class. In order to perform formula optimization, classical methods in sensory analysis can be participated in, in this thesis, focus group, check-all-that applies (CATA) and a simple ideal profile method were considered.

### Aims

The main aim of this thesis, was to develop and optimize new cricket-based cookies using formula optimization, by changing the mix proportion of cricket flour, wheat flour and butter. A secondary aim was to investigate the Italian consumers' readiness to adopt insect-based food and to understand the expected acceptance of the new cricket-based cookies by showing their images to consumers.

### Materials and methods

Wheat flour (WF), cricket flour (CF) and butter (F) were chosen to be the independent variables in mixture design with a total fix proportion of 67%. Other ingredients including cocoa fiber accounted for 37%. (Fig. 1)



**Figure 1. Cookie formulas with variable ingredients**

Formula design, focus group interviews, check-all-that-apply (CATA), simplified ideal profile method (IPM) and ideal formula optimization were tools for the sensory evaluations. Google Forms were used for the consumer audit (Fig. 2).

## Results and discussions

### 1. Formula Optimization

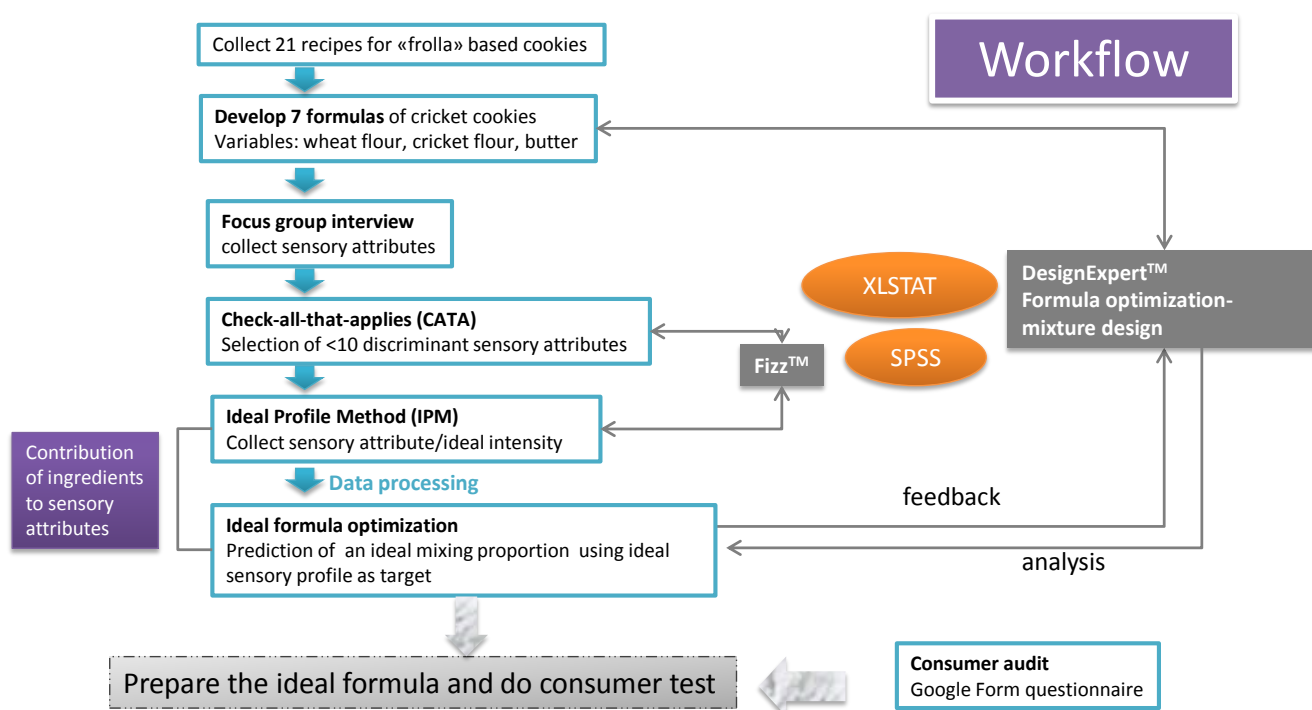
In CATA analysis 10 discriminant sensory attributes were chosen (Table 1) to proceed to IPM. Samples with the same formula showed consistency in sensory perception.

The results of principal component analysis (PCA) of the data in the matrix formulations x sensory attributes and ingredients (as supplementary variables) are shown in Fig. 3. The ingredient cricket flour is positively related to attributes which were darkness, bitterness, cricket flour flavor and umami; negatively related to sweetness. Butter is positively related to oiliness, friability and butter flavor; negatively related to hardness and dryness. Friability and oiliness are opposite to hardness and dryness, similar results were shown in CATA results.

Ideal intensities were collected during IPM sessions (Table 1) and were set to be target

**Table 1 Ideal intensity of sensory attributes from consumers participating in the IPM excluding outliers**

attributes	darkness	oiliness	bitterness	sweetness	umami
Ideal score	5,5	3,3	2,5	6,8	1,3
attributes	cricket flour flavor	butter flavor	friability	hardness	dryness
Ideal score	1,7	4,5	6,7	4,2	2,9



**Figure 2. Workflow of experiments and analysis**

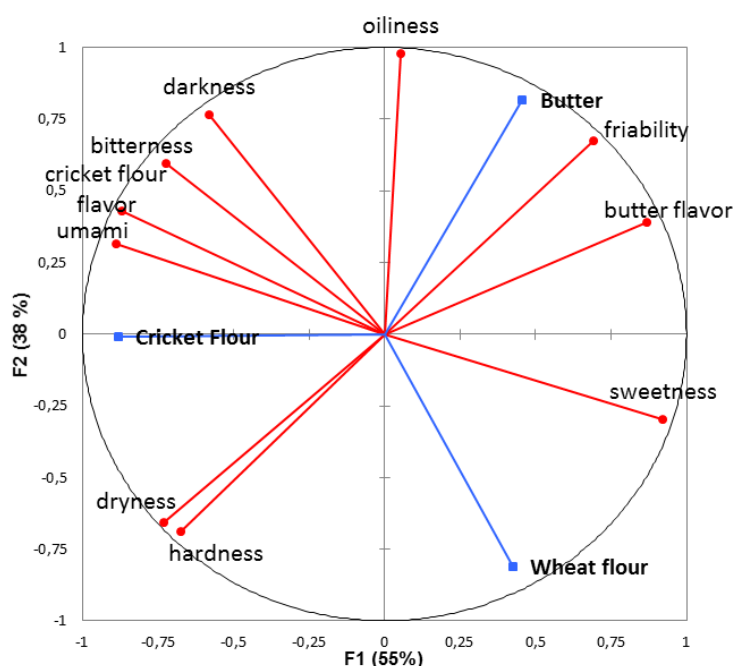


Figure 3. PCA result of IPM

value for optimization in software Design Expert.

A predicted formula with highest desirability 0.6 (max=1) were shown in Table 2 .

Table 2 Predicted formula achieving highest product desirability

Wheat flour (%)	Cricket flour (%)	Butter (%)
37.744	5.413	23.843

## 2. Consumer survey

Consumers who perceive “all insects are disgusting (AIAD)” believe that eating insect is contraindicated, disgusting and not pleasant comparing to consumers who perceive “Some insects are disgusting (SIAD)” and “I am indifferent to insects (IAII)”. However, belief values related to “not expensive”, “healthy”, “nutritious”, and “sustainable for the environment” were not significantly different among the three groups.

When asked about general insect-based food liking, willingness to try, willingness to buy/pay more, and expectation for insect as new protein source, AIAD consumer showed signifi-

cantly low interests. Similar phenomenon was observed when showing our cookie image in the online survey.

## Conclusion

- I. The effects of cricket flour, wheat flour and butter in cookie-based formula design to sensory attributes were defined. A final formula with 0.6 desirability was predicted according to the liking of Italian customers.
- II. Careful selection of the attribute inputs to formula optimization can increase desirability by decreasing possibility of dilemmas.
- III. Perceptions for insects can divide customers for marketing segmentation and consumer education in insect-based food promotion

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## Study to examine iron absorption in a matrix of young child formula

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Iron deficiency is the most common nutrient deficiency in the world. Even though iron deficiency affects large numbers of children and women in developing countries, it is surprisingly a common health problem which still has a significant impact in industrialized countries. Research revealed that iron deficiency even without anemia during early life (0-36 months) can have a long-term adverse influence on neurodevelopment and behavior and those effects might be irreversible. Although milk formula is one of the main food sources during early life and it is fortified with iron, the actual fraction absorbed is low compared with human milk.

Based on in-depth understanding of iron absorption mechanism and factors of influence on iron absorption varying from intrinsic physiological factors to external factors including product matrix, this work was aimed to understand how to optimize the iron source in a format that can enhance its physiological and nutritional benefits. Different iron fortified milk formulas were investigated, and the properties (e.g. binding and release) of iron was tested in the milk matrix in conjunction with static digestion.

*Confidential topic*



## Effect of antimicrobial activation of a compostable package on the shelf life of selected food products

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### Introduction

In spite of modern advances in technology, the preservation of foods is still a debated issue. One major challenge faced by the food industry is loss of food quality as a result of microbial contamination. Microbial contamination reduces the shelf life of foods and has led to recent outbreaks in foodborne illnesses caused by pathogens like *E. coli*, *Salmonella* and *Listeria* (Quintavalla and Vicini, 2002; Jin and Zhang, 2008).

The globalization of food trade and distribution of food from centralized processing has necessitated modern food industry's dependence on a range of preservative technologies to ensure that food is maintained at an acceptable level of quality from the time of manufacture through to the time of consumption.

Chemical additives have generally been used to combat specific microorganisms. But consumers of today are particularly aware of the health concerns regarding chemical food additives as well as the health benefits of “natural” and “traditional” foods, processed with no added chemical preservatives. As a result, consumer demand for high quality, long shelf-life, ready-to-eat foods have increased in the last decades. Food producers are therefore faced with the challenge of finding natural alternative antimicrobial treatments to ensure food safety.

One emerging technology receiving considerable attention is antimicrobial packaging

which involves incorporating antimicrobial peptides like nisin into/on food packages in a way which ensures the package interacts with the food product and the headspace to reduce microbial load (Soares et al., 2009) .

The aim of this study was to investigate the potential to increase the shelf life of selected food products using a compostable package activated with nisin.

### Research objectives

- i. To study the antimicrobial efficacy of 3 different concentrations of nisin in order to select the most appropriate concentration for the study
- ii. To activate a compostable package by spraying with the selected nisin solution
- iii. To evaluate the antimicrobial effect of the activated package on the *Brochothrix thermosphacta* 7R1 inoculated in orange juice, olive brine and mozzarella storage liquid.

### Methodology

#### Selecting the best nisin solution

The antimicrobial efficacy of 3 different concentrations of nisin (0.11 g/ml, 0.20 g/ml and 0.40 g/ml) were evaluated using two methods namely:

Agar diffusion test

Ringer challenge test

This was done in order to select the best nisin solution for study in the food products.

#### Use of the best nisin solution in foods

Four compostable packages were obtained. Three were activated by spraying the surface of their internal walls with the best nisin solution and one was left unactivated to serve as the control.

Each of the 3 food products (orange juice, olive brine and mozzarella storage liquid) was inoculated at a level of  $10^5$  to  $10^6$  with a spoilage microorganism called *Brochothrix thermosphacta* 7R1 after which 1ml was sampled (Ti). The inoculated food product was then divided into four equal portions, one for each of the 4 compostable packages. After pouring into each of the four packages, each of them was swirled and then 1ml of the inoculated food was sampled (T0). All four packages were covered with aluminum foil and refrigerated at 5°C until further sampling.

Serial dilutions were carried out for Ti and T0 followed by pour plating using TSA media. The plates were incubated at 26°C  $\pm$  2 for 48hrs and observed colonies were counted after that.

The orange juice experiment was monitored for 7 days [T0, T1, T2, T24, T48, T72, T144 (day 6) and T168 (day 7)] while the olive brine and mozzarella liquid experiments were monitored for 2days (T0, T1, T2, T20, T24, and T48).

### Results and discussions

#### Selecting the best nisin solution

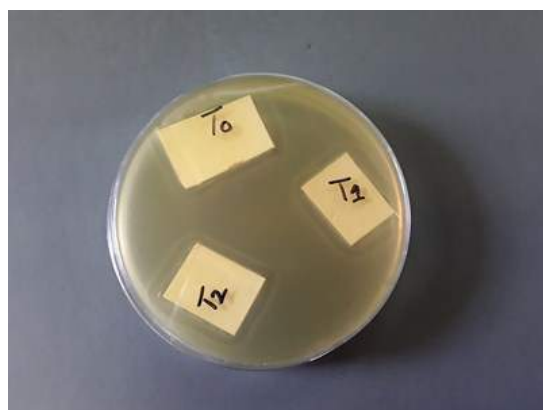
The principle behind the agar diffusion test is that nisin diffuses from the activated surface into the TSA media containing the test microorganism (*Brochothrix thermosphacta* 7R1). The inhibition of the test microorganism results in the creation of an inhibition zone within the vicinity of the activated surface. The bigger and clearer the inhibition zone, the better the antimicrobial activity.

Of the 3 nisin solutions tested, 0.20 g/ml nisin solution had the clearest and biggest inhibition zone.



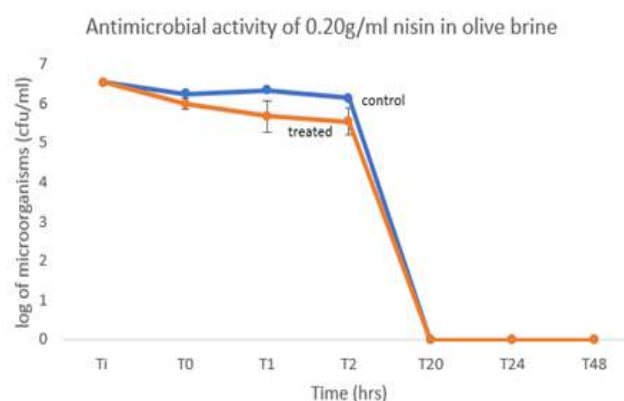
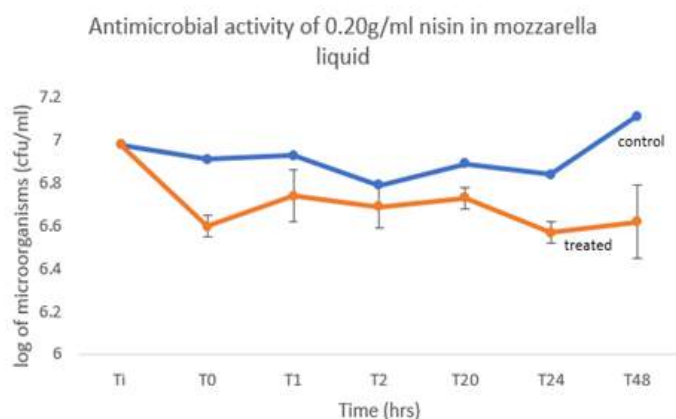
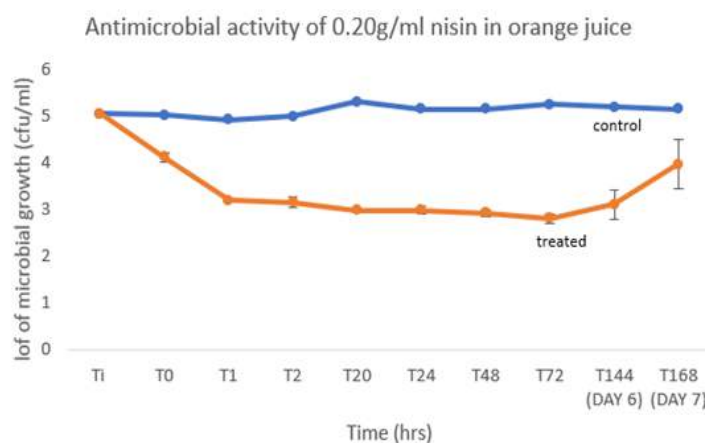
In the ringer challenge test, 0.20 g/ml nisin recorded the highest log reduction (0.20 g/ml = 3.73 > 0.11 g/ml = 3.67, > 0.40 g/ml = 2.49)

Based on both results, 0.20 g/ml nisin solution was selected as the best solution for studying nisin's antimicrobial activity in the food products under study.



**Figure 1: agar diffusion test showing inhibition zone for 0.20 g/ml nisin**

### Activity of 0.20 g/ml nisin in foods



Among the 3 food products, the highest log reduction (2.24) was observed in the orange juice, specifically at T72 after which microbial load increased. Log reduction was less than one (0.36) in the mozzarella storage liquid. Antimicrobial activity could not be followed in the olive brine because a reduction in the level of *Brochothrix thermosphacta* was observed right after inoculation. *Brochothrix thermosphacta* was completely inhibited after T20.

According to Ray and Daeschel, (1992) food related factors like pH, NaCl, proteolytic enzymes and lipid content have an influence on the activity of nisin. In this study, 2 predominant food factors which affected the activity of both nisin and the test microorganism were pH and NaCl content.

For pH, Liu and Hansen (1990) reported that the solubility of nisin decreases with increasing pH, implying that the antimicrobial activity of nisin decreases with increasing pH. Results observed in this study was therefore consistent with this studies since the orange juice which was the most acidic food product (pH=3.7) recorded the highest log reduction while

mozzarella storage liquid which was the least acidic (pH=4.7) recorded the least antimicrobial activity.

Though the pH of the olive brine was slightly more acidic than the mozzarella storage liquid, antimicrobial activity could not be followed because of the inhibition in growth of *Brochothrix thermosphacta*. According to Leroi et al., (2012) *Brochothrix thermosphacta* does not require NaCl for its growth. NaCl concentration of 60-65 g/L can inhibit its growth. It is therefore deduced that the NaCl content of the olive brine was possibly above the tolerable level required for the growth of *Brochothrix thermosphacta* leading to the inhibition of its growth.

### Conclusions and recommendations

The spray coating method used was effective in activating the compostable package.

The activity of 0.20 g/ml nisin was highest in orange juice, mild in the mozzarella storage liquid and impossible to follow in the olive brine because of the inhibition of the growth of the test microorganism.

The activated compostable package was able to exert antimicrobial activity in the orange juice for 3 days. In order to improve the antimicrobial activity of nisin in the orange juice, it is recommended for further studies, that a hurdle technology like pasteurization be combined with the use of the same concentration of nisin. Based on results obtained from the olive brine test, and results from previous studies suggesting that the physico-chemical properties of a food product can affect the activity of nisin and the target microorganism, it is recommended that the antimicrobial activity of the same concentration of nisin on the spoilage microorganisms naturally associated with the selected food products be evaluated.

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## Evaluating the behaviour of probiotic *Lactobacillus plantarum* 299v in non-dairy oat based yogurt using two different packaging materials

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### Introduction

Oatly AB, a Swedish food company founded in the 1990s, produces vegan milk-product alternatives made from oats. It caters to the appeal for veganism and vegan products, which is on a rise. This shift of food preference goes beyond a niche group who avoid animal meat for ethical purposes, towards consumers looking for a cleaner and healthier diet (Lea et al. 2006). With increased consumer awareness in recent years, food products are not only being consumed to satisfy hunger and basic nutritional requirements but also to enhance the quality of physical and mental wellbeing (Siro et al. 2008). This has paved way for the concept of 'functional foods', which include ingredients with additional health benefits or that can support specific body functions that conventional nutrition models do not address (Buttriss 2000; Menrad 2003). Probiotics represent a major segment of this functional food market (Granato et al. 2010).

Probiotics are live microorganisms that are natural inhabitants of the human gastrointestinal tract. The viability of probiotic strains in dairy products like yogurt has been studied extensively, however the research on non-dairy food matrices is limited. Among probiotic dairy products, yogurt has a wider consumer market (Siró et al. 2008) and hence, Oatly's spoonable oat based yogurt called 'oatgurt' was selected as the test product for the experiment (Figure 1). When probiotic strain is incorporated in certain quantities into food matrices and ingested, they can potentially improve the health of the host especially by

contributing to intestinal microbial balance (FAO/WHO 2002; Grajek et al. 2004). The survivability and functionality of the probiotic culture is strain specific and depends on several factors including method of incorporation, temperature (Mokarram et al. 2009), pH, composition of the food matrix and level of available oxygen (Tripathi & Giri 2014)



**Figure 1.** Oatly's vanilla/blueberry oatgurt in polypropylene cup.

## Objectives

This study aimed to investigate the behaviour of a probiotic strain obtained from Probi AB, *Lactobacillus plantarum* 299v (*L. plantarum* 299v), in Oatly's non-dairy oat-based yogurt called 'oatgurt'. The food product selected was Oatly's blueberry/vanilla oatgurt (Figure 1).

- ⇒ To study the viability of *L. plantarum* 299v in oatgurt
- ⇒ To evaluate the change in the physico-chemical properties (pH and colour) of the probiotic oatgurt during storage.
- ⇒ To evaluate if incorporating *L. plantarum* 299v into oatgurt packed in polypropylene (PP) cups would be viable for Oatly

The two main influencing factors included how the strain was incorporated into the oatgurt and presence of oxygen in the packaging units.

## Materials and methods

The viability of *L. plantarum* 299v (Probi AB, 2017) and changes in the oatgurts' physico-chemical properties (pH and colour) was studied with respect to the two main influencing factors. Each factor had two levels that was analysed (Table 1).

### Factor 1: Step of probiotic strain incorporation into the oatgurt

**Fresh Oatgurt:** *L. plantarum* 299v was added along with starter culture and fermented together. This was prepared without other additives like the stabilizers and fruit.

**Commercial Oatgurt:** *L. plantarum* 299v was added directly into ready-to-eat flavoured finished vanilla/blueberry oatgurt, post fermentation and cooling.

### Factor 2: Presence of oxygen in packaging unit during storage

**Polypropylene cups:** The packaging unit currently used at Oatly are 1mm thickness polypropylene (PP) cups. They have an oxygen transmission rate (OTR) of around 150-200 ml/m<sup>2</sup>.day.atm (Buntinx et al. 2014). Thermosealable aluminium foil was used to seal the cup. Analysis of the gas in the headspace of sealed PP cups over time showed a gradually increase in O<sub>2</sub>%.

**Glass jars:** To evaluate the effect of oxygen that is present in the PP cups, glass, which is impermeable to oxygen (Jayamanne & Adams 2004) was used as a packaging unit for comparative analysis. Glass jars were sealed using heat sealed metal screw caps that maintained anaerobic condition inside the unit.



**Figure 2.** Manually packed commercial and fresh oatgurt samples in PP cups and glass jars.

The four samples were: Commercial oatgurt in PP cup, PPcomm; commercial oatgurt in

**Table 1. Experimental design with two different packaging materials and food matrices.**

Food Matrix	Polypropylene				Glass			
	Viability	pH	O <sub>2</sub>	Colour	Viability	pH	O <sub>2</sub>	Colour
Commercial Oatgurt								
Fresh Oatgurt**								

\*\* prepared without additives (colour, flavour, stabilisers)

glass jar, Gcomm; fresh oatgurt in PP cup, PPfr; and fresh oatgurt in glass jar, Gfr. The samples were prepared and packaged manually (Figure 2). They were stored at 8°C in a closed incubator with no light. Different tests (Table 1) were carried out for every sample in triplicates once a week for a period of eight weeks and analysed.

**Viability of *L. plantarum* 299v:** To study the viability of the four samples, standard spread plate method was used (NMKL 140). The samples at different dilutions were spread on solid MRS agar and incubated at 37°C for 48 hours followed by the enumeration of the colony forming units (cfu).

**Oatgurt pH:** The change in pH over time was evaluated to understand probiotic strain behaviour in all four samples.

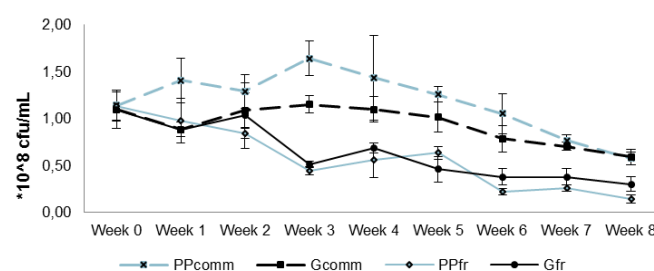
**Colour Stability:** The colour stability was measured using Spectrophotometer-CM Food (Konica Minolta Colorimeter, Japan). L\*a\*b colour system was used and the change in visual perception,  $\Delta E^*$  was calculated.

## Results and discussions

As a food matrix, the oatgurt manufactured by Oatly AB appeared to be suitable for probiotic strain incorporation. It is rich in beta-glucan that is known to be a prebiotic (Mårtensson et al. 2002) and has a pH of 4.2 that most probiotic strains are tolerant to.

**Viability:** In all four samples, PPcomm, Gcomm, PPfr and Gfr, the strain viability from an initial concentration of  $1.14 \pm 0.16 \times 10^8$  cfu/mL remained above recommended dosage of 107

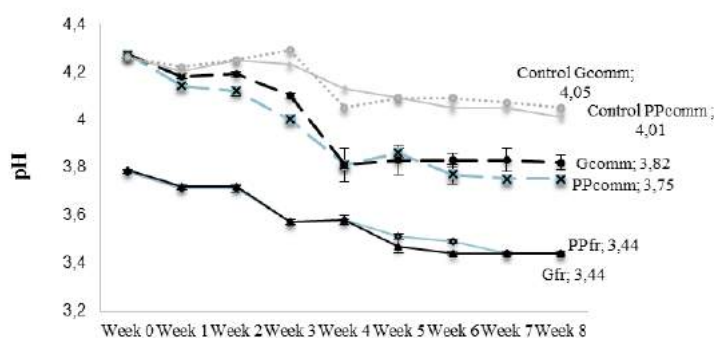
cfu/mL well after the storage period (Figure 3). There was however, a statistically significant difference in strain viability between fresh and commercial oatgurt from week 2 of analysis. The increased stress during fermentation during processing and presence of starter culture in the fresh oatgurt sample could explain the comparatively lower stability in viability of the probiotic strain in fresh sample. With the resulting data it was not



**Figure 3. Viability of *L. plantarum* 299v in commercial and fresh oatgurt sample.**

possible to establish a significant effect that the packaging material had on the viability.

**pH:** The reduction in pH of the samples could mainly be attributed to the probiotic strain activity, as the pH of the control sample declined at a much slower rate (pH 4.26 to pH 4.01). There was a statistically significant reduction in pH over time in fresh and commercial samples; however, the effect of packaging material on the pH could not be established. Although the strain viability is not affected by the pH decline, there could be an influence on product flavour (taste).



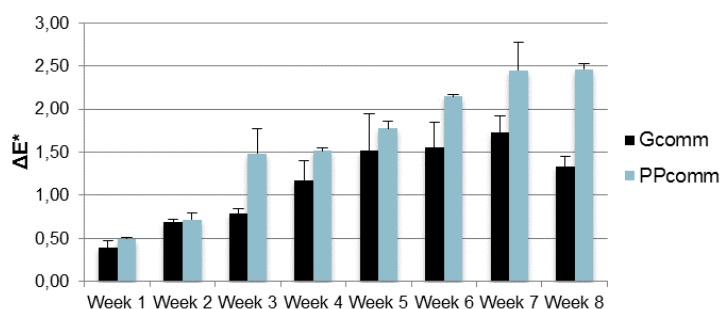
**Figure 4. Probiotic and control sample pH measured over eight weeks of incubation.**



The fresh oatgurt had a comparatively low initial (start) pH and therefore the subsequent decline could have a bigger impact on product sensory properties.

**Colour Stability:** The change in colour in the vanilla/blueberry oatgurt specifically was a concern for the manufacturer. The presence of *L. plantarum* 299v in the product did not have an obvious affect on the product colour. The packaging material, however, seemed to have an impact on the change in colour, or change in visual perception ( $\Delta E^*$ ) of the product. The colour of week 0 sample was considered as the reference colour that was accepted by the manufacturer (desired value).

There was a steeper increase in total change in colour of oatgurt in PP compared to glass (Figure 5). In PPcomm after week 5,  $\Delta E^*$  was above 2, indicating that the change in colour compared to desired reference would be perceptible at a glance. Till week 5 the change in colour in PP cups was only perceptible through close observation.



**Figure 5. Change in colour perception compared to desired reference over eight weeks of incubation at 8°C.**

## Conclusion

The study presents the possibility of commercially incorporating probiotic strain *L. plantarum* 299v into Oatly's oat-based yogurt

(oatgurt) and packaging it in PP cups. Oatly's oatgurt was able to sustain *L. plantarum* 299v stably for over eight weeks when stored in cold chain. The current packaging material used for the commercially available non-probiotic oatgurt, PP, could be used for probiotic oatgurt as well since it sustains the viability of *L. plantarum* 299v over the incubation period well above recommended dosage. The colour of the oatgurt could be improved with a packaging material having higher oxygen barrier, however PP could ensure acceptable colour stability for at least five weeks of storage.

In order to further advance the development of this probiotic product, sensory analysis for appearance and change in flavour should be conducted. The organic acid profile due to the incorporation of *L. plantarum* 299v should be studied to understand its metabolic activity in this food matrix. Pilot scale experiments are required to ensure viability of the bacteria is not compromised because of other process and production related factors. With limited research on probiotic non-dairy food products, there is a need for several studies to establish concrete commercial product development strategies.

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**Table 2. Overall conclusion of experimental results.**

Packaging Material		Polypropylene				Glass			
Commercial Oatgurt		Viability	pH	O <sub>2</sub>	Colour	Viability	pH	O <sub>2</sub>	Colour
Fresh Oatgurt**		Viability	pH	O <sub>2</sub>	-	Viability	pH	O <sub>2</sub>	-

Note: Green - Favourable; Yellow - Acceptable; Red - Unacceptable

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## Potential functionality of protein hydrolysate-enriched biscuits for glycaemia control

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### Introduction

The control of blood glucose is associated with a reduced risk of Type 2 Diabetes. Many nutritional factors can affect blood glucose levels (Thomas & Elliott, 2010). A strategy to control post-prandial glucose is the consumption of foods with low glycaemic index. The glycaemic index of foods indicates the elevation of the blood glucose after consumption of 50g of available carbohydrate from the food compared to a reference food. Intrinsic and extrinsic factors that alter the rate of gastrointestinal motility, digestion and absorption, and the nature of the starch, cooking method, particle size, and the presence of fiber, fat, and proteins are all found to influence the glycemic index. A low post-prandial glycaemia with a low glycemic index food can also reduce the postprandial rise in gut hormones like incretins and insulin (Jenkins et al., 2002).

Incretins are hormones that are released from the gut into the bloodstream in response to ingestion of food and modulate the insulin response. Specific nutrients generated by food digestion stimulate the release into the circulation of a specific pattern of incretins such as glucagon-like peptide-1 (GLP-1), oxyntomodulin, pancreatic polypeptides, peptide tyrosine tyrosine (PYY), and cholecystokinin (CCK), that work as gut hormones and can also modulate appetite (Crespo et al., 2014). Among macronutrients proteins are recognized as the

most satiating one and it has been reported that protein induced satiety coincides with a relatively high GLP-1 release (Veldhorst et al., 2008).

Dipeptidyl Peptidase IV (DPP4) is an enzyme more widely known for its ability to inactivate the incretins glucose-dependent insulino-tropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). The finding that DPP4 is responsible for more than 95% of the inactivation of GLP-1 has attracted considerable research interest in the inhibition of this enzyme (Lacroix & Li-Chan, 2015). Incretin-based therapies providing DPP4 inhibiting molecules (such as gliptins) are effective to lower blood glucose levels in T2D (Kim & Egan, 2008).

Natural alternatives to DPP4 inhibitory drugs are some dietary proteins that are source of bioactive peptides with ability to inhibit DPP4. To date, animal proteins and mainly milk proteins (casein and whey fractions) are the most studied (Nongonierma & FitzGerald, 2015). However, vegetal proteins providing bioactive peptides include soy, pulses, oat, wheat, hemp seed, canola, and flaxseed (Udenigwe & Aluko, 2012).

*In vitro* methods simulating digestion processes are widely used to study the gastrointestinal behavior of food or pharmaceuticals. Although human nutritional studies are still considered the “gold standard”, *in vitro* methods have the advantage of being more rapid, less expensive, less labor intensive, and do not have ethical restrictions. Simulated digestion methods typically include the oral, gastric and small intestinal phases. They try to mimic physiological conditions *in vivo* taking into account the presence of digestive enzymes, pH, digestion time, and salts (Minekus et al., 2014).

## Research objectives

The general aim of this study is to evaluate the potential functionality towards glycaemic response resulting from the incorporation of protein hydrolysates in a biscuit model. The specific aims are:

1. To evaluate the degree and type of inhibition of two commercially available protein hydrolysates on dipeptidyl peptidase IV (DPP4) enzymatic activity.
2. To study the DPP4 inhibition effect of the same protein hydrolysates within a model biscuit after an *in vitro* simulated gastrointestinal digestion.
3. To determine the potential effect of the protein hydrolysates on the (*in vitro*) glycaemic index of the model biscuits.

## Methodology

### Protein hydrolysate enriched biscuits

Biscuits were developed using the formulation proposed by Kong et al. (2016) with some modifications in order to obtain biscuits containing 12% and 20% energy coming from either casein or soy proteins.

### Dipeptidyl Peptidase IV Inhibition Assays

The effect of the protein hydrolysates on DPP4 activity was determined using a modified version of the inhibition assay described by Lacroix & Li-Chan, (2013). The protocol was the same except for the samples were reconstituted to concentrations from 0.25 to 25 mg/mL. And the concentration of the substrate Gly-Pro-p-nitroanilide was 6 mM and for DPP4 it was 0.01 units/mL. In the case of the biscuit digesta samples, enzyme concentration was 0.05 U/ml and substrate was 3 mM and the incubation was done for 1 hour (instead of 0.1, 6 mM and 30

minutes, respectively) the rest of the protocol was the same.

Each test sample was analyzed in triplicate, and the absorbance values were corrected with sample blanks. The positive 100% reaction control and negative 0% reaction control were prepared by using Tris-HCl buffer in place of the sample and in place of the sample and DPP4 solution, respectively. The percent DPP4 inhibition was calculated using the absorbance values as follows:

$$\%inhibition = \{ [(100\% - 0\%) - (sample - blank)] / (100\% - 0\%) \} * 100$$

The IC<sub>50</sub> values (concentrations of hydrolysate required to cause a 50% inhibition of the enzyme activity) were determined from the regression equations of the percentage of DPP4 inhibition against hydrolysate concentrations. The tripeptide Diprotin A (Ile-Pro-Ile) was used as a reference inhibitor.

For the Lineweaver Burk assays the protocol used was as described by Nongonierma & FitzGerald, (2013). The measurements for absorbance of the samples were performed every minute for a period of 20 minutes. The concentration of enzyme and the volumes of substrate, enzyme, inhibitor (or TRIS blank) and sodium acetate were the same as the ones used for the inhibition assays. The K<sub>m</sub> and V<sub>max</sub> values were determined from the double reciprocal plots and the mode of inhibition was analyzed by comparing K<sub>m</sub> and V<sub>max</sub> obtained in the presence and absence of the DPP4 inhibitory peptide.

### ***In vitro* simulated digestion of protein hydrolysate enriched biscuits**

Biscuit samples underwent a simulated gastrointestinal digestion protocol. Samples were incubated in a shaking water bath at 37 °C and different enzymes were added for each of the three phases: salivary phase with amylase, gastric phase with pepsin and intestinal phase using pancreatin (Minekus et al., 2014).

### **Glycaemic Index Determination**

The protocol described by Kim & White, (2012) with some modifications was used. 100 mg of dried biscuits, reduced into powder, and the control (white bread) were put into 50 mL tubes with 10 glass beads. A 2 mL acid solution of pepsin was added to each tube and incubated at 37 °C in a shaking water bath for 30 min. Then a 4 mL of sodium acetate buffer (0.5 M, pH 5.2) and a freshly prepared enzyme solution (1 mL) containing porcine pancreatin and amyloglucosidase, were added to increase the pH and to allow the starch hydrolysis, respectively. During incubation at 37 °C aliquots (100 µL) were taken at 0, 30, 60, 90, 120, and 180 min intervals and mixed with 50% ethanol (1 mL). The samples were centrifuged and the hydrolyzed glucose content of the supernatant was measured by using the glucose oxidase-peroxidase assay procedure (Sigma Aldrich). According Goñi et al., (1997), to transform the glucose into starch, a factor of 0.9 was used. Total starch hydrolysis (%) was calculated and the GI was then estimated by using the following equation of Goñi et al., (1997):  $GI = 39.71 + 0.549HI$ .

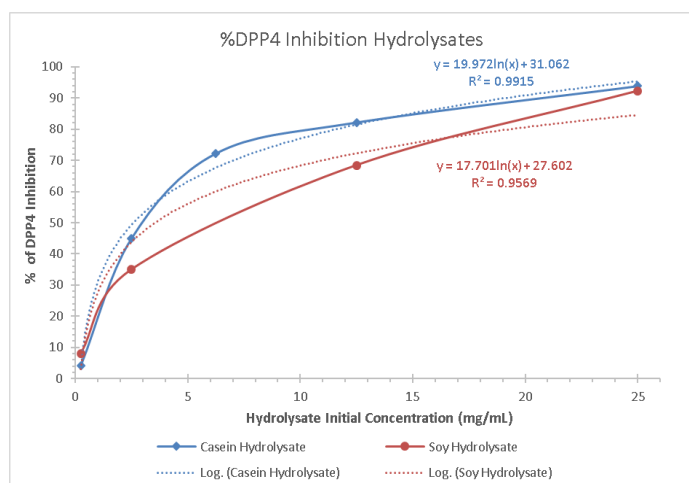
All analyses were done in triplicate, and the values were averaged. Data on the replicate preparations were analyzed by using the analysis of variance (ANOVA) and then compared by a T-test for paired means

when applicable. All statistical analysis done on the results of this project are based on a 95% confidence level ( $\alpha = 0.05$ ).

## Results and Discussion

### Effect of hydrolysates on DPP 4 inhibition

The inhibitory activity of casein and soy hydrolysates towards DPP4 is shown in **Figure 1**.



**Figure 1. DPP4 inhibition and IC<sub>50</sub> for casein and soy hydrolysates ( $p < 0.05$ ).**

In general it shows that soy hydrolysate was less effective compared to casein. This was confirmed by the measure of IC<sub>50</sub> that was  $3.56 \pm 0.42$  for soy and  $2.59 \pm 0.34$  for casein ( $P < 0.05$ ). The different IC<sub>50</sub> between the hydrolysates can be explained by differences in the sequence of the contained peptides. Previous studies suggested that the specific amino acid sequence is the predominant factor determining DPP4 inhibitory activity. In silico studies have shown that potent inhibitory peptides generally contain a branched-chain amino acid or an aromatic residue with a polar group in the side-chain at their N-terminal and/or a proline residue at their P1 position. Also the presence of a tryptophan residue at the N-terminal position has been reported in several of the most

potent peptides (Lacroix & Li-Chan, 2016). In particular, looking at results from a recent study it could be hypothesized that casein peptides contained higher trypsin and tryptophan fractions than soy (Nongonierma & Fitzgerald, 2015).

Regarding the type of inhibition the results of the Lineweaver Burk assay showed that soy hydrolysate act as a competitive inhibitor (similarly to DiProtin A) whereas casein hydrolysate had a mixed type of inhibition (both competitive and non competitive). This could be confirmed by the lower V<sub>max</sub> value calculated for the casein than for soy. This can be seen by comparing the ratio of K<sub>m</sub>/V<sub>max</sub>, which was  $1.70E+03$  for Diprotin A, followed by  $1.10E+03$  for soy; then  $8.02E+02$  for casein and last  $2.07E+02$  for the samples with no inhibitor.

The binding of many food derived peptides with competitive DPP4 inhibitors has been described at the active site of DPP4. However, a secondary binding site for DPP-IV inhibitors has also been described, located close to the active site and results in a linear mixed-type or parabolic mixed-type inhibition of DPP4. Also, several peptides coming from bovine milk sources have been identified as having a non competitive or mixed type of inhibition (Nongonierma & Fitzgerald, 2013).

### Hydrolysate-enriched Biscuits

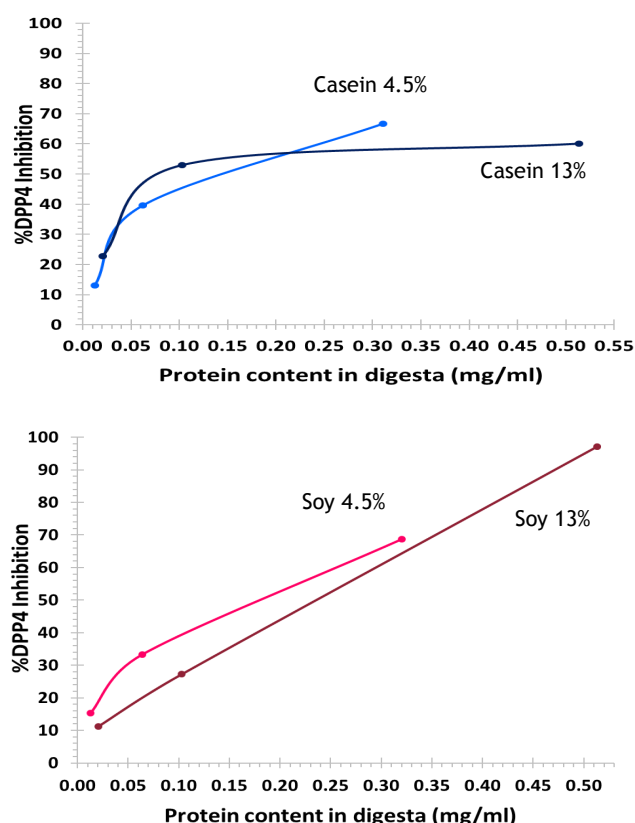
The nutritional composition of the biscuits developed in this study is reported in **Table 1**.

**Table1: Nutritional composition of the formulated biscuits**

Amounts per 100 g	Casein 4.5%	Casein 13%	Soy 4.5%	Soy 13%	Control Biscuit
Energy (kJ)	1620	1600	1640	1640	1620
(kcal)	387.2	382.4	392.3	392.3	382.2
Protein (g)	12.7	20.0	13.1	20.9	8.7
Fat (g)	0.9	0.8	1.2	1.5	1.0
Saturated fats	0.2	0.1	0.2	0.3	0.2
Carbohydrate	79.2	71.1	79.8	71.3	83.1
Fibers (g)	2.2	1.4	2.5	1.2	3.2
Sugars (g)	24.0	23.6	24.3	24.0	24.1
Starch	53.0	46.1	53.0	46.1	55.8
Sodium (mg)	280	640	361	872	84
%Energy from protein	13.12%	20.92%	13.34%	21.30%	8.99%

### Potential effect of the biscuits on DPP 4 Inhibition

Figure 2 presents the results for the digesta assays.



**Figure 2. DPP4 inhibition of biscuit digesta**

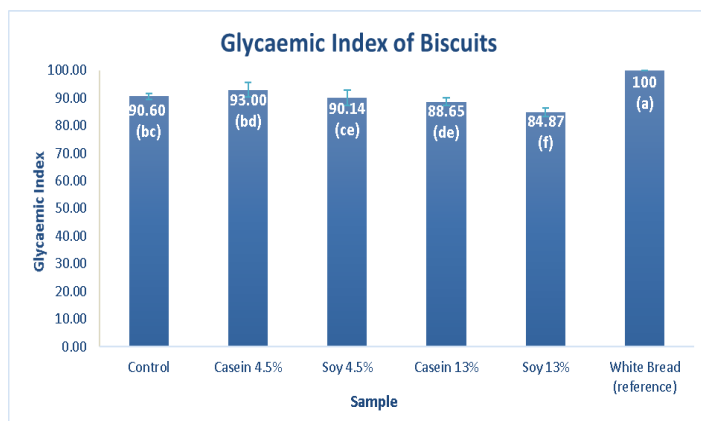
The IC<sub>50</sub> for each biscuit digesta was calculated (the concentration needed to achieve 50%) and the results show a trend of the soy biscuits generating a lower IC<sub>50</sub> than the casein digesta, which means its inhibition potency was higher. It was  $0.150 \pm 0.031$  for casein 4.5%,  $0.157 \pm 0.049$  for casein 13%,  $0.139 \pm 0.034$  for soy 4.5% and  $0.127 \pm 0.023$  for soy 13%.

Nongonierma & FitzGerald (2015), evaluated the DPP4 inhibitory activity of several plant derived hydrolysates after simulated gastrointestinal digestion. They found significant protein breakdown following SGID of the plant protein isolates. Moreover, they observed that certain tryptophan-containing peptides were further degraded by the proteolytic action of the gastrointestinal enzymes. This is proof that the peptide profile of a hydrolysate can be significantly changed during gastrointestinal digestion and the rate of the change of DPP4 inhibition capacity will also depend on the origin and composition of the initial hydrolysate.

Previous results of DPP4 inhibition of food digesta in human subjects is not yet reported in present studies. Among the few published studies, Capriotti et al., (2015) concluded that the presence of protease inhibitors in soy protein reduces the effect of gastric proteolysis and generates larger peptides that are still compatible with the absorption of peptides in the human gastrointestinal lumen and can act as substrate-like inhibitors of DPP4. On the other hand Zhang et al., (2016), report a high level of hydrolysis of caprine and bovine casein fractions when treated with gastric enzymes, and also a slight but statistically significant increase of DPP4 inhibition in the hydrolyzed fractions with lower molecular weight distributions.

## Glycaemic Index and Starch Hydrolysis

The following figure presents the results obtained by the *in vitro* determination of the Glycaemic Index of the hydrolysate biscuits.



**Figure 3. *in vitro* Glycaemic Index of hydrolysate biscuits.**

Initially, the soy 13% formulation was significantly different from all the other samples and it had the lowest glycaemic index of all. A two way ANOVA of the results from the hydrolysate biscuits indicated that when the percentage of protein is higher, the GI is lower, and it also indicated that the soy hydrolysate biscuits have lower GI than the casein ones. From these results and based on the principle of the method used, it can be hypothesized that the soy hydrolysate used in biscuits generated a lower GI probably due to a higher protein content.

The carbohydrate content of each formulation can be contrasted with the GI. The biscuits containing the lower amount of hydrolysates had a higher carbohydrate percentage, that was mainly composed of sugars and starch. These two carbohydrates are the source of the glucose measured in the GI index assay and therefore it can be expected that the higher their content is, the higher is the *in vitro* GI of the food product. The previous affirmations are supported by Manders et al, (2009), who

mention that increasing the amount of dietary protein has been suggested to increase satiety and reduce total energy intake, and that this can be explained partially on the basis that dietary protein is increased at the expense of carbohydrate.



## Conclusions

1. When evaluating the inhibitory activity of the raw hydrolysates on DPP4, the casein hydrolysate presented a lower IC50 value of 2.59 mg/ml in comparison to the soy hydrolysate with 3.56 mg/ml. This might indicate that the casein hydrolysate has a peptide profile content that is more efficient in the inhibition of DPP4.
2. When evaluating the mode of inhibition of the raw hydrolysates, soy presented a type of inhibition more similar to that of a competitive inhibitor. Casein on the other hand seemed to have a mixed/non competitive type of inhibition. This again can be attributed to differences in the peptide profile, in particular the specific sequences or types of amino acids present in the two types of hydrolysates coming from different sources.
3. The glycaemic index (GI) of the biscuits shows that the biscuit formulation that had more clear decrease in the GI was the biscuit containing soy at 13% hydrolysate. This shows that the addition of proteins in a biscuit formulation in a higher percentage (13% or possibly more) as a substitution of wheat flour can potentially decrease the GI of the biscuit and with this generate a food product with potential to generate a lower rise in post prandial glycaemia.



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# Impact of biobased packaging materials on quality of fully-baked frozen bread

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## Introduction

Global plastic production increases every year in which almost 40% of plastic demand in Europe was intended for packaging purposes (PlasticEurope, 2016). Reducing amount of fossil resources and environmental consideration from excavating it led many industries to look for more renewable resources to make plastic. Moreover, consumers, retails, and government regulation urge for a more sustainable packaging (McQuilken, 2016).

Biobased packaging materials could be one of the solutions. However, suitability of biobased packaging application on specific food product needs to be evaluated to ensure the satisfactory of minimum product protection while improving the environmental impact of the packaging

In this study, application of biobased packaging materials for fully-baked frozen bread was evaluated. Hotdog buns and hamburger buns were the types of bread chosen for the study provided by Lantmännen Unibake, Sweden. These breads undergo freezing in the package after baking and have 7-day shelf-life in retail shelves after thawing. It is an interest to understand the effect of the biobased packaging materials on the packed buns. The current packaging for these buns is petroleum-based polyethylene (PE).

## Research objectives

1. To identify the characteristics of different biobased packaging materials (film) that exist in the market.
2. To evaluate how biobased packaging

material would maintain the quality of the hotdog buns and hamburger buns for 7-day shelf life after thawing in comparison with reference PE bag.

## Methodology

Total of six different packaging materials were used in this study. The five biobased packaging materials were biobased polyethylene (bio-PE), Biodolomer®, polylactic acid (PLA), polybutylene succinate-co-adipate (PBSA), and paper laminated with biobased polyethylene terephthalate (paper/bio-PET). Polyethylene (PE) is the reference packaging. Packaging water vapor transmission rate (WVTR) and puncture resistance were measured.















Fresh half-cut hotdog buns and hamburger bun were repacked manually and sealed with a clip. The buns were frozen at -18 °C for at least 72 hours. Thawing was done at room temperature for 24 h. Quality of bread in the package along shelf-life was evaluated i.e. weight loss, textural properties (crust hardness, crumb hardness, and crumb springiness), water activity, and appearance. Evaluations were performed day 1, 3, and 7 after the buns were thawed. Triangle test was performed to investigate if there is a significant difference between hotdog buns in bio-PE and hotdog buns in PE reference.

## Results and discussion

### Packaging evaluations

Transparency of the packaging is important for this product application. Bio-PE, PLA, and PBSA are transparent (Table 1), providing good visibility of the product. On the other side, Biodolomer® is less transparent and paper/bio-PET is opaque. Less visibility of the product might affect how consumer perceive the overall product.

Water vapor transmission rate (WVTR) is an important parameter in packaging selection. WVTR value of bio-PE was not significantly dif-

Packaging material	Picture on text	Picture of packed buns	Biobased content*	Biodegradability
PE reference hotdog buns			0%	Non-bio-degradable
PE reference hamburger buns			0%	Non-bio-degradable
Bio-PE			50-85%	Non-bio-degradable
Biodolomer®			50-85%	Home compostable
PLA			>85%	Industrial compostable
PBSA			20-50%	Home compostable
Paper/bio-PET			50-85%	PET part is non-bio-degradable

**Table 1 Characteristics of packaging materials used**

ferent compared to both PE reference, 3.96-6.37 g/m<sup>2</sup> day. Biodolomer®, PLA, PBSA, and paper/PET WVTR values were significantly higher than PE reference, 71.1, 61.70, 140.12, and 25.09 g/m<sup>2</sup> day respectively.

Puncture resistance is a characteristic of flexible packaging materials that is related with packaging durability against impact from external penetration (Lange et. al., 2002). All the biobased packaging materials evaluated in this study had similar or higher puncture resistance compared to reference. Therefore, the performance against eternal penetration is expected to be similar or better.



### Packed buns evaluations

Weight loss of hotdog buns and hamburger buns in bio-PE were not significantly different compared to PE reference day 7 after thawing. Paper/bio-PET could only maintain the

weight loss of hamburger buns. Other packaging materials could not maintain weight loss of the bun even in the first day after thawing. Higher weight loss might be caused by the higher packaging WVTR value.

In term of textural properties, hotdog buns and hamburger buns packed with bio-PE had the most similar textural properties to hotdog buns in PE reference. These results correspond with the result of water activity and appearance of the buns (no dry edge observed). The triangle test confirmed that there was no significant difference between hotdog buns packed with bio-PE and hotdog buns packed with PE reference.

Paper/PET could limit the dry edge of the buns. However, it could not maintain crumb hardness (hotdog bun) and crumb springiness (hamburger bun). Dry edge was observed on the buns packed with Biodolomer®, PLA, and PBSA day 7 after thawing (Figure 1). In fact, these packaging could not maintain at least two out of three bun textural properties measured in this study.

Biobased packaging materials	Hotdog bun			Hamburger bun		
	Weight loss	Textural properties	Appearance	Weight loss	Textural properties	Appearance
Bio-PE	NS	NS		NS	NS	
Biodolomer®	***	Harder crust & crumb		***	Harder crumb	
PLA	***	Harder crust & crumb		***	Lower springiness	
PBSA	***	Harder crust & crumb		***	Harder crust & crumb	
Paper/bio-PET	**	Softer crumb		NS	Lower springiness	

**Figure 1** Evaluation results on the quality of packed buns day 7 in comparison with buns in the reference packaging

NS: not significant

\*: significant

## Conclusions

Biobased packaging materials used in this study have different characteristics and barrier properties. Bio-PE had similar characteristics with the PE reference. Other biobased packag-

ing materials had higher WVTR values and some of them are not transparent i.e. Biodolomer® and paper/bio-PET.

Bio-PE could maintain the quality of hotdog buns and hamburger buns in 7-day shelf life after thawing. A triangle test confirmed that there was no significant difference between hotdog buns packed in bio-PE and PE reference. On the other side, Biodolomer®, PLA, and PBSA did not perform well for this type of application which showed in the higher weight loss and dry edge of the buns in day 7 after thawing. Paper/bio-PET is an interesting biobased packaging material since it could maintain some of the quality parameters.

## Future studies

It might be interesting to evaluate the performance of other commercial biobased packaging materials and the effect of different material thickness. Biobased packaging materials application for other type of products might be an interest, for example pastries, which require a more breathable packaging. Packaging for frozen bread can be another application to be evaluated since the water loss during freezing is minimum.

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## The Effect of Added Multicomponent Antioxidant Formulations in Sunflower Oil based Model and Food Systems

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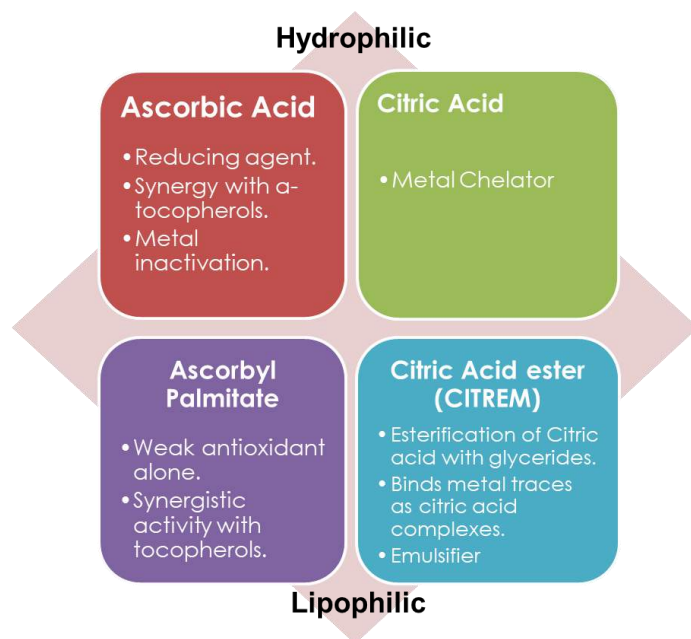
### Introduction

Rancidity in edible oil is a serious problem in many sectors of food industry, because of the increasing emphasis of the use of polyunsaturated vegetable and fish oils, discontinuing the use of synthetic antioxidants and the fortification of iron in food products. Oxidation of lipids produces undesirable rancid flavours, decrease of the nutritional quality and safety, lose of essential fatty acids and the production of toxic compounds as free radicals. Oxidative stability is the resistance to oxidation during processing and storage. The resistance to oxidation can be expressed as the period of time necessary to attain the critical point of oxidation, whether it is a sensorial change or a sudden acceleration of the oxidative process.

Lipid oxidation can also occur in emulsions, which are complex, heterophasic food systems, separated by an interface composed by amphiphilic compounds. Lipid oxidation is a “surface” reaction dependent on the rate of oxygen diffusion, pH, droplet size, physical stability and many other factors related to the composition of the emulsion and their interactions.

Antioxidants can be added to food products to slow down the oxidation rate. They can scavenge free radicals, control transition metals, quench singlet oxygen and inactivate sensitizers. In this research, the activity of ascorbic acid, citric acid, ascorbyl palmitate





and citric acid ester were investigated to reduce lipid oxidation in sunflower bulk oil and three food emulsions.

### Research objectives

The general objective was to evaluate the activity of two hydrophilic antioxidants (ascorbic acid and citric acid) and their lipophilic counterparts (ascorbyl palmitate and citric acid ester) in a model and three food systems based on sunflower oil.

Specific objectives:

- A better understanding of the “polar paradox” in bulk oil and different type emulsions based on oxidative stability.
- Determination of the best antioxidant formulations for every food system.

### Methodology

#### 1. Model System

The model system was built in order to determine the most efficient concentration for ascorbic acid, citric acid ascorbyl palmitate, citric acid ester that provides the best oxidation stability in sunflower bulk oil. The antioxidants were

added with a determined concentration of  $\alpha$ -tocopherols and lecithin, according to Palsgaard®’s standard antioxidant formula. The oxidation stability was evaluated independently for every antioxidant with the following concentrations: 0.00, 0.13, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.50 and 3.00  $\mu\text{mol/g}$  using Rancimat at 100°C in duplicate.

#### 2. Food Systems Production

Series of food systems:

**Trial 1:** Control 1, **Trial 2:** AA-CA (Ascorbic acid and Citric Acid), **Trial 3:** AA-CAE (Ascorbic acid and Citric Acid Ester), **Trial 4:** AP-CA (Ascorbyl Palmitate and Citric Acid), **Trial 5:** AP-CAE (Ascorbic acid and Citric Acid Ester) and, **Trial 6:** Control 2.

Mayonnaise (O/W, 70% fat) was produced in cold process using Homogenizer at 3000 rpm.

Margarine (W/O, 70% fat) and shortening (100% crystalized oil) were produced in a chilling tube, a pin rotor machine and other two chilling tubes. Chilling tubes were set to 18°C and 500rpm. C3 was set to 400 rpm. For PRM, the conditions were 22°C and 120 rpm.

#### 3. Analysis of Food Systems

After production, samples of Mayonnaise, Margarine and Shortening were stored for 14 days in a Climate test Chamber at 50°C and held in the dark for 14 days. A pair of samples were withdrawn on day 0, 3, 6, 10 and day 14 for chemical and physical analysis (**Rancimat, pH, Microscope, GC-MS and Peroxide Value**) to determine the oxidative stability of the food systems with different antioxidant formulations.

## Results and discussions

### Model System

For the ascorbic acid/ascorbyl palmitate, both antioxidants showed a similar activity in all concentrations, presenting a disagreement with that Polar paradox states. Ascorbic acid is known for being a metal chelator and working in synergy as reducing agent, regenerating  $\alpha$ -tocopherols during the oxidation (Frankel, 2012).

As a single antioxidant in the system, ascorbyl palmitate could be expected to have a poor antioxidant activity. Because  $\alpha$ -tocopherol is added into the oil, ascorbyl palmitate can work as a reducing agent and perform as good as its polar counterpart. For both antioxidants the induction time is no longer prolonged after  $1\mu\text{mol/g}$ .

At the moment of evaluating citric acid and citric acid ester, it was necessary to add 10 ppm Fe(III) to the bulk oil in order to determine the real chelating activity of these two antioxidants. Citric acid displayed a higher antioxidant activity than its apolar counterpart citric acid ester over the full concentration range. As a polar compound, citric acid locates in the interface between oil and traces of water, being able to form insoluble metal complexes with  $\text{Fe}^{3+}$  and reduce oxidation. On the other hand, CITREM can also bind metals as citric acid complexes, but because of its polarity, it can remain in solution in the oil and it is not able to bind metals in order to reduce oxidation.

After evaluating the induction time of every antioxidant, it was selected a concentration of  $1.00\mu\text{mol/g}$  for ascorbic acid, ascorbyl palmitate, citric acid ester and  $0.50\mu\text{mol/g}$  for citric acid to be added in the food systems.

### Food Systems

#### - pH

The change of the pH was measured for the days 0, 6 and 14. For the three different food systems produced, the control samples (1 and 2) presented the highest pH of each system and a minor change of it over time. The reason of this might be because no acidic antioxidant was added as on the other samples. The mayonnaise presented the lowest pH compared to the other systems. This food complex was the only one produced with acetic acid. Also, the mayonnaises with the different antioxidant formulations showed major changes of pH over time than margarine and shortening. The storage at  $50^{\circ}\text{C}$  and the type of emulsion might induce a possible decomposition of the glycerides, acids or other components present. It was not found a trend of pH related to antioxidant formulation in the food systems.

#### - Emulsion structure

The use of antioxidants with different polarities influences the lipid oxidation, structure and physical stability of the emulsions, depending of the dispersion and interactions of these compounds in the interface.

At the end of the 14 days, the mayonnaises were observed again using the microscope and it was found that the control samples were still stable with a homogeneous distribution of the oil droplets. On the other hand, the samples with antioxidant formulations added were totally unstable and considered to be flocculated. It can be said that the addition of the stabilizer did not prevent the oil droplets to move and merge.

On the other hand, margarine and shortening presented stable and homogeneous emulsions from day 0 to day 14 stored at  $50^{\circ}\text{C}$  with minor changes.

At the day 14, the margarine produced with

AP-CAE presented a broken emulsion. Ascorbyl palmitate and citric acid (CITREM) are also emulsifiers, amphiphilic compounds with ramified structures; due to that, they might have created a competition between emulsifiers and had as a result a thin and weak interface, making the emulsion to break.

#### - Peroxide Value

Peroxide value (PV) was used as an indicator for the primary oxidation of food systems after 14 days of storage at 50°C.

The most important observation for this analysis was the difference in values from the Samples Control 1 and 2. Process and formulation was the same for both samples. Therefore, it was supposed to obtain similar results, including Peroxide Value. Because the trial Control 2 was the last one done, it can be said that the difference in the results for Control 2 is due to traces of antioxidants left in the machinery, impacting in the PV, but not in the general oxidation results, as it will be further discussed.

#### - Oxidative Stability

Oxidative stability of oils is the resistance to oxidation. It can be expressed as the period of time necessary to attain the critical point of oxidation, whether it is a sensorial change or a sudden acceleration of the oxidative process. In this section, it will be discussed which is the best antioxidant formulation for every food system based on the oxidative stability.

The control 1 and 2 samples displayed initial induction time of 15 hours at day 0.

After 10 days, the induction time is reduced to 10 hours, for every food system. This meant that, no matter the system, the activity of the  $\alpha$ -tocopherols and lecithin in the mayonnaise, margarine and shortening are not affected by the emulsion structure or ingredients added in

each complex over time. Also, because the emulsion structure is stable (for mayonnaise and margarine) there is a higher interface area, in which oil, water and other components present can interact with each other and facilitate oxidation reactions.

#### Ascorbic acid and citric acid (AA-CA)

The addition of ascorbic and citric acid showed the shortest induction time comparing the others antioxidant formulations. However, for day 6, margarine showed the best induction time compared to all the other formulations for this food system. As polar compounds, ascorbic acid and citric acid had a better performance of their antioxidant activity. This formulation cannot be considered as a real improvement, because the induction time of the three food applications is really close to the control trials (1 and 2), except for margarine with a positive result on day 6.

#### Ascorbic acid and citric acid ester (AA-CAE)

Mayonnaise showed the same oxidation behavior as when AA-CA were added. Margarine showed as poor results as mayonnaise. It is probable that ascorbic acid is participating in side reactions or even as prooxidant, not providing enough oxidative protection in the food emulsions. On the other side, with the addition of citric acid ester, it can be noticed an improvement in the oxidation stability of shortening. It can be said that shortening, as a crystallized oil system, shows low mobility of components that promote oxidation and citric acid ester just behaves as a fatty acid. For shortening, the AA-CAE formulation is the optimum, showing the best oxidation stability for day 6.

### Ascorbyl palmitate and citric acid (AP-CA)

When this formulation was added in the food systems, mayonnaise showed a better performance over time compared to the other formulations with ascorbic acid. In an emulsion oil in water, ascorbyl palmitate showed better protection against oxidation, being present in the oil-water interface, working as a reducing agent with tocopherol and reducing oxidation in side reactions. In the case of margarine and shortening, ascorbyl palmitate also showed good oxidation stability. The activity of citric acid in the food systems cannot be seen, probably because the concentration of transition metals is low. This antioxidant combination can be considered the best for the mayonnaise system.

### Ascorbyl palmitate and citric acid ester (AP-CAE)

Even if the combination of these two antioxidants showed better oxidative stability compared to the others formulations, the physical stability of the emulsions was not present in mayonnaise and margarine. Due to this, this antioxidant formulation cannot be taken in consideration as an improvement of the oxidative stability.

### Secondary Oxidation Products

The analysis of gas chromatography and mass spectrometry was done for all the samples in order to evaluate the production of secondary oxidation products during storage at 50°C for 14 days. Only for the mayonnaise, known secondary oxidation products were recognized. For margarine and shortening, the products of interest were so small, that the software did not consider them. However, a manual determination was done and it was found some secondary oxidation products, but only in margarine. In the previous results, it has been shown that

shortening showed the best oxidation stability. Probably in the GC-MS analysis, the oxidation was in an early stage that only hydroperoxides were present and none secondary oxidation products were produced yet.

### Conclusion

The results of this study evidence a partial confirmation of the Polar Paradox statement for bulk oil, W/O and O/W emulsions based on sunflower oil. In the Model System, when bulk oil was evaluated, Citric acid showed better performance in induction time than Citric Acid Ester. In contrast, Ascorbyl Palmitate showed as good oxidative stability as its polar counterpart, ascorbic acid. When antioxidants were added to the food systems, the combination of ascorbyl palmitate and citric acid showed the best results for Mayonnaise, even though this system was really unstable. For Margarine, the polar antioxidants (ascorbic acid and citric acid) represent the best compounds for a water in oil emulsions. The addition of ascorbic acid and citric acid ester is the optimum formulation for shortening in order to have a stable product over time.

For the production of the food systems with the multicomponent antioxidant formulations, poor physical stability of the system impacted positively in the oxidative stability, as the case when Ascorbyl Palmitate and Citric Acid Ester were added in Mayonnaise and Margarine. As contrast of the Model System, ascorbic acid displayed a higher antioxidant activity than ascorbyl palmitate regardless the food system. The mayonnaise might be a food complex not suitable for oxidation analyses when it is stored in a Climate Chamber

at medium temperature (50°C), due to its weak emulsion structure.

Over time, at the end of the 14 days of study, most of the food systems with different antioxidant formulations, showed a similar oxidative stability. Finally, ascorbic acid might act as a prooxidant when it is added with citric acid and citric acid ester in sunflower bulk oil and the food systems.

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## Future of Fermentation – Idea Generation & Development of Prototypes

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### Introduction

The objective of the internship was to introduce the concept of prototyping in the idea generation phase and have a holistic approach of product development phases. This objective was focussed mainly on the concept of fermentation, to look for new insights in the domain of early life nutrition and to develop ideas into live prototypes that could be evaluated.

### Research objectives

The objective of research was to identify new health benefits, new species and strains, trends in the market and strategies used in the market that were all interesting for product development for the future.

### Methodology

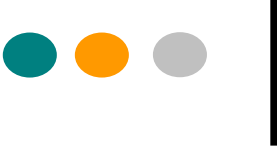

Literature, competitors' and patent review was done. This helped in selection of a few important ideas that could prove to be fruitful for the business. The ideas were brainstormed and developed with the help of fermentation and further processing to produce infant formula prototypes. Analysis of the prototypes to study the sensorial, microbiological and biochemical composition was done.

### Results and discussions

Five prototypes were developed and analysed. The prototypes confirmed technical feasibility and were studied for potential health benefits with literature.

### Conclusion

The concept of prototyping in upstream innovation process showed great potential in assessing technical feasibility of the ideas.



With the help of development of prototypes and literature review, ideas could be easily analysed for their potential in the future, and idea generation could be further structured for future business prospects.

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***Confidential topic***

# Evaluation of the Corrugated Box Strength Performance in Supply Chains : A Case Study of Duni AB

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## Introduction

Duni, a Swedish company that is focusing on high quality table setting concept produces about 70% of their goods at their own manufacturing establishments across Europe. About 30% of the goods are sourced from all over the world, mostly from their Asian and European suppliers. Longer distances between the producer and consumers leads to strength reduction of Duni corrugated boxes along the supply chain.

## Purpose & Goal

**Purpose:** To understand the interconnection between logistic condition, practices and activities that can eventually reduce the strength of the corrugated box.

**Goal:** To propose to Duni on how the safety factors of their corrugated box can be

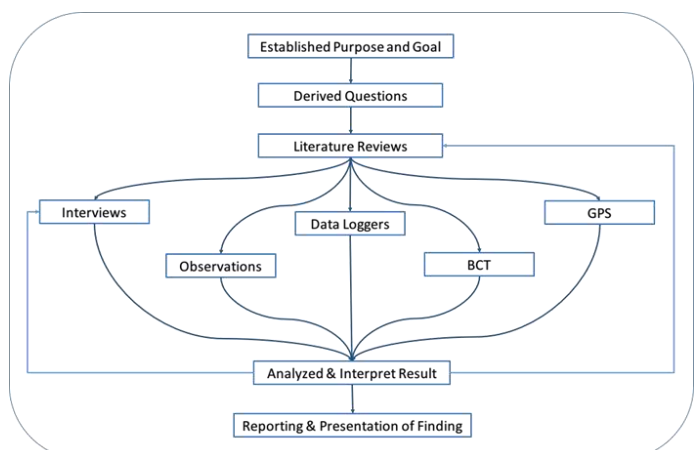


Figure 1. Overall methodology approach

further revised and improved.

## Methodology

Type of measurement	Description
Literature reviews	Familiarization with the topic and problem.
Interviews	Conducted individually and in a group to get a clearer picture of the problem with packaging strength
Observations	Participant (on-site visit) and non-participant observation to observe in real life, the current situation, practices and activities conducted at each actor
Data Logger	To measure shock, vibration, relative humidity (RH) and temperature (T) introduced to the packaging system
Global Positioning System (GPS) tracker	To detect the location of the packaging
Box Compression Test (BCT)	To see how much strength of the corrugated box would be reduced across the supply chain.

## Overview

Three products were selected to represent Duni manufacturing facilities in Poznan and Bramsche and their supplier A in Shenzhen, China. The packaging system of the selected products

Product	Origin	Description
Product A	Poznan	Napkins 3-ply 33cm 20 pieces
Product B	Bramsche	Napkins 2-ply 40cm 125 pieces
Product C	Shenzhen	Stirrer box 112mm white

were studied in details and supply chain mappings were provided for better visualization.

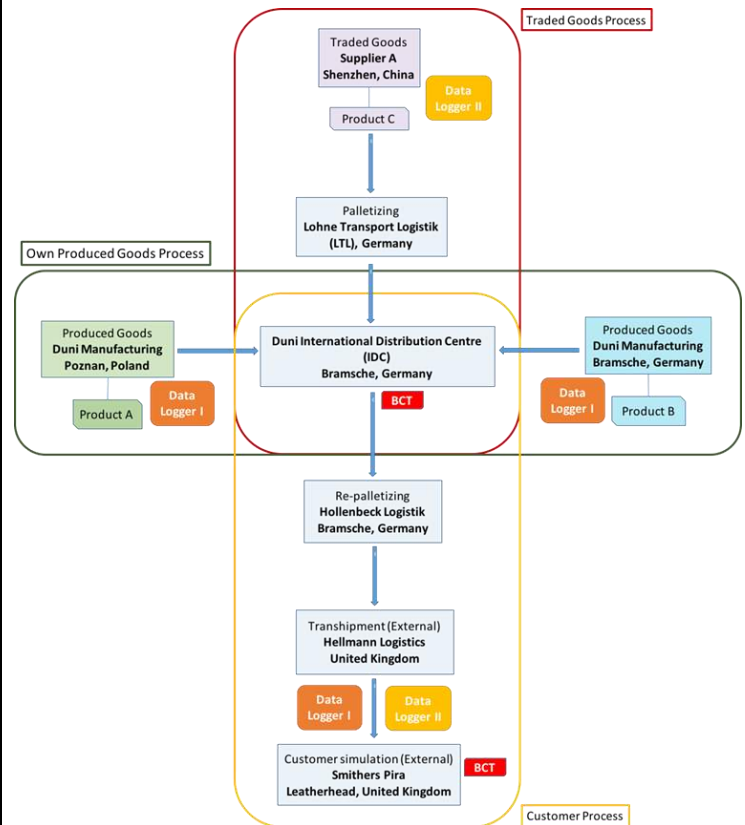


Figure 2. Overview on the scope covers by this master thesis

Data logger I is placed inside the secondary packaging of product A and B. Both products were transported or transferred from their manufacturing establishments to International Distribution Centre (IDC) in Bramsche. Data logger II is placed inside the packaging of product C. Product C was transported as loose load inside a cargo vessel from Shenzhen, China to Lohne Transport Logistik (LTL) in Germany for palletizing. As all products were meant for United Kingdom (UK) customers, they were undergoing re-palletizing process to change the load carrier from Euro pallet (1200 x 800 mm) to UK pallet (1200mm x 1000m). After that, all prod-

ucts with data loggers I and II inside their packaging were transported to Hellmann Logistics warehouses (transshipment point) in Osnabrück, Germany, in Litchfield, UK and in Colne Brook, UK. Smithers Pira in Leatherhead acted as the customer in simulation for this project.

## Results and discussions

### A. Relevant finding during observation

<i>Finding</i>	<i>Observation</i>
Pallet pattern/ configurations	Mistakes during manual stacking – boxes were not in the centre of the pallet, misalignment, gap between boxes. Wrong pallet scheme with unrealistic box arrangement was issued
Basic condition in supply chain	“Container rain” was observed inside the container from China Triple and quadruple pallets stacking observed inside warehouses
Design of the secondary	Corrugated boxes with dented vertical edge due to excessive space or setting of the stretch wrapping ma-

### B. Shock/Vibration

- Both manual handling and automated systemable to cause great amount of shocks or vibrations to the boxes.
- Transportation by truck, on the road, gave the least shock or vibration impact compared to other logistics activities.
- Overall, most of the shocks and vibrations recorded during loading and unloading activities of the pallets across the actors in the supply chain.
- The corrugated box located at the bottom layer of the pallet always exposed to higher shocks or vibrations impact compared to other layers.

### C. Relative Humidity (RH) and Temperature (T)

By benchmarking ISO standard test atmosphere for testing of pulp, paper and board which

is 50% RH, 23 °C, average relative humidity of <50% was measured in Europe particularly during the project timeline can be considered as good.

### D. Box Compression Test (BCT)

<i>Type of measure-</i>	<i>Description</i>
Unused corrugated box	Demonstrated the initial strength value possess by the corrugated box before it is being used
Unfilled corrugated box	Conducted by removing out the product from the box, thus the box will be emptied
Filled corrugated box	Conducted to see the contribution of the product or primary packaging inside the corrugated box in supporting the box towards given compression

- Strength of the corrugated box reduced as the packaging goes further along the supply chain flow.
- Filled box of product A does not help in improving the corrugated box resistant towards the compression – excessive box space.
- Products or primary packaging (filled box) of product B & C give a good support to the corrugated boxes from the given compression.

### E. Recommendations to revise the safety factor (SF)

Decision tree was provided, emphasized on:

- If the content of the corrugated box (primary packaging or products) helps in providing mechanical support to the corrugated box from the given compression, hence, the corrugated box is well designed and can have minimum SF of 3 for newly produced corrugated box.

- ii. If the filled corrugated box performed poorly than unused corrugated box or there is problem such as self open perforation, the corrugated box needs to be re-design.

### Conclusion & Future Research

To achieve appropriate safety factor for corrugated box, Duni could follow the recommended decision tree that derived from the thorough data collections of shocks and vibrations, relative humidity and Box Compression Test (BCT) in the supply chain.

Future studies can be focused in three aspects:

- i. Studies to see the interconnection and interaction of product, primary packaging and secondary packaging along the supply chain, in order to improve the design and functionality of each entity.
- ii. Study the possibilities to minimize the number of supply chain actors. This would promote a leaner supply chain with less loading and unloading processes and thus a lower shock impact.
- iii. Study on the ageing effects of the corrugated box by conducting a project that following an actual lead time of the products. To make it more interesting, the study can be conducted outside Europe, in a different continent and with various mode of transportations.





## Effect of the variation of the formulation ingredients and process parameters on the stability and sensory properties of aerated desserts

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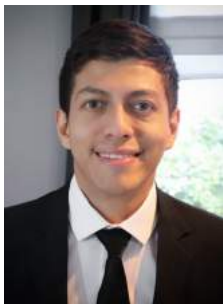
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The purpose of this project is to improve the formulation of the whipped topping of *Li geois* type dessert, leading to a reformulated product with 1. Longer stability in shelves 2. Better nutritional profile (due to a fat content reduction) 3. None or best organoleptic impact and 4. Price improvement. The scope of the project covers also the production process, since its parameters should be adjusted to the new formulation.

***Confidential topic***



## Comparative Study of Different Characterization Methods of the Cooking Level in Cereal-based Products

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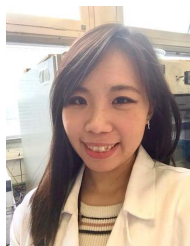
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### Master Thesis tutor :

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Dr. Stéphanie ROUX



### Introduction

The usual manufacture of cereal-based products involves baking. Among all biochemical reactions, the Maillard reaction (MR), together with sugar caramelization, is the main chemical event during the baking process. During the course of the reaction, development of color pigments, fluorescence and cross-linking products are observed (Matiacevich & Pilar Buera, 2006), which give rise to different shades of color, desired flavor and aroma as well as the increase of toxicity (Purlis, 2010). The mechanisms attributed to the final quality of baked products depends on the physico-chemical characteristics of the raw dough (i.e. water content, pH, content of sugars and amino acid) and on the operating environment during baking (i.e. temperature, air speed, relative humidity, mode of heat transfer) (Zanoni, Peri, & Bruno, 1995).

In order to recognize an appropriate cooking level corresponding to desired sensorial properties of the products, a critical index should be proposed as a function of state parameter during baking. Due to the complexity of the reaction pathways and the difficulty to measure physical-chemical changes rates within the products, few successful real-time methods for baking products are proposed. To comply with this objective, several approaches are taken into account with the consideration of accuracy, repeatability, and flexibility.

Firstly, the surface color is the primary quality parameter evaluated by consumers and is critical in the acceptance of baking products (Broyart et al. 1998; Purlis 2010). Compared to visual evaluation by human inspection, the objective measurement systems used in the industry are determined by different approaches: (i) CIE  $L^*a^*b^*$  scale by tristimulus colorimeter (Broyart et al. 1998; Bosmans et al. 2013; Capuano et al. 2008); (ii) measurement of color production at 420 nm with or without pronase enzymatic hydrolysis and aqueous extraction (Farroni & Buera 2012; González-Mateo et al. 2009; Rufián-Henares et al. 2006); (iii) computer vision-based image analysis (Čáslavková et al. 2015; Gökmen et al. 2007).

It is also interesting to evaluate the employment of fluorescence to assess cooking level of the products, due to its sensitivity and direct link with the presence of advanced MR compounds and raw bakery ingredients. To determine fluorescence, there are three different ways: (i) measuring the total pool of fluorescent compounds (which is composed of those free in the matrix and the link-to-protein fraction) by sample extraction and further enzymatic treatment using pronase (Delgado-Andrade et al. 2006, 2008; Farroni & Buera 2012; Przygodzka et al. 2016; Rufián-Henares et al. 2006); (ii) measuring the extracted free fluorescent compounds (Delgado-Andrade et al. 2006, 2008); (iii) applying a front-face fluorescence spectroscopy directly on samples (Botosoa et al., 2013; Rizkallah et al., 2008).

Several characterization techniques are presented above. It is necessary to narrow down the bulk of selected methods which will be tested further while taking into consideration of the objective. To the best of our knowledge, non-destructive methods on intact samples without homogeneous surface (i.e. tristimulus colorime-

try and front-face fluorescence) are selected to compare with the same measurements on grounded samples with expected uniformity. The computer vision analysis possible to investigate the whole surface colorimetric characteristics is also studied.

### Objective

The purpose of the study was to find convenient, repeatable, and discriminant methods to evaluate the cooking level of cereal products. Not a suitable cooking level but an appropriate way of measuring would be determined for a sponge cake model. Solid knowledge on characterization techniques would enable to measure the level of cooking in an appropriate way, through proper selection of processing condition.

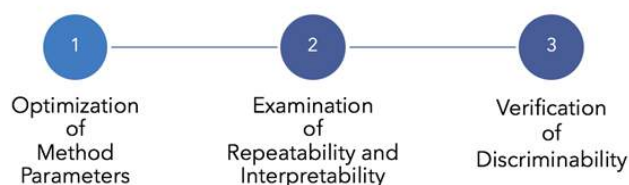


Figure 1. Experimental strategy for the determination of suitable characterization methods of the cooking level.

## Materials & methods

### Sample Preparation

Sponge cake model preparation was adapted from Cepeda-Vázquez et al. (2017): 500 g of batter was made up of 45% whole eggs, 25% flour, 25% sucrose, 4% non-hydrogenated palm oil and 1% NaCl. The samples were baked at 170 °C with a forced convection mode for 10, 15, 20, 25, 30, 35, 40, 45 and 50 minutes separately. In order to ensure that all parameters associated to browning were identical to each sample, radiation (temperature of radiant surface is 155 °C) and convection heat transfer abilities (convective heat transfer coefficient is in the

range of 16-18 W·m<sup>-2</sup>·K<sup>-1</sup>) were defined.

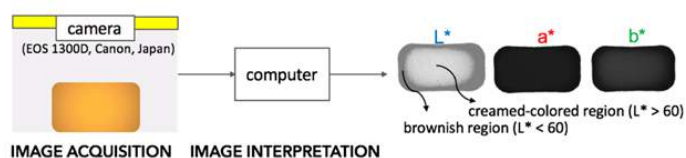
### Experimental Flow

In the first step, the prepared sponge cakes were measured by non-destructive techniques (i.e. tristimulus colorimeter, and computer vision, and front-face fluorescence) in 2 days after baking. Then, the samples were ground into powders and the L\*, a\*, b\* color parameters and surface fluorescence properties were again measured on the powder.

### Tristimulus Colorimeter

The mean of the values triply measured on the centers was used to represent the color characteristics of the crusts after justification by a Wilcoxon-Mann-Whitney test. On the other hand, measurements on ground samples were carried out with the expectation of color uniformity. Powders were placed in a covered transparent bowl and measured at 3 locations randomly. Their mean values were calculated to represent the color values.

### Computer Vision-based Image Analysis



**Figure 2. Schematic of the computer vision-based image analysis.**

The camera was positioned vertically using the following setting: manual mode with the lens aperture at  $f = 4.0$ , speed 1/200, and ISO100. With controlled illumination conditions, after precisely selecting the whole sample as the desired area by thresholding technique, images were analyzed in L\*, a\*, b\* units. Inspired by the previous study (Gökmen, Açar, Arribas-

Lorenzo, & Morales, 2008), browning ratio was calculated as: pixels of brownish regions divided by total pixels, where the brownish region was defined to have pixels whose L\* is lower than 60 units, and the opposite is the creamed-colored region.

### Front-face Fluorescence

Based on the excitation-emission-matrix (EEM) which was measured by means of a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, CA, U.S.A.), 6 fluorescence wavelength couples (FC) which showed corresponding tendencies along with time of cooking were identified as following: FC1 ( $\lambda_{exc}$  290 nm and  $\lambda_{em}$  330 nm), FC2 ( $\lambda_{exc}$  370 nm and  $\lambda_{em}$  424 nm), FC3 ( $\lambda_{exc}$  405 nm and  $\lambda_{em}$  485 nm), FC4 ( $\lambda_{exc}$  425 nm and  $\lambda_{em}$  485 nm), FC5 ( $\lambda_{exc}$  470 nm and  $\lambda_{em}$  530 nm), and FC6 ( $\lambda_{exc}$  495 nm and  $\lambda_{em}$  544 nm). FC1 and FC2 were measured at 520 V and the rest was measured at 540 V applying for all times of cooking. The single value of the center was considered to represent the product because no statistical difference was found between the average of 7 points and the value of the center. On the other hand, in order to reduce color inconsistency and structure interference, the powdered samples placed in a quartz cuvette were measured at the 6 defined wavelength couples.

## Results and discussions

### Method Reproducibility Verification

There were 4 trials carried out on different dates. Each trial was considered as independent data and a Kruskal-Wallis test was used to compare the L\*, a\*, b\* values, intensities of the 6 wavelength couples, and values of browning ratio between the 4 trials. There

was no significant difference ( $\alpha = 0.01$ ) between samples of each trial.

## Discriminability Verification

### A. Tristimulus colorimeter

1. The variation of lightness could be divided into 2 stages with cooking times. The darkening behavior after 10 min supported the development of browning. From 80 to 30, it followed linear regression ( $R^2=0.97$ ).
2. The change of hue was similar to lightness. After 10 min when the dough was shaped in the mold, it followed a zero-order kinetics ( $R^2=0.99$ ) which represent the change from yellow to red regardless to lightness and vividness.
3. The colorimetric results of the powders showed the same tendency as the intact samples. However, it was noticed that the variations of  $L^*$ ,  $a^*$ ,  $b^*$  values of powdered samples were more flattened due to different heat-moisture dynamics. The intensity of color was diluted by the inner part because the rate of color formation was slower.

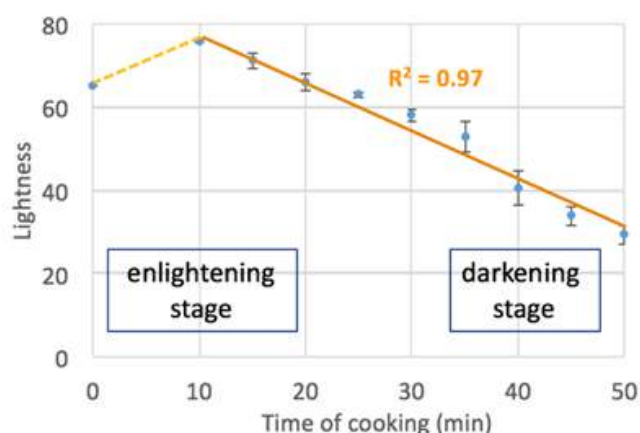


Figure 3. Lightness change during cooking.

Figure 6. Variation of fluorescence intensity at the selected couple wavelength ( $\lambda_{exc}$  290 and  $\lambda_{em}$  330 nm).

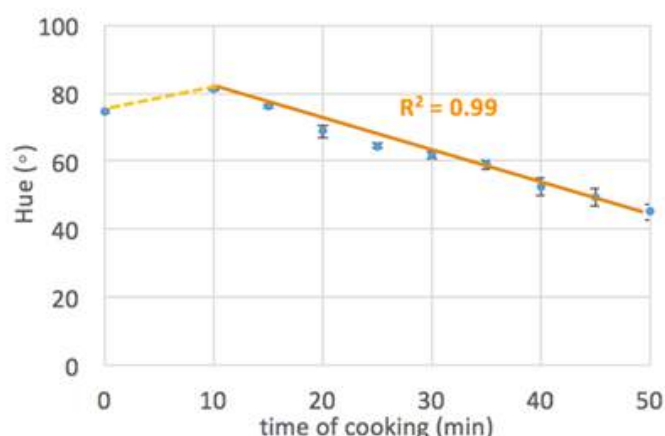


Figure 4. Hue change during cooking.

### B. Computer Vision Image-based Analysis

The browning ratio had a promising relation with times of cooking. The dynamic spread of brownish pixels could be followed from the edge to the center all along the kinetic.

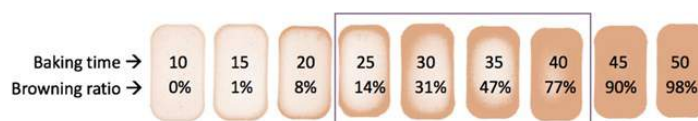
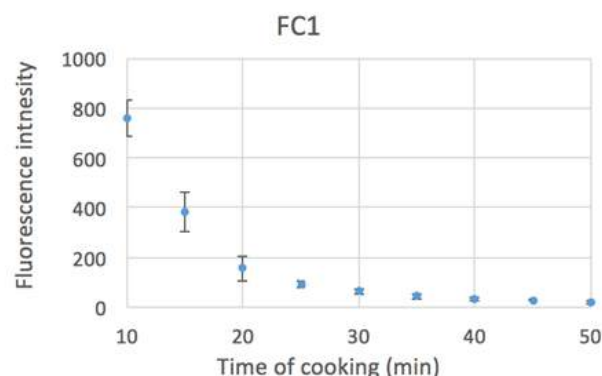


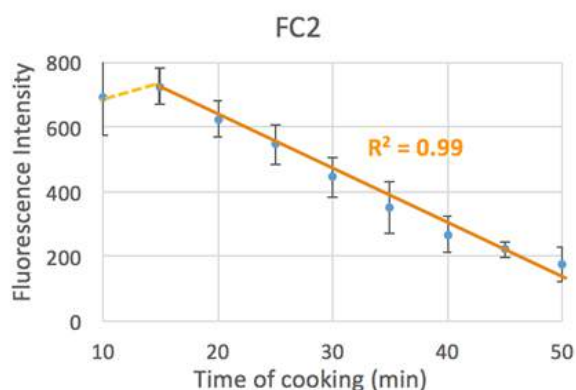
Figure 5. Illustration of segmented images of sponge cookies baked for different times with calculated percentages of browning ratio.

### Front-face Fluorescence

1. FC1 was recognized as the tryptophan due to its wavelengths and its presence at the beginning of cooking.
2. FC2 was recognized as ferulic acid. After around 15 min, the amount decreased until the end of cooking following a simple linear correlation ( $R^2=0.99$ ).







**Figure 7. Time course of fluorescence intensity at the selected couple wavelength ( $\lambda_{exc}$  370 and  $\lambda_{em}$  424 nm).**

3. The rest of FCs were hard to identified to a specific compound. While by their behaviors, we could relate them to advance or final Maillard reaction compounds.
4. The results of fluorimetric characterization of powders didn't meet the expectation of sample consistency because of differences between quartz cuvettes.

## Conclusion

Measuring on the cake without grinding was recommended for the three techniques.

Tristimulus colorimetry was simple, fast and highly linked to what consumers would see. The two zero-order kinetics of lightness and hue after sample solidification provided strong differentiability.

Computer vision allowed samples to be analyzed without contact. The approach could be easily modified by the thresholding of  $L^*$  parameter to expand its application for other products.

Front-face fluorescence was rapid and sensitive, providing spectral signatures in a few seconds, as long as the measurement parameters are determined. Each FC has typical trend along with baking times enabling to serve as the cooking level markers at different stages of cooking.

It should be notice that the results presented in the study were specifically defined for the sponge cake model. These markers and values could be reconsidered before implemented for similar products. Applying these novel methods coupled with sensory analysis, the food industry could effectively estimate product characteristics and control baking process.

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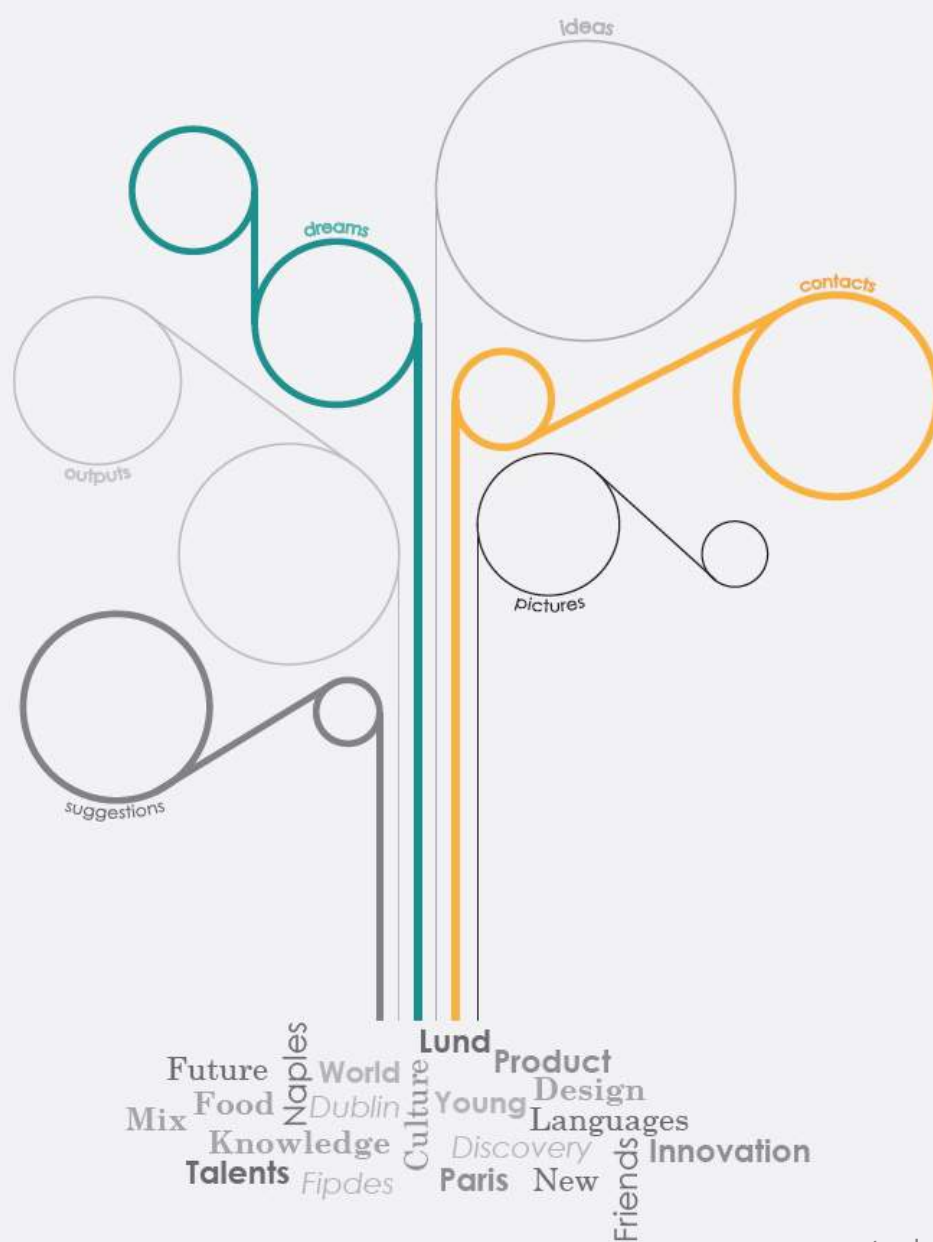
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