

Food Innovation & Product Design

New horizons for interdisciplinary food challenges

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Congratulation to the FIPDes Cohort 4 !



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Welcome to the FIPDes community!

Dear FIPDes Graduates of 2016,

Welcome back to Paris for your graduation! It is such an honor to be able to write this message to you as you complete your Master's degree in Food Innovation and Product Design.

You have been on such an amazing academic and personal journey. The Erasmus Mundus program is truly one-of-a-kind in offering a food development degree taught in four European countries. And as a class, you are a global community representing 14 countries. This combination represents the power and uniqueness of the FIPDes degree program, i.e., a multicultural perspective from both the academic locations and the diversity of the student body.

The degree itself is a diverse course of study merging the science and the art of food design which I think leads to the most important word within your degree - innovation. This Masters provides a strong background for innovation within the global food industry, a role which is becoming ever more important in meeting the food supply needs of the future. The food industry has to evolve, and I am confident you are well prepared to help in that evolution.

I was fortunate to teach you about sensory and consumer science in Dublin in June of 2015. I was not sure what to expect of a group of students from such diverse backgrounds and experiences, and studying far from their home countries. But I must say I was quite impressed with your passion for this program, your willingness to explore new approaches to evaluating food, and your engagement level overall. These qualities will surely serve you well as you move on in your careers, and they will serve the industry well in helping to feed the world.

I wish you all the best and look forward to hearing about your successful careers!



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Generation of furanic compounds in model sponge cakes with respect to changes in formulation and baking conditions

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Introduction

Bakery products are the rich source of aroma compounds and it has been reported that they produce more than 540 volatile compounds during baking (Cho and Peterson, 2010). Several of these volatiles have an important role in the quality of the product. Furan and Furfural are two important furanic compounds responsible for safety and sensorial aspects of product quality respectively. Occurrence of furan in food is a matter of safety concern as the International Agency for Research on Cancer (IARC, 1995) has classified furan as “possibly carcinogenic to humans” -Group 2B. On the other hand furfural imparts characteristic flavor and aroma to the product (Pico, Bernal, & Gómez, 2015). Furan and furfural are closely linked compounds, which makes it important to understand the evolution of these compounds during the course of baking. Formation of these two compounds can be due to several mechanisms occurring simultaneously during baking depending upon the formulation and baking conditions. Furan, furfural and other furanic compounds in bakery products are majorly formed due to caramelisation and Maillard reaction but there are other reactions like thermal degradation of certain amino acids and thermal oxidation of ascorbic acid & polyunsaturated fatty acids which can also contribute to these compounds as well. The complexity of formulation always makes it difficult to pin point the exact mechanism behind the generation of these compounds as tracing the reaction pathway is difficult. There are different other factors like structure of

the matrix, moisture content, time and temperature which are closely linked with reactions involved. To understand the role of specific precursors along with other physical factors it's important to control certain parameters like structure of the cake, moisture content, time and precursors.

The aim of this master thesis was to explore the possibility of using an inert model cake designed from non reactive ingredients (Bousquieres et al., 2016) for studying the effect of different physical parameters and precursors on furan and furfural generation during baking.

Objectives

The main objectives of this study were:

- To evaluate the inertness of the model towards furan and furfural generation during the course of baking.
- To evaluate the generation of furan and furfural in the model cakes added with precursors like D-glucose and leucine.

An Overview

In this study, model sponge cakes were developed according to recipe by Bousquieres et al., 2016. The model was developed using corn starch and a hydrocolloid solution containing hydroxypropylmethylcellulose and methylcellulose. All these ingredients are stable at baking temperature and hence were considered suitable for developing a model to study chemical reactivity.

Apart from native model two other models were designed in order to facilitate specific reaction pathways: (i) Caramelization in model (G) containing only glucose and (ii) Maillard and Caramelization reactions in model (G+L), containing leucine along with glucose.

This study utilized a novel Headspace Trap-GC/MS method for furan analysis. It was introduced

by Huault et al. (2016) and was further developed and optimized by Cepeda-Vazquez et al. (2016) which facilitated the simultaneous screening of furan and furfural. The quantification method used in this study was internal standard method using a mix solution of deuterium labeled isotopes of furan and furfural in water.

The volatiles were extracted in headspace by equilibrating the temperature. The compounds were trapped on an air monitoring trap. The compounds then were desorbed into GC. Volatile analytes were separated on a column using helium as a carrier gas with constant flow rate followed by mass spectrometric detection.

Evaluation of inertness of the model

Model's inertness towards generation of furan and furfural during baking is an important aspect as it decides the feasibility of this model to study chemical reactivity of different precursors. The models without any precursors were baked at different baking conditions to cover large process range.

The results obtained show that the models do not produce any furan and furfural on its own as the levels were found below the detection and quantification limits of the method, until certain heat load is applied. Only furan was found in trace amount (close to the range of LOQ), when the baking time was extended.

Quantification of furan and furfural generated in different models cakes during the course of baking

Being inert, the model cake proved to be very appropriate for studying the generation of furan and furfural with respect to different precursors and other physical parameters during the course of baking. Both the models were indeed found to produce furan and furfural during the baking on addition of specif-

ic precursors.

Furan and furfural both were observed earlier for G+L model as compared to the other G model. Although the rate of reaction was different in two models, furan and furfural concentration increased with time in both the cases. Since the model with glucose had D-glucose as the sole precursor, the only reaction involved in this case is caramelization. The reaction occurs at slow rate as it requires low moisture content and temperature over 120°C for the dehydration of glucose (Purlis, 2010), which explains the late appearance of furan and furfural during the baking.

In the model G+L, the presence of leucine along with glucose provides free amino groups to react with reducing sugar through the Maillard reaction. Appearance of early browning on the surface suggests that the loss of moisture also triggers the Maillard reaction but earlier and at a faster rate than caramelization. Maillard reaction is usually followed by caramelization reaction at higher temperature and much lower moisture content leading to more intense browning (Hadiyanto et al., 2007; Purlis & Salvadori, 2009).

Conclusion

This study demonstrates the suitability of the developed model cake for studying the chemical reactivity of different precursors in the solid food like the sponge cake. Both physical and chemical factors have evident effect on governing thermal reactions like Maillard and caramelization reaction. Loss of moisture content from the surface triggers the reactions. It is an important factor which determines the rate of reaction. Also in a complex cascade of reactions, it is important to track the intermediates (dicarbonyl compounds). These compounds can have a catalytic role and thus can help to find the exact links between the reactions.

Future Prospects

This work has set the base for studying kinetics of formation of furanic compounds along with the degradation of precursors and even the formation of specific key intermediates. Different hypothesis regarding the role of precursors on reaction pathways occurring in solid food can be easily elucidated. Also, the possibility to change and control the structure is an important characteristic that will make it possible to study the impact of the structure on reactivity. The kinetic parameters so generated will be used to model the link between chemical reactions and physical variables of the process and product.

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Alternative packaging insulation Material solutions for a meal kit Subscription Box industry leader

Participatory action research and a case study for material identification, testing and implementation

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Introduction

The grocery market is one of the few remaining major commercial retail industries to transition to an online model. This sector represents an enormous area of opportunity for online retailers as it is the largest retail category and the second largest expenditure per household, second to only home mortgages or rent payments. Globally, the food industry is more than two trillion dollars. Food retail represents half of this (Noah Advisors, 2015). The Boston Consulting Group estimates that global egroceries will be a \$100 billion business as soon as 2018 if customer adoption rates continue at current pace (Segran, 2015) (Brandau, 2015).

A meal kit subscription box (MKSb) is a weekly egrocery service that is comprised of a box packed with a specific number of meals for a set number of people delivered to the customer's doorstep. Each ingredient is pre-portioned into the exact quantities required for nutritionally balanced and chef curated recipes created to cater to that specific consumer segment's preferences.

HelloFresh Ag & Co (HF) is one of the largest start-ups to come out of Europe within the last five years. Today, the Berlin-based company is the leading provider in the MKSB industry with more than 7.2 million meals delivered monthly to more than 500,000 subscribers. HF has operations in Germany (DE), Austria, The Netherlands, Belgium, the United Kingdom (UK), United States of America, Switzerland, Australia

and Canada. This master thesis work focuses specifically on operations in DE where a majority of boxes are delivered to customers via United Parcel Service (UPS). Currently, HF DE uses an insulated secondary package solution made from sheep wool from UK company Woolcool.

Objectives

This thesis work serves as an investigation and testing of packaging solutions for last mile delivery of perishable food products sold via ecommerce channels, specifically within the meal kit subscription box industry.

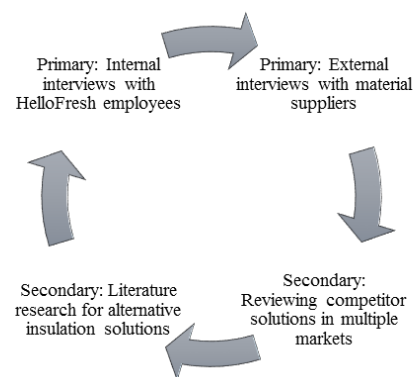
The study is done within the context of the case study subject's (the industrial partner) product, production, and logistics' requirements. The industrial partner commissioned a review and determination of alternative insulation materials from sustainable sources to insulate their food products during delivery to the end customer. Additionally, the industrial partner required coolant usage recommendations in order to ensure products are adequately cooled during transport through their logistics streams to fulfil legislative and food safety requirements. In order to achieve these objectives, the research questions (RQs) were framed as follows:

- RQ 1. What packaging solutions and materials are currently being used by competitors within this industry to chill their products?
- RQ 2. What kinds of alternative insulation materials could be employed?
- RQ 3. How is the performance of said insulation materials when introduced into the industrial partner's current packaging system?
- RQ 4. What temperatures are endured by the industrial partner's boxes while in transit?
- RQ 5. How much PCM should be placed inside the package to ensure products stay sufficiently chilled during the entire transit period?

Materials and Methods

The study was carried out via participatory action research that included qualitative and quantitative methods.

Primary research included interviews, participant observation and a case study by the author. Interviewees included internal employees, external current and potential packaging material suppliers, and academics in the area of packaging and materials science. Internal stakeholder interviews were key in deriving information and constructing the case study. External interviews with packaging and material suppliers aided material identification and procurement of material samples for testing. Academic professionals interviewed helped the author to better understand package heat transfer and the test method employed.



Participant observation was employed by the author throughout the entire course of the study, specifically within the production facility where the author participated in the pick and pack (PnP) and quality assurance (QA) systems' activities on a regular basis.

The insulating capability of procured package materials and systems were tested via a pragmatic method called a heat penetration test. Packages were packed with 2 kg of ice and held at a constant ambient temperature for a known period of time. After the test period, the remaining amount of ice was

weighed. This allowed for the determination of the package's h^* (heat penetration rate) value. This method is similar to ASTM D3103. The equation to derive h^* is adapted from the Fourier equation for steady-state heat flow through a flat surface via conduction.

$$Q = -kA \frac{\Delta T}{\Delta x}$$

The symbol k is the thermal conductivity of the barrier material, Q is the total amount of heat transfer, A is the surface area over which heat is passing (meters squared, m^2), ΔT is the temperature gradient across the barrier material (i.e. the outside temperature versus the temperature inside the package; degrees kelvin, K), and Δx is the thickness of the barrier material (meters, m). As the area, thickness, and thermal conductivity of the package system are fixed variables, they can be combined into one term representing the overall package system. This value is denoted by h^* with units of W/K (watts per degree kelvin).

$$h^* = k * \frac{A}{\Delta x} = \frac{W}{mK} * m^2 * \frac{1}{m} = \frac{W}{K}$$

In order to determine Q , the amount of heat that penetrates a package system, the amount of melted ice (m_{ice}) is multiplied by the latent heat of fusion of ice melting to water which equals 334 joules (J) per gram (g). One joule is equal to one watt (W) per second (t). The latent heat is “energy absorbed or released by a substance during a change in its physical state that occurs without changing its temperature” (Encyclopedia Britannica, 2016). While ice melts, it remains at zero degrees Celsius and the water that is produced from melting is also zero degrees Celsius.

The calculated penetrated heat is then divided by the total time (t , seconds) the ice was melting and the temperature difference (ΔT) between the ambient air outside the package and

$0^\circ C$ (the temperature of the melting ice and melt water). This then generates the h^* value of the package system.

$$h^* = \frac{m_{ice} \Delta H^\circ}{t \Delta T} = \frac{g * J}{t * K} = W/K$$

A smaller h^* (heat penetration rate) value is an indicator of a better insulating system. Considering the equation of h^* , the less heat that is able to pass through the insulation (ΔH) per unit time (t) given a specific temperature gradient (ΔT) will result in a colder packaged, insulated product.

Temperatures within logistics were collected using data loggers and compared to historical weather data via regression analysis.



Figure 1. Example of the datalogger used for collecting logistics temperatures: EasyLog 21CFR-Compatible Temperature Data Logger with LCD Screen, Manufactured by Lascar Electronics

Results and Discussion

RQ #1

Insulated packaging solutions of key MKSB and grocery competitors across the globe were reviewed. The overwhelming trend was towards disposable packaging. However, consumers are increasingly sensitive towards



Figure 2. An insulated shipping box lined with hay from German packaging company LandPack, and a reusable insulated shipping container employed by FreshRealm (USA).

the amount and source materials of the packaging they receive with their food deliveries. Customers perceive the required packaging for portioning and shipping these ingredients as very wasteful (Konrad, 2015). According to a study done by the ECC Cologne, 36% of German consumers would be more inclined to order groceries and food online regularly if the food was packaged in environmentally friendly packaging and with less packaging waste (lfhkoeln.de, 2015). In order to compensate for this, materials that are made from recycled materials, 100% recyclable, or 100% biodegradable are used and touted in their marketing material.

For example, Plated – another major US competitor – funded the packaging startup Temperpack in order to source 100% plant-based liners for their boxes. Additionally, many of these MKSB businesses provide recycling programs where the customer may ship back their ice packs, insulation, and any other packaging. However, customers complain that this can be even more cumbersome than just going to the grocery store and buying the ingredients themselves (Lazzaro, 2016).

RQ #2

Both natural and unnatural materials were considered. As e-groceries and the corresponding insulated food box is a relatively new industry, other sectors and corresponding material research studies were reviewed in order to derive alternatives, such as the housing and automobile industries. The most promising materials—in terms of price and availability—included recycled polyethylene terephthalate (PET), hemp, jute, wooden wool, cellulose and sheep wool. These were then tested for performance in RQ #3.

RQ #3

Materials were procured from local providers and introduced into the HF current packaging

system. This is comprised of a flat piece of insulation lined in perforated plastic, then folded and placed inside an external plastic envelope to form an insulated “pouch.” Suppliers that were capable of providing their material in a complete solution had their submissions tested in their existing state. The packages and materials were then subjected to the heat penetration test as described in the methods section. Varying densities and thicknesses were tested.



RQ #4

In order to garner a better understanding of the temperatures boxes are subjected to during transport as well as to ensure temperatures of chilled products are maintained within safe, mandated temperatures, temperature dataloggers are routinely included in weekly test boxes shipped from the production facility to the company’s headquarters. This historic data was compiled and analysed. Average temperature in transit versus historic weather data was analysed via regression to generate a predictive average temperature model. By being able to estimate the temperatures that would be endured and knowing the transit times, these data points were then entered into the calculation (in RQ #5) to determine the amount of coolant to pack into the customer boxes to ensure they are delivered properly chilled.

RQ #5

A constant question and struggle for the industrial partner was that of how to determine the proper amount of coolant to pack the box with. The amount of coolant affects margin due to the cost of the gel or ice pack itself, plus the increased cost of shipping the additional weight. Furthermore, it is an operational burden to repeatedly review packing schemes and estimating—based on past experience—the amount of coolant to use.

By utilizing the packages test results from the heat penetration rate test method from RQ #3 (h^*), the average transit temperatures from RQ #4 (uncooled temp), the latent heat of fusion of ice, and the known logistics times (time) and routes (cooled temp), one can then safely calculate the coolant amount quickly and simply.

$$\begin{aligned} \text{total coolant required (medium Woolcool pouch)} = & \frac{h^*(\text{time}_{\text{seconds,cooled}})(\text{cooled temp.})}{\text{latent heat of fusion}} + \frac{h^*(\text{time}_{\text{seconds,uncooled}})(\text{uncooled temp.})}{\text{latent heat of fusion}} = \\ & \frac{\left(0.2826 \frac{\text{joules}}{\text{sec} \cdot ^\circ\text{C}}\right)(46800 \text{ sec})(7^\circ\text{C})}{\frac{334\text{J}}{\text{g}}} + \frac{\left(0.2826 \frac{\text{joules}}{\text{sec} \cdot ^\circ\text{C}}\right)(61200 \text{ sec})(18.1^\circ\text{C})}{\frac{334\text{J}}{\text{g}}} = \\ & \mathbf{1214.44 \text{ grams of ice}} \end{aligned}$$

Conclusion

In a survey conducted by Bitkom, the digital association of Germany representing more than 2300 companies in digital economy, the reasons German consumers do not buy groceries online include the wait for delivery, doubts regarding product freshness, shipping costs, and privacy concerns (Ecommerce News, 2016). Additionally, as previously mentioned, German MKSB customers are sensitive to packaging material quantity and source.

Meanwhile, the cost of the materials utilized to maintain the required product temperatures during last mile delivery are the costliest features of the secondary packaging solution. These materials include the insulation material

itself, associated plastic packaging with said insulation, and all PCMs (phase change materials, such as ice or gel packs) required to control the temperature in transport. However, the resulting solution for any type of packaging should be very inexpensive. Meal kit subscription box (MKSB) companies pursue a margin of 10% while traditional grocery stores have a margin between 2-4% (Fallgren and Sundborg, 2013).

This thesis work sought to solve these problems via determination and testing of alternative sustainable materials that are also cost competitive combined with a simple method for calculating coolant usage to ensure safe food delivery and avoid overpacking chilled products.

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Packaging solutions to reduce discoloration in spices due to light exposure

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Introduction

Light exposure causes discoloration in some of the spices from Santa Maria Company. Consumers have identified the problem in shop shelves at retailers. Additionally the company analyses the option of adding LED magnetic lights in their displays to create an improved ambiance: Also it was relevant to consider that the European (EuP) Directive 2005/32/EC, which took effect in 2016, promotes the use of more LED bulbs due to its lower environmental impact. In order to understand which packaging material could provide sufficient protection to reduce the pigment discoloration process in Santa Maria spices; different scenarios were considered, in the form of 3 different arrangements representing controlled conditions and a simulation of the retail environment. Also the most relevant spices for the company were selected to conduct the study.

Research objectives

1. To suggest good practices regarding the display of the spices on the shelves.
2. To determine under which type of light conditions and packaging solutions, the selected spices are less susceptible to present discoloration.
3. To provide Santa Maria with insights to make an informed decision when implementing an innovative packaging solution.

Delimitations

The study was limited to the perceived visual changes in the selected spices: Paprika,

Basil, Cayenne Pepper, Parsley and Grillkrydda (a blend of spices). And to use of different provided packaging materials.

The changes in the molecules present in the spices whereas how the flavor and taste might be affected were not considered in this study. Water activity was only measured for spices that were stored without packages.

Methodology

A mixed methods strategy was used for this study, which consisted of a qualitative phase, formed by semi-structured interviews and observations; and a quantitative phase including 3 different set ups or arrangements. These arrangements combined the use of different packaging materials and the storage under the exposure to different light conditions.

The simulation of different storage conditions was possible due to the use of different facilities; those facilities were located in Ljus Lab in IKDC building for arrangement 1 and 2 and in Santa Maria facilities for arrangement 3, the reasons why each arrangement was used are described below.

Arrangement 1: Without packaging in and under 5 different light sources.

Arrangement 2: With packaging protection against light and reproducing a controlled scenario.

Arrangement 3: The most similar scenario to conditions in the retailer environment, where spices were stored with different packaging materials in a shelf display.

The following figure gathers the most relevant points considered in each phase as the asked questions and the test performed to the spices exposed under the different conditions of each arrangement.

The Eq-1 was used to correlate the obtained results from the colorimeter into a value that

allowed comparing the changes in color between the spices.

$$\Delta E^* = \sqrt{(L_0^* - L^{*ref})^2 + (a_0^* - a^{*ref})^2 + (b_0^* - b^{*ref})^2}$$

EQ-1

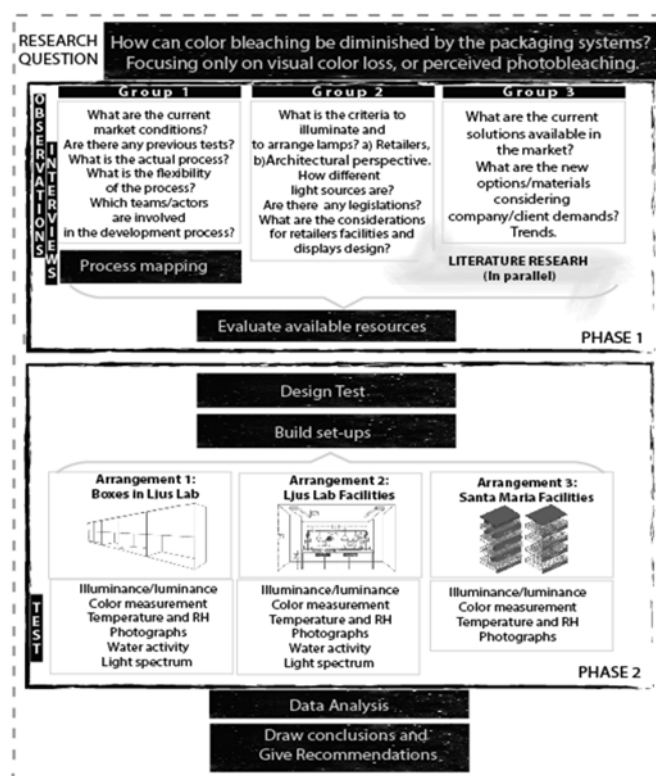


Figure 1. Research question and thesis outline. Phase 1 consisted of a qualitative research and Phase 2 a quantitative research based on insights from phase 1.

Hypothesis

Based on the initial screening the following hypothesis was made:

A combination of packaging materials would increase the protection against the light, without modifying greatly the manufacturing process and the actual supply chain.

Results

Figure 1 shows the process mapping summarizing the insights obtained from phase 1, these results identify where the contact with light causes more damages to the color of

the selected spices, and guided the focus of the study for phase 2 to only the retailers and households light exposure.

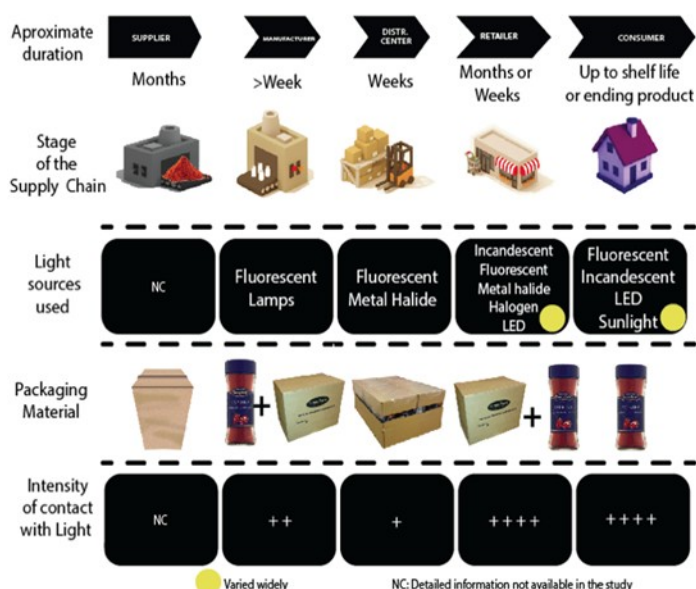
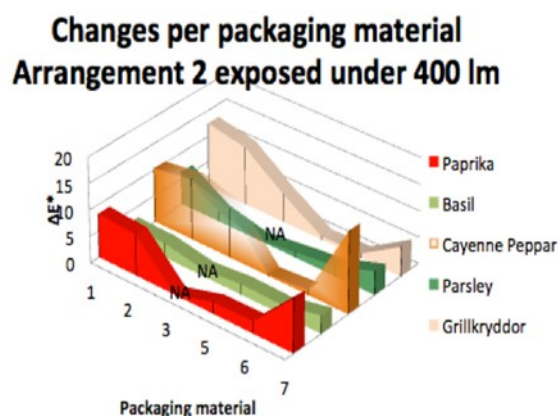


Figure 2. Process mapping, results from observations in warehouses, retailers, and households. Semi-structured interviews with experts and academia

Based on the EQ-1 the values ΔE were calculated for every condition on every arrangement, below in table 1 (only arrangement 2: LED lights of 400 lm.) The "X" axis represents the packaging material in which the spices were contained (1:P1 detailed in Figure 4). The packaging materials offering less protection were the P1: glass jar, followed by the P6: PET bottle:

The spices were placed at the same distance to the luminaries, having a similar exposure to light. The combination of materials P1 and P6 provided an added protection, which was represented by P7: Glass jar+ PET plastic sleeve. The spice with less color changes was Basil.

Table 1. Values of ΔE per packaging material and spice variety.



The velocity of spices discoloration under the studied light arrangements depended on: 1) The type of light source, being Sunlight and LED the most harmful, 2) The distance to the light source, being the closest the most dangerous, and 3) The spice variety, being the red spices the most sensitive. Thanks to the performed measurements it was observed that the order of color change on the 5 studied spices, remained the same regardless the arrangement. Figure 2 shows the spices and its sensibility to light, being "1" the most sensitive and "5" the less sensitive.



Figure 3. Studied spices and their sensitivity to light. Being the red spices (with presence of carotenoids) the most sensitive

The color bleaching was directly proportional to the amount of light passing through the used packaging material. Figure 4 shows that if more light was passing, more pronounced was the color bleaching observed in the contained spice (the blue color represents the light coming inside the packaging material and the percentages are only explanatory).

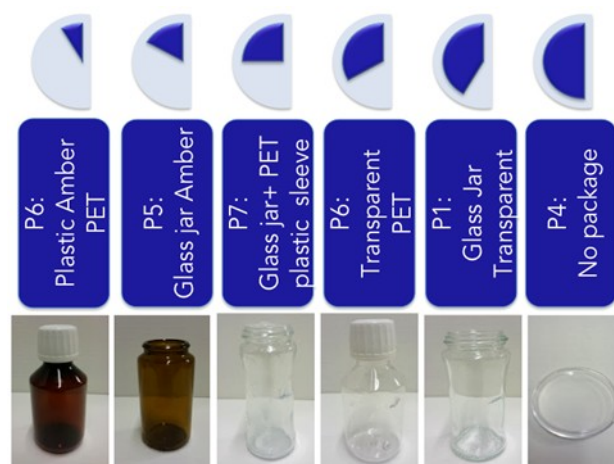


Figure 4. Light passing through each package

Conclusion

Only opaque packaging materials presented a sufficient barrier against the detrimental effects of lighting. The combination of packaging materials (PET + glass) presented an improved effect as stated in the initial hypothesis. Nevertheless, the light blockage was not in the same amount as opaque packaging materials. In the case of the studied spices, red spices (Paprika, Cayenne Pepper and Gryllkrydda) bleached faster and in a more visually detectable way than green spices. (Basil and Parsley) as stated by Klein(1984). All the important aspects observed in this study are gathered according to the research objective they are answering. Also a recommendation for a new packaging solution and it can be seen below,

Answering to Objective 1:

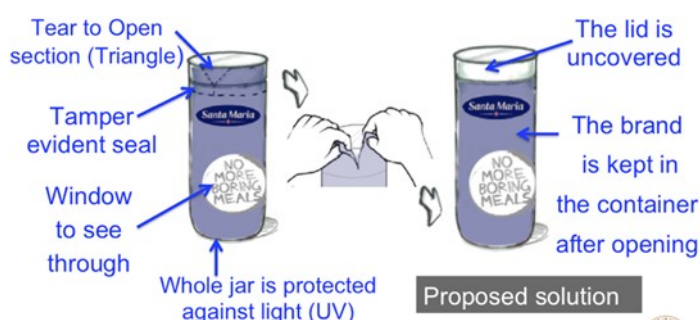


Figure 5. Proposed solution

Answering to Objective 2:

- Artificial LED light had a stronger effect than other environmental conditions regarding colour bleaching.
- LED light accelerates the bleaching process in the studied spices.
- The closer the luminaries were placed, the strongest the effect on color bleaching, confirming what was referred by Cuttle (2015) with other materials. (Demonstrated mostly with arrangement 3).

Answering to Objective 3:

- Red spices bleached faster, being consistent with Klein et al, (1984) about carotenoid and chlorophylls degradation. Is important to consider these spices for light protection tests.
- Only opaque materials presented a robust protection against light in all different arrangements, (P5 and P6).
- P2 suggested that a correct combination of materials could provide the desired protection against light.

PET recycling chain is very developed in Sweden, which means the suggested packaging solution with a bigger plastic sleeve wont have an increased Carbon footprint as the material is already in use for the current temper evident seal, and the label will need to be eliminated.

Keywords

Spices, Color Bleaching, Packaging, Light effect, Shelf display, Retailer's arrangements, glass, PET, LED lights effect on foodstuffs.

More information

<https://www.youtube.com/watch?v=5QSwpf0Uqaw>

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(More references in full text)



Impact of two different modalities of milk preparation on a new soft cheese technology and its optimization

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Profile in a nutshell:

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Chr. Hansen is working on the development of a new technology for a specific soft cheese variety. It aims to bring several industrial benefits such as greater flexibility in production planning because of process simplification and lower investment costs for equipment. The technology brings in change in some steps in the production process. It also includes development of a new starter culture to complement the changes in the process.

This project was focused on application of the traditional and the new cheese-making technology to two different milk types- 'traditional' and 'modern'. The two milk types differ in composition and also the preparation process. The aim was to compare the reference fabrication (Traditional milk + Traditional technology) with the other three in terms of biochemical composition and acidification kinetics, and make changes for technology optimization to close the existing differences between the cheeses.

The preliminary pilot productions were done at Chr. Hansen soft cheese pilot plant which was followed by the main trials at INRA, Rennes. Compositional analysis was performed for the milks used and the cheeses. The cheeses were analysed and compared with respect to MFFB% (Moisture Fat Free Basis), Ca/SNF% and pH at demoulding. The milk preparation had an important impact on the fabrications. Based on the results obtained, certain technological parameters were optimized. The optimization showed effect of



improvement in certain analysis. The study shows the approach to be taken for further application of this new technology.

Confidential Topic



Determination of brewing conditions for developing an innovative ready to drink tea product

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Green tea contains high content of polyphenols with potential health benefits. Tea beverages have recently gained a lot of popularity. In particular, ready to drink teas are a growing segment in the beverage category, brought about by improvements in the flavor of these products and healthy market trends driven by consumers. In this context, a start-up in progress wants to develop an innovative ready to drink tea beverage to provide consumers health benefits and original taste. The aim of this work was thus to determine the brewing conditions contributing to high polyphenols content and antioxidant activity, and to study the effect of adding other polyphenol-rich ingredients, in particular by-products from food industry.

Confidential topic



Development of New Easy to Open Cheese Packaging for Elderly in Sweden

A Design Thinking Approach

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Introduction

The population is ageing, especially in western countries. The baby boomers that were born post-World War II, are in their fifties now – if not older – and serve a large group in the market. Increased demands on convenience along with environmental sustainability are among the biggest trends nowadays. And when it comes to food, prepacked food that has been growing over time has to encounter a big challenge in its packaging, especially the primary packaging that will be handled by the consumers.

With a big leap in the 1970s when there was a campaign of six to eight sandwiches a day by the Brödinstitutet (Swedish Institute of Bread), the cheese consumption in Sweden keeps on increasing over time. This made cheese considered a staple in Sweden, especially the range of Swedish block cheeses. These cheeses are packed in colour coded packaging, putting a strict limitation for graphical design. Having a distinct feature on the packaging can be a way to stand out in the market.



Figure 1. Color coded Swedish cheese packaging

Considering the need of convenience, easy to open is one aspect that is widely observed. This is also supported by the fact that ISO just established ISO 17480:2015 on

Ease of Opening in the end of 2015. Applying easy to open feature on block cheese packaging in Swedish market can be a way to stand out in the market.



Figure 2. PopPack-- easy opening solution with bubble compartment (source: <http://www.poppack.com/>)

PopPack® is a novel easy opening technology involving an additional bubble compartment within the packaging. The main idea is to burst the seal around the bubble as it is popped to provide separated opening tabs to grip.

Design thinking approach is used in this project. Since primary packaging is a part of the consumption unit, human factor is crucial in this project. Given its nature of being a human-centred methodology, design thinking is deemed a good fit for this project.

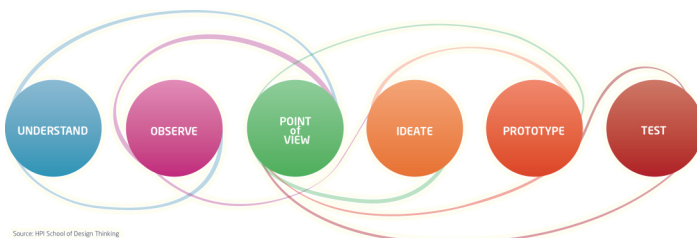


Figure 3. Design thinking process by Hasso-Plattner-Institut School of Design Thinking (used in this project)

Objectives

This project is carried out in collaboration with Flextrus AB, a packaging supplier in Sweden, who has the authority to implement the Pop-Pack® technology on their products. With a focus on block cheese product, the main objective of this project is to develop a new easy to open packaging solution to cater the needs of elderly consumers in Swedish market. The elderly market is chosen based on the assumption that if the solution works well for them, it will work for other user group as well; also because of the reason of ageing population in western countries.

Evaluation of design thinking approach in the field of food packaging development was also an important objective in this project. This makes this project unique, because design thinking in the field of food packaging development has never been reported in an academic document and is considered a new approach in the industrial partner's development process.

Method: Iterative process of Design Thinking

| Stage | Aim | Activities |
|---------------|--|---|
| UNDERSTAND | To assess the aspect and nature of the problem | <ol style="list-style-type: none"> 1. Literature studies on food packaging trends, cheese, and ergonomic requirements 2. Expert interviews with Managing Director of Pop-Pack LLC and Sales Expert |
| OBSERVE | To find out the user needs through direct observation and interaction with the customers | Customer test with 30 elderly panellists (above 65 years old) in ÅR Packaging Pension Meetup and Lund University Area. |
| POINT OF VIEW | To analyse findings from Understand and Observe stages and establish the main desires to address | <ol style="list-style-type: none"> 1. Development of Consumer Persona (profile) 2. Building User Journey Map 3. Main properties establishment |
| IDEATE | To generate ideas in order to address the main properties established in Point of View stage | <ol style="list-style-type: none"> 1. Brainstorming with participants from different divisions in Flextrus AB (product expert, machinery expert, business manager, and product developer) 2. Idea selection (scoring and mapping) |
| PROTOTYPE | To make a physical prototype of the winning idea generated in the Ideate phase | <ol style="list-style-type: none"> 1. Manual prototyping (laboratory scale) 2. Automated prototyping (pilot scale) with form-fill-seal machine |

| | | |
|-----------|--|--|
| | To evaluate the prototype's performance in both qualitative and quantitative manners | 1. Qualitative Tests a. Scoring Test with 15 panellist (staffs in Flextrus AB and students in Lund University) per sample b. Popping Success Rate measurement by popping 20 replicates per sample 2. Quantitative Tests a. Popping Force measurement by using the Mecmesin Mutitest 10-i machine b. Peel Strength analysis by using INSTRON equipment |
| PROTOTYPE | To iterate the best performing prototype from the previous step, based on the results of the tests | Mounting the rubber plate on the sealing part of form-fill-seal machine to manipulate the seal strength |
| TEST | To evaluate the desirability of the product in the customer's side | Pitching the idea to the cheese manufacturer (Arla Foods Denmark) |

Results and Discussion

In the understand phase, the researcher gained understanding about the current cheese packaging solution, trends in food technology, as well as the understanding upon the PopPack® technology. To enrich her knowledge, the researcher did a direct contact and observe the consumer behaviour toward the current cheese packaging solution and the PopPack® Prototype 0 during the observe phase.



Figure 4. Observation tests with panelists aged above 65 years old in ÅR Packaging's Pension Meetup

Having all the needs gathered, researcher built a list of needs expressed in persona and consumer journey map in the point of view phase. In the end of this phase, a problem fields were established, giving the further direction for the next phase. The problem fields are the main aspects to be considered in making the solution, they are listed as follows;

- Easy to open function (popping)
- Good popping sound
- Low peel force
- Attractiveness (fun shape, aesthetic standpoints)
- Easy to understand concepts

The problem fields were then turned into brainstorming questions in the ideate phase. The ideas generated were then clustered into six different groups as follows;

- Commercial and widgets inside bubble
- Graphical designs
- Bubble designs
- Bubble position
- Materials
- Sealing/Peel

After a voting, the ideas that will be looked into further is the bubble designs, then the ideas in this cluster was mapped into an impact-effort matrix. The best ideas were then discussed further for the technical details on prototyping. Four bubble designs were decided to be tested in the prototypes.

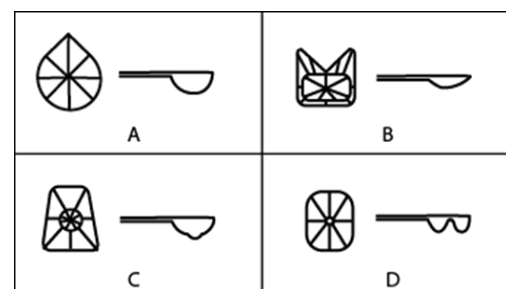


Figure 5. Bubble designs from ideation session (left: top view; right: side view)

The prototype phase started with manual prototyping in Flextrus AB's prototyping laboratory. The attempts taken here were not working because of the sealing failure. Then the prototyping was continued on the pilot scale, utilising the form-fill-seal machine in PackDesign AB's facility. Only three bubble designs were included in the prototype due to the machine limitation. There were three different material combination and/or process conditions tested, giving this project three different prototypes with three bubbles on each, resulted on nine samples to be tested (bubble A in P1, P2, and P3; bubble B in P1, P2, and P3; bubble C in P1, P2, and P3).

| Code | Bottom | Top Web |
|------|--|--|
| P1 | Nylon 70/100 Forming 5.8 seconds at 105°C, 6.5 bars | Ecobar 12/40 AF Peel Sealing 2 seconds at 120°C, 3 |
| P2 | | OPET/PEP Peel Sealing 2 seconds at 115°C, 3 bars |
| P3 | | OPET/PEP Peel Sealing 2.5 seconds at 120°C, 1.5 bars |



Figure 6. (left to right) P1, P2, and P3

In first test phase, there were two qualitative tests and two quantitative tests carried out. The first qualitative test was the scoring test, resulting on the compatibility of each sample to the problem fields established before. The second qualitative test was the popping test as suggested by PopPack LLC. This test gives the re-

sult on which design is the most success in the term of making the popping sound and bursting the seal. From these qualitative tests, the best performing sample is bubble A on P2.

The quantitative tests include popping force and peeling strength. Both were done on machines, resulting on a graph of force needed to pop the bubble or peel the packaging (separate top and bottom web). Unfortunately, the best performing sample in these tests were not quite conclusive, due to lack of actual range of nominal forces desirable. But based on several approaches around it and insights gathered from experts, it was decided that bubble A on P2 still performs better than the others.

Bubble A on P2 is the winning idea. To improve this sample, this project was sent back to prototype phase. A technical iteration was carried out in order to manipulate the seal strength around the bubble. As illustrated on Figure 7, the sealing rubber plate was mounted except for the part around the bubble, in order to weaken the strength of seal in this area. Given the limited duration, similar tests could not be carried out for this iterated solution as it was done from the previous samples. Instead, on the last test phase, the iterated prototype was shown to the cheese manufacturer and feedbacks were gathered for further development of this project.

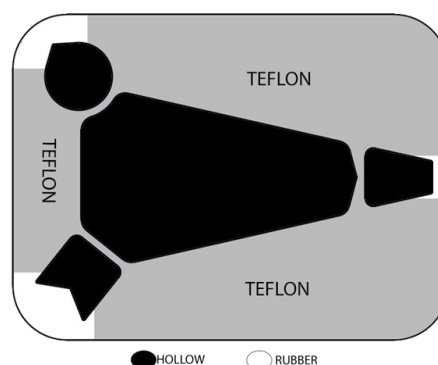


Figure 7. Modification on the rubber plate at the sealing compartment

Conclusion and Recommendation for Further Research

Among the three tested bubble designs, bubble A performs the best among the other bubble designs and P2 gives the best material combination and process conditions.

Design thinking approach is a good method to be used in this kind of project, resulting on an in-depth understanding about consumer needs, therefore giving the project an orientation to start with. Some challenges were faced during the ideation/brainstorming session but the whole process was worth the experience of introducing design thinking into this project.

Further studies can be carried out in the exploration of applying the PopPack® on to reclosable packaging material, since there was a demand of reclosability in the cheese packaging through the consumer test. Trying it on a semi rigid packaging solution can also be a way to broaden the range of products in which PopPack® can be implemented.

Suggested by the cheese manufacturer, the bubble should be located in the sharp corner of the cheese packaging. This will impact the angle of peeling, therefore it is necessary to proof that bubble A will still perform as good in different angle.

Investigating the possibility of implementing the solution on the packaging line of block cheese, including the machine flexibility to assist the change of reel size is important. Another solution could be by changing the machine in packaging line, or changing the size of the cheese. Both alternatives are costly; therefore an investigation needs to be done.

Establishing a range of the nominal force so that the solution has a more defined goal for the development is also important. Another consumer study to see the acceptance of the solution, as well as testing methods of educating consumer (i.e. imitating commercial videos)

are necessary before the solution is launched.

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Master Thesis document can be found at the URL below:

<http://www.lunduniversity.lu.se/lup/publication/8884318>





Technological and Functional Characterization of Cricket Powder for New Food Product Development

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Profile in a nutshell:

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Introduction

Increasing world population and protein demand results large amount of available agricultural land occupied and providing these needs is only possible in sustainable ways. Beside conventional protein sources, novel protein sources (like insects, algae, and duckweed) are proposed in recent research as an alternative for animal-derived proteins to enter the European feed and food market (Yi, 2015).). It is estimated that insects form part of the traditional diets of at least 2 billion people. More than 1900 species have reportedly been used as food (FAO, 2013). Today, there is an increasing interest in the academic field to discover all the potential that edible insects offer regarding many topics, among them nutritional value keeps an important space. an important space.

Insects are good source of protein, lipids, certain vitamins, and minerals such as calci-

with animals showed that quality of insect protein is superior than of soy and casein (Ekpo, 2011; Finke et al. 1989). The fat content of the widely consumed insect groups range between 13.41 to 33.4 % of the total nutrient content (Rumpold and Schlüter, 2013). Edible insects are reported to contain a good quality fatty acid particularly long chain omega-3 fatty acids such as alpha-linoleic acid (Micek, 2014).

The most important functional properties of proteins in food applications can be listed

as: protein solubility, water holding capacity, foam stability, gelling and emulsifying capacity and fat binding properties. When a protein ingredient is added to a formula it is often adding more than just protein. Moreover, protein ingredients are one of several ingredients in foods and therefore must “function” in a complex system (Foegeding, 2011). The challenge for food product innovation involves the introduction of new ingredients that improve food functionality and nutrition while retaining the familiarity of conventional foods (Krishnan and Darly-Kindelspire, 2013).

Objectives

The objective of this thesis is to evaluate the possibility to use insect powder as functional ingredient in bakery foods. In particular, in this thesis the experiments are carried out on *Gylodes sigillatus*, also called “The banded cricket”. Its technological properties including the solubility, water absorption capacity, foaming and emulsifying properties are firstly evaluated. Then a recipe for “cracker” prototype including insect proteins is formulated and the consumer acceptability of the produced crackers is determined with a sensory panel test involving 20 participants. Among functional properties of the insect protein-enriched crackers the Glycaemic Index (GI) of the prototype is measured *in vitro*.

Materials and Methods

1. Technological Properties

Solubility

Solubility of the protein-rich ingredients was estimated using the procedure of Mishra and Rai (2006) and Martinez et al. (2011) with slight modifications.

Water Absorption Capacity

Water absorption capacity was measured as described by Gani et al. (2012).

Foaming Properties

The capacity and stability of foams were determined by the method that was described by Kaur and Singh (2005).

Emulsifying Properties

Emulsifying activity and stability were measured according to the method that was described by Neto et al. (2001) with slight modifications.

2. Product Development

Recipe Formulation

Several recipes are formulated and the ingredients are chosen accordingly. “Crackers”. Wheat and soy crackers are also prepared in respect to the formulation of cricket crackers as they are only used as control samples in glycaemic index analyses. Among 50:50 and 70:30 ratios, 70:30 is chosen as the perfect formulation (wheat flour: cricket powder / soy flour).

Sensory Analyses and Survey

A 5-point rating scale system is used to gain insight on consumer attitudes towards to edible insects. A 9-point rating scale system is applied for the sensory analysis of the product. Participants are asked to evaluate the certain attributes such as colour, taste and crispiness of the product.

3. In vitro Glycaemic Index

The in vitro starch digestibility was determined by the method of Kim and White (2012) with slight modifications.

4. Statistical Analyses

The data obtained were statistically analyzed with Open Office Statistics, using one way analysis of variance (ANOVA). Student’s t-test was applied to measure the significance between the samples at $P \leq 0.05$.

Results and Discussion

1. Technological Properties

Solubility of cricket powder was found 21% and water absorption capacity was measured as 101.2%; these values were higher than wheat flour. Emulsion capacity of cricket powder (10.5%) was lower than wheat flour (33.8%) however greater emulsion stability occurred with cricket powder (84.7%) than with wheat flour (50%). Similar results were obtained for the foaming properties. Foaming capacity of cricket powder was 28% lower than wheat flour despite a higher stability of foam ($>85\%$) compared to wheat flour ($\geq 75\%$). Cricket crackers were developed by replacing 30% of wheat flour.

2. Sensory Analyses and Survey

Results of survey suggested that those who are open to trying novel protein sources are also willing to adopt insects in their daily diets. Sensory analysis results showed that the crispiness was the most liked attribute of the insect-enriched crackers while the color and the taste were not appreciated by the panellists.

3. In vitro Glycaemic Index

GI of the cricket crackers was estimated at 62.3 which classifies this product among the Intermediate GI foods.

Conclusions

In this study for the first time technological properties of *Gyllodes sigillatus*, also called "The banded cricket" powder were tested. Moreover a prototype of cracker containing 30% of the insect powder was developed and sensory analysis was carried out. The glycaemic index in vitro was compared to soy-enriched cracker and 100% wheat cracker. All in all data showed that despite the technological properties of the cricket powder tested in this study and its applicability for new cracker development, the

new food formulation should be improved in future studies in order to improve sensory properties of the final product and/or obtaining a new food that can also be more favourable for the GI.

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A study of milk protein digestibility: an *in vitro* approach

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Introduction

Unlike any other animal, humans continue to consume milk beyond infancy, particularly cow's milk. However, as the composition of each species' milk differs to meet the specific needs of its young, this can prompt nutritional, digestive, and immunological complications. Depending on the body's reaction or immune response, this can manifest as cow's milk allergy, CMA, or cow's milk protein intolerance, CMPI.

Reduction in allergenicity or intolerance can be achieved by altering the protein structures in milk. This can be completed through heating processes, fermentation, where by-products may aid in the digestion of milk proteins, and enzymatic proteolysis.

Current products on the market to address CMA and CMPI are commonly aimed towards infants and toddlers, such as powdered formulas, as this population group is the most affected. However, one Finnish dairy company released a milk product to target consumers who suffer from mild gastrointestinal discomfort due to milk intake. This product is both lactose free and with partially hydrolyzed milk proteins. It is proven to better relieve digestive issues than standard lactose free products, and highlights a market opportunity for such digestive-ease dairy products with hydrolyzed proteins (Sibakov, Tossavainen, 2013).

Finally, the current published studies on this topic focus on protein hydrolysis in powdered or isolated protein sources, rather than in a complete milk system.

Objectives

The aim of this thesis was to set up lab scale conditions to develop a highly digestible milk through the use of enzymatic hydrolysis in a whole milk system. This process will be used to create a milk that is more digestible for those with digestive sensitivities, intolerances, or allergies to milk proteins.

To this effect, the time, temperature, and enzyme concentration were manipulated to optimize milk stability and peptide formation. Furthermore, the effect of milk types on stability and peptide synthesis were observed.

Materials and Methods

The selected enzyme is classified as an endopeptidase and is characterized by broad amino acid specificity. For confidentiality, it was coded as TS13.

The enzyme activity was tested on the following milks:

- UHT partially skimmed fat (UHT/PS)
- UHT lactose-free partially skimmed fat (UHT/LF/PS)
- Pasteurized partially skimmed fat (P/PS)
- Pasteurized lactose-free whole fat (P/LF/W)
- Microfiltered lactose-free partially skimmed fat (MF/LF/PS)

The experimental methods have been omitted for confidentiality purposes.

Stability analysis included observations of color change, measurement of pH, and performance of the dairy-industry alcohol test. Peptides were extracted via Strata C18 2ml column and analyzed by LC/HRMS and orbitrap. A marker peptide was selected to track milk digestibility. This marker peptide will remain confidential.

Results

1. For stability, UHT/PS and MF/LF/PS remained stable at higher levels of TS13 than the other milks
2. For optimum peptide synthesis to achieve reduced allergenicity, 0.0003% TS13 in UHT/PS provided the most desirable amount of marker peptide. However, this sample is unstable.
3. For stability and peptide synthesis, UHT/PS milk was found to be the most suitable overall.
4. Lengthening hydrolysis time to 15 minutes at 0.00015% TS13 shows a noticeable increase in marker peptide formation, and is stable. However, it does not create levels of the marker peptide that match the market reference milk. Therefore, the reduction of allergenicity or intolerance should be further investigated.

Conclusion and Future Prospects

Future studies should focus on TS13 concentrations above 0.00015% and below 0.0003% at hydrolysis lengths of greater than 10 minutes and less than 20 minutes.

Lastly, it may be of interest to explore the synthesis and utilization of calcium-binding bioactive peptides to ameliorate colloid stability issues resultant of protein hydrolysis.

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Extraction of Functional Oligosaccharides from Spent Coffee Grounds

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Introduction

Coffee is one of the most important commodities worldwide. It ranks 2nd in terms of trade only after petroleum¹⁰. An industry with such dimensions generates a large amount of residues that are liberated along the production chain. Only 6% of the harvested crop is used for the production of the beverage while the remaining 94% is by-products²⁶. The main sources of these include: husks, skin, pulp, mucilage, parchment, and silverskin, in addition to discarded beans that don't enter the process⁷. Additionally, once the brew is prepared, more waste is generated such as the Spent Coffee Grounds (SCG). These are the constituents of the water insoluble fraction that remains after extraction with hot water either with the aim to produce instant/soluble coffee or in order to get the coffee beverage at commercial or home scale. SCG have received an increased amount of attention due to the fact they constitute a source of functional and bioactive compounds.

Briefly, they contain a significant amount of protein (8.5-13%) with an amino acid composition similar to soybean meal. with a high Fischer ratio²⁰. Additionally, the non protein nitrogenous fraction in SCG includes small compounds that are highly valued such as: caffeine, theobromine, theophylline, paraxanthine and other alkaloids and melanoidins¹⁸. In regards to their lipid fraction, the content ranges from 15-20% which is mainly linoleic, palmitic, stearic and oleic acids and sterols such as sitosterol, stigmasterol, and campesterol known

for their serum cholesterol lowering and diterpenes like cafestol and kahweol that have applications in the pharmacological and cosmetic industry⁷. Furthermore, SCG contains phenolic compounds such as chlorogenic and caffeoylquinic acid as well as being rich in potassium¹⁷.

The carbohydrate fraction is the focus of this research. They surround 50% of the dry weight of the SCG and are mainly composed by water insoluble polysaccharides, namely: 40 to 50% galactomannan, 32% arabinogalactan, 18% cellulose and 0.5 to 1% of xylan and other minor sugars¹⁴. Galactomannan has the potential to yield oligosaccharides with functional properties that are called manno-oligosaccharides (MOS) and are composed principally of mannose with a degree of polymerization (DP) of 2 to 10 units of mannose³. MOS have been correlated to creating positive effects on health acting as prebiotics¹. Some studies report the following benefits: in shrimp MOS improved feed conversion, modulated intestinal microflora and enhanced the resistance against pathogens⁸; in rats, they reduced fat absorption and blood pressure¹, reduced the level of abdominal and subcutaneous fat and adipose tissue in the liver, reduced fat absorption in the intestines and increased the amount of fat excreted²⁴ and in humans MOS improved defecating conditions by increasing volume and Bifidobacteria²⁵.

Currently, the techniques aimed at the extraction of these oligosaccharides from SCG comprise the following: thermal, enzymatic and microbial hydrolysis, alkali treatments, microwave assisted extractions, supercritical fluid extractions and acid catalyzed hydrolysis which will be the method used in this research.

Objectives

The general objective of this research was to evaluate an alternative way to obtain a valuable product from a common waste sourced from the food industry such as SCG. More specifically,

it was aimed to perform an acid catalyzed extraction on the material left after espresso brewing, to analyze the resulting products in order to identify valuable oligosaccharide chains and finally, to assess their functionality by carrying out tests on the growth of common probiotic bacteria.

Materials and Methods

Raw material and preparation of the sample

SCG obtained from brewing with a commercial espresso machine (Rancilio Classe 10 USB 2, Italia). The SCG were subject to a reduction of the moisture from 53.97% to 3.69% by drying at 60°C for 8h in a stove (MMM Group IP20, Germany). The lipids were extracted by using overnight agitation with hexane (Sigma-Aldrich) with a 1:4 ratio (m/V). This was performed twice and the recovered material subject to evaporation of the residual hexane.

Acid catalyzed extraction

The method consists in an adaptation of¹¹ and¹. Defatted SCG were suspended in distilled water to form a 5% (w/w) slurry. Its pH was adjusted to 3.8 with HCL (2M). The slurry was then introduced in the reactor. It consisted of a stainless steel cylindrical chamber with a volume of $2 \times 10^{-4} \text{ m}^3$. The chamber was introduced in heated purified sand (silicon dioxide, Sigma-Aldrich, Italy) with a temperature of 200°C. The residence time of the chamber was split in two periods: the 1st consisted in the necessary amount for the slurry to reach the thermal equilibrium with the system whereas the 2nd consisted in the reaction time. The first period was of approximately $4 \times 10^2 \text{ s}$ for all the experiments while the reaction time consisted of 30, 60 and 90s depending on the sample. Once the time was reached, the chamber was removed and cooled. Next, the slurry was neutralized with

NaOH (1M). The solution was centrifuged and the hydrolysate, was recuperated

Characterization - HILIC HRMS detection

The identification of hexoses was performed according ⁶ and ⁴ without derivatization. Briefly, 10 µL of each extract were added to 90µL of Merck water and were subsequently injected into the LC-HRMS system. The mass of the hexoses to be identified ranged from C₁₈H₃₂O₁₆ (504.437Da) to C₆₀H₁₀₂O₅₁ (1639.421Da)

Hexoses separation was achieved on a U-HPLC Accela system 1250 (Thermo Fisher Scientific, Bremen, Germany) and obtained by using a Luna amino column (100x 2.0 mm, 3.0 µm; Phenomenex, Torrance, CA) operating in HILIC mode. To set up the optimal condition, an aqueous solution of stachyose (10 µg/mL) was infused directly into the Exactive Orbitrap HRMS system (Thermo Fisher Scientific, Bremen, Germany) equipped with a heated electrospray interface operating in the positive and negative mode and scanning the m/z range of 60–1200.

Functionality assessment

It evaluated the growth of selected bacteria in different mediums in which the source of carbohydrates was replaced by the SCG hydrolysate. They corresponded to 4 strains of Lactobacillus: L. delbrueckii (YOV5), L. johnsonii LA5 (LA5), L. johnsonii LA3 (LA3) and L. reuterii DSM 17938 (REU). The medium preparation consisted of 6 different MRS broths with diverse source of carbohydrates. MRS 1 to 4 replaced glucose with a fixed volume of the SCG hydrolysate that came from the 4 samples. MRS 5 corresponded to a standard formulation made with glucose and MRS 6 replaced glucose with inulin. Bacteria were inoculated in triplicate in test tubes into a chamber (Memmert UN160, Germany) set at 37°C for a period of 24h. Their growth was assessed by a turbidimetric method that consisted in measuring the optical density (OD) of each sample with the

use of a spectrophotometer (Eppendorf Bio-Spectrometer Basic, Canada) at 650nm.

Results and Discussion

Extraction procedure

The main goal of a rapid, high temperature and low pH hydrolysis was achieved by performing the hydrolysis at 200°C with a pH of around 3.8 for 30 to 90s. The process carried out in this experiment evidenced that after 30s of extraction the differences in the products obtained aren't significant from the ones attained at 60s and 90s meaning that, in this procedure in particular, there is no further hydrolysis after 30s. This can be seen in the figure 1 which shows that the only procedure that yielded a lower amount of oligosaccharides was the one where the SCG were used without the removal of the fat. An interesting advantage of processes like this relies on the short time needed to achieve the cleavage of the long chains as opposed to other techniques that have the same goal. Even though the process is carried out in a batch the time needed is very short making it suitable for a potential scale-up. In order to be successful, the conditions should be controlled more thoroughly ⁵, especially the temperature inside the reactor. Even though acid hydrolysis is one of the most effective methods for hydrolyzing polysaccharides¹⁷, it is imperative to avoid long periods to prevent hydrolysis to monomers.

Composition of the extracts

The extraction yielded a mixture of 7 molecules of hexoses with a DP from 3 to 6 (see figure2). Two isomeric compounds were found in the case of chains of 3, 4 and 5 hexoses and one for a chain of 6 hexoses. Experimentally, it could not be concluded the exact characterization for these isomeric compounds; however, based on bibliographical research some inferences can be made.

The process and composition make it suitable for attaining either galactomannose or mannose-only chains from the main galactomannan polymer. Galactomannans consist of a main chain of 1→4 linked mannan with galactose unit side chains linked at C-6 with different degrees of branching. In a polymeric structure the side chains are released first followed by the cleavage of the main chains^{21,17,23}. These assumptions lead to infer that it is highly probable to have obtained oligomers from the cleavage of the galactomannan polymer at sites where the chain was branched with galactose as well as at sites where there was not a substitution

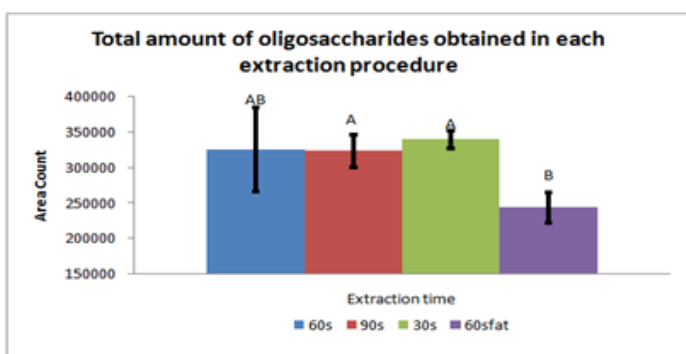


Figure 1 : Total amount of oligosaccharides obtained depending on the extraction procedure

The extracts had the tendency to yield a high amount of trisaccharides (>70%) followed by tetrasaccharides (15-20%) and low quantities of pentasaccharides (4-7%) and hexasaccharides (<1%). More trisaccharides and less hexasaccharides were produced compared to previous results¹¹. The extract where the fat was not previously removed and was subject to a 60s extraction, sample 60sfat, yielded significantly lower amount of carbohydrates as well as had a different composition (Figure 3). 60sfat is significantly different from the others in its amount of trisaccharides, which is lower, and in the amount of 4-hex-A and hexasaccharides in which is higher. The other 3 extracts don't present considerable differences among themselves in terms of composition. The profile of sample 60sfat shows significantly lower

amount of more simple sugars, higher presence of longer chain molecules, as well as the relatively lower yield which leads to conclude that the presence of the fat in the SCG caused a marked composition towards bigger chains. This can be an evidence that the fat interfered in the extraction thus limiting the overall amount of oligosaccharides obtained and reducing the degree of cleavage of the chains. It could be hypothesized that its presence caused the matrix of the SCG to be more complex thus, the energy required is higher to achieve the same results as when the fat was removed.

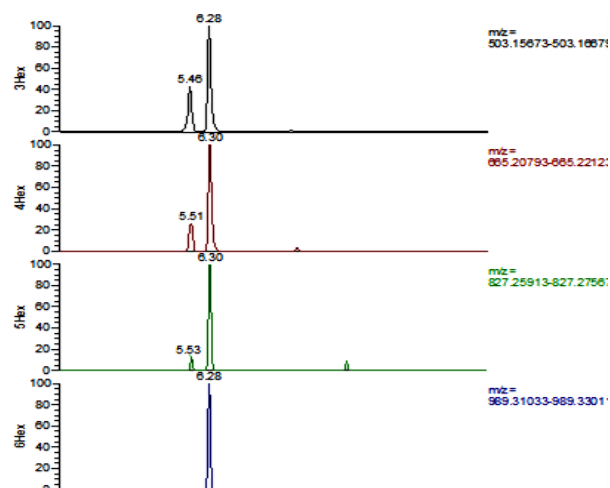


Figure 2 : HILIC HRMS identification

Functionality

Lactobacilli present a high preference of substrates. Within this genus, the capacity of individual strains and species for oligosaccharide metabolism differs substantially⁹. Although its range of fermentable carbohydrates and selectivity is very broad, virtually all lactobacilli metabolize α-glucans. Pathways for disaccharide metabolism also enable the metabolism of tri and tetrasaccharides; nevertheless, the metabolism of oligosaccharide is limited by transport¹². Their metabolic preferences are towards compounds that are composed of 2 to 10 monosaccharide residues^{22,12}. This preference of

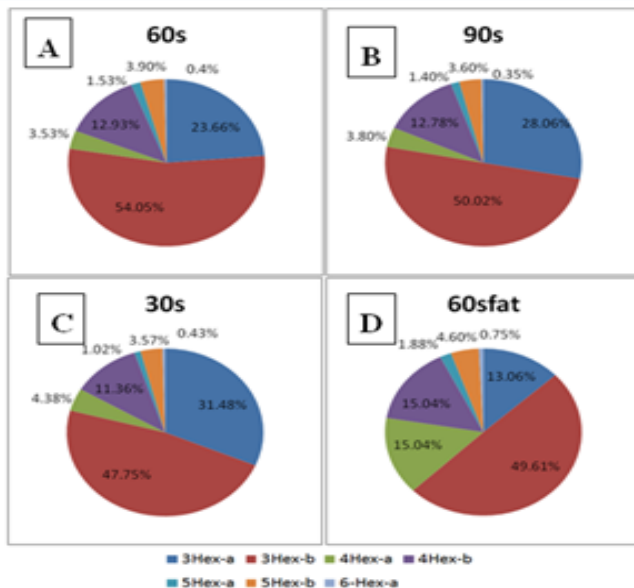


Figure 3 : Relative amount of each hexose identified

substrates is evidenced in the results in which there was an overall tendency to prefer any of the media prepared with the extracts as opposed to the MRS prepared with inulin which is composed of fructose molecules linked with terminal glucose residue with a DP that can range from 10 to 65 fructose units being 12 to 15 the most common¹⁵. This study corroborated that there is a preference of *Lactobacillus* for fermenting shorter chains as opposed to longer chains. Other researchers also tested inulin and other prebiotics on probiotic bacteria and reached the similar conclusions²⁴, longer time incubation time is needed before inulin is fermented as opposed to shorter chain oligosaccharides which are readily used in the upper part of the GIT²⁷ and that, some lactobacilli have a low potential to metabolize higher degree oligosaccharides^{13,16}.

Nonetheless, the behavior of all the bacteria of this study was not the same, as seen in figure 4. This behavior is attributed to the high selectivity and particular metabolism of each strain. The fermentation substrates of *L. johnsonii* are largely restricted to mono, di, and trisaccharides thanks to the abundance of PTS sugar transporters and galactosidases and because it lacks xylanases, amylases, and other enzymes that depolymerize higher polysaccharides¹⁹. This

supports the fact that both strains of this specie were the ones that developed the best in the three media as opposed to (Yov5) and (Reu). The case of Yov5 is special since this strain developed equally poor in every medium. This strain is better at consuming substrates rich in sugars with a DP 4-5¹⁶. All the extracts were relatively poor in these oligosaccharides thus it's hypothesized that their composition was not useful for Yov5. REU did not develop as well as *L. johnsonii* either. This strain is characterized by being one of the most representative species to have a narrow spectrum of oligosaccharide ferment-

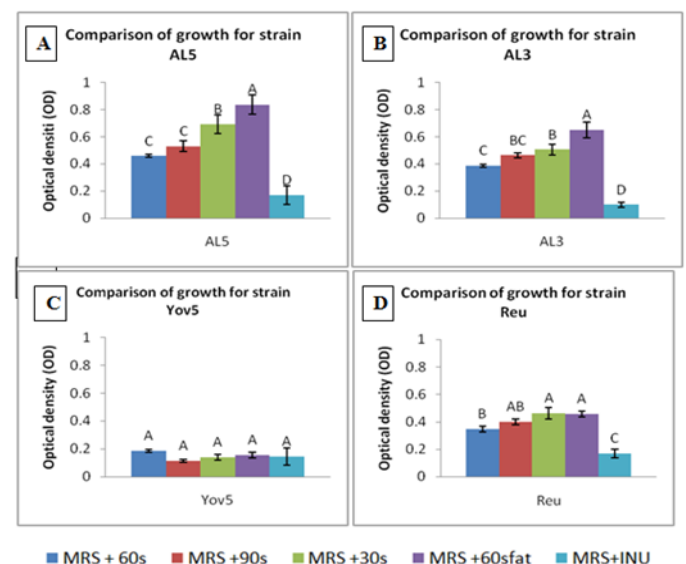


Figure 4 Comparison of the bacterial growth of each strain in the different mediums (Glucose not taken into

Figure 4 : Comparison of the bacterial strain growth in the different media

tation in the GIT. Its high selectivity on the sugars it ferments cues to conjecture that that is the reason behind its lack of proper development in these media. Also there was a statistical difference in the growth of AL5 and AL3 in the medium that contained 60sfat. Since still this sample contained a high amount of trisaccharides (71.60%), one of the preferred substrates of *L. johnsonii*, the better development of the strain can be attributed to the presence of other compounds that were not identified. There's a

chance that the effects of the presence of the lipids in the SCG matrix at the time of extraction generated or maintained other compounds that yielded beneficial for the growth of this strains; however, a more in dept analysis of this hypothesis should be further explored.

Conclusion

This research consisted in an exploratory approach of the possible uses of food waste, namely, spent coffee grounds. The acid catalyzed hydrolysis implemented which relied in short time, low pH and high temperatures in a batch reactor, poses as a faster and simpler alternative to obtain water soluble extracts composed of oligosaccharide hexose chains with a DP from 3 to 6. Additionally, the main products were obtained just after the 30s making it unnecessary to treat further. These extracts are potential sources of prebiotic carbohydrates for *Lactobacillus* strains that showed to be more effective, in these conditions, than inulin. It was also shown that there is an effect on the composition and functionality of the extracts when the lipids are not removed causing a different behavior due to its known fragments as well as some other unidentified components.

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Evaluation of vegetable raw ingredients for the development of fermented yogurt-like products

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Introduction

This study was aimed at understanding how to develop fermented yogurt-like products based on vegetable sources. Multiple raw ingredients were characterized in terms of chemical composition and technological functionality. The multiple products obtained were submitted to rheological and fermentation analysis.

Confidential topic



Recommend a Specification for Potato Flakes to Deliver Product Quality and Process Efficiency in Savory Snacks

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When doing new product development involving the use of innovative raw materials, some challenges can be faced regarding process efficiency and final product quality. The lack of studies and knowledge about the novel ingredients can make it difficult to design the specification sheets for suppliers as the impact of the characteristics of the raw material in processing and quality remains a question.

This problem was faced by Mondelez International, when using potato flakes in one of its snack products. Therefore, one of the objectives of this project was to study and characterize this ingredient using analytical techniques. Furthermore, another objective of the research was to find the relevant criteria in order to select the potato flakes, taking into account their performance in the final product at pilot plant scale. The results of this project were useful in the design of a more adjusted specification sheet that will help to deliver process efficiency and assure consistent finished product quality.

Confidential topic



Exploitation of ozone-based processing technology for the development of high quality melon fruit products

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Introduction

The increasing demand for healthful foods has been growing in recent years and the food industry is constantly looking for new sources of nutritional and healthful components. Besides numerous scientific investigations point fruits as rich sources of antioxidants, only few of them involve waste parts of the products. Great quantities of waste and by-products of fruits are formed during industrial processing. Melon fruit is highly consumed worldwide in a variety of forms, and also well known as a healthy and nutritious product. Few works have studied the main characteristics of melon [1, 2]. Only limited information on the nutritive value and bioactive compounds is available, and the existing is especially focused on Cantaloupe melon. However, no studies have been reported concerning the presence of these healthier compounds in the fruit waste parts. Bioactive components of some fruits waste parts have been quantified and compared with their edible parts, which corroborate the potential of these by-products as sources of antioxidants (carotenoids and polyphenols) [3, 4]. A careful assessment of the transformation process is crucial, seeking development of new strategies for adding value to fruit products. Ozone-based treatment is a promising technology that is gaining interest in the fruit industry. The efficacy and usefulness of ozone has been proved over the years with its widespread application in the treatment of water and food [5]. The effect of gaseous ozone on the quality of Cantaloupe melons has showed

no impact on quality attributes [6, 7]. Studies on the efficiency of ozone application in fruit juices have also been done, mainly in apple and orange juices [8]. The majority of published works concluded that ozone mainly affected the juice colour and that juice quality degradation is highly dependent on the type of juice, treatment time and ozone concentration applied [9, 10]. This work points out ozone as a potential non-thermal technology that can be considered as an alternative to conventional thermal processing in order to achieve high quality fruit products.

Objectives

The first objective of this research was to characterize Piel de Sapo (*Cucumis melo* L. var. *inodorus*) and Cantaloupe (*Cucumis melo* L. var. *reticulatus*) melons in terms of physico-chemical, bioactive compounds and total antioxidant activity in edible (juice and pulp) and non-edible (peel and seeds) parts. The second objective was to study the effect of gaseous ozone on juice, pulp and seeds and peel purees of Cantaloupe melon. Different ozone treatment times (30 and 60 minutes) were applied and SSC, titratable acidity (TA), pH, colour and water activity (aw) were determined. The characterization in terms of total phenolics (TPC), chlorophylls, total carotenoids and antioxidant activity (AOX) was also carried out in fresh and treated samples. With this research, it is expected to understand the efficiency of ozone treatment in order to obtain high quality fruit products. This work also aims to reveal the potential practical applications of enhanced edible fractions and to give added value to wasted materials, which is important from industrial and economic perspectives.

Materials and Methods

Fruit material and samples preparation

Nine 'Piel de Sapo' and 'Cantaloupe' melons were obtained at a local supermarket, at com-

mercial maturity stage and stored overnight at 4 °C. For each replicate, three melons, of each variety, were randomly selected for average weight assessment of each fruit. The non-edible parts (peel and seeds) were triturated for 3 min with a domestic blender to obtain a homogenized puree (n = 3). Edible parts (juice and pulp) were obtained by a domestic centrifuge (n = 3).

Physico-chemical analysis

SSC was measured with a Palette PR-32 digital refractometer (Atago, Tokyo, Japan) (n = 3). Titration was conducted with a 0.1M NaOH to pH 8.1 (n = 3). pH was measured using a pH meter (GLP 22, Crison Instruments, Spain) (n = 3). The colour values (L^* , a^* , b^*) of the fresh and ozone treated constituents were assessed using a Minolta CR-400 colorimeter (Konica-Minolta, Osaka, Japan) (n = 3). aw determinations were performed with a dew point hygrometer (Aqualab – Series 3, Decagon Devices Inc., USA) at $22 \pm 1^\circ\text{C}$ (n = 3).

Bioactive compounds content & Total antioxidant activity

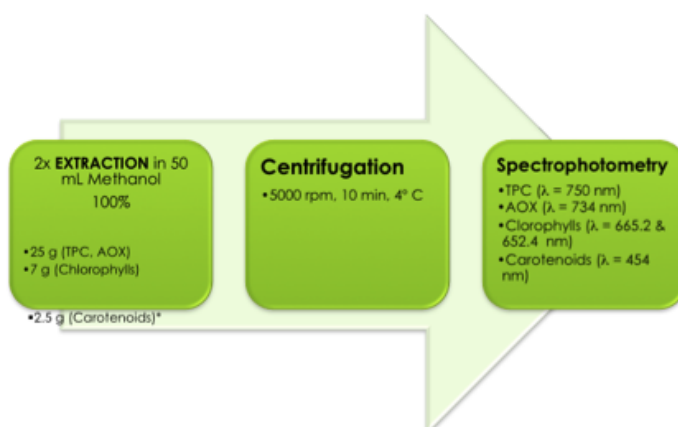


Figure 1. Methodology for the determination of TPC, AOX, total chlorophylls and carotenoids.

*Extraction in 10 mL of cooled ethanol 100% + 10 mL of n-hexane 100%

Ozone treatment

Ozone treatments were performed in a pilot plant. O₂ was passed through a corona discharge generator (OZ5, SPO3 - Sociedade Portuguesa de Ozono, Lda., Porto, Portugal) to produce ozone at 5 g/h. O₃ was continuously bubbled into 50 mL of juice (4° C) or a hermetic plastic box (12 x 27 x 17 cm) with a tick film of 50 g of sample puree (4° C) for peel, pulp or seeds (n = 3) at treatment times of 30 and 60 minutes. Ozone concentrations in g/L were determined by the iodometric titration method [11].

Table 1. Ozone concentrations applied in Cantaloupe edible and waste parts.

| Melon fractions | O ₃ (g/L) |
|-----------------|----------------------|
| Juice | 7 |
| Pulp | 9 |
| Peel | 29 |
| Seeds | 31 |

Results and Discussion

Piel de Sapo and Cantaloupe melon characterization

Physic-chemical parameters

Waste parts represent up to 30% of total weight. SSC is significantly lower in peel than the other three melon parts, which means that this fruit component is less rich in sugars. In contrast, Piel de Sapo seeds had the highest SSC (12.83 ± 0.32 °Brix), probably because they contain a reserve supply that can be used for the first plant development stage. Waste parts presented higher TA values, possibly because they have more organic acids quantity. Since seeds pH is also higher, their organic acids might be weaker than the ones present in juice, pulp and peel. Cantaloupe edible parts (juice and pulp) presented values around 6.13, which are lower than the ones obtained for Piel de Sapo. The lower pH value (5.17 ± 0.01) ob-

tained for Cantaloupe peel possibly means that the organic acids present are stronger than in seeds. Regarding colour, the higher L* values obtained for Piel de Sapo seeds and pulp indicate a lighter colour of these fruit portions. The a* coordinate showed the lowest value for peel, as it was expected since the green colour of this fruit part. The b* coordinate displayed an increase of yellowness for peel and seeds in Piel de Sapo. In Cantaloupe melon, colour parameters indicated an orange colour, probably due to carotenoids content. Water activity was similar for all melon parts and close to 1.

Bioactive compounds content and total antioxidant activity

In Piel de Sapo melon, peel and seeds stand out by their higher levels of TPC when compared with the edible parts. Although Cantaloupe peel had significantly higher total phenolics concentration, seeds stand out with a higher value (229.13 ± 20.92 µg/g). This is in agreement with previous findings [3]. The higher concentration of phenolic compounds in external tissue (peel) is believed to be associated with their main natural function: protection against environmental stress and pathogens [4]. Chlorophylls are strongly related to green colour and were only detected on peel. Chlorophylls content was similar in both varieties. As was expected, melon edible part had higher concentration of total carotenoids, which is related with its orange-fleshed colour. Peel was the melon part with less total carotenoids concentration. This result can be due to losses during the saponification process, since peel is rich in chlorophylls [12]. No results were obtained for Piel de Sapo melon.

AOX values for Piel de Sapo juice and pulp are similar and significantly lower than data for peel and seeds (493.01 and 475.40 µg/g, respectively). However, these results for melon edible parts are consistent with the one

sreported by [16]. Related to Cantaloupe melon, antioxidant activity had the lowest value for pulp, followed by juice, peel and seeds. The values obtained for this melon edible part are in agreement with the ones reported by [1]. The value obtained for seeds ($653.67 \pm 169.20 \mu\text{g/g}$) was significantly higher than the others. This is in agreement with a previous work [13] that pointed seeds as an important source of natural antioxidants.

Effect of Gaseous Ozone in Cantaloupe edible and non-edible parts

Effect on physic-chemical parameters

Only treated juice colour was significantly different from fresh sample after 30 min, probably due to its liquid state. pH decreased in juice and pulp after 60 minutes of ozone treatment while in seeds this parameter significantly changed right after 30 minutes. These increases in the acidity level are probably due to ozone oxidation reactions. SSC is not affected by ozone in most parts, which is in accordance with literature. However, significant differences were obtained for seeds after 30 minutes, which can be a consequence of the conversion of sucrose into glucose and fructose.

Effect on bioactive compounds and total antioxidant activity

As can be observed in Figure 2, in general, the gas affected significantly both parameters. These results are in agreement with previous works [14, 15]. TPC had a significant increase for juice and peel after 30 minutes of ozone treatment. Although it can be expected the loss of antioxidant compounds due to the strong oxidizing activity of ozone, its oxidative stress may induce some defense mechanisms in the product leading to an increase of phenolic compounds. This accumulation probably happened due to the activation of pre-existing enzymes [14]. The prolonged exposure of the fruits to ozone evokes a stronger system response in-

volving transport of antioxidants from internal tissues to the site of oxidative stress [16]. However, the antioxidant response of phenolic compounds is dependent on the fruit product matrix. In fact, a significant reduction of nearly 50 % was obtained in phenolics content for pulp after 30 minutes of ozone treatment. This degradation may be related to several possible chemical responses, which may be direct reactions of ozone with the target compound or with its intermediates, and reactions between hydroxyl radicals produced through ozone decomposition catalyzed mainly by the hydroxide ion [15]. AOX significantly decreased after 30 minutes of ozone treatment in juice, peel and seeds. The reduction may be caused by ozone and ozone-reaction products inducing decomposition of proteins, including enzymes [17].

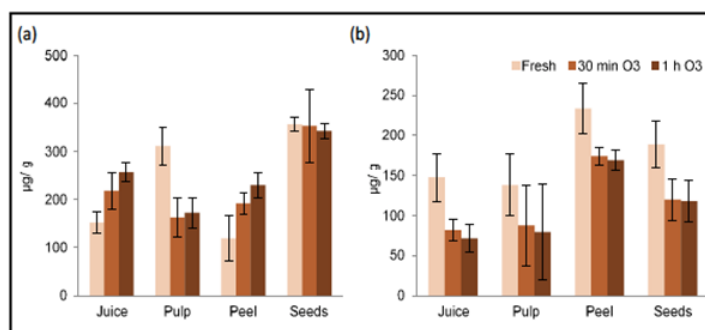


Figure 2. Impact of ozone treatment on total phenolics content (a) and antioxidant activity (b) of edible and non-edible Cantaloupe melon parts. Data represents the mean values and bars the confidence interval at 95%.



Total chlorophylls content is not affected by ozone in cantaloupe peel after treatments. The presence of antioxidant compounds may have a protective effect on chlorophylls [18]. In juice, ozone significantly reduced total carotenoids content right after 30 minutes, probably due to the reduction of beta-carotene and other antioxidants. This can explain the changes attained in colour for fresh and ozone treated juice [19].

Conclusion

This research demonstrated that melon edible parts, juice and pulp, had a similar physicochemical profile, with the exception of colour. These fractions also presented similar results for all bioactive compounds analysed. Melon waste parts should be considered as a potential source of different bioactive compounds and antioxidant capacity, and represent a potential opportunity for the development of new products with functional properties. Ozone treatments were performed in Cantaloupe melon parts, since characterisation pointed out this variety as richer in antioxidant activity and total carotenoids. In general, juice is the fraction more affected by ozone in terms of quality, while peel is just affected in terms of bioactive compounds. It would be convenient to work at lower exposure time to avoid undesirable changes (changes in colour and/or pH) or loss of valuable compounds (carotenoids). Treated juice is the most important edible fraction, being an important source of TPC and AOX. Treated pulp is an important source of AOX by its high content of TPC and carotenoids, and therefore can be a suitable ingredient for food formulations. Ozonated peel is a fraction with high potential, because it contains highest levels of chlorophylls and AOX/TPC. Treated seeds, which are rich on sugars, contain high AOX and the greatest TPC. Consequently, waste parts could be used in the elaboration of cosmetics, pharmaceutical and food products as beverages, capsules and/or colorants. In conclusion, the evaluation of ozone treatment reflects important advantages to be explored.

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Sensory drivers and barriers to consumption of food with high plant protein content

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How to feed nine billion people while:

↓ 10 x 🌱 🚰 💧 and ↑ 😊 ?

Introduction

One of today's challenges in food and agriculture is to try to answer to the question "How to feed the world in 2050".

One obvious solution toward a sustainable food system is to decrease the consumption of animal origin proteins and to increase the proportion of plant-based food rich in proteins (IFT, 2010).

However, previous studies have shown that the usage of plant-origin proteins in food is limited by the emergence of some unaccepted sensory characteristics.

For example pea proteins are claimed to introduce unpleasant flavor (bitter, burnt, earthy etc.) as well as displeasing mouthfeel (gritty, pasty, astringent etc.) (Gleski, 2014).

Objectives

In this context, the research objectives were:

- I. Determine **drivers, barriers and expectations** for the consumption of products rich in plant-origin proteins.
- II. Describe the **sensory parameters** of developed products with **high pea protein** content and identify potential **sensory defaults**.
- III. Suggest a preliminary **aromatization strategy** for plant-origin products and test whether projective aroma preferences hold true in developed products.

Materials and Methods

To answer to these objectives, the work was divided into three different parts (Figure 1):

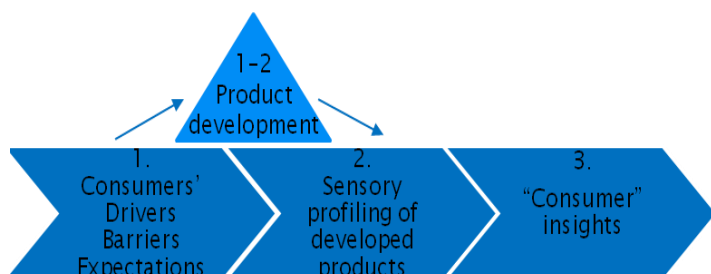


Figure 1. Workflow in three steps

In the first part an elaborated questionnaire was performed with 150 French consumers to determine their drivers, barriers and expectations related to plant-origin products in the context of reducing their consumption of animal origin proteins.

Secondly, the obtained insights were used to perform sensory-led development of products high in pea proteins. Furthermore, the sensory characteristics of developed, non-aromatized products were determined to identify the global profile and temporal sensations of texture, aroma and taste.

In the last part, the work was concentrated on one of the sensory dimensions. Here the consumers' theoretical aroma expectations were confronted with the developed non-aromatized and aromatized products using 10 "aroma pens" (equally representing the meat and plant universe) (Figure 2).



Figure 2. Aroma pens, provided by Firmenich

Results and Discussion

The main drivers & barriers for consumption of products rich in plant-origin proteins:

- + Experiment with new products (84%)
- + Diversify nutrition, preserve health (83%)
- + Experiment with taste (68%)
- Lack of variety (46%)
- Too expensive (42%)
- Unknown taste (39%), ingredients (37%).

Sensory characterization results show that the texture of developed snacks high in pea proteins was described as "crunchy", "crispy", "hard" and "dry".

Furthermore, the products were determined to possess an aroma resembling "fresh green pea" and "pea".

Resulting from previous, the main sensory barriers for consumption were identified to be the unappealing "pea" aroma and "dry" texture related mouthfeel.

Out of the 10 proposed aromas participants preferred "Herbes de Provence" aroma to be present in future plant origin alternatives (Figure 3). Furthermore, based on the hedonic aroma pens test the participants expected plant origin products to taste like "plants" and not like "meat".

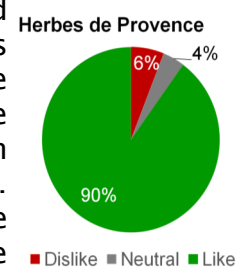


Figure 3. Aroma test

Conclusion

Current research identified various drivers and barriers related to the consumption of products high in plant origin proteins. In addition, innovative snacks high in pea proteins were developed. The sensory characterization of developed products revealed potential sensory defaults such as "dry" mouthfeel and "pea" aroma: Regarding the expectations, it was discovered that French consumers expected plant origin products to taste like "plants" and not like "meat" both in theory and in developed products high in pea proteins.

Future Perspectives

Determine other nationalities drivers and barriers for the consumption of products high in plant origin proteins.

Optimize developed products formulation and characterize obtained products sensory properties. Then re-evaluate products with pre-screened consumers to determine the increase or decrease of preference.

Use other pulse flours (lentil, chickpea) for the development of innovative, sustainable and highly preferred products rich in plant origin proteins.

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Investigation of high-barrier materials development for long shelf-life dairy based products with enhanced properties

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Introduction

In modern world, food packaging plays a defining role in preserving the quality of the product throughout its lifecycle, from manufacturing to the consumption. The requirements of packaging material are dependent on the type of food product it will contain. In regard to dairy-based products, the maximum protection against oxygen permeation is imperative, as it causes the oxidation process with the consequent loss of product nutritive and organoleptic qualities.

Medical nutrition products developed by Danone Nutricia Research are, in majority, presented by dairy-based liquids and characterized by a fat content reaching up to 52% in some of them. High fatty oils' content makes Nutricia products highly subjected to oxidation, with the group of risk including LCPUFA (Long Chain Poly-Unsaturated Fatty Acids). The latter enters into the reaction with oxygen with a consequent formation of components that are responsible for rancid odors and flavors, as well as the deterioration of structure. As a result, due to oxidative reactions the shelf life of the product shortens considerably. Two main forms of packaging for product portfolio currently in place at Nutricia are: rigid packaging (plastic bottle) and flexible packaging (pouch laminate). Danone Nutricia realizes the importance of moving in the direction of eco-sustainability, optimization of packaging physical parameter, such as weight and thickness that are resulting into down gauging and cost reduction.

Speaking objectively, the main obstacle for

the implementation of new packaging technologies is thwarted by the very first hurdle - a lack of thorough comparative research of a general scope of packaging materials currently being present on the market. The situation is further complicated by scarce research on how the modification of the physical parameters of currently used materials will impact the package performance and shelf-life of the product it contains. This case refers to the currently accepted standard for the thickness of EVOH as an oxygen barrier material.

Aims

The principal goal was described as follows:

- * The thorough investigation on the recent and ongoing developments, as well as prevailing trends on the market of high barrier materials for oxygen-sensitive foods, particularly applicable for dairy-based foods.

The completion of main goal allows to achieve several sub-goals such as:

- * Selection of alternatives for the current flexible and rigid packaging of Nutricia products, based on the preliminary analysis of barrier performance
- * Theoretical and experimental study on the barrier performance impact via modification of current barrier material (EVOH) for rigid packaging.
- * Exploration of the alternative ways rather than the modification of barrier material in existing rigid packaging, with the purpose of the extension of shelf-life

Methodology

The study flow needed to be organized in a way that would allow the accomplishment of the main goal and sub-goals. The foremost intention of this research was to understand what are the changes, trends and promising developments on the market of barrier materials for

food packaging, with a particular focus on oxygen-sensitive foods. For that, the study method had to facilitate the extensive and thorough surveying through the vast amount of information to identify the points of exclusive interest. Therefore, the technology scouting approach chosen as the most appropriate to undertake. The steps of the technology scouting process integrate the components of qualitative and quantitative research. The Figure 1 demonstrates the data collection and analysis flow as the determinants of the technology scouting evolution.

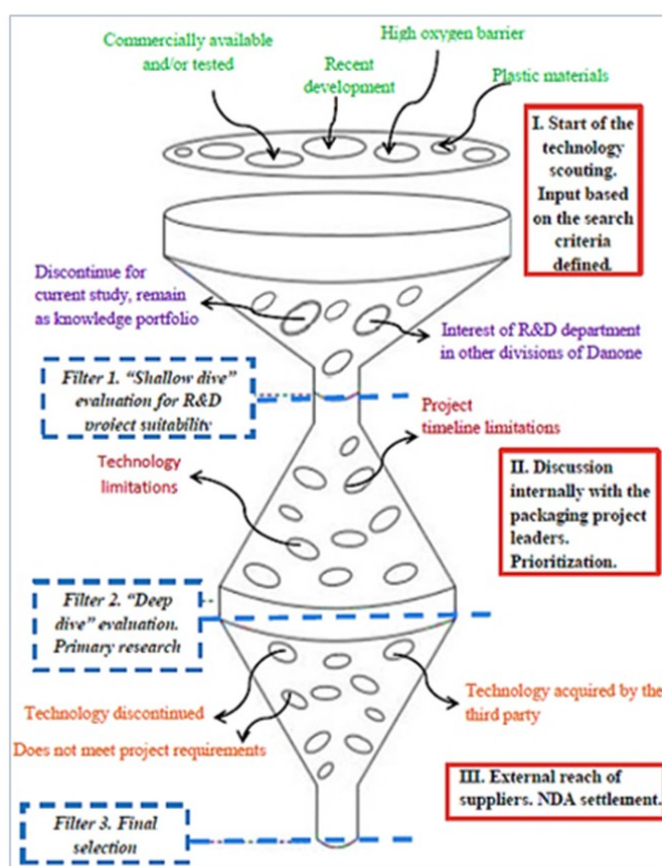
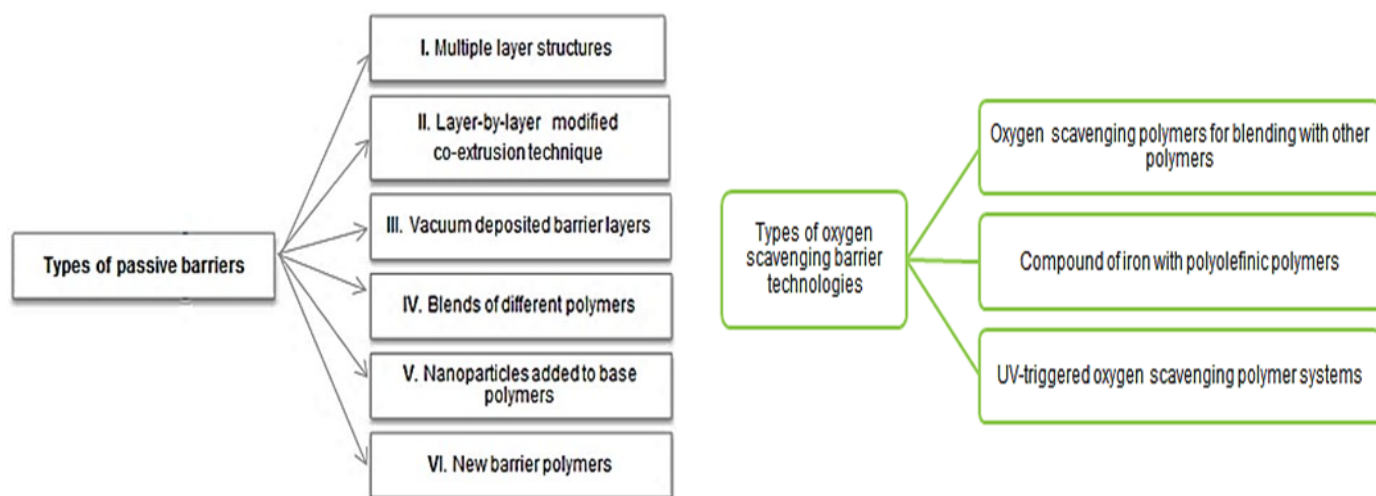


Figure 1. Evolution of technology scouting. Data collection and analysis stage.

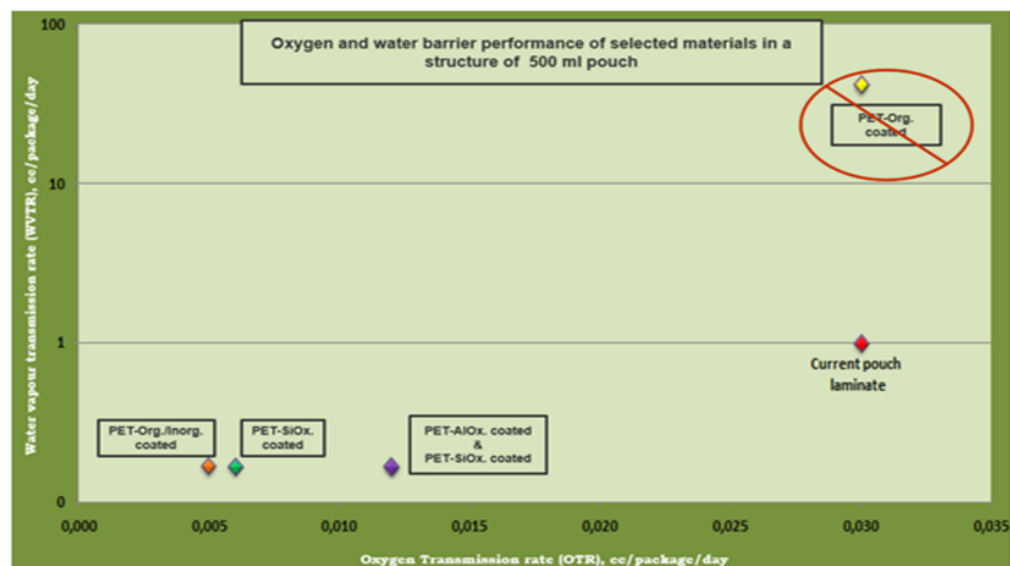
Results

Secondary research phase of technology scouting.

As the outcome, two major group of plastic barrier materials have been identified: passive and active barrier materials. The names



imply their mechanisms of protection: passive materials are simply serving as an “armor” against permeating oxygen, while active barriers have so-called oxygen-scavengers, presented by substances in material structure that can absorb permeating oxygen. The findings have been grouped by author of the thesis, based on their barrier mechanism and nature of materials (Figure 2)



Primary research phase of technology scouting.

a. Flexible packaging

Based on the findings of the first phase, the list of alternative structure to pouch laminate has been proposed. The benefits expected from proposed alternatives were: replacement of aluminum foil, down gauging of the packaging (the reduction of film thickness), extension of shelf-life of the

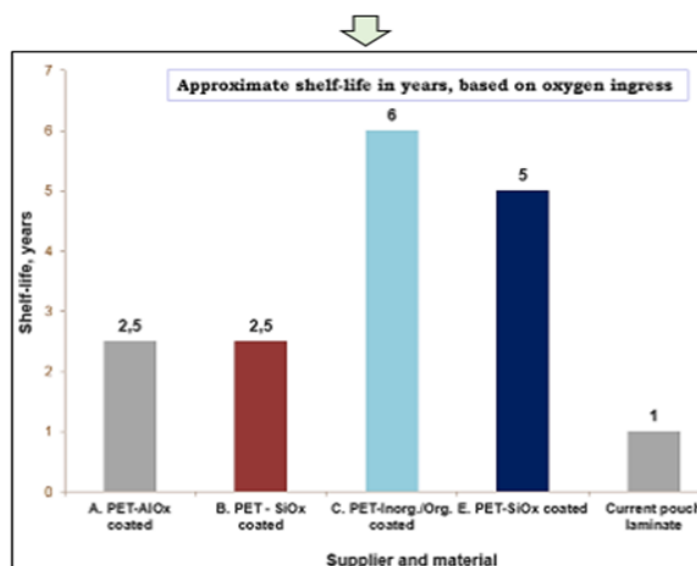


Figure 3. The initial selection of oxygen barrier materials for pouch laminate

product from 12 months to 15-18 months based on the oxygen ingress. The comparative graph of alternatives versus current pouch was prepared in regard to oxygen and water barrier properties (Figure 3).

b. Rigid packaging

For bottle, the relationship between the thicknesses of the barrier layer, in this case EVOH, was shown to be inversely proportional to the oxygen transmission rate. The evaluation of oxygen barrier performance has been conducted on Nutricia bottles with 5 different thicknesses of EVOH – 10, 15, 20 (the current thickness used at Nutricia), 25 and 30 microns. (Refer to Appendix C.1). Thus, the difference in EVOH thickness lied in a range of -50% to + 50%. However, the results were not consistent and noticeable differences exist within and between each of the methods used (Figure 4). It can be attributed to testing conditions, distribution of EVOH layer in the bottle structure and equipment failure.

Currently used EVOH grade for Nutricia bottles has 32mol% of polyethylene content. However, the lower polyethylene content results in the stronger oxygen barrier properties. It was compared to EVOH with 27 mol% PE using Norner OTR calculator (Figure 5). The results backed up the assumption that bottle oxygen barrier is stronger with EVOH 27%mol. Further in the study, the experimental testing is imperative to confirm the theoretical results.

Apart from the influence of packaging materials on the oxygen ingress, other factors have been examined, such as the headspace volume and oxygen content in a headspace after the filling. Currently, headspace volume is equal to 29 ml with 7% of residual oxygen, that is almost 3 times higher than the O₂ ingress over 1 year. It proves the fact, that headspace plays a critical role in oxygen content contributing to a shelf-life loss of the product. Therefore, the influence of headspace volume on shelf-life has been modelled, based only on oxygen ingress (Figure 6).

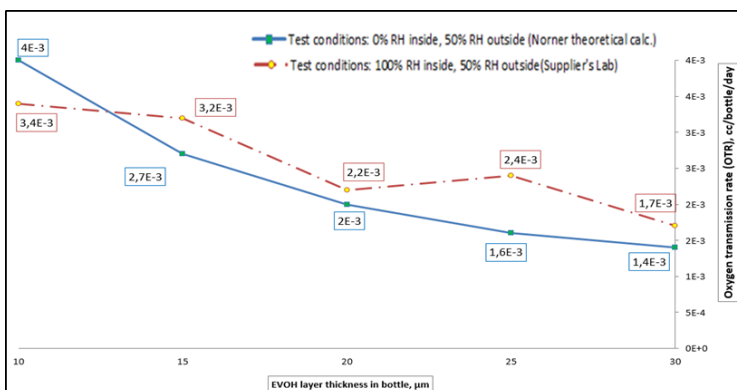


Figure 4. Oxygen barrier of bottles VS EVOH layer thickness

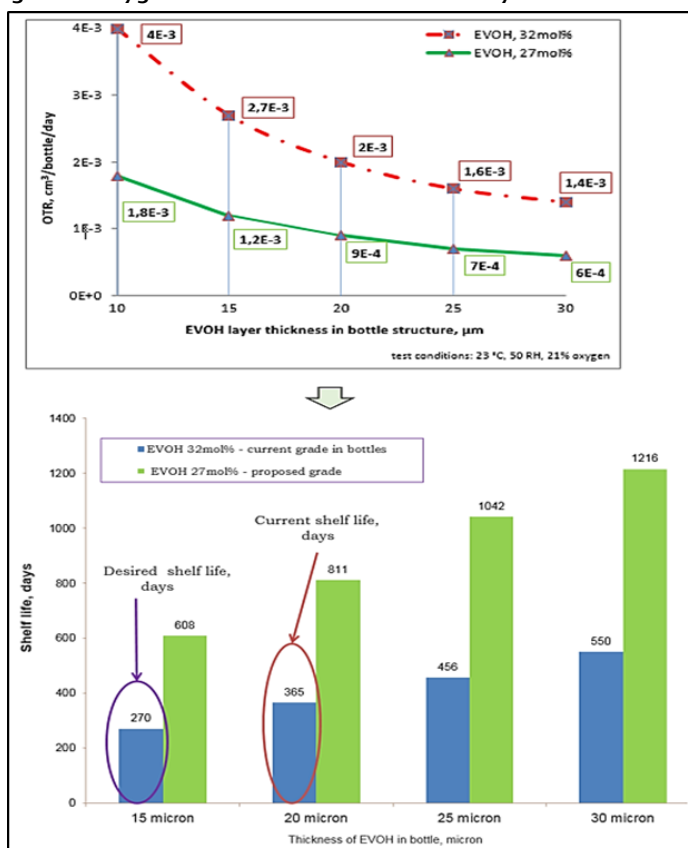


Figure 5. Oxygen barrier of bottles VS EVOH grade and layer thickness

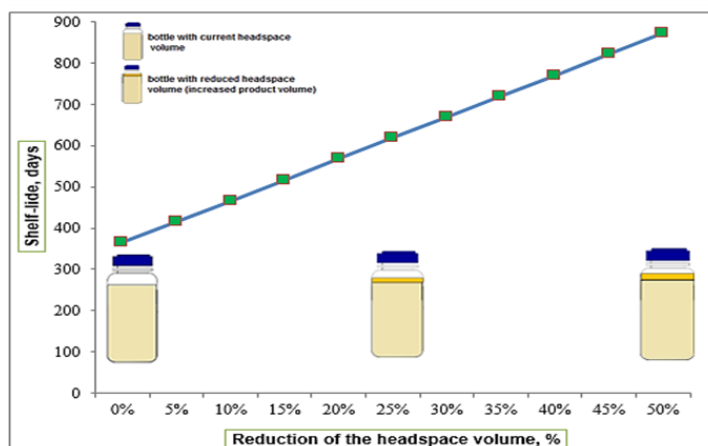


Figure 6. Reduction of headspace volume VS oxygen shelf-life

The next step was to see how the decrease of the oxygen % in headspace will influence shelf-life. Currently, it is equal to 7%, but reduction as small as by 2% can give the shelf-life even longer than the desirable 550 days (Figure 7).

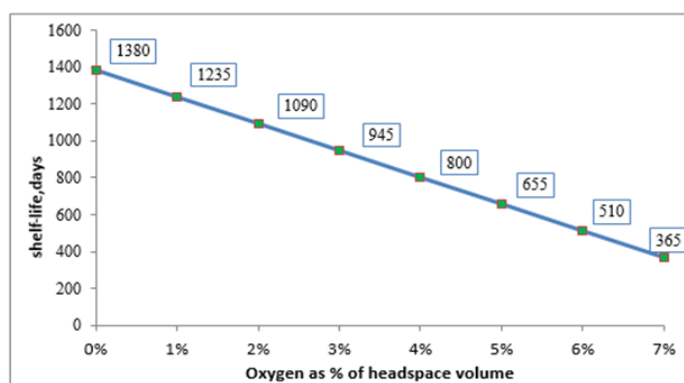


Figure 7. Reduction of headspace oxygen VS oxygen shelf-life.

Conclusion

Flexible packaging

Among the latest developments for high barrier flexible packaging, thin barrier coatings on polymer base, such as silicon oxide and aluminum oxide represent the significant part. The first estimations led to the conclusion that most of the considered alternatives exhibit stronger oxygen and water barrier properties when compared to the current pouch structure. Further evaluations must be scheduled by Nutricia, both for OTR and WVTR, as well as other tests such as drop test. Moreover, any of the tests must be performed on a sample as similar to the current pouch as possible, meaning the same volume and dimensions. So far, all OTR and WVTR values received from suppliers, are obtained on the evaluations of pouches significantly smaller in size comparing to Nutricia pouch. The study has also revealed a necessity to conduct the product shelf-life tests with any of the packaging considered.

Rigid packaging

As theoretical and experimental evaluations have shown, there is an effect of the thickness of EVOH on oxygen barrier. However, more

tests must be scheduled due to the inconsistency of results between measurements conducted and it is early to draw the conclusion. Other approach, such as the decrease of the empty headspace volume implies two options: filling the bottle with more product or changing the bottle size. Both of these options must be investigated in depth to understand the required investment and possible consequences. One assumption is that the first approach with more product may require the optimization of the filling process to avoid the risk of overfilling with subsequent negative impact on bottle sealing. Secondly, the consumer category as vulnerable as medical patients has many restrictions to comply with. The consequences of adding the excessive volume of product are not known and must be investigated. The second approach is to change the size of the bottle mold in extrusion blow molding machine. This solution can require large capital investments and the separate study must be scheduled to investigate the benefits and drawback of such change.

Bottle closures with oxygen-scavenging sealing layer can be one of the promising options to investigate deeply. It was not considered in this study, but it is strongly recommended for further research. Based on the same principle, as any of the oxygen scavenging packaging, the closures of this type remove the oxygen out of the headspace right after the filling process. Its consumption by scavenger can bring the expected shelf-life extension, with no further modifications required to headspace volume or packaging material.

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<http://www.plog.lth.se/education/fipdes/>



Understanding the effect of different emulsification technologies on fresh dairy products

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Dairy is one of the most important industries in the food sector that provides processed milk, cream, yogurt, cheese and ice creams. There are many important unit operations involved throughout the processing of dairy products. One of such operations is emulsification which is performed in most of the manufacturing process of dairy products. It involves the stabilization of the emulsion by altering the structure of the fat globules, which when not carried out results in creaming and therefore undesired final product.

However the real challenge is in selecting the appropriate emulsification technology, for achieving the required functionalization in order to obtain the final product. Careful selection of emulsification technology will lead to improved functionalization process thereby resulting in reduced cost and high quality end product.

Based on the preliminary bibliography studies, few emulsification technologies of interest were chosen. The effects of these technologies on products were measured mainly in terms of granulometry, rheological and textural properties.

Confidential Topic



Rapid sensory procedure for quality control of widely consumed foods: the case of canned peeled tomatoes

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Introduction

Sensory analysis and evaluation used by companies have been recognized as a valuable way to obtain actionable data on consumer perceptions and needs that can be translated and applied in functions like quality control; and consequently, increasing product acceptability to consumers. Generally, consumer response to a food is affected by a combination of factors: sensory properties of the product, the positive or negative responses to the product, the existing knowledge or opinions about the product, and the willingness of the consumer in doing something in a specific situation (Costell, Tarrega, & Bayarri, 2010). Furthermore, perceived quality is the result of expected quality by the consumer and then their experienced quality (Verdu Jover, Llorens Montes, & Fuentes Fuentes, 2004). The concept of expectations and experiences that lead to an individual's perception on quality is highly affected by sensory characteristics that are sought by the consumer. It should be noted that each individual may not be able to specifically define their measure of quality as each person has their own internal measure of what a product's quality consists of (Moskowitz, 1995). However, classical sensory profiling tools often require considerable investment of resources, particularly time and money that make them less adaptable to growing and changing demands (Varela & Ares, 2012). To address the mentioned constraints, various other methods called rapid sensory profiling have come into practice. It is,

therefore, of interest to understand whether they can be effectively applied as alternatives.

The most widely used classical descriptive method for product stability evaluation, quality assurance, and quality control is the quantitative descriptive analysis (QDA®) (Dairou & Sieffermann, 2002). In practice, it has been found that conventional profiling is consuming in terms of time and costs (Varela & Ares, 2012). The evolution and adaptation of methodology addresses these problems. Various novel methodologies have been developed for rapid evaluation and analysis. The rapidity of these new tools is often under the concept that they do not need an extensive training phase for judges (Valentin, Chollet, & Abdi, 2012). These methods allow each judge to generate different sensory spaces that reflect the differences in how they perceive and assess samples (Vidal, Antúñez, Giménez, Varela, Deliza, & Ares, 2016).

Objectives

The scope of this thesis involves evaluating two novel rapid sensory methodologies in order to determine the most effective method that has the ability to determine discriminative sensory attributes for perceived product quality. This study established whether Napping with supplementary attributes or FP is an appropriate methodology that allow for more time and resource efficient evaluation in sensory analysis while allowing for discriminative ability. Sensory data was also compared to instrumental measures as well as market research results.

Materials and Methods

Samples

Our experiments involved ten different brands of canned peeled whole tomatoes available in Italy (Table 1).

Three different sample preparations were done 1) a raw mixture blended with 0.5% salt;

| Table 1. Product Brands | |
|-------------------------|-------------|
| Annalisa | La Torrente |
| Cirio | Mutti |
| La Carmela | Pomilia |
| La Fiammante | Valfrutta |
| La Rosina | Vitale |

2) a cooked mixture blended with 0.25% salt and 1.0% olive oil heated on the stove for 10 minutes;

3) a whole intact peeled tomato.

Judges

The novel rapid methodologies applied in this study do not require trained sensory judges. The judges that were recruited and selected were students or researchers familiar with descriptive sensory procedures, but not trained on the specific product.

Methodology

For each evaluation, coded sample set consisting of one of each brand with a repeated blind control were presented to the judges simultaneously. The first evaluation of each experiment test was used to conduct the Napping method. Judges evaluated the samples based on the similarities or differences they perceived to position samples on the bi-dimensional space provided on the software Fizz 2.4 program (Biosystems, France). Judges also recorded sensory descriptors they used to differentiate the samples. All attributes were then compiled to create a global sensory attribute list. The second session was the beginning of the FP procedure. The global attribute list so that judges could reassess their chosen attributes used for sample discrimination; overall, a maximum of ten attributes were allowed. On a blank form, judges were

allowed to freely define the meaning of their attributes as well as the technique they used to evaluate each attribute. The third and fourth evaluations consisted of two replications of FP for ranking samples. Judges were each given a form consisting of blank scales for each attribute they had chosen in the previous session to discriminate samples within the set given. They were then asked to evaluate the sample set and rank the samples from low to high intensity (left to right) for each attribute they chose to use. It was made clear that samples could be ranked together if judges deemed them to be the same for a specific attribute.

Instrumental measurements were also performed on each type of sample preparation to compare against the perceived attributes by judges. Measurements taken include Brix (α Atago Dr-A1 Refractometer), color (Minolta CR-300), pH (model), and water activity (Aqualad – Dewpoint Water Activity Meter 4TH). It should be noted that for the whole peeled tomatoes, only the liquid medium was used for instrumental measurements.

A market research investigation was carried out to understand the purchasing and consumption habits of tomato products by pizzerias. A short questionnaire was conducted during the "Tutto Pizza" event.

Data Analysis

Multiple Variable Analysis (MFA) was performed on Napping data with attributes as supplementary data taste (Le, Le, & Cadore, 2015). General Procrustes Analysis (GPA) was performed on the FP data (Delarue, 2015). ANOVA and Duncan's test were performed on instrumental data.

Results and Discussion

Elicited Sensory Attributes

Overall, it was observed that due to the way at-

tributes were elicited and chosen, the diversity of attributes were much more in FP than in Napping. The elicited attributes were also categorized into appearance, flavor, and texture. For the whole tomato samples, appearance and texture attributes were used more. For the raw and cook tomato mixtures, mostly flavor attributes were used. This may be due the fact that the former sample type was more representative of the original product; whereas, the latter two were representative of the product in use.

Napping vs. FP

Overall, the explained variance and the ability to position the repeated samples as well as Cirio and Valfrutta together were better in FP than in Napping. As discussed previously, this may be due to the two repetitions done for FP; but, it may also be due to the fact that Napping is done previous FP in the evaluation process of each type of sample. Napping has been shown to help familiarised judges with the product space and attributes in subsequent evaluations of FP. For both methodologies, there were not many overlapping significant attributes that were used to assess all three types of samples. However, if based on Flash Profiling, it can be seen that Cirio, Mutti, and Valfrutta becomes more viscous or dense after cooking. It can also be noted that in terms of the whole peeled tomatoes, La Carmela, La Fiammante, Pomilia, and Vitale have less tomato peel than the rest. However, this attribute was not consistent between the raw and cooked tomato mixture.

Instrumental vs Sensory Data

This may be due to various reasons: 1) differences amongst samples were not overtly perceivable; and 2) perceptions of samples take into account many factors like interactions between the properties of the

sample, whereas instrumental measures mostly evaluate one parameter of the sample independently of others.

Market Research Results

Almost all pizzerias interviewed used canned peeled tomatoes. The most frequently used brands are La Torrente, La Fiammante, and Ciao. The main attributes that pizzerias used to choose the brand to make pizzas included consistency, "acidity" and fresh tomato "odor/flavor".

Conclusion

Results from Napping and FP were compared and it was found that FP was the better of the two in terms of discriminative ability in all three cases of sample preparation. This may be due to the fact that FP requires 2 replications of evaluations and as indicated in literature, Napping done in prior to FP increases the judges familiarity of the product space and samples (Liu, Gronbeck, Di Monaco, & Giacalone, 2016). Validation of sensory data to instrumental measures could not be effectively achieved in the case of this study and further instrumental measures that are correlated with attributes related to attributes like flavor and texture should be performed. From the market research, the sensory attributes used to decide tomato products used for pizza were also attributes elicited by judges in the evaluation sessions for the raw tomato mixtures, indicating that these attributes are important to the acceptance of canned peeled tomato products.

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Effects of a new tool implementation on Project and Portfolio Management in a worldwide Food Company

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Beatrice MORNET (DANONE)



The food company deployed a tool for a success management of projects and portfolios.

A Quantitative and Qualitative evaluation was implemented worldwide in order to identify the performance of the tool and how it impacted on project and portfolio management in the organization.

Confidential topic



Characterization of casein micelles and sodium caseinate in dense suspensions

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Introduction

Casein is the most abundant protein found in milk making up around 80% of the total milk content. Casein is a key functional and nutritional component in a wide variety of dairy products both within milk as well as as an additional ingredient in an isolated form such as micellar casein isolate or sodium caseinate. In milk, the majority of the casein proteins are found in large aggregates called micelles. It is this micellar structure that makes casein such an important component or ingredient in dairy products. Decades of research have been dedicated to understanding the structure of the casein micelle and how this structure interacts and reacts to its environment; however, the exact nature of the structure is still unknown. This thesis is part of an ongoing research project between Professor Taco Nicolai, director of research of the Polymères, Colloïds, and Interfaces (PCI) lab within the Institut des Molécules et des Matériaux du Mans (IMMM) and the Dutch dairy cooperative, FrieslandCampina. Proposed by FrieslandCampina, this project aims to improve the knowledge base concerning the rheological behavior of dense suspensions of three casein ingredients produced by FrieslandCampina in relations to changes in concentration, temperature and pH. Due to confidentiality issues, this thesis will only discuss the work done on two of those ingredients, micellar casein isolate and sodium caseinate. By improving the knowledge base concerning the rheological behavior of their ingredients and how this behavior

changes in response to certain variables, FrieslandCampina will be able to improve a wide variety of products and processes within their business and allow for the continued innovation needed to grow and expand.

Objectives

In order to enhance the knowledge concerning the behavioral properties of casein-based ingredients and enable FrieslandCampina to better produce, process and control these ingredients, the following goals were set:

- The primary objective of this thesis is to characterize the rheological properties of dense suspensions of sodium caseinate and micellar casein with respect to changes in concentration, pH and temperature in order to better control/manipulate them in an industrial setting.
- In order to complete this primary objective, it is also necessary to determine the effects of temperature and concentration on the pH and the relationship between pH and charge density (α).

Methods

It was determined early on that using pH as a variable did not best represent changes in rheological behavior when other parameters such as temperature and concentration are varied due to the pH being affected by these parameters. Instead, it is more relevant to observe changes based on changes in charge density or α expressed in charges per protein. This ratio is not affected by changes in temperature or concentration and is more closely connected to changes in the protein structure or behavior than pH. This relationship and the importance of using α when looking at dynamic systems like casein has been previously reported on by Kharlamova et al. (2016). In order to better understand the relationship between pH and charge density, a series of titrations was performed. Second, another series of tests was performed to demonstrate the difference be-

tween pH and charge density and how each of these is affected by changes in concentration and temperature. Once this relationship was established and explained, it was decided that all of the rheological measurements would be observed as a function of $\Delta\alpha$ with the reference $\Delta\alpha=0$ point being the unaltered α of the powder.

The rheological behavior and the changes in this behavior due to temperature, pH and concentration were observed primarily through looking at the zero shear viscosity (h_0). This was measured using a rheometer over a range of $\Delta\alpha=-3-20$ and concentrations from 200g/L-100g/L were observed at 5°C 20°C and 40°C for both sodium caseinate and micellar casein suspensions. These results were plotted using sigmaplot to allow for the visualization of any behavioral trends.

Results

Relationship between pH and α

The results of the titration curves performed allowed for the relationship between pH and α to be determined at 10g/l. Comparisons between sodium caseinate and casein micelles in terms of the number of charges needed to adjust the pH was also done and suggests the difference is due to the presence of the calcium phosphate nanoclusters. When observing the changes in pH due to concentration, it was determined that the pH decreased with increased concentration for both sodium caseinate and micellar casein with the effect being greater for micellar casein. Changes in pH due to temperature were also observed for casein micelles dispersions, but not for those of sodium caseinate. The differences in the effects for sodium caseinate and casein micelles have been attributed to the presence of calcium phosphate.

Rheological behavior

The results of the rheological testing showed a sharp increase in h_0 as a function of increased concentration for both sodium caseinate and casein micelle suspensions as previously reported and discussed in various literature sources. (Pitowski et al., 2008), (Thomar, (2016) (Boucheaux et al., 2009) and (L. Dahbi et al., 2009). Additionally, as the temperature is increased in the range of 5-40°C, the viscosity of both sodium caseinate and micellar casein suspensions is decreased. Effects of the temperature on the h_0 for suspensions of sodium caseinate and micellar casein are due to the decrease in the effective volume fraction as a result of the weakened hydrogen bonding and electrostatic interactions due to heat. When observing the sodium caseinate suspensions as a function of $\Delta\alpha$, an increase in h_0 was observed as the $\Delta\alpha$ was increased.

The h_0 results were plotted as a function of $\Delta\alpha$ to better visualize the effects as seen in Figure 1.

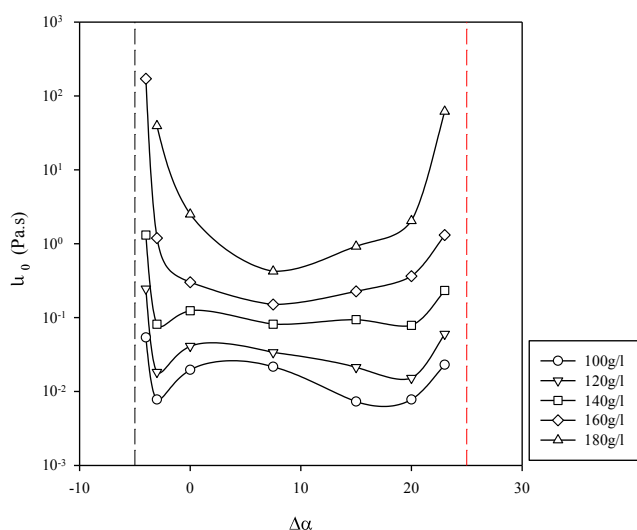


Figure 1: Zero Shear Viscosity (h_0) of casein micelles as a function of $\Delta\alpha$ at 20°C with differing concentrations with vertical lines denoting the critical $\Delta\alpha$

This is due to a decrease in the net charge density of the micelles and the progressive dissociation of calcium phosphate leading to micellar instability and aggregation with gelation close

to the critical $\Delta\alpha$. The impact of the changes in $\Delta\alpha$ for casein micelle suspension is more complex with a number of factors likely playing a role. The forced close packing of the micelles due to concentration along with the steric repulsion and the shifting electrostatic repulsion and charge density all play a role in the stability and structure of the suspension. Taking all of these different elements into account it can be hypothesized that at 140g/L, the micelles have been forced into close contact due to the concentration, with the relative distance between particles counteracting the effects of local densification due to an increase in attractive forces between micelles. At concentrations below 140g/L, there is a decrease of viscosity when the density locally increases due to attraction between micelles. At concentrations above 140g/L, local densification is no longer sufficient and the micelles remain close packed with the attractive forces leading to aggregation. A visual representation of this behavior can see in Figure 2.

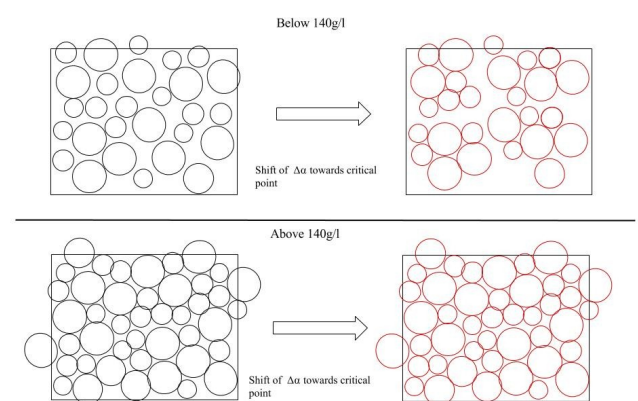


Figure 2: Visual representation of the change in rheological behavior of casein micelles above and below 140g/L due to shifts in $\Delta\alpha$ with red representing increased attractive forces

This hypothesis is also supported by the shift in behavior from that more closely resembling solid sphere to that of soft spheres

or microgels characterized by Boucheaux et al. (2009) and L. Dahbi et al. (2009). As the charge density nears the two critical values, a rapid increase in viscosity is observed for all concentrations. This is likely due to the breakdown of these counteracting effects leading to the instability of the micelles and a shift towards aggregation and gel formation. A visual representation of this behavior can be seen in Figure 3.

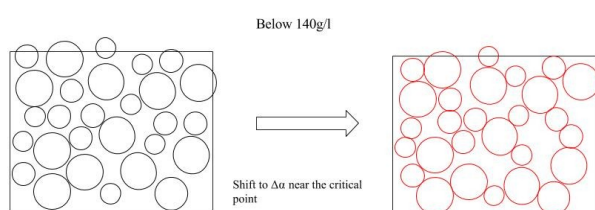


Figure 3: Visual representation of the the change in rheological behavior of casein micelles below 140g/L as the $\Delta\alpha$ nears the critical point with red representing increased attractive forces

Additionally, alkaline gelation was observed at $\Delta\alpha=-5$, a behavior not observed or described in the literature as far as we know. This is likely caused by micelles instability due to the deprotonation of phosphate and its resulting interactions with calcium and the micelles.

Conclusion

This work discussed here has provided some new insights as to how the rheological behavior of dense suspensions of sodium caseinate and micellar casein changes with respect to changes in concentration, pH and temperature. The confirmed importance of the charge density (α) and its relationship to pH for both sodium caseinate and micellar casein provides a new way of looking at casein systems and provides some insight as to the interactions that govern micellar stability in dense systems. Although alkalization is rarely used by the food industry, the observance of gelation due to alkalization of dense suspensions of casein micelles could potentially have industrially applications not yet

discovered. While some of the results and conclusions reinforce previous work concerning dense suspensions, they also have demonstrated some previously unexplored behaviors and raise new questions to be considered. Studying sodium caseinate systems with added calcium phosphate could help to better understand the role of calcium phosphate in the relationship of pH and α for casein micelles. Comparison of the gels formed by acidification and alkalization as well as further exploration as to the mechanisms behind this alkaline gel formation should also be considered for further study. The continued study of casein micelles and their structure and behavior will allow for more efficient production of technofunctionally specific ingredients and products allowing the food industry to continue to innovate and improve.

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Bovine Milk Oligosaccharides as Functional Ingredient: Chemical Reactivity and Nutritional Implications in Milk Model System

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Introduction

Bovine Milk Oligosaccharides (BMOs) have recently captured the interests of researchers due to their prospective application as alternative sources of complex oligosaccharides (OS) with close structural resemblance to the functional species of Human Milk Oligosaccharides (HMOs), a clear advantage over currently used linear OS (e.g. galactooligosaccharides, fructooligosaccharides) [3]. In mammalian cells, both BMOs and HMOs are produced in the same biological synthesis site thus justifying their congruence in nature [3]. Predominant forms of BMO exist in tri- and tetrasaccharides form with either lactose or lactosamine cores. However, typically, they could range from having 2-10 monosaccharide constituents linked by covalent, glycosidic bonds [1]. Depending on their constituents, BMOs can be categorized as sialylated, neutral, fucosylated or with terminal lactosamine residues [9]. With these structural resemblances, researchers found that BMOs may likely be able to replicate the functional benefits of HMOs in humans, principally for neonates, in terms of their prebiotic effect, role in brain development and their contribution on improved immunity among others [1]. However, taking into account their diverse structures, incorporating BMOs in food demands the understanding of how they will behave within a food matrix. Additional effect of the employed processing technique, such as thermal treatment, could also influence BMOs to some degree.

With the targeted application geared to-

wards milk and infant formulas, gaining insight of the reactivity of BMO towards heat-induced chemical reactions such as Maillard reaction (MR) is necessary as this could have nutritional and quality implications in the food such as losses of essential amino acids, formation of glycation products and development of undesirable compounds to name a few [12][14]. Certainly, the chemical nature of BMOs plays a major role on this. MR in milk involving mono- (e.g. galactose, glucose) and disaccharides (e.g. lactose) had been well studied but little had been known about the fate of OS [10]. Apparently, increased polymerization and complexity of OS consequently decrease their general reactivity towards MR and also alter their reaction pathways (Wedzicha & Kedward, 1995). Two mechanistic studies of OS degradation proposed that OS degradation can either be thermally induced [5][7] and/or proceed via a “peeling off mechanism” due to their interaction with an amino group [7]. Considering these, it is possible that these mechanisms may also supervise BMO behaviour within a food matrix.

Objectives

The overall aim of this study was to investigate the potential utilization of BMO as an emerging functional ingredient. In particular, this study aimed to individuate the governing chemical mechanisms of BMO and their prospective nutritional impact in thermally-treated milk model systems (raw milk, commercial cow milk-based infant formula and BMO-enriched infant formulas) as governed by MR.

Materials and Methods

Milk Model Systems. Five milliliters of thawed raw milk (Napoli, Italy) samples were thermally treated in hermetically sealed headspace vials at $180 \pm 2^\circ\text{C}$ in a preheated air convection oven for 2, 4, 6, 8 and 10 minutes. One millilitre of reconstituted infant formula (Infant formula: 3g/20 mL; BMO-Enriched Infant formula (3g/20 mL infant powder + 12% w/w BMO powder) were thermally treated in closed glass pressure vials (13x100) using microwave heating

(Whirlpool, 900W M420 Double Emission System) set at 160 W for 0, 20, 40, and 60 seconds. Samples were then immediately cooled in an iced water bath (10°C) and were stored at 4°C until analysis.

Oligosaccharide extraction. The method for fat and protein removal was adapted from Liu, *et al.*, (2014) who reported a higher OS recovery and better measurement reproducibility with ultrafiltration method. Prior to ultrafiltration, 1 ml of control and heat-treated milk samples were spiked with 10 μL of 1000 ppm stachyose solution. Fat removal was achieved through centrifugation of milk samples at 14800 rpm for 20 minutes at 5°C . Equal volumes of the defatted milk and water were then transferred to 2-mL centrifugal filters (Amicon Ultra 15, Ultracel 2K, Regenerated Cellulose, 3000N/MWL) and were further centrifuged for 30 minutes at 4000 rpm (10°C). Filtered solution were directly transferred to vials for subsequent analysis.

HILIC-MS Analysis of Oligosaccharide and Amadori Products. The identification of BMO was performed according to modified method of Fong and Bajad (2011). Ten microliter of supernatant retrieved from the milk samples were injected into the LC-HRMS system. BMO separation using Luna amino column (100 x 2.0 mm, 3.0 μm ; Phenomenex, Torrance, CA) operating in HILIC mode was achieved in U-HPLC Accela system 1250 (Thermo Fisher Scientific, Germany) ran through the following solvent compositions: Mobile phase A (Acetonitrile) and Mobile phase B (3 mM, pH 9.2 adjusted with 30 mM ammonium hydroxide). The following gradient of solvent B (min/%B) at a flowrate of 300 $\mu\text{L}/\text{min}$: (0/15), (2/15), (6/100), (11/100) were established. The U-HPLC system was directly interfaced to an Exactive Orbitrap high-resolution mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with heated electrospray interface operating in both positive and negative mode with a scanning m/z range of 60-1200.

Free Amino Acids Analysis. Free amino acids were quantified according to optimized method Troise, *et al.*, (2015). Mobile phases were formic acid in water (solvent A) and

0.1% formic acid in acetonitrile (solvent B). The following gradient was set at a flow rate of 200 $\mu\text{L}/\text{min}$ at an injection volume of 5 μL : (min/% B): (0/5), (4/5), (7/50). Analytes were detected through heated electrospray interface (HESI) operating in positive mode with a scanning m/z range of (60-400).

Software and Tools: Relative quantification of peaks were extracted and performed through XCalibur software. Graphical analysis were performed on GraphPad 6.0.

Results and Discussion

The BMOs identified in this study were based on the BMO library provided by Barile and coworkers [1]. In raw milk, total of 13 OS were detected with 9 species showing quantifiable peaks. In control infant formula, 17 BMOs were detected with 10 showing quantifiable peaks. Upon BMO enrichment of infant formula, 27 BMOs were detected with 15 being quantifiable. Data of this study highlight an overall decrease of the majority of BMOs during heat treatment of milk clearly showing that these sugars do degrade as a result of elevated temperature (**Figure 1, Figure 2, Figure 3**). Although, there was an observed increase in areas of the lower MW BMOs, this behaviour was more of a consequence of the degradation of higher MW sugars rather than a reformation as the latter requires a higher activation energy to proceed [6].

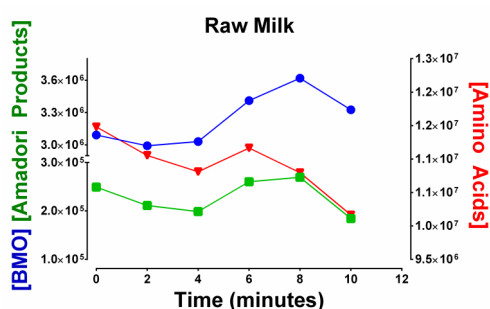


Figure 1: Overall chemical reactivity of BMO in Raw Milk Model Systems (n=4)

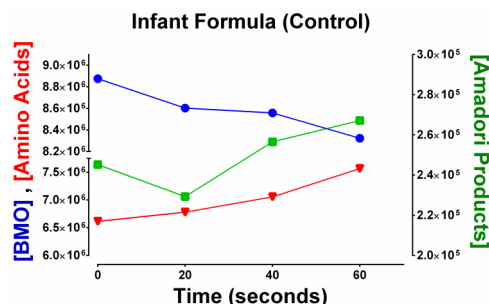


Figure 2: Overall chemical reactivity of BMO in Infant Formula Model Systems (n=4)

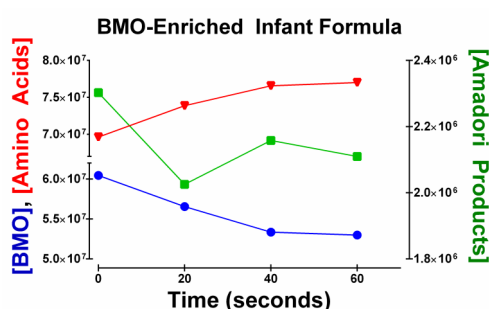


Figure 3: Overall chemical reactivity of BMO in BMO-Enriched Infant Formula (n=4)

Published studies propose that in pure aqueous models, sugar fragmentation is proton-catalyzed, driven by hydrolytic, glycosidic cleavage [5][6]. In this study, the observed degradation pattern of BMOs fits within the above-mentioned oligomer thermolysis wherein OS breaks down to their lower molecular weight counterparts. Short glucans ($\text{dp} \leq 3$), for example, could yield through thermolysis their non-volatile monomer and dimer counterparts. At elevated temperatures and/or favourable conditions though, BMOs could potentially isomerize or form anhydrosugars and transglycosylated compounds but these were no longer confirmed in the study [6]. Certainly, components of the milk model systems influenced the behaviour of the BMOs as supported by the formation of APs. AP formation was indicative of the ability of BMO to participate in MR despite having inferior number of reducing ends [6].

The AP from BMOs observed in all of the milk models was fucosyllactose-glycine. Then, there was formation of disiallylactose-glycine in infant formulas. The BMOs which formed adducts with glycine have monomer constituents ranging from 2-4, all with lactose cores. This demonstrated that tetramers (disiallylactose) and trimers (fucosyllactose) could form adducts with amino acids. In the study conducted by Capitan and coworkers (2015), formation of non-covalent bond between β -lactoglobulin and maltooligosaccharide was observed in aqueous matrices while in this study, both the oligomeric sugar and the degradation products formed covalent bonds with the amino groups [6]. The mechanism behind the participation of BMOs in MR seem to fit within the proposed *peeling off mechanism* of Kroh's team (2000). Initially, there was the formation of APs in the milk followed by the appearance of degradation products containing one monosaccharide less than the original OS. In the case of fucosyllactose, its steric properties resembled that of a triose thus explaining its favourable reaction towards glycine. *Peeling off* of fucosyllactose-glycine could be explained by the elimination of a monomer constituent at the reducing hexose or fucose end giving rise to Hex₁Fuc₁ or Fuc₁. Both of which could further react with other amino acids. This postulation was supported by the observed formation of Fuc-Gly and Hex₁Fuc₁-Gly adducts in the milk models (Data not shown). Solidification of these observations could be further done by an in depth study of the formation of α -dicarbonyls on which future studies will address. The characterization of these intermediate/end products is important as these dicarbonyls could alter the course of MR and eventually influence the array of flavours and compounds formation in BMO-fortified milk. Nevertheless, in a quantitative perspective, sugar reactions such as isomerization, degradation and rearrangement proceed significantly than MR particularly in highly aqueous matrices [14].

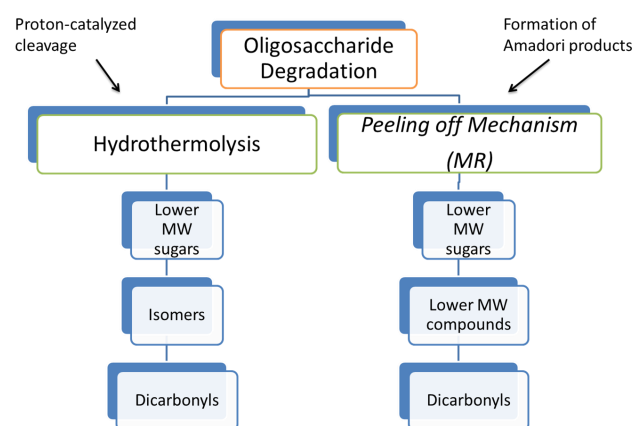


Figure 4: Proposed governing mechanistic degradation pathways of BMO in Milk.

As known, MR in milk model systems involved not only the degradation of the sugar but also the losses of the essential amino acids. In this study though, amino acids were not affected, in terms of depletion, with the addition of BMO. The reduction of amino acids in raw milk was expected as this has already been observed by several researchers. While the commercial infant formula did not exhibit a reduction of majority of the amino acids, it has the major losses in terms of lysine 53% compared to the 7% and 12% of fortified infant formula and raw milk respectively (Data not shown). Moreover, the observed increase of amino acids in infant formulas could be attributed to the liberation of amino acids from the initial glycated products formed in the commercial powder as well as the protein hydrolysis driven by the application of microwave thermal treatment in closed systems [11]. Aligned with the expectations, enrichment of infant milk increased the functional OS (e.g. of 3'sialyllactose (3'SL)) in the BMO-enriched formula (63-160 $\mu\text{g/mL}$). Clearly there are significant losses of 3'SL during processing of infant formulas (5-8 $\mu\text{g/mL}$) as their concentration is significantly ($p < 0.05$) lower than raw milk (91-121 $\mu\text{g/mL}$). However, the loss of this 3'SL during processing of milk could provide a window for fortification using pure BMOs sourced from dairy waste streams [8].

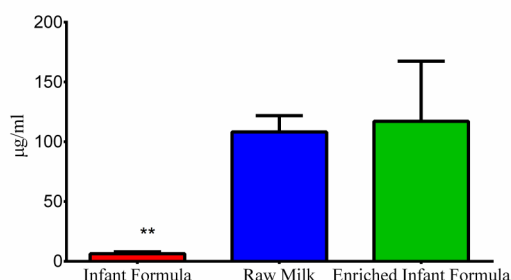


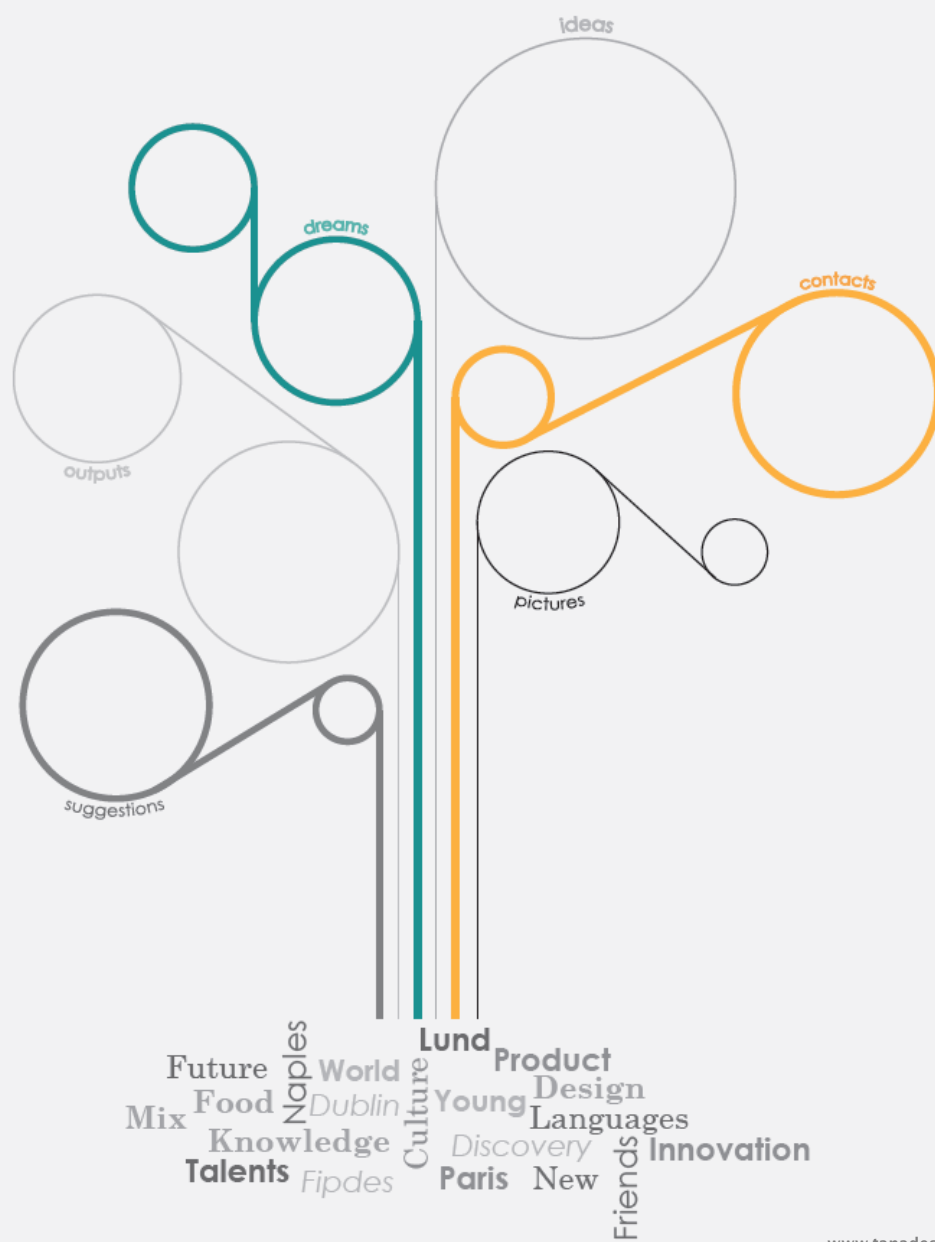
Figure 5: 3'Sialyllactose concentration in milk Model Systems using One-Way ANOVA ($\alpha=0.05$). Post hoc test performed using Tukey Kramer.

Conclusion

Results of this study showed: BMO chemical reactivity in milk model systems could be governed by two possible mechanistic pathways: regular hydrothermolysis and/or *peeling off mechanism* initiated by the formation of BMO-Amino acid adduct. BMO fortification vividly provides the advantage of adding functionality in milk in terms of addition of OS with more complex and specific structures similar to that of HMO without significantly resulting to losses of amino acids

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