

FIPDes Day 2014
International talents in Food
Innovation and Product Design

Students' Book of Executive Summaries







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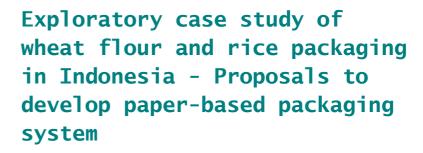
Let's make our future not be happened, but prepared.

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Evaluation of current supply chain and consumers insights of wheat flour and packaged rice in Indonesia were done to introduce sustainable and functional paperbased packaging solution to Indonesian market. After considerations OΠ the marketing, product. logistics. environmental, and consumers' requirements, two paper-based packaging proposals for wheat flour and packaged rice were developed.

#### Introduction

Indonesia as tropical country do not produce strong and long paper fibers like the ones produced in four-seasons country. The lack of raw materials to produce strong paper bag packaging can be solved with the involvement of BillerudKorsnäs as paper supplier from Sweden.

The logistical challenges in Indonesia (poor infrastructures and vast land distance) and the tropical climate (high temperature and humidity) pose challenges especially in applying paper-based packaging. Manufacturers in developing countries have a tendency to reduce costs while developing packaging without knowing the probable negative economic impacts along the supply chain. To evaluate the overall supply chain and get customers insights are beneficial in developing the optimum packaging system.

#### **Objective**

The purpose of this thesis is to evaluate the packaged rice and wheat flour supply chain in Indonesia and develop an overall paper-based packaging system solution from holistic (manufacturers to consumers) point of view.



The method being used is case study with two embedded analysis units, wheat flour and packaged rice. Different analysis types are summarized on the table below.

| Types of analysis | Qualitative analysis of  | Qualitative analysis on consumers                                |  |  |
|-------------------|--|--|--|--|
| Method            | Direct observation<br>(POEMS method)   | Indirect<br>observation<br>(packaging<br>scorecard)              | Semi-structured interview                                    | Direct observation with structured interviews                        |
| Objective         | Explore, describe<br>and explain how<br>each SC actor<br>interacts with<br>packaging | Evaluate the performance for the packaging within a supply chain | Explain the importance of packaging design for each SC actor | Evaluate the the usage habit of the product related to the packaging |

Table 1. Thesis methodology summary

The supply chain actors and consumers being analysed are summarised in Figure 1.

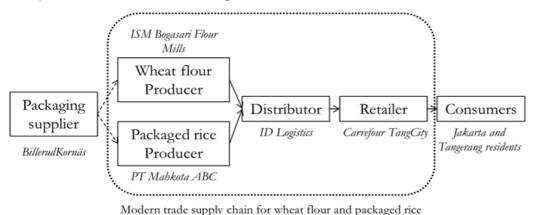


Figure 1. Study focus on supply chain and the consumers of wheat flour and packaged

## Results and discussion

Modern trade Supply chain of modern trade is simpler with single manufacturer, distributor and retailer (Figure 1). The case study for two different products, wheat flour and packaged rice shows two different manufacturer types.

Wheat flour manufacturer, PT ISM Bogasari Flour, is the biggest wheat flour producer in Indonesia. Bogasari initiates environmental marketing campaign and new packaging formats. Packaged rice manufacturer, PT Mahkota ABC, is one of the many packaged rice producers in Indonesia with most being local producers. Mahkota ABC produces

modern retailers' private label products and tends to follow the packaging trend in the packaged rice category. The distributor, ID Logistics, is the distributor for Carrefour retailer. The retailer in focus is Carrefour store in Tangerang City Mall. The packaging system level (primary packaging as the packaging being bought by customer, secondary packaging as a group of primary packaging, and tertiary packaging as pallet) interaction with the logistics activities along the supply chain can be found below.

packaging system levels and over-stacking as main concerns. The distributor arranges mixed-products pallets according to the stores' orders. The distributor feels that this method is correct and causes 0.03% loss due to packaging breakage during transportation to stores. The current 1kg wheat flour and 5kg packaged rice products need more time to be displayed in the store shelves. The stores feel that the handling ability and display ability of the products can be improved.

|             | Supply Chain<br>Members   | Manuf           | acturer             | Transport |                   | DC              |                  | Transport | Reta      | il Outlet            | Reuse       |
|-------------|---|-----------------|---------------------|-----------|-------------------|-----------------|------------------|-----------|-----------|----------------------|-------------|
| Product     | Packaging<br>System (row) \<br>Logistics<br>Processes<br>(column) | Filling process | Warehousing process | ort       | Receiving process | Picking process | Shipping process | ort       | Receiving | Replenishing process | and Recycle |
| Wheat flour | Primary   | Х               |                     |           |                   |                 |                  |           |           | Х                    |             |
|             | Secondary   | Х               |                     | Х         | Х                 | Х               |                  |           | X         | Х                    | Х           |
|             | Tertiary  | Х               | Х                   |           |                   | Х               | Х                | Х         | X         |                      | X           |
| Rice        | Primary   | Х               |                     | Х         | Х                 | Х               |                  |           | X         | X                    |             |
|             | Tertiary  | Х               | Х                   |           |                   | Х               | Х                | Χ         | X         |                      | X           |

Table 2. Packaging system and logistics activities interaction along the supply chain

The primary packaging for packaged rice is 5kg LDLPE and nylon bag and for wheat flour is 1kg PE bag. The secondary packaging for wheat flour is double fluted cardboard box with 12 primary packages. The tertiary packaging for the wheat flour is internal plastic pallet with 50 secondary packages on it. Sixty rice bags are stacked to the internal wooden pallet as its tertiary packaging, without the use of secondary packaging.

The direct observation, semi-structured interviews, and packaging scorecards of the supply chain actors found that the manufacturers have the most concerns about the packaging, with modularity among

The consumers store certain amount of rice as each Indonesian consumes around 500 grams of rice per day. The consumers are influenced to consume more western foods such as cake and bread which drives the consumption of wheat flour. Indonesian consumers stores the rice in rice boxes, in various containers, or in its own packaging. The storage of wheat flour are normally in its own packaging with rubber band to secure the opening or in an air-tight container to keep the wheat flour from clumping.



supply chain actors feel that the introduction of paper bag packaging for wheat flour and packaged rice is potential, but certain measures such as barrier ability of the packaging and the modularity within the packaging level are enough to protect the products during the transportation in less than ideal infrastructures and in the humid tropical climates. The supply chain actors and their needs are taken into consideration developing new paper-bags packaging system. The holistic analysis approach and integrated packaging development can optimise the packaging system to gain more logistical efficiency, especially where the logistics expenditure can be up to .

The packaging solutions implementations can be faster if they are based on manufacturer financial ability and innovation involvement, consumers' usage habits, and negotiation

position with the modern retailers. The solutions still need to answer to product, environmental logistics. marketing, and Two different paper-based requirements. packaging solutions are proposed to cater to the wheat flour and packaged rice products. The primary packaging proposals can be found on Figure 2. The secondary packaging of the wheat flour (corrugated box) is dimensionally modular with the primary and tertiary packages. The box has tearable section and is ready to be displayed in modern retail shelf. The secondary packaging of the packaged rice will be cardboard tray and plastic shrink wrap. This will be modular with the retail display shelf and the pallets in the factory and during distribution.



Figure 1. (a) Primary packaging of wheat flour based on FibreForm with easy-to-pour spout and reclose able. The cap can be used as measurement device (cup or 100gram) (b) Primary packaging of rice with easy-to-carry feature that doubles as resealing tool

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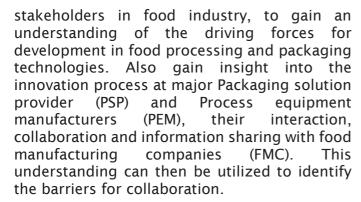


In the dynamic economic environment where knowledge is vastly distributed companies can no longer rely on their own research and are pushed to utilize outside sources to sustain growth (Saguy, 2011), thus pushing the industry towards collaboration in R&D and innovation.

The food industry involves large number of horizontal and vertical relationships, the very dynamic nature of these relationships play role in innovation (Cannon T. 1994). The role suppliers and their relation with manufacturers in improvement process has long been recognized (Petroni & Panciroli, 2002). Numerous studies recognize that supplier-customer collaboration in product development (NPD) has a positive impact on product quality, cost and time to market (Clark, 1989). In order to fully capitalize on supplier-customer collaboration it becomes vital to understand the dynamic relation between packaging and processing industry and need to operate closely, develop ways to identify good partners and create & maintain fruitful collaboration (Birkinshaw et al, 2007).

Based on the understanding that the role of suppliers is crucial for technical innovation in food industry, the study is based around understanding of the interaction between food manufacturing companies (FMC) and their suppliers. There has been emphasis and prior research with focus on the role of ingredient suppliers retailers and innovation, as they induce most visible innovations and a market push (Van der Valk & Wynstra, 2005; Traill & Meulenberg 2002). However the research on collaboration with actors on the other side of food system including packaging and processing equipment industries as well as academia is rather limited.

The primary purpose of the research is to study interactions and relations between



The interactions can be defined in terms of involvement in NPD (new product development), research collaboration, sharing of production data. Previous research has focused on quantitative evaluation of food manufacturing industry and their suppliers (Ettlit, 1983; Petroni & Pancioroli, 2002). In this study a qualitative approach was adopted which relyies on the nature of interaction, degree of interaction as well as at what level the interaction is carried out.

#### Methodology

The research follows an inductive approach which starts with a premise and structure is built around the conceptual framework and the research objectives. The research design is in between tight pre-structured one and loose emergent one. Secondary data collected through literature survey was utilized to develop a conceptual model.

Primary data was collected through interviews with experts from the industry and academia who have experience in working with innovation and collaboration. A non-probability sampling technique was adopted selecting the experts based on three criteria purposive, strategic and convenience. Semi-structured interview technique was followed where respondents were asked for their opinion on specific open-ended questions. The responses

are interpreted along the way and used to investigate further with a sub-question. The interviews were transcribed to text and categorized under common themes which for analysis and comparison. To ascertain the credibility of the data it was triangulated and compared to literature.

### **Results and Discussion**

Idea generation is at the front end of innovation, in the majority of food companies these new product development processes are still based on internal innovation factor (Bigliardi et al. 2013). One expert from Tetra Pak Processing AB mention that traditionally the ideas came from academia or from the technical staff within the company and a research project started with a technical solution in mind. The success rate for such projects is very low and in the competitive market situations companies are forced to reconsider this approach to innovation. There has been realization that innovation is about problem solving and thus now the front end of innovation is based on need finding, market push, competitor products as well as advances in institutional research. This shift call for a better understanding of the customers as well as end users end consumers, collaboration with suppliers and research organization.

While the idea generation and collaboration process in larger companies is more complex, to understand the innovation idea generation process in a multi-national packaging company (> 10000 employees) an aspect communication came to fore. There exist communication channels. system a requirements from customer, channelize market and competitors, translating it into requirements, prioritizing it and using them to define research projects. The process is illustrated in below **Figure 1**.



Figure 1: Communication channels for customer interaction in a multinational food packaging company

Communication is on a global scale, where ideas and needs from different markets are collected into a central marketing function, converted into requirements, prioritized and finally fed to the centralized R&D. In this system the requirement owners i.e. the research and development team seldom comes in contact with the need owners (customers). In that sense communication channels hamper personal relations and thus a hindrance for collaboration.

Based on the understanding of the communication channels in multinational food packaging companies, flow of innovation projects and inputs from literature, an understanding of the innovation process was built. The innovation process is illustrated in **Figure 2** below.

Most companies today operate on a global scale, supported by a trend of consolidation by merger and acquisitions in the food industry (Returners, 2014). Multinational companies having central R&D cater to customers in every corner of the world; this has let companies to develop innovation process to gather, filter and prioritize the ideas to lead. Also create partnerships and collaborate with other stakeholders.

In the above model ideas are collected from customers, market demands, competitor development, suppliers, consultants and

technological advances in the industry and fed innovation funnel. Usually marketing department filters these ideas, checks for feasibility and builds a business case around them. Ideas with a strong business case are prioritized and passed on to the research and development department. They work on the technical developments collaboration with suppliers. The nature of this collaboration is mostly 'contractual'. Academic or research institutes are engaged if any fundamental or basic research needs to be conducted. As the developments usually take several years to commercialize and owing to the dynamic market situation these is a need to check and reiterate the market needs as well as the business rational being the project. After the product is developed it is tested with an industrial partner or a trusted customer, finetuned and launched in the market. The actors are involved in the early stages of product development especially customers academia (technology scouting). Their role in strategic development is limited and underdeveloped.

Further factors that limit the role of suppliers in collaboration and hinder collaboration were identified. Some common barriers to collaboration identified are legal hassles, documentation, and ownership of the research, agreements and setting up a legal framework

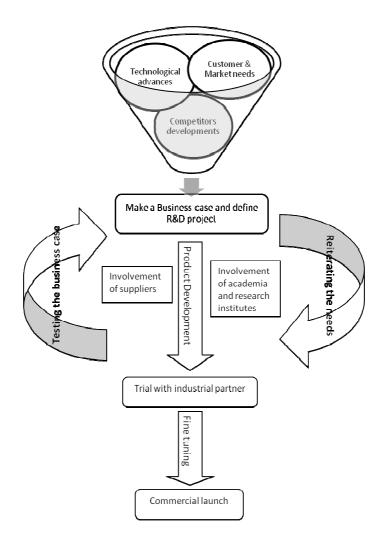


Figure 2: The innovation process for processing and packaging companies

(Sagay, 2011). Food manufacturers, especially suppliers their as important collaborator for innovation, in their relationship there is exchange of market knowledge and ideas. The reasons cited by food manufacturing companies for limited collaboration with packaging are high cost of capital intensive and trial cost is also more, time consuming. It is much faster and cheaper to work with ingredients for new product development and develop new products new for the market. Another reason that prevents manufacturers to experiment with new processing and packaging technologies is skepticism about safety and the perception amongst consumers.

#### Conclusion

The views of industry experts strongly reflect the role of suppliers of processing and packaging in food industry is "contractual" in nature, whereas ingredient suppliers tend to be more mature partners in the innovation process. Petroni and Panciroli (2002) in their research on innovation as a determinant of suppliers' roles and performance found a need for food machinery suppliers to make it their goal to move up from a "contractual" to "mature" or even a "strategic" partner in NPD and innovation.

Documenting the innovation process from industry perspective and role of suppliers in innovation, it can be noted that, the innovation process at major food machinery and

packaging company corresponds well to the 'food-machinery framework of open innovation (Bigliardi et al., 2010). It is apparent that food industry is taking steps to integrate external knowledge sources in the innovation process, still suppliers continues to play limited strategic role in innovation.

Gain insight into barriers to collaboration; some of the barriers to collaboration were identified and they can be grouped into two types: technical and perspective. Technical factors constitute lack of technical expertise amongst food manufacturer, requirement for legal framework and difficulty in predicting future needs. But the more imperative barriers are lack of trust, skepticism about new technologies and conflict of interest Trust continues to be the major barrier for collaboration. Especially with process equipment manufacturer and packaging solution provider where technological edge accounts for their market advantage. The development for framework for engaging actors should be aimed at fostering trust and build a transparent as well as symbiotic relations. This is a major challenge that needs attention for further research.

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# Investigating the impact of saliva on aroma release

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The relationship between transglutaminase (TG) and volatile release was investigated in *vitro* in a model system miming differences in TG levels detected in human whole resting saliva from obese and normal weight people. TG interaction with three volatiles - benzaldehyde, ethyl acetate and 2-phenylethanol, were analyzed in the dynamic headspace of an artificial saliva solution by SPME-GC/MS. The resulting lower aroma release in saliva from obese compared to normal-weight subjects can be attributable to volatile concentration. polarity, the interaction between polarity and concentration, and interestingly to TG.

#### Introduction

Results from studies suggest that the impression people get from the sensory properties of foods plays a very important role in the way they select their food and how much they eat. Seen in the light of the continuing increase in the prevalence of overweight and obesity in large parts of the world, it is important to identify all aspects of human appetite regulation to understand this trend, and to prevent it from progressing. Investigating the link between the sensory perception of food and human eating behaviour is thus crucial.

During eating/drinking, food is mixed with saliva and the products of the food-saliva interaction are perceived rather than the food itself. Therefore, together with its main functions (e.g. speech, maintaining oral and general health, and food processing), saliva has a role in the appreciation and acceptance of food/beverage. During eating/drinking, all kinds of oral sensation (taste, viscosity, astringency, etc.) modulated by saliva. In addition, the olfactory perception, arising retronasal when the odorants interact with odour receptors by migrating from the mouth to

the nasal cavity via the nasopharynx, is significantly affected by the interaction with saliva. Given all the known facts about saliva, this thesis within the framework of this question: Is it possible that the change in saliva composition due to a pathology can affect sensory perception and ultimately food preference, perception and therefore personal diet selection. This study question if diseases resulting in an alteration of saliva composition modify the food/beverage sensory perception of an individual and consequently their preferences, choices and habits.

In light of evidences from studies, the hypothesis that in obese individuals, salivary enzyme transglutaminase could be responsible for an alteration of the retro-nasal aroma volatilization was tested. This could have an impact on the delay of satiation due to a lower olfactory stimulation although only in vivo studies could support this hypothesis. A possible implication of saliva on the amount of food intake in obese people is supported by previous research, which shows that slower habituation of salivary responses to food stimuli is related to greater energy intake, and that obese individuals habituate slower than normal-weight. These findings suggest that decline in sensory responding to food occurs more slowly in obese individuals, possibly contributing to delayed satiation and greater food intake.

#### **Aims**

The aim of this research was to test (*in vitro* by model solutions) the impact of a specific salivary component, transglutaminase, on the retronasal aroma release in the following ways:

To investigate the interaction of volatile compounds with this component in normal and obese artificial saliva:

To assess three different volatile compounds representing: (1) volatiles with proven potential to behave differently after interaction with saliva from obese versus from normal weight subjects; (2) different degrees of polarity, covering a wide range of log P values; (3) from different kinds of chemical classes; and

To understand what other factors can affect said saliva-volatile interaction.

#### Materials and methods

#### I. Sampling

Saliva was collected from 28 O and 28 N male individuals aged between 20 and 68 years.

# **II. Proteins: Biochemical analyses**

For protein profiles analysis, samples were separated by 12% SDS-PAGE electrophoresis performed by using the Mini PROTEAN and PROTEAN xi 2-D cell systems (Biorad). Selected protein bands showing the major variability were identified by Peptide mass fingerprinting (PMF) by MALDI-TOF mass spectrometry (MS).

#### **III. Volatiles**

#### Sample Preparation

Solutions of volatiles were prepared with appropriate concentrations of a single volatile according to their corresponding odor detection thresholds in water (Table 1). These volatiles were dissolved in a 90/10 (v/v) water/ethanol solution. An internal standard was also prepared using alcoholic solution of 2-octanol (50 mg in 250 mL ehtanol).

Table 1. Volatiles, their Properties, and Concentrations

| Volatile        | Molecular | LogP (o/w) | Odor Thresholds | Concent | rations Test | ted (mg/L) |
|-----------------|-----------|------------|-----------------|---------|--------------|------------|
|                 | Weight    | (pH=7)*    | (mg/L)          | c1      | c2           | c3         |
| 2-phenylethanol | 122.16    | 1.51       | 14              | 28      | 42           | 56         |
| benzaldehyde    | 106.12    | 1.60       | 7.5             | 15      | 23           | 31         |
| ethyl acetate   | 88.11     | 0.71       | 3.5             | 7       | 11           | 14         |

Artificial saliva (AS) was composed of the chemicals listed in Table 2. TG was then added in different quantities, based on the amount for N and O subjects. For N saliva samples, concentration of TG was fixed at 83 µL TG solution in 50 mL AS while for O saliva, TG concentration was computed to be 137 µL TG solution in 50 mL AS, using the data obtained from the study of Piombino et al. (2014). No TG was added to one of the artificial saliva solutions to serve as the control. One-mL aliquots of the artificial saliva (AS) solutions (for control, N, and O) were then

prepared and stored in the freezer (-20°C), to be thawed out for 30 minutes just in time for each analysis.

Table 2. Components of Artificial Saliva Solution (Genovese et al., 2009; van Ruth et al., 2000)

| Solute                                 | Quantity Required |
|--|-------------------|
| calcium chloride dehydrate             | 0.441 g           |
| sodium bicarbonate                     | 5.208 g           |
| potassium phosphate dibasic trihydrate | 1.369 g           |
| sodium chloride                        | 0.877 g           |
| potassium chloride                     | 0.477 g           |
| sodium azide                           | 0.500 g           |
| milli-Q water                          | 1.0 L             |

# Release and Isolation of Aroma Compounds by Dynamic Headspace/Solid Phase Microextraction (DH/SPME)

Artificial saliva + volatile + internal standard were added to a 4-mL vial (15x45 mm) at pH=7 and equilibrated for 40 minutes at 37°C (to mimic normal body temperature) under a water bath while being continuously mixed by a magnetic stirrer. The SPME (solid-phase microextraction) fiber was inserted into the vial and then exposed to the headspace of the mixture for 10 minutes. for volatile compounds to be trapped on the SPME fibre. Each experiment was performed in two replicates.

# <u>High Resolution Gas Chromatography-Mass</u> Spectrometry (HRGC/MS)

A GCMS-QP2010S mass spectrometer (Shimadzu, Kyoto, Japan) coupled with the gaschromatograph and equipped with a DB-Wax fused capillary column (60 m x 0.25 i.d., 0.25 µm film thickness) was used.

#### IV. Data Analysis

Significant difference of the TG content between the two study groups (O vs N) was determined by means of the non-parametric Kolmogorov-Smirnov Test. For the comparison of data obtained from the GC-MS runs for volatile release. statistical analysis was performed using XLSTAT software. Data were analyzed both by one-way ANOVA followed by multiple comparisons tests and two-way ANOVA with interactions. For all analyses, a difference was considered to be significant at a level of P<0.05.

#### **Results and discussion**

# I. TG Variability Between Whole Resting Human Saliva from O and N Subjects

One of the proteins showing a major variability between the two groups was identified to be Transglutaminase (TG). TG was significantly higher (Figure 1) in the O compared to N samples. This higher variability observed for N samples is likely linked to the high degree of inter-individual variability in human saliva composition. The common trend observed in TG levels for O samples can be linked to a common alteration of the saliva composition occurring in cases of obesity.

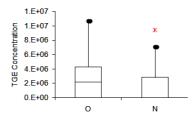


Figure 1. TG variability in salivary samples of O and N subjects. \*P<0.05

# II. Study of the Influence of Salivary TG on Volatile Partitioning from Model Solutions

The Partial Least Square Discriminate Analysis (PLS-DA) performed from the study of Piombino et al. revealed that the detected difference in TG levels was among the most relevant variables in terms of explained variance for the observed general reduction of in vitro volatile release between O and N saliva.

Among the volatiles showing a significant diminution in concentration after interacting with saliva from O with respect to saliva from N, 3 are those taken into account for further investigations aimed to understand the impact of different levels of TG on their release. These compounds are: ethyl acetate, 2phenylethanol and benzaldehyde. Among the affected volatiles, most the headspace concentration of ethyl acetate, benzaldehyde 2-phenylethanol significantly P<0.001 to P<0.05) fall down: 49 %, 21 % and 60 %, respectively. Different behaviors were observed for the three volatiles tested. No impact of the TG presence and concentration

was observed in the case of benzaldehyde independently from its concentration. Ethyl acetate showed the most consistent trend with regards to the effect of TG on volatiles release at the highest TG concentration in the artificial saliva, there corresponded a lower headspace level of ethyl acetate and this trend seemed to follow a linear behavior. In spite of a not very constant trend, the impact of TG on the release reduction was shown to be significant for 2-phenylethanol and also at the highest concentration tested. At 22% change in reduction of aroma release for O vs. N, the impact was higher for the more hydrophobic compound. However, with the hydrophobic volatile tested (Bzh), no impact of the TG was observed. This variance can be attributed to the inhibiting effect of an aldehyde to the enzyme transglutaminase. Aldehydes form stable covalent bonds with the active site cysteine upon the reaction of aldehyde with the active site cysteine thiol of the enzyme. Higher quantities of aldehyde may inhibit more TG so that for O saliva, the volatile release is higher than N samples, which is the reverse of the expected effect of the protein. Hydrophobic compounds are more likely to be affected by the presence of TG in the saliva.

To further understand the impact of TG on the volatile release from saliva interaction, a two-way ANOVA was performed for all three volatiles tested (Table 3). Results showed that TG can indeed affect volatile release, as well as the quantity and polarity of the volatile present, and the interaction between concentration and polarity of the volatile.

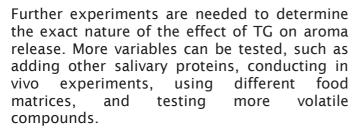
| Fonte         | F       | $P_r > F$ |
|---------------|---------|-----------|
| TG            | 4.601   | 0.019     |
| ConcV         | 95.848  | < 0,0001  |
| Log P         | 201.274 | < 0,0001  |
| TG*ConcV      | 1.754   | 0.167     |
| TG*Log P      | 2.235   | 0.092     |
| ConcV*Log P   | 42.718  | < 0,0001  |
| TG*ConcV*LogP | 1.166   | 0.355     |

Table 3. Two-way ANOVA with interactions for Volatile

#### **Conclusions**

The relationship between TG and volatile release was investigated in a model system miming differences in TG levels detected in human whole resting saliva from obese and normal weight people. A lower inter-individual variability and a significant high mean value of TG was detected in saliva samples from obese with respect to lean subjects, showing a recurrent alteration of salivary TG correlated the pathology. Multivariate statistical analyses indicated the altered TG levels as one of the factors negatively affecting the in vitro aroma release from a liquid food matrix (white wine) after interaction with saliva from obese donors. Upon further in vitro tests, the variability of aroma release between the two subject groups can be attributable to the volatile concentration, polarity of the volatile, interaction between polarity concentration, and more interestingly to TG.

This study supports the initial hypothesis, suggesting that transglutaminase can be one the salivary components that responsible for a possible alteration of the retronasal aroma release for obese individuals, but in vivo analyses are needed. In spite of the simplified model conditions, the results of this study are of interest because they allow adding further knowledge to a very complex research subject. It has reported that a lower extent of aroma release corresponds to a higher amount of ad libitum food intake, and this is considered one of the bases of behavioral choices towards food consumption in obese people. As further highlights, at the best of our knowledge, no previous results are available in literature about the impact of TG on the aroma portioning in water solutions. Results from the study seem of interest not only for the understanding of why an altered sensory perception was observed in case of obesity, but also in the field of food technology. Indeed, this basic knowledge may constitute the starting point for a future production of food specifically designed to "compensate" the possible altered perception of people affected by pathologies (obesity as the focus of this study, but also celiac disease) responsible for an altered amount of TG in saliva.



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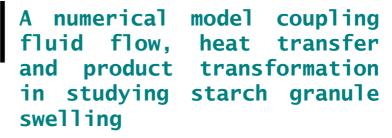
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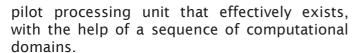


#### Introduction

Modeling is becoming widely used to simulate many processes in the food industry, because it reduces the number of experiments, time and expenses. It also provide more information that may not even be possible with experimentation and is used for process optimization like thermal treatment to which many liquid food are subjected not only as a way for improving their safety and extending their shelf life also for inducina product transformation like starch gelatinization (Datta, 2008).

The evolution of starch granules under continuous thermal treatment involves the occurrence of fluid flow, heat transfer and product transformation. These phenomena are coupled along their continuous thermal processing. When modeling the starch granule swelling under continuous flow, a two way dependence has to be considered. Firstly, fluid flow and heat transfer drive shear rate and temperature fields, which affect the product transformation. Secondly, the own transformation can modify the properties (as its product apparent viscosity). Inside a heat exchanger, the progressive occurrence of larger swollen starch granules increases the product viscosity, slowing down the fluid parcels near the walls and hence exposing them to additional heating and consequent transformation.

In this study we demonstrate the feasibility of modeling the swelling of starch granules under continuous flow through numerical simulations by developing a computational fluid dynamics (CFD) model, for solving the coupled problem of fluid flow, heat transfer and starch granules swelling, where the latter is represented by the help of reaction kinetics of order 2 and by representing a



The following strategy will be considered. Firstly, the evolution of starch is observed under laboratory conditions, in submitting it to thermal treatments characterized by different duration. Secondly, the study of the product thermal history in relation to its transformation state enables the estimation of kinetic parameters which allow us to present the transformation of the product inside the model. In parallel, the rheological behavior associated with the product is described as a function of its transformation state. The last step is the comparison between model predictions and measurements at pilot scale and laboratory scale.

# Methodology

The evolution of a starch suspension under heat treatment and continuous flow is hereafter studied with the help of numerical simulations. For compressible fluid, the conservation equations for mass, momentum and energy under steady-state conditions can be written:

(1) 
$$\vec{\nabla} \cdot (\rho \vec{u}) = 0$$

(2) 
$$\rho(\vec{u}.\vec{\nabla})\vec{u} = \vec{\nabla}\left(-pI + \eta(\vec{\nabla}\vec{u} + (\vec{\nabla}\vec{u})^T) - \frac{2}{3}\eta(\vec{\nabla}\vec{u})I\right) + \vec{F}$$

(3) 
$$\rho C_p(\vec{u}.\vec{\nabla})T = \vec{\nabla}.(K\vec{\nabla}T) + Q$$

The mean volume diameter ( $D_{[4,3])}$  associated with the granule size distribution can be chosen as an indicator of the product transformation state at a given time t. Values of mean volume diameter deserving particular interest are  $D_{[4,3]0}$  before any heating, and  $D_{[4,3]m}$  after the strongest thermal treatment. The transformation state of a starch suspension can be characterized by the swelling degree of starch granules in water which can be defined as a dimensionless variable, bounded between 0 (no swelling at all) and 1 (full swelling) (Lagarrique et al., 2008):

(4) 
$$S = \frac{\left(D_{[4,3]} - D_{[4,3]0}\right)}{\left(D_{f4,3]m} - D_{f4,330}\right)}$$

Its variation with time can be reasonably be represented through a second-order kinetics equation (Lagarrigue et al., 2008):

$$\frac{dS}{dt} = V(1-S)^2$$

Where V is the reaction rate constant which is estimated through a threshold linear function. Experimental work has indicated, since the 1940's (French, 1944) that during the heating process, there is a temperature called Ta bellow which there is no swelling. Ta is the swelling starting temperature. The rate constant V was considered equal to zero below the swelling temperature and increasing linearly versus temperature above it.

(6) 
$$V(T) = 0 \quad \text{if } T < Ta$$

$$V(T) = V_a (T - T_a) \quad \text{if } T > T_a$$

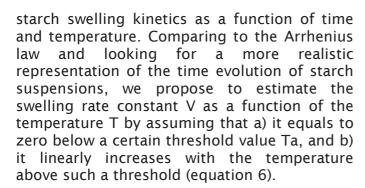
The equation (2) requires the apparent viscosity associated with the aqueous suspension of starch granules. The equation (6) requires the kinetic parameters Ta and Va. Both kinetic and rheological parameters were estimated from measurements performed at laboratory scale as detailed below.

# Estimation of parameters from laboratory measurements

Kinetic and rheological parameters were identified in the case of stabilized and cross-linked waxy maize starch (3.42%) from measurements performed at laboratory scale (rheological and laser granulometry measurements).

Two major sets of parameters are required in the scope of this study.

The first set concerns the parameters associated with the representation of the



Kinetic parameters were estimated by minimizing:

(7) 
$$Min \left\{ \sum_{tests} \left( D_{end}^{measured} - D_{end}^{estimated} \right)^2 \right\}$$
  $D_{end}^{estimated} = D_{[4,3]0} + \left( D_{[4,3]m} - D_{[4,3]0} \right) \left( 1 - \frac{1}{1 + \int\limits_{0}^{t_{end}} V dt} \right)$ 

The second set of parameters involves the representation of the apparent viscosity, more precisely its approximation as a function of key variables: the shear rate, the temperature, and an indicator of the transformation state.

(8) 
$$\eta_a = A\dot{\gamma}^{(n_a + (1 - n_a) \exp(-B(\phi - \phi_0))) - 1} \exp\left(\frac{C}{RT}\right) \exp(D\phi)$$

Parameters A, B, C, D and n were carefully determined from measurements of apparent viscosity conducted at 20 and 40°C, after restricting our interest to shear rate ranges corresponding to a well defined behavior for the apparent viscosity: between 5 and 20 s-1 for no heating time, and between 10 and 500 s-1 for heating times 4 and 8 minutes.

# Representation of the pilot processing unit through a sequence of computational domains

The pilot OMEV (Officine MEccaniche Vadesi) (HTST/UHT System) is composed of 4 heating sections and 4 cooling sections. Each section was represented as the figure 1:

Realistic geometrical conditions were assumed, associated with a tubular heat exchanger which is being employed in our laboratory; namely, we take into account the radius (4.5 mm) and the length of each of heating, holding, and cooling sections (overall length about 7.5 m).

Realistic boundary conditions were assumed, corresponding to selected flow and thermal operating conditions with that heat exchanger: volume flow rate of 15 liters per hour, and product temperature of about 44 °C at the exchanger inlet and about 70 °C at the last heating section's outlet. Further, a fully developed parabolic flow profile is assumed at the exchanger inlet.

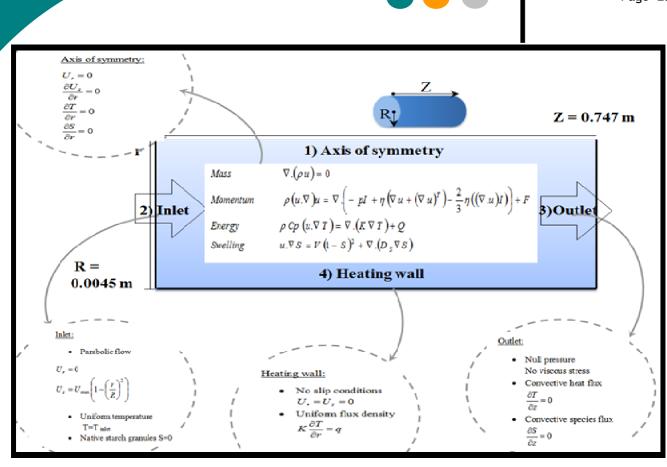


Figure 1: Computational domain of interest (section 1), which constitutes the two-dimension, axi symmetric representation of a cylindrical heat exchanger. Boundary conditions are summarized for the axial Uz and radial Ur components of the velocity, the temperature T and the swelling degree S.

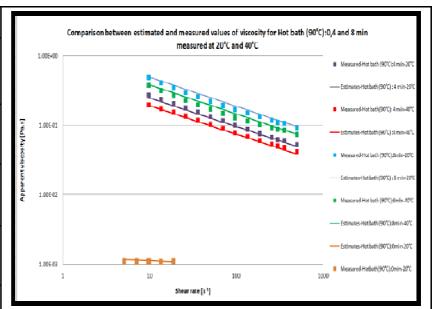
### **Results and discussion**

### **Kinetic Parameters estimations**

| Parameters          | Values            |   |
|---------------------|-------------------|---|
|                     |                   | Kinetic parameters determination  Hot bath (90°C): I min (1) Hot bath (90°C): 2min(2) Hot bath (90°C): 2min(2)  |
| D <sub>[4,3]m</sub> | 43.25 ±0.1 μm     | Hot bath (90°C): 1 min (1) Hot bath (90°C): 2 min(1) Hot bath (90°C): 2 min(2) Hot bath (90°C): 4 min(1) Hot bath (90°C): 4 min(2) Hot bath (90°C): 8 min  1:1 45 |
|                     |                   | [H 40]  |
| D <sub>[4,3]0</sub> | 15.53 ± 0.5 μm    | 25 33 E   |
|                     |                   | Mean volume diameter 25 20 20 20 20 20 20 20 20 20 20 20 20 20  |
| Va                  | 3.7 10-3 s-1 °C-1 | 15) 25  |
|                     |                   | N 20  |
|                     |                   | 15 20 25 30 35 40 45  |
| Ta                  | 62.1 °C = 335.25K | Do+(Dmax-Do)*(1-1/(1+integral( Va*(T{t}-Ta)*dt )) [μm]  |
|                     |                   |   |

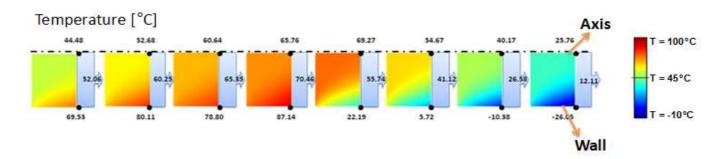
# Rheological parameters estimation

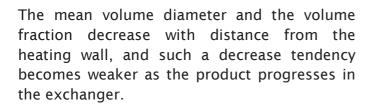
| Parameters | Values                                     |
|------------|--|
| n∞         | 0.55                                       |
| В          | 3.8  |
| С          | 9.7 10 <sup>3</sup><br>J.mol- <sup>1</sup> |
| А          | 1.44 10 <sup>-5</sup> Pa.s <sup>n</sup>    |
| D          | 10.1                                       |



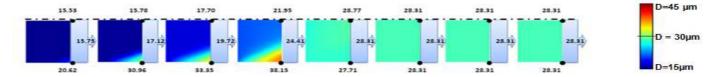
#### **Model Results**

Fluid parcels moving near the wall are slowed down while those running at the axis are accelerated justifying the higher maximum axial velocity. We observe weak values of shear rate near the exchanger center and low values at the wall, between them two progressively increasing values. The temperature increases faster near the wall than the axis of symmetry. The temperature increases along the domain as a consequence of developing thermal boundary layer from the heating wall towards the exchanger center. The thermal boundary layer, along which the T decreases linearly with distance from the heating wall, becomes wider.





Mean volume diameter [µm]



Near the wall the overall result is the increase of suspension viscosity due to the increase of solid volume fraction. Such an increase slows down the velocity near the wall.

Experiments on a pilot scale tubular heat exchanger were used to compare results with those obtained at the laboratory scale and with model predictions. The deviation between laboratory and pilot measurement was about 2.6 % for the final mean volume diameter. The deviation between model prediction and pilot measurements was about 20% with perfect mixing hypothesis.

#### Conclusion

A numerical model was developed to study the evolution of starch suspension in a heat exchanger. A second-order kinetic equation is assumed to describe the evolution of the swelling degree. Kinetic rate constant is assumed to increase linearly with temperature above a threshold temperature. rheological model was established to predict the apparent viscosity as a function of granule's volume fraction, temperature and shear rate. Kinetic and rheological parameters were identified in the case of stabilized and cross-linked waxy maize starch (3.42%) from rheological and laser granulometry measurements. Experiments on a pilot scale tubular heat exchanger were used to compare results with those obtained at the laboratory scale and with model predictions.

deviation between laboratory and pilot measurement was about 2.6 % for the final mean volume diameter. The deviation between model prediction and pilot measurements was about 20%.

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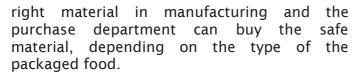
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The corrugated board is considered as the most used transportation packaging in the world. percent of Fortv corrugated packaging is used for packing food products but only 4% of this is actually used in direct contact with foods, while the rest is used as secondary packaging (European Corrugated Industry, 2014). Corrugated packaging is used to protect food products to help minimize food Cardboards are widely used in food; however, only a small percentage is used for direct food contact as shown in recent statistics; examples of these include pizza cartons or boxes for hamburgers and sandwiches. The examples stated could be considered as high risk applications due to relatively high temperature of the food when initially packaged and the high fat content of the food products, which can contribute to faster migration rates of unwanted substances from the packaging to the food (Albu, 2011).

It is still a challenge for food and packaging companies to address all known and unknown substances and use the right material for each product. There is a responsibility for packaging manufacturers to ensure that cardboards meet all the requirements of food safety and hygiene. Companies should have a system to identify the risks and to choose the appropriate material which can be used when food contact is the case. Another issue is the internal communication between different departments inside the company (e.g., sales, design, purchasing and production) to identify whether the produced packaging will have direct food contact or not from early stages of development. If internal communication works properly, the sales department can ask the right questions to the customers, and the design department can make the proposals that suit each product's needs. Production can use the



# Purpose of the research

The main goal of this thesis is to assess the current packaging development process in one of the leading Swedish cardboard packaging companies, from a food safety perspective focusing on the following questions:

- 1. Does the process in early stages identify whether the packaging to be developed is suitable for direct food contact?
- 2. What is lacking in the process in order to ensure that direct food contact requirements are sufficiently considered in development projects where food safety requirements apply?

To fulfill the purpose, the following subobjectives have been identified:

- Design a supportive tool in the form of a template to help designers and sales representatives to ask the right questions before or during the project, in order to recognize if direct food contacts is the case.
- Explore the current system and give suggestions on how to improve the data collection and internal communication between different departments within the company (e.g., sales, design, purchase, production).

# Methodology

study was based on qualitative methodology research. The first part of the study was obtained through a review of books. publications. relevant literature, doctoral theses, journals in the field of new packaging development and food safety of corrugated boards. Another type of data was collected through careful selection interviewees. Ten semi-structured interviews were conducted, which included:

(1) Main departments involved in the packaging development process. (2) Several packaging experts working in food companies in Sweden and (3) Professors and academic

experts in the field of packaging and food industry.

All interviewees were selected based on their expertise in the field. All interviews were performed face to face. Before each interview a copy of the questionnaire was sent to each interviewee in advance by email. A short presentation of the project was performed and its purpose was stated before starting the interview. The length of each interview was approximately 50 minutes. The researcher recorded and transcribed each interview, and interviewees were kept anonymous and were coded to specify their position and years of experience, among other details.

Thematic analysis was used to categorize data into different topics. It was also used to analyze and describe the data in profound ways and to build a proper structure to the research paper.

#### **Results and Discussion**

According to the respondents, there are four departments involved in the packaging development process within the packaging company (Sales, Design, Production, and Purchase). Sales and design have a follow up communication and involvement in the new packaging development and have a close communication with their customers (food companies) to identify the project and customer needs for different products.

There was a need to understand the role of department in order to produce packages with direct food contact. As each food product has its own requirement, the company needs to answer some food questions about the nature of the product, product requirements and customer requirements. A tool was developed to assist packaging companies to ask the appropriate questions. The author found that it would be beneficial for the sales department to use a checklist for food products to ask specific questions to the food producers (customers), order to determine the appropriate packaging material.

The author suggested a template to improve internal communication, which can be added to the system as a tab specifically for food packaging requirements and inputs different products. This tab on the wizard can called 'Critical information for food products and food safety' where all the necessary information (about the packed product, temperature, contact with packaging etc.) was developed. Interviewees pointed that answering *auestions* by those understating the nature of the product, which will reflect the need for specific materials and processes to use in the production to avoid all possible risks, and to produce safer packages.

Regarding the used material, (company P) uses only virgin fibers for direct food contact. Interviewees from food companies mentioned that recycled material should be considered, as it is a sustainable resource, but it must be handled correctly to avoid all the possible risks. Other interviewees mentioned that in some cases, there is a need for higher quality

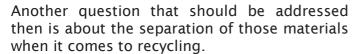
of materials to minimize the risk, and debates show that packaging and food companies have to be more vigilant towards this, and not only be cost driven.

The EU does not have a harmonized legislation for the use of food contact paper and board materials (Albu & Buculei, 2011). More work is needed by food authorities and researchers to help packaging and food companies produce safer packages for foods in the future. Interviewees from food companies mentioned that if legislations get stricter that will help them to be more careful during production.

Food companies as well as the final consumers will be willing to pay more if packages were proved to be safer than its competitors, and this can open new markets to packaging companies and increase their sales. The author believes that efficient functional barriers which can be, for example, PET or other functional barriers, can be used.

Summary of the company's current situation and the author's recommendation on department responsibilities

|                          | Current situation of responsibilities  | Recommendations for direct food contact packaging  |
|--------------------------|--|--|
| Sales<br>department      | Collects all needed information and customer requirements  | Ask the appropriate questions regarding food safety requirements, depending on the nature of each product  |
| Design<br>department     | <ul> <li>Packaging concept</li> <li>Design</li> <li>Printing process</li> </ul>  | Migration data     Amount of ink applied to package.     Adhesive and ink selection depending on product requirements.  Cood bygions and manufacturing practices including.  |
| Production<br>department | <ul> <li>Packaging production</li> <li>Production risk assessment</li> <li>Quality assurance</li> </ul>                                | <ul> <li>Good hygiene and manufacturing practices including equipment cleaning and personnel hygiene.</li> <li>Production sequence (produce package which have direct food contact first before the packages without direct food contact</li> <li>Control all microbiological, physical, chemical hazards when producing packages with direct food contact.</li> <li>Machine setup for production of direct food contact.</li> <li>Applying food safety standards</li> </ul> |
| Purchase<br>department   | <ul> <li>Purchase orders for the needed raw<br/>material from suppliers with the best<br/>cost</li> <li>Checking compliance</li> </ul> | Coordinate with quality control to insure that regulations are compiled well.  |



It should also be considered that only using different materials will not prevent all the possible risks of contamination (Biedermann, 2011), as contamination can occur from other sources, e.g. through transportation or machinery, which can have dangerous levels of mineral oils or heavy metals that might transfer to packages, then afterwards to the food itself. Thus, the best solution is to have a complete system which assesses all the possible risks.

The author assessed the current situation within the packaging company and suggested further responsibilities which can be added to each department involved in the packaging development process, in order to produce safe packages for direct food contact.

# **Concluding remarks**

The focus of this study was to assess the current process of cardboard packaging development at company 'P' (in Sweden) from a food safety perspective, to identify what is lacking in the process to ensure that the requirements for direct food contact packaging are sufficiently considered in development projects, as well as to get a holistic view of food companies' and experts' opinions about using cardboard (corrugated and solid) in the food industry as a primary and secondary packaging.

There are more guidelines needed in the production of packaging intended for direct food contact in order to produce safer packages Responsibilities were suggested to each department to help produce safer products. Different departments within the company are recommended to be more aware act based on these responsibilities. The company is recommended to also have increased awareness if the responsibilities changes in are to be implemented, as well as greater control of their revised functions or processes.

Each food product has specific packaging requirements. To help in identifying and addressing these requirements, a template for sales, logical diagram and system template for various departments were designed facilitate and improve internal communication within the company. Additionally, templates were constructed to help the packaging company in their development process, such as asking the appropriate questions to the customers, identifying the needs of each product, and sourcing the right material to be used for the packaging.

#### Recommendations for further research

study was done, through After this investigating and assessing the packaging development process at a packaging company from a food safety prospective, further research is needed to test different food products (inside solid and corrugated packaging) through the supply chain to identify more possible risks that can affect different physical, chemical and microbiological hazards.

More rigorous studies are also needed to improve the developed tools (logic diagram and template), which have been created for the packaging companies in this research. Through this, food and packaging companies will be able to implement better procedures throughout the supply chain, from manufacturing until consumption, as well as in choosing the best possible packaging material which varies from one product to another.



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Biedermann, M.; Ingenhoff, J.; Barbanera, M.; Garbini, D. & Grob, K. (2011). Migration of Mineral Oil into Noodles from Recycled Fibres in the Paperboard Box and the Corrugated Board Transport Box as well as Printing Inks: A Case Study. Packaging Technology and Science. 24 (5): 281-290.

#### **External links**

**European Corrugated Industry (2014).** The 7 rules of efficient packaging. Available at: <a href="https://www.fefco.org">www.fefco.org</a>.

Industry Guideline for the Compliance of Paper & Board Materials and Articles for Food Contact. (Issue 2, Sep. 2012). Available at: <a href="http://www.citpa-europe.org/sites/default/files/Industry%20guideline-updated2012final.pdf">http://www.citpa-europe.org/sites/default/files/Industry%20guideline-updated2012final.pdf</a>.



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- Engineering degree in Food Biotechnology from Kemerovo Technological Institute of Food Industry (Russia), 2001-2006

Master Thesis hosting lab: Polymères, Colloïdes, Interfaces, UMR CNRS Université du Maine (France)

#### Master Thesis tutor:

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#### Introduction

WPI (whey protein isolate) is a powder obtained by spray drying the milk whey. Its main components are two globular proteins: β-lactoglobulin and  $\alpha$ -lactalbumin (Mahmoudi et al., 2007; Bolder et al., 2006). When globular proteins are heated in water solutions, the structure of their molecules changes. Active chemical groups hidden within the protein globules become exposed and available for interaction with other proteins. As a result, the formation of a of protein suspension aggregates observed (Donald, 2008; Phan-Xuan et al., 2011). These aggregates may be of different shapes and sizes depending on the conditions during the heating process (pH, protein concentration, presence of salts etc.). Above a critical protein concentration aggregation leads to the formation of a gel (Baussay et al., 2004).

Globular protein aggregates are interesting for application in the food industry such as to induce gelation of food products, stabilization of foams or emulsions, and encapsulation (Nicolai et al., 2011).

Protein aggregates are especially interesting for new product development due to the fact that consumers in Europe tend to choose additive-free products with simple labels and 'transparent', wholesome components. Milk proteins are generally perceived as healthy ingredients and they do not require safety assessment and approval by the European Food Safety Authority, so they need not to be indicated by another E number on the packaging.

However, the use of protein aggregates in mass-scale food manufacturing requires detailed understanding of the way they modify the structure of food products. Many scientific publications that have already been published deal with the mechanism of

aggregation and the aggregate structure at different conditions. A number of publications report on the process of so-called cold gelation by which a gel is formed when a salt (or an acid) is added to a prepared suspension of protein aggregates. This process happens already at room temperature, but is accelerated at higher temperatures (Nicolai et al., 2011). However, a more systematic study is needed of the rheological properties of the systems prepared with WPI aggregates.

As a part of the master thesis project we conducted rheological measurements on suspensions and solutions of WPI aggregates, and also on the gels formed by WPI aggregates (WPIA) in mixtures with CaCl<sub>2</sub> and after decrease of the pH.

# **Methodology and Results**

The following procedure was used to prepare WPI aggregates. The protein powder was diluted in distilled water with an antibacterial agent and stirred overnight. The stock solution was filtered two times (0.45  $\mu$ m and 0.2  $\mu$ m pore size) and the pH was adjusted to 7. The solution was further diluted to the required protein concentration and placed in sealable glass bottles. The bottles were heated in a thermostated waterbath at 80 °C for 24 hours.

WPIA suspensions prepared at different protein concentrations (from 20 g/L to 90 g/L) are presented in Picture 1.



Picture 1. WPI aggregates prepared at protein concentrations of 20, 30, 40, 50, 60, 70, 80 and 90 g/L (from the left to the right).

The aggregates prepared at different concentrations differ in size and molecular weight. The aggregates prepared at 20 g/L are much smaller compared to the aggregates prepared at 90 g/L. Close to the point of gelation ( $C_g = 93.5 \text{ g/L}$ ) the size of the aggregates increases dramatically, which is demonstrated in Figure 1.

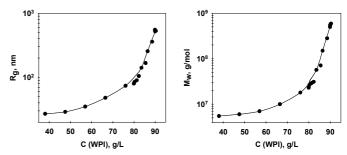
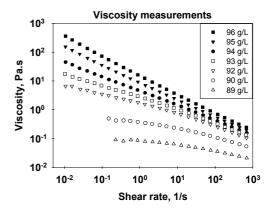


Figure 1. Dependence of  $R_{\rm g}$  and  $M_{\rm w}$  on the concentration of WPI. The solid lines are guides to the eye. The data were kindly provided by Walailuk Inthavong.

We conducted measurements of the shear dependent viscosity and the frequency dependent shear moduli for suspensions of WPI aggregates prepared at different protein concentrations. The results are presented in Figure 2.



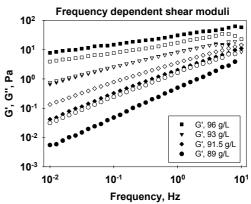


Figure 2. Shear rate dependent viscosity and frequency dependent shear moduli of WPI aggregates prepared at different initial protein concentrations close to the point of gelation ( $C_g = 93.5 \text{ g/L}$ ). Closed symbols in the left graph represent systems that formed a gel (not soluble in water), while open symbols represent suspensions of soluble WPI aggregates. Closed symbols in the right graph correspond to the storage (G) modulus, and open symbols correspond to the loss (G") modulus.

The graphs show that with increasing protein concentration (and, consequently, increasing of the size of the aggregates), the viscosity of the suspensions also increased steeply. The behavior of the systems changed from liquid-like at 89 g/L to gel-like at 96 g/L. The systems demonstrated increasingly shear-thinning behavior with increasing protein concentrations.

We also conducted measurements on dilutions of WPI aggregates. Two suspensions of WPI aggregates formed at 90 and 93 g/L were diluted in water to concentrations ranging from 30 to 90 g/L and the viscosity of the dilutions was measured. The structure of the aggregates doesn't change upon dilution (Ako et al., 2010). The values of the viscosity at shear rate 1 s<sup>-1</sup> for WPIA dilutions were compared with the values for WPI aggregates prepared at different protein concentrations. The results are presented in Figure 3.

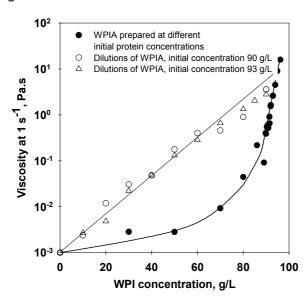


Figure 3. Values of the viscosity at shear rate 1 s<sup>-1</sup> for WPI aggregates prepared at different initial protein concentrations (closed symbols) and dilutions of two WPI suspensions (open symbols).

The graph demonstrates that at the same protein concentrations the viscosity of the two systems is very different. For WPIA dilutions the size and the molecular weight of the aggregates stay the same and the viscosity depends only on the concentration and an exponential increase is observed. However, for WPIA prepared at different concentrations the aggregate size

increases dramatically with increasing protein concentration (as shown in Figure 1), that is why the increase is much stronger. It is clear that if the aggregates are to be used as thickeners in food products, they should be produced at high protein concentrations.

To investigate the effect of calcium (an essential ingredient of dairy products) on WPIA we prepared mixtures of the aggregates with CaCl<sub>2</sub> at different concentrations and heated in sealed vials at 80 °C for 15 minutes. Three different states were observed: liquid, homogeneous gel, heterogeneous system (gel+syneresis). The results are summarized in the state diagrams presented in Figure 4.

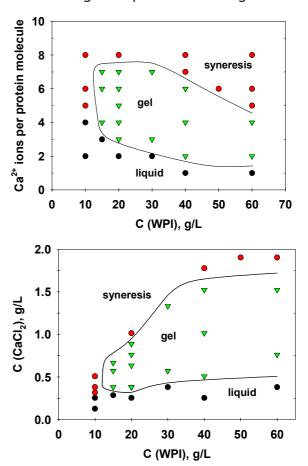


Figure 4. State diagrams for WPIA+CaCl<sub>2</sub> mixtures after heating at 80 °C for 15 minutes. The graph on the left shows the results as a function of the molar ratio and on the right – as a function of the CaCl<sub>2</sub> concentration. The lines are guides to the eye indicating the area within which a homogeneous gel is formed.

We conducted the rheological measurements on the gels formed by WPI aggregates in the presence of CaCl<sub>2</sub>. Freshly prepared mixtures were heated in the rheometer at 80 °C. For most systems gels were formed within a few minutes, but the elastic modulus continued to increase with time. Therefore the systems were heated for 60 minutes, before cooling to 20 °C. The decrease of the temperature after heating resulted in increase of the values of shear moduli by about a factor of 3. We compare the values of the storage modulus for different gels at 20 °C in Figure 5.

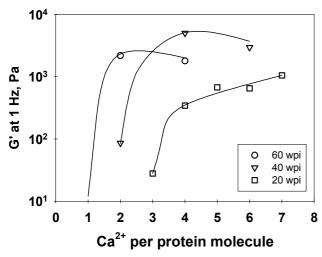


Figure 5. Values of the storage modulus at 1 Hz at 20 °C for gels formed by WPI aggregates as a function of the CaCl<sub>2</sub> concentration after one hour of heating at 80 °C. The solid lines are guides to the eye.

The storage modulus reached values up to 4 · 10³ Pa, which is much higher that values found for yogurts in the literature (100-300 Pa) (Ramírez-Sucre and Vélez-Ruiz, 2013; Hussain et al., 2011). Thus, by varying the concentrations of WPI aggregates and CaCl₂ one can form gels with different characteristics, which opens possibilities for new product development.

We also conducted a study of the effect of acidification on WPIA suspensions and found that H<sup>+</sup> is less effective than Ca<sup>2+</sup> for the formation of a homogeneous gel. Homogeneous gels are formed in a much smaller area of the H<sup>+</sup> - WPI state diagram and the gels are less stiff.

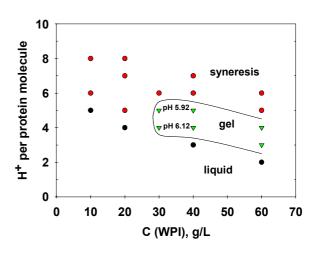


Figure 6. State diagram for WPIA + H+ systems after heating at 80 oC for 15 minutes. The lines are guides to the eye indicating the area within which a homogeneous gel is formed.

# **Conclusions and perspectives**

The general objective of the research presented here was to help food manufacturers to develop new dairy products with modified textures using just dairy ingredients.

Globular proteins are a promising source of 'clean label' texturizing ingredients due to their exceptional functional characteristics. The aggregates of the proteins can be used as an ingredient modifying the viscosity of liquid systems (thickeners). WPIA easily form strong gels in mixtures with salts or upon acidification, so the aggregates prove to be effective gelling agents that might be potentially used to create new textures in food products.

The application of the aggregates requires further investigation of their interactions with other components of dairy products, such as caseine proteins and minerals.



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# Liquid food to manage dysphagia: A rheological perspective

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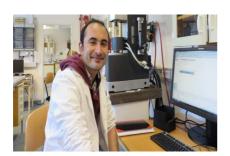
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Dysphagia refers to the difficulty in swallowing. Normal eating process involves chewing and mastication. During these processes food experiences a number of structural transformations. These transformations include both shear and extension. These changes are aimed at reducing the particle size of the food material. Non-Newtonian fluids have been highlighted as helpful for safe swallowing. It also been pointed out that the extensional properties of the food may help improve the swallowing process. However, literature lacks any consistent data which relates specifically these extensional properties to safer swallowing. In the present project we created three food grade model fluids with Newtonian (constant shear viscosity), Boger (constant shear viscosity and elastic) and shear thinning dependent and (shear rate elastic) properties to probe the effect of elasticity on safe swallowing in patients suffering from dysphagia. Preliminary results suggested that elastic properties of the fluids promote a safer swallowing at least in pharyngeal oral and phases swallowing.

# Introduction

Dysphagia is a serious concern especially in the elderly population which often leads to malnutrition2. About 50% of the elderly (65 years or above) people in nursing homes suffer from swallowing disorders. elderly population in US alone is expected to rise from 39 to 69 million by 2030. Swallowing disorders are more pronounced for low viscosity foods<sup>3</sup>. Innovative food companies have been studying the rheology of the food to manage dysphagia. Commonly the texture of a food product is adjusted which changes the rheological properties of the food. Various food used to thickeners achieve this are

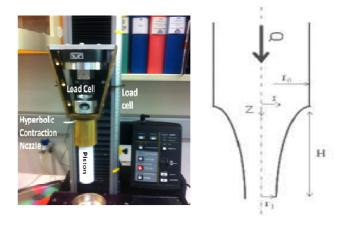
objective<sup>6</sup>. Due to limited knowledge of rheology in relation to swallowing, evidence of its success barely exists. A normal swallow consists of three phases; oral, and esophageal. Swallowing pharyngeal, begins in the oral cavity in order to transfer food from mouth to the stomach<sup>1</sup>. Normal process involves chewing eating mastication. During these processes food experiences number of structural a transformations. People suffering swallowing disorders may have an entirely different oropharyngeal food processing. The key factor often ignored is the stage of swallowing and elasticity in the Therefore swallowed product. more comprehensive knowledge of the swallowing process in relation to the product's elastic properties is required<sup>6</sup>.

The current project was designed to study if rheological properties (in particular the elastic or stretching properties) of the liquids foods promote safe swallowing in those suffering from dysphagia.

# Materials and methods

Maltodextrin was used here as newtonian polymer to enhance the viscosity of the model fluids and make the elastic affect more distinguishable. Xanthan gum is molecular weight polysaccharide. It was used in the study to provide elasticity to the model fluids. Contrast media facilitates in x-ray visualization of the vascular system. Iohexol which is an iodinated contrast media was used in this project. Maltodextrin was added slowly to the water at constant stirring to ensure complete dispersion. Xanthan gum was dissolved in Iodine directly. The calculated amount of xanthan gum was added slowly to magnetic stirrer with predetermined amount of iodinated contrast media. All the ingredients were mixed slowly on magnetic stirrer to achieve complete dispersion. The model fluids were characterized by both shear and extensional rheology using ARES G, and Hyperbolic contraction flow system of the **INSTRON®** respectively. For extensional properties, a specially designed technique hyperbolic contraction flow was used (fig 01

left). The nozzle has a higher inlet (r<sub>0</sub>) than the outlet (r<sub>1</sub>) diameter which provides maximum extension proportional to the amount of elasticity in the sample. The small volume of the sample at the inlet of nozzle reaches its maximum extension at the outlet nozzle. The extension viscosity is measured by subtracting the shear factor from the measured stress to get the corrected true extensional viscosity (equation 1).



$$\eta_e = \frac{\sigma_{measured} - \sigma_{Shear}}{\varepsilon}$$

Fig 01: Hyperbolic contraction flow system and shape of Hyperbolic contraction nozzle of the INSTRON

Clinical trials were performed at the Neurological clinic/dysphagia laboratory, Malmö Hospital, Sweden. The study was performed in the presence of a speech language pathologist (SLP) and radiologist. Oral Transit Time (OTT, Phayrngeal transit time (PTT) and Pharyngeal retention were recorded.

#### **Results and discussion**

Three different kinds of model fluids that varied in the degree of elasticity were created (fig. 01). They were characterized both in the shear and extension rheology.

# Characterization of the fluids in shear rheology

Figure 02 Shows the model fluids created with the contrast media. The sample with no xanthan has straight plateau qum a throughout the applied shear rate depicting Newtonian behavior. 200 ppm of xanthan gum contained sample has been selected as the Boger fluid. It has a slight shear thinning trend at lower shear rates while the trend becomes Newtonian with a constant viscosity of 0.5 Pa.s as the shear rate is increased. This slight shear thinning has been reported before in the literature by Stokes et al., 2001. The sample with 500 ppm xanthan gum has a viscosity of ~ 4.5 Pa.s which decreases sharply with the increase in shear rate (typical shear thinning behavior). In order to show the samples had elasticity in the shear experiments, the first Normal stress (N<sub>1</sub>) is plotted as a function of shear rate (Fig 01 right). The normal stress rises as the shear rate is increased. This is due to the Weissenberg affect also called the rod climbing phenomenon. The knowledge of Weissenberg effect (N<sub>1</sub>) is a good tool to differentiate the Newtonian and non-Newtonian fluids<sup>1</sup>. Shear thinning fluid has the highest N, increase with increasing shear rate followed by the Boger. N1 is zero for the Newtonian fluid due to the lack of elasticity.

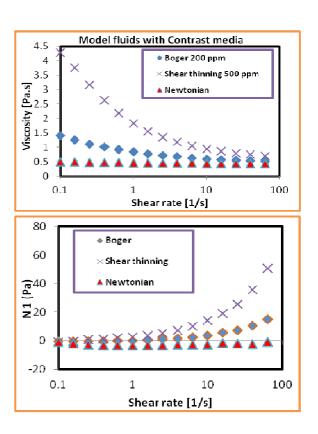


Fig 02. (Top) Shear rate dependance of the model fluids with contast media that were used in the clinical trials. First Normal stress diffrence  $(N_1)$  (fig. bottom) of the model fluids at different shear rates (1/s)

# Characterization of model fluids in extension rheology using hyperbolic contraction flow

The extension viscosity of samples decreased with increasing rate of extension depicting extension thinning behavior. Slight variation in the behavior is visible. This is due to the nature of the technique where every time the piston that provides the extension has to be operated manually. This is opposite to the viscosity measurement where parameters are set in advance and the instrument works automatically till the end. The purpose of the results, however is to show that the model fluids have elasticity in them and that the degree of elasticity increases with increasing the amount of xanthan gum. It was observed here that 500 ppm xanthan gum sample (shear thinning from the shear experiments) is more elastic than 200 ppm xanthan gum sample (Boger from the shear experiments).

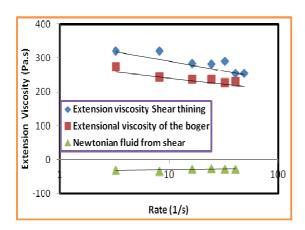
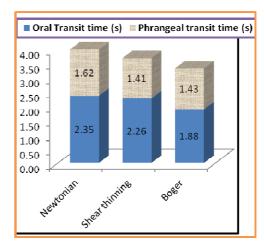


Fig 03: Stretching properties of the model fluids during hyperbolic contraction flow

#### Clinical trials

A faster OTT is generally associated with a safer swallow (Chen and Loliveret, 2011). Our results (Fig 04 left) indicate elastic samples have been swallowed with much ease than the Newtonian ones since the elastic fluids had a comparatively faster OTT. The average oral transit time for Newtonian fluid was the highest 2.35±0.99 seconds followed by shear thinning fluids 2.26±1.22s. Boger fluid has the least OTT with a value of only 1.88±1.09s. Five patients had the OTT faster with Boger, four with shear thinning and only three had the OTT faster with the Newtonian fluids.

Pharyngeal transit time: A longer PTT indicates motility problems in patients<sup>2</sup>. The average PTT was shorter for shear thinning and Boger fluids (1.41 and 1.43 s respectively) compared to the Newtonian fluids. The difference were however not significant (P = 0.05) statistically. Out of the 12 patients examined five had the least PTT with shear thinning fluids followed by four with Boger. Only three patients had a shorter PTT with the Newtonian fluids. Overall the OTT and PTT shows that the regardless of the degree, the samples with elasticity were easy to swallow than the Newtonian sample. These differences are however not significantly different (P= 0.05) due to a large variation in the nature of subjects.



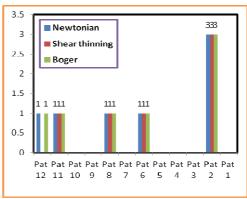


Fig 04: Average oral and pharyngeal transit time (s) of the model fluid during clinical trials (top). Pharyngeal retention: (bottom) it is defined as the contrast media pooling into the vallecule or /and the pyriform sinuses. It is measured as; 0= no, 1= mild, 2= moderate, 3 = severe

Pharyngeal retention indicates the activity of pharyngeal constrictor muscles. **Impaired** pharyngeal constrictor activity leads pharyngeal retention. No difference was noticed in the degree of pharyngeal retention among the patients examined (fig 04 right). Four out of five patients experienced mild pharyngeal retention. One of the patients had the severe pharyngeal retention. The model fluids, seems not to have any significant impact in the later stage of swallow. The physiological health status of the individual patients seems to be the dominant factor in determining the safety of swallowing than the fluids itself.

# Sensory analysis

Sensory analysis (fig. 04) was based on a single question if the liquid was very easy (rank 5) to very difficult to swallow (rank 1). The result showed fluids with elasticity were comparatively easy to swallow. Shear thinning sample was the easiest to swallow with a score of 37 followed by Boger fluid with nearly similar score of 36. Newtonian fluid in this study was comparatively harder to swallow. The sensory results are consistent with two of the four parameters measured in the clinical trials (OTT and PTT) for the safer swallowing with elastic liquids. The reason for a simple sensory analysis was the nature of the subjects involved in the study.

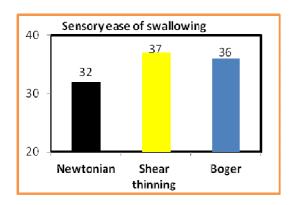


Fig. 07: Ease or difficulty of the model fluids during swallowing examination

#### **Conclusions**

Edible model fluids with elastic properties and radio-opacity were successfully developed. Elastic properties of the fluids promote safe swallowing during oral and pharyngeal phases in those suffering from dysphagia. The link between elasticity and safe swallowing however could not have been established in the later stages of swallowing. This is due to an overall slower OTT causing the fluids to reach the body temperature thereby losing its elastic properties. Moreover the use of 50% contrast media to achieve radio-opacity further reduced the elastic and viscous properties of the fluids. This is not necessary in products available in the market. Hence highly elastic product without contrast media in the market may assist the management of dysphagia.

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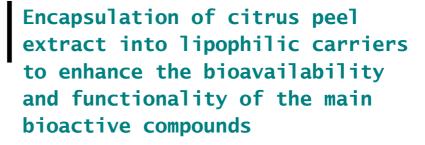
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#### Profile in a nutshell:

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In this research we demonstrated that encapsulation of citrus peel extracts rich in polymethoxyflavones (PMFs-nobiletin, sinensetin and tangeritin) into lipid carriers using particles from gas saturated solutions (PGSS) could be considered very promising improving their incorporation functional ingredients in food products. We developed PMF rich citrus peel extracts using supercritical fluid extraction (CO<sub>2</sub> and ethanol as co-solvent), a high pressure technology with minimal environmental impact. Six citrus peels (clementine, lemon. lime, orange, red grapefruit and tangerine) were studied and we showed that tangerine peel was the richest in terms of the total PMFs of interest and also showed that tangerine and orange peel extract had the highest anti-proliferative effect on HT29 colon cancer cell mono-layers which we correlated with the contents of PMF particularly nobiletin and sinensetin.

Three lipid carrier systems successfully developed consisting of a solid lipid alone (glyceryl monostearate-GMS) and a mix of solid and liquid lipids (glyceryly mono-oleate-GMO and sunflower oil-SFO). The particles morphology was observed by scanning electron microscopy. The phase transition and melting point of the particles was studied bv differential scanning calorimetry and the storage stability was evaluated. The lipid particles were incorporated into aqueous based beverage systems. The systems incorporated with glyceryl monostearate (GMS) and glyceryl mono-oleate (GMO) particles were more stable. The total phenolic content monitored during accelerated storage for seven days showed that there was no expulsion of citrus peel extract into the system during storage.



There is an increased focus and interest on both the nutritional and additional health benefits of foods which has led to a growing foods number of researches into compounds they contain which are likely to confer a health benefit. Generally, foods with health benefits additional are functional foods however, over the years different names have emerged such nutraceuticals, there is also an increased awareness by consumers on the potential health benefits that foods possess and the possible risk reduction of certain diseases therefore this plays a significant role in their food choices. There has been an accelerating knowledge and studies on phenolic compounds (a class of phytochemicals) which are considered to be amongst the most desirable phytochemicals because of their antioxidant activity and as a large group they also have diverse biological functions and recently flavonoids one of the classes of phenols has gained much attention.

Polymethoxyflavones (PMFs) a class of highly lipophilic flavonoids (sub-class of polyphenols) are recognized to have potent anti-cancer activity, and pre-dominantly exist in citrus peels however, they are highly hydrophobic compounds with poor solubility in oil and water at ambient and body temperature thus limiting their bioavailability and incorporation as functional ingredients in food products or use as nutraceuticals. Recently, several studies have focused on encapsulation of PMFs to improve their bioavailability and incorporation in food products. Previous studies in which delivery systems consisting of nano-emulsions or visco-elastic emulsions have been explored to encapsulate PMFs though successful were reported to be unstable in terms of PMFs tendency to crystallize, sediment or precipitate out of the oil phase over time. It is therefore necessary to further explore other delivery systems aimed at not only overcoming this challenge but also improving bioavailability by increasing lymphatic uptake of PMFs in the GI tract and also improve their incorporation in food products and use as nutraceuticals.

# **Objectives**

The principal objective of this research is to explore the possibility of encapsulating citrus peel extracts into lipid carriers to enhance the bioavailability of polymethoxyflavones as well as their functionality and to achieve this our research was divided into three parts:

- Development of PMF rich extracts from citrus peels using supercritical fluid technology
- Development of lipophilic carriers for encapsulation of PMF to enhance their bioavailability and functionality.
- Develop formulations to improve their incorporation in nutraceuticals and functional foods.

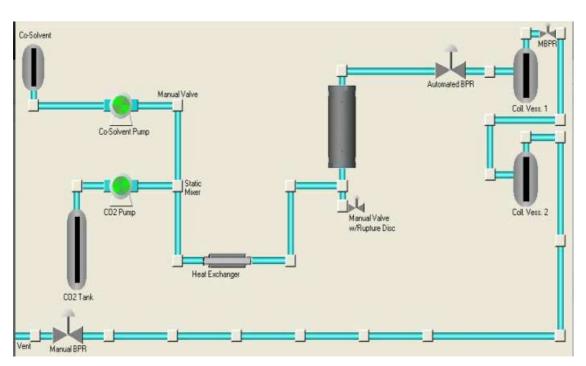
# Strategy/methods

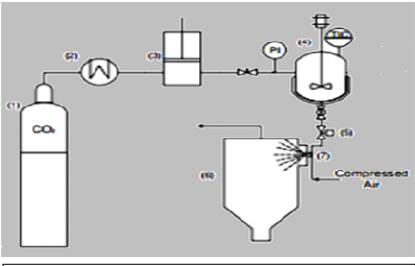
Clementines, Lemons, Limes, Oranges, Red grapefruits and Tangerines were purchased from a local fruit shop in Oeiras, Portugal. The fruits were of good eating quality. The juice was extracted. The residues after extraction and the peels were separated. The peels were crushed and freeze-dried for a minimum of 48 hours. The freeze-dried peels were extracted by conventional methods (solvent-ethanol and methanol) and supercritical fluid extraction with CO<sub>2</sub> as supercritical fluid and 23% (w/w) ethanol as co-solvent at 275bar (27.5MPa) and 50°C for two hours. The extracts were characterized in terms of PMF content namely nobiletin, sinensetin and tangeritin using HPLC-DAD technique and the anti-proliferative activity evaluated in a 2D colon cancer cell model.

Particles from gas saturated solutions (PGSS) a high pressure technology which is based on the high solubility of supercritical carbon dioxide in many molten fats, lipids or polymers at moderate pressures was used to produce lipophilic forms of PMF-rich citrus peel extracts using solid lipid (GMS-glyceryl monostearate) and a blend of solid-liquid lipid (GMO-glyceryl mono-oleate and SFO-sunflower oil) carriers. The experiments were carried out at different pressures, temperatures and carrier to extract load ratio conditions.

The particles produced by PGSS were analyzed by differential scanning calorimetry (DSC) to monitor phase transition and melting point and scanning electron microscopy (SEM) for morphology characterization. The particles storage stability was also evaluated by comparing their oxygen radical absorbance capacity (ORAC) after storage at 4°C, in the dark (ambient temperature) and in the light (ambient temperature).

The particles were incorporated commercially available juices and their dispersion behavior observed. The total phenolic content of the juice before and after incorporation with lipophilic particles was also compared to determine if there was expulsion of citrus peel extracts into the juice after incorporation and during accelerated storage for seven days.





(1) CO2 cylinder (2) cryostate (3) pneumatic piston pump (4) stirred vessel (electrically thermostated) (5) automated depressurization valve (6) recovery vessel (7) nozzle

Fig 1: Schematic diagram of supercritical fluid extractor (A) and PGSS apparatus (B)



Tangerine peel extract was the richest in total PMFs characterized followed by clementine and then orange. The PMF yields were higher for supercritical fluid extract than conventional extract confirming high selectivity of supercritical fluid extraction for isolating PMFs (Toledo-Guillén, Higuera-Ciapara, García-Navarrete, & de la Fuente, 2010).

# **Anti-proliferative activity**

The highest anti-proliferative effect was structures and formed aggregates. The obtained with tangerine and orange which could particles were smaller and more clustered be correlated with PMF content particularly together for systems consisting of solid-liquid nobiletin and sinensetin.

We successfully developed solid lipid and solid-liquid lipid particles. The percentage recovery was higher for systems consisting of solid and liquid lipid only when the extract load was minimized. The recovery seemed to decrease as the system became more lipophilic.

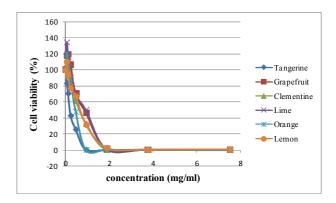
| Citrus Peel    | Sinensetin(mg/g<br>of extract) | Nobiletin(mg/g<br>of extract) | Tangeritin(mg/g<br>of extract) |
|----------------|--------------------------------|-------------------------------|--------------------------------|
| Clementine     | 2.15                           | 22.95                         | 4.41                           |
| Lemon          | 1.21                           | < 0.01                        | < 0.01                         |
| Lime           | < 0.01                         | < 0.01                        | < 0.01                         |
| Orange         | 6.57                           | 15.44                         | 1.43                           |
| Red grapefruit |                                |                               |                                |
|                | < 0.01                         | 1.79                          | 0.35                           |
| Tangerine      | 6.98                           | 79.43                         | 35.66                          |

Values reported as mgPMF/g of extract

Table 1: PMF quantification supercritical fluid extraction 1st fraction (15mins)

ORAC values decreased significantly when the particles were stored in the light at ambient temperature denoting a decrease in antioxidant activity as a result of decrease in capacity to quench peroxyl radicals.

The melting point of the particles decreased for the systems consisting of solid and liquid lipid carriers. The particle morphology of the carriers loaded with citrus peel extract was not so different from that of the carriers alone. The particles had amorphous wool-like structures and formed aggregates. The particles were smaller and more clustered together for systems consisting of solid-liquid lipid carriers.



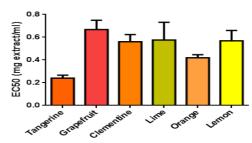
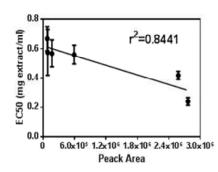
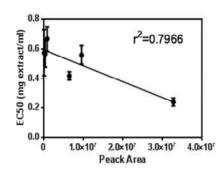


Fig.4. Anti-proliferative effect of PMF rich extracts in 2D colon cancer cell model





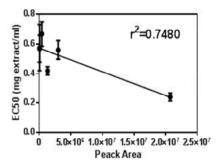


Fig. 5: Correlation between PMF content and EC50

| Citrus peel extract | Lipid<br>carriers  | Carrier to extract ratio | % Recovery |
|---------------------|--------------------|--------------------------|------------|
| Red grapefruit      | GMS                | 50:50                    | 27         |
| Clementine          | GMS +<br>GMO (3:1) | 80:20                    | 44         |
| Orange              | GMS+SFO<br>(3:1)   | 95:5                     | 42         |
| Tangerine           | GMS                | 50:50                    | 30         |
| Tangerine           | GMS+GMO<br>(3:1)   | 50:50                    | 8          |
| Tangerine           | GMS+SFO<br>(3:1)   | 50:50                    | 13         |

Table 2: Encapsulation by PGSS

lipophilic The particles produced incorporation in juice formed a separate layer phase indicating separation. The incorporated with particles consisting of GMS+GMO were more stable as we observed only a slight phase separation however, significant improvement was observed for juice consisting of all three GMS+GMO, and GMS+SFO) lipophilic particles after re-incorporation with hydroxypropylmethylcellulose (HPMC) as a stabilizer particularly for the GMS+GMO system.

The total phenolic content of the juice after incorporation of particles and during accelerated storage for seven days did not vary significantly hence indicating there was no expulciosn of citrus peel extract into the juice.

# **Conclusions**

We successfully developed PMF rich extracts from citrus peels using supercritical fluid extraction a high pressure technology. We also demonstrated that this method under the conditions employed was highly selective for isolating PMFs. We also confirmed the antiproliferative activity of citrus peel extracts obtained in the frist 15 mintes of supercritical fluid extraction and showed that tangerine and orange peel had the highest effect which was correlated with their rich contents of nobiletin and sinensetin. Particles from gas saturated successfully solutions was explored producing lipophilic forms of citrus peel extracts using three lipid carrier systems (GMS, GMS+GMO and GMS+SFO), and successfully varied the carrier to extract ratio.

The lipophilic particles showed promising results when incorporated in aqueous based environment indicating that encapsulation of citrus peel extract into lipid carriers using PGSS could enhance the incorporation of PMFs as functional ingredients in food products or as nutraceuticals and possibly improve bioavailability.

In future, we suggest studies to optimize the carrier to extract ratio of the particles and determine the load with the most effective encapsulation efficiency particularly for the systems consisting of GMS+GMO and GMS+SFO. Also develop assays to mimic the ingestion and digestion of the lipopilic particles with a view of monitoring the release of PMFs and their bioavailability.

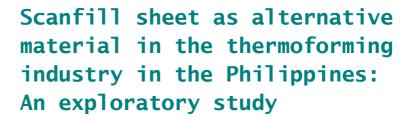
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#### Introduction

Rigid plastic packaging, the end-product of thermoforming, has become ubiquitous as it offers convenience and ease of transport in today's society. However, ambivalence to its use remain. On one hand, it offers a wide range of functions like food packaging applications while on the other, its durability has raised concerns about their end-of-life disposal (Philp, et.al., 2012). Companies and research agencies alike continue to intensify their efforts to formulate solutions to help minimize the impact of this packaging category.

# **Objectives**

The overall purpose of the study is to identify and describe the factors that could influence the adoption of an alternative raw material for thermoformed products used as food packaging in the Philippines

The following objectives were formulated to meet the overall purpose within the context of Philippine thermoforming industry, which are:

- Outline the different steps in manufacturing thermoformed products
- Investigate similarities and differences in quality and performance of cups using Scanfill material in comparison with current polypropylene (PP) material.
- Formulate recommendations for future studies.

# Methodology

A mixed method approach combining case study and action research was used in this study. A case study that was explorative in nature focused on the contemporary phenomenon within the context of the Philippine manufacturing industry for rigid

plastic packaging. A logic model was used to describe the inputs, activities, outputs, short-term-outcomes, intermediate-term outcomes and long-term outcomes of the study. An action research strategy was also applied to gather and analyze quantitative data that relates to the technical aspect within the thermoforming industry.

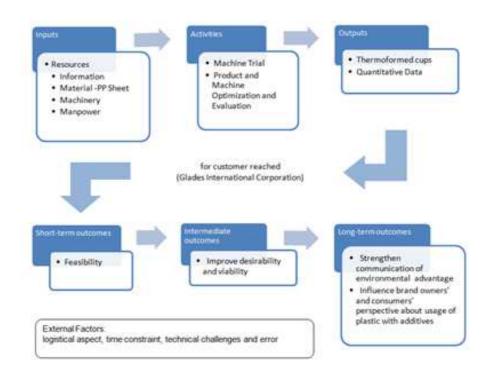


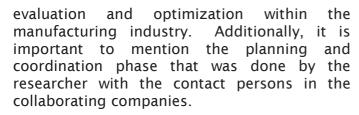
Figure 1. Elements of the study based on logic model

# **Results**

# **Logic Model Findings**

The elements of the study were embedded within the logic model. The *inputs* included the resources provided by the collaborating provided companies: Scanfill AB information about their raw materials, the innovation potential of their products and applications technologies, its in food packaging and technical expertise since the beginning of the study. Scanfill AB also the connection to company, Polykemi Compounds (Kunshan) Co. Ltd. The Polykemi Group's subsidiary in China made it possible to produce the Scanfill sheets in a more proximate location and also handle the logistical aspect of trasporting the sheets via sea freight to the Philippines. On the other Glades International Corporation provided the warehousing facility to receive the sheets, the thermoforming machine and mold for the trial as well as the manpower assistance to carry out the trial (QA and Process Technician). The management of the company also gave support by arranging with the planning department about the proposed schedule of the trial. The laboratory facilities and instruments were also utilized to gather quantitative data after the cup samples were collected. Suggestions and recommendations by the personnel who the researcher worked with during the trials were also noted.

The *activities* included the thermoforming machine trial, as well as product and machine



The *outputs* included the thermoforming cups and the quantitative data gathered. The data from the cups were compared and analyzed to that of the existing standards.

The *short-term outcome* showed the feasibility of using Scanfill sheet. It is possible to use the new material using the existing technology and machineries in the thermoforming company, although modifications in the machine and mold (i.e. optimizing parameter settings and sharpening the cutting plate) might be necessary.

The *intermediate outcomes*, on the other hand, showed that there are still measures or modifications that need to be done in order to improve the desirability and viability of the material being studied. The desirability can be improved by improving the quality of the cups (i.e. producing a smooth rim and weight requirements). The viability, on the other hand, deals with the business and financial aspect of the product, so it can be mutually beneficial to all the companies concerned. The pricing, demand and added value of the material should be further discussed. To study whether capital expenditure is worth allocating for the thermoforming company (i.e. predryer) can be considered. In the comparison of industry processes of the collaborating companies, it was mentioned that both are capable of extrusion operation. In the future, the supplier could explore the possibility of formulating a Scanfill granulate that would not require predrying so it can also be used in the extrusion operation as well.

The *long-term outcomes* could include the need for strengthening of the communication

of environmental advantages especially to manufacturing industries, brand owners and consumers. This, in turn, could influence brand owners and consumers to prefer the use of plastics with non-oil based additives. the Philippines undeveloped in the area of sustainability and recent events have driven legislative changes in the use of rigid plastic packaging even up to imposing its ban in the cities in the surrounding the capital, opportunities for innovative packaging solutions still prevail. This can also be seen as an educate opportunity and to alternative packaging materials.

The external factors such as logistical aspect, time constraint and technical challenges had to be managed in order to carry out the study successfully. The logistical aspect included the shipment of the materials from one country to another, so the knowledge and coordination of import and export personnel from both sides were essential. The time constraint was also part of the challenge of the study. A total of 5 hours was the time period allocated for the trial. The reason for this is that machines and molds for the sole purpose of pilot studies are not available, only commercialscale were. The same equipment are also used produce packaging products for customers who are expecting timely deliveries, so extension was not possible. Lastly, during the machine trial, challenges were faced such as having to start on a trial and error basis to configure the machine parameter settings, which eventually was solved within the given time frame. The researcher also regrettably committed an error by referring to the the wrong standard for the weight of cups during the trials, therefore instructing the process technician to adjust parameter settings based on the assumed target weight.

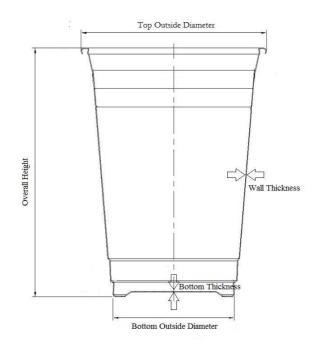


Figure 2. 16 oz cup product drawing and attributes

# Material Specifications, Machine Parameters and Cup Attributes

The quality of the Scanfill sheet was verified by checking the specifications. The sheet was opaque white and had glossy or matte texture on either side. The sheet had a thickness and width profile of 1.10~mm~x~580~mm. The measurements showed that the sheet was within  $\pm~5\%$  tolerance, which was between 1.045~mm to 1.155~mm range.

The machine parameters were recorded to compare if there was significant difference between the cups that used clear PP sheet (existing, reference group) and Scanfill sheet calcium carbonate). (white PP with Thermoforming machine heater temperatures and cycle time were tabulated. The results showed lower machine heater temperatures were required to process Scanfill sheet as compared to the existing clear PP sheet in the thermoforming company. An average difference of 36.88 °C for the upper heaters and 21.88 °C for the bottom heaters were observed. This could be an indication of lower electricity consumption and thus energy savings. On the other hand, there was no difference observed in the cycle time for the both clear PP sheet and white Scanfill sheet which was at 13 shots per minute.

Lastly, the attributes of the cups that used Scanfill sheet material were measured and analyzed. The resulting cups were opaque white, glossy on the outside and matte (almost paper-like) texture on the inside. There was one notable quality issue that was observed in the cups, which was the rough rim. The rough rim was caused by the dull cutting of the thermoforming mold during processing. The mold is currently allocated for PP clear, and the added stiffness of the Scanfill sheet made it somewhat difficult for the mold to compensate and achieve smooth rim for the cups.

The results showed that all the cups were above the weight range of  $9.7 \pm 0.3$  g. Unfortunately, there was a major source of error identified by the author only after the trial. The researcher referred to the product specifications of one of the products which had the same sheet and product profile except for the weight, as a consequence the process technician modified the machine parameters to meet the assumed weight.

The overall height for all cups were below the minimum tolerance of 130.70 mm. The wall thickness was within the acceptable range of 0.20 mm to 0.30 mm. The top outside diameter for all cups were above the maximum tolerance of 93.80 mm. This is due to the excess plastic material caused by the rough rim. The bottom outside diameter for all cavities was above the maximum tolerance of 59.00 mm. Finally, the bottom thickness of cups from cavities 2 and 3 were within the acceptable limits of 0.25 mm to 0.35 mm while cups from cavities 3, 4 and 5 were above the maximum limit of 0.35 mm. The rigidity of all the cups were also measured and was higher than the existing PP cups, which means a stiffer product. The suitability for food contact was also verified as Scanfill sheet, along with other material grades from Scanfill AB, is approved for direct contact with food (Scanfill, 2014). The material complies with EU Regulations 1935/2004, 2023/26 and 10/2011, as evidenced by their Certificate of Compliance (CoC) for food contact materials. Material Safety Data Sheet (MSDS) Technical Data Sheets (TDS) are also provided.



The quality and performance of Scanfill sheet were evaluated in relation to the standards and capabilities of the Philippine thermoforming industry. The sheet dimensions were measured and the texture requirements were found to be at par with the standards.

The different steps in manufacturing thermoformed products were outlined and described. Similarities and differences in quality and performance of machine parameters and cups were investigated, using the control/reference group (existing PP clear sheet) and the test group (Scanfill white sheet).

The thermoforming machine's parameter setting showed that using Scanfill sheet required a lower temperature for the heating plates as compared with existing PP clear sheet. This could translate to lower electricity consumption and thus energy savings. However, the machine did not exhibit any difference in cycle time as compared when the PP clear sheet is ran, and both were at around 13 cycles or shots per minute.

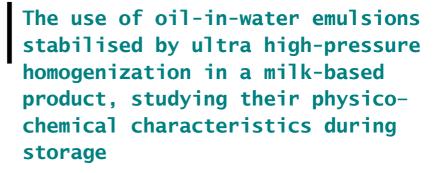
The cups produced using Scanfill sheet were acceptable in terms of aesthetic value with regards to gloss and matte texture. Cup attributes such as wall thickness and rigidity were at par with the standards. Rigidity of the cups was also found to be higher than the existing PP clear, which indicates a better and stiffer cup. The material used also complies with regulatory requirements for food safety. However, other cup attributes such as smooth rim, weight, height, top outside diameter and bottom outside diameter were not within set specifications. One of the factors might have contributed to this result such as non-optimal machine parameter setting. Also, since the thermoforming mold was originally intended for PP clear, the cutting plate of the tooling may not be sharp enough to cut the rim smoothly.

In conclusion, this study has proven the suitability and feasibility of the use of Scanfill sheet in the thermoforming industry in the Philippines. However, other issues such as desirability of the cups as well as viability can be improved and further studied.

As for suggestions for further study, it can be an objective and future work to optimize the performance thermoforming machine specifically in terms of cycle time. A machine run time that would be longer than what was carried out in this study (i.e. > 5 hours) can be quantification considered. The of environmental impact of Scanfill sheet (i.e. savings in electricity, fresh water use) in the Philippine setting can serve as a better communication tool to convey the advantages of the material. Another interesting topic in the future could be an in-depth study of consumer behavior and acceptance of the cups produced using Scanfill sheet and compare them with existing rigid food packaging in the market.

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#### Introduction

The interest in functional food products is growing thanks to their role in the maintenance of health and well-being and in the prevention of diseases. Recognition of the potential benefits linked to omega-3 fatty acids increased the demand for the supplementation of foods with these lipids and recently fortification with omega-3 fatty acid is one of the fastest growing trends in the food industry. Fortification of food products with fish oil is a good way to increase the intake of omega-3 without changing the eating habits (1).

Omega-3 fatty acids may easily undergo oxidative damage, as they are sensitive to heat, oxygen, light and metal ions. The oxidation is a main reason of deterioration in food products that can diminish nutritional value and enhance formation of toxic compounds, off-flavours and off-odours, thus altering flavour, aroma, texture, shelf life, and colour of food. Therefore, the incorporation of omega-3 fatty acids into food is a difficult task regarding formulation and processing of food products (2).

The oxidative damage of omega-3 fatty can be diminished encapsulation, which can reduce the contact with environment that may enhance the deterioration process. For better protection, can be combined with substances, like other oils, polysaccharides, and proteins (3). A good method to incorporate encapsulated omega-3 fatty acids into foods is in the form of lipid dispersions. The oil encapsulation efficiency of emulsion can be optimized by controlling its droplet size, as emulsions with small droplet size distribution showed higher oil

encapsulation efficiencies (4). Microstructure of emulsion is mostly determined by droplet size and droplet size distribution. The size of droplet has a great impact on many emulsion physical properties, such as microbiological stability, rheological and optical characteristics, bioavailability and taste. Often the emulsifying process is aimed at fabrication of as fine droplets as possible in order to ensure stability of the emulsion (5). homogenization Ultra-high-pressure technology that enables production of fine and stable emulsions (6).

The aim of the present study was to investigate the impact of the storage time on the physico-chemical properties of milk-based product with the emulsion enriched with omega-3, fabricated using ultra-high-pressure treatment, in comparison to the product with emulsion produced by conventional homogenization.

# **Methods**

Coarse emulsion (pre-emulsion) was fabricated by homogenizing the mix of oils and whey protein dispersion. The emulsion consisted of whey protein isolate (4%) and mix of oils (containing 20% of oil, 0.6 % of omega-3 and the proportion of sunflower to olive oil 3:1). To fine emulsions conventional homogenization and ultra-high-pressure homogenization were applied. The emulsion treated by conventional homogenization (CH) produced by processing the coarse emulsion at 15 MPa. The ultra-high-pressure treated (UHPH) emulsion was fabricated by subjecting the coarse emulsion to 200 MPa. To produce two milk-based products, skim milk was mixed with CH emulsion and UHPH emulsion to obtain fat content of 2% in the final product. To both products indirect UHT treatment was applied and they aseptically packed in 200 ml slim containers. Products were held in 21 °C during 10 weeks of storage period.

After the production particle size of both products was analysed, as well as fat content, total protein content and total solids content. During the storage period physical stability was examined visually and by the analysis of backscattering (BS) profile of milk-based

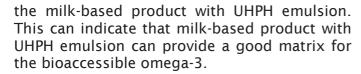
product sample. For the quantification of the bioavailable fraction of fatty acids, milk samples were subjected to in vitro digestion. To assess the rheological behaviour the consistency coefficient (K) and flow behaviour index (n) were calculated. The colour and pH of samples during storage also measured. The oxidative stability was determined measuring primary oxidation products hydroperoxides (PV) and secondary oxidation thiobarbituric acid-reactive products substances (TBARS).

#### **Results and discussion**

Milk-based product with CH emulsion showed a trimodal particle distribution characteristic, while product with UHPH emulsion showed narrow monomodal distribiution. The surface moment mean D [3,2] as well as volume moment mean D [4,3] were higher for the product with CH emulsion, milk-based compared the product with UHPH emulsion. It was previously shown that in milk fortified omega-3 both homogenization temperature and pressure influenced the size of the fat droplets. The largest droplet size was detected in the emulsion prepared at low pressure, whereas temperature and smallest droplet size was obtained in the emulsion prepared at higher temperature and pressure (7).

There was no difference in fat content between two products, but dry mass content and protein content was higher in the milk-based product with CH emulsion. This difference can be due to the homogenization conditions that affected those parameters.

The ability to control lipid digestibility within the human gastrointestinal tract is a very important aspect of the development of functional foods. The main barrier for the bioaccessibility of emulsions as lipid-based delivery systems is the stability and the absorption efficiency within the Therefore, gastrointestinal tract. the understanding and controlling the digestibility emulsified lipids within the gastrointestinal tract is of a great significance (8). The in vitro digestion of milk-based product sample showed, that the percentage of free fatty acids released was higher from



Milk-based products with CH and UHPH emulsion exhibited a Newtonian behaviour ( $n\approx1$ ) during the storage, with apparent viscosity between 2 -3 mPa  $\times$  s. There was no significant difference between the values of K and n of both products during the 10 weeks period.

During the visual analysis of products it could be observed, that there was formation of cream layer on the top of the milk-based product with CH emulsion. The visible cream layer was detected on day 23 of the storage and creaming was increasing during 71-days storage period. However, no visible creaming was detected in the milk-based product with UHPH emulsion during storage.

The alteration in backscattering profile (BS), measured as a function of storage time, gives a good indication of creaming, flocculation and other destabilisation processes as BS varies with the change in particle size (9). The decrease in the BS in the middle of the sample occurred at a higher rate in the product with CH emulsion, which can imply that flocculation was progressing faster in the product with CH emulsion. The BS values on the top pf the sample were higher for the product with CH emulsion, what demonstrates that creaming was slower in the product with UHPH emulsion. The decrease in the BS on the bottom was higher for the product with CH emulsion. It suggests an increase in the creaming, as particles migrate from the bottom to the top. These results show that the product with UHPH emulsion was more physically stable that the product with CH emulsion, as creaming occurred at slower pace.

The instability mechanisms depended on the characteristics of the emulsion used. In conventional emulsion the movement of particles is dominated by gravity, because of the relatively large size of particles. Nanoemulsions usually are more stable to droplet aggregation than conventional emulsions, because of the impact of the small particle size on colloidal interactions (10).

For both products lightness (L\*) decreased and colour intensity (a\* and b\*) progressively increased during storage. The development of parameters L and b\* was found to faster in product with CH emulsiosn. An increase in the magnitude of the positive b\*-value indicative of an increment of yellowness, the development of this colour may be the consequence of oxidizing lipids in the presence of protein during storage (11). Lower b\* value of the milk-based product with UHPH emulsion and its slower development during storage, can indicate delayed oil oxidation and therefore improved oxidative stability of fish oil in this product. The change of colour was developing faster in the milk-based product with CH emulsion. The overall change in colour during storage was relatively small in all of the samples ( $\Delta E < 10$ ).

The pH of both milk-based products didn't change significantly during storage, there was no difference in pH values between both products.

Initial stages of lipid oxidation in milk-based products were monitored by analysing lipid peroxides. The initial concentration of lipid hydroperoxides was higher in the product with CH emulsion. The content of lipid hydroperoxides increased during the storage period in both products, slightly faster in the product with CH emulsion, but the difference was not significant between both products. However, the PV differed among the products on each day. There was an irregular behaviour with increasing and decreasing periods, as peroxides are unstable primary oxidation products and they transform quickly into secondary oxidation products. As a result of decomposition reactions of primary oxidation products many secondary oxidation products formed. Thiobarbituric acid are reactive substances (TBARS) are quantified in order to analyse secondary lipid oxidation products. The difference of the TBARS values between both products during storage was significant, with higher values recorded for the product with CH emulsion.

The homogenization conditions affect the droplet size and thereby the total interfacial

in emulsions. Therefore a general expectation is that a large interfacial area can lead to increased oxidation, due to an increased contact area between the oil and possible pro-oxidants present in the water phase of the emulsion (7). Some studies on emulsions confirm this theory, because an increase in total interfacial area has been shown to accelerate lipid oxidation (12). But other studies showed the opposite. It can be seen, that the processing conditions can influence the oxidation rate in the product. In the milk-based product with CH emulsion, where the homogenization conditions were less harsh, with lower temperature and pressure, the measured TBARS value was higher in comparison to milk-based product with UHPH emulsion. The reason for the lower oxidation rate may be the smaller particle size of the product with UHPH emulsion and the properties of the interface that were created the ultra-high-pressure homogenization, as the higher temperature and pressure could have ensured better stability of the product.

# **Conclusions**

The results of this study show, that there was a difference in the physico-chemical stability during storage time between milk-based products with omega-3 enriched emulsion prepared by conventional and ultra-highpressure homogenization. Milk-based product with UHPH emulsion was found to be less susceptible to physical instability, as creaming rate was much slower than in the product with CH emulsion. The change of colour also developed slower in the product with UHPH emulsion, which can be an indication of occurring oxidative deterioration. The results of TBARS showed that the rate of oxidation was higher in the milk-based product with CH emulsion. The bioaccesibility of lipids was higher from the product with UHPH emulsion. The difference in those parameters may be due to the particle size that was much smaller in the product with UHPH emulsion, and because of the processing conditions during fabrication of emulsions. This suggests that the milkbased product with emulsion prepared by ultra-high-pressure homogenization provide a more stable food matrix for omega-3 fatty acids, than the product with emulsion prepared by conventional homogenization

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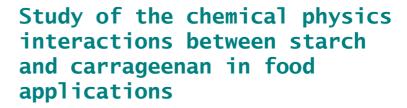


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Dairy products as various desserts are popular among consumers. These products are popular as ready-made cold or frozen items in supermarkets. Shelf space provided for these products is a good measure of how important they can be in companies' profits. There is a similar situation in restaurants, where desserts command far higher prices than the other items in the menu.

In production, they have a large range of texture and mouth feel. The textures are categorized as custard, soft gel, vla and light custard (Zobel & Stephen, 1995). Structural components of dairy dessert are made 84-90% milk, 8-12% sugar, 2-4% starch and 0.1-0.3% carrageenan. Their small amount against other components only shows their importance in the final texture and body of the dairy desserts.

Their ratio of combination seems to be very influential on the rheological and textural properties (Huc al.. et 2014). Understanding the interaction between starch and carrageenan has been subject to many studies. These findings highlight a particular behaviour and interaction mechanism between starch carrageenan. Starch characteristics such as gelatinization temperature. sizes granules obtained after pasting or amount of leached polymers during pasting were shown to be possibly impacted by the presence of carrageenan (Kim & BeMiller, Matignon, Barey, Desprairies, Mauduit, & Sieffermann, 2014; Tecante, A Doublier, 1999)

The objective of this study was a better understanding of starch / carrageenan interactions and further studies the nature of the mechanism by using Confocal Laser Scanning Microscopy (CLSM).



Starch pasting behaviour when occurs temperature reach a certain degree known as gelatinization temperature. The granules lose their native crystalline order and swell irreversibly. By increasing temperature and time, the molecular components (mostly amylose) seep out of swollen granules and eventually disrupt the granule structure. At the end of the thermo-mechanical treatment, a starch paste/gel is obtained. The amount and nature of leached molecular components and of the level starch granule disruption determine the microstructure and the rheological properties of a starch paste/gel (Alloncle, Lefebvre, Llamas, & Doublier, 1989; Atkin, Abeysekera, & Robards, 1998).

Carrageenans are a family of linear polymeric sulphated galac-tans. Kappa, iota and lambda carrageenan are composed of one, two and three sulphated group per disaccharid, respectively. They go under a coil to helix transition depending on both the temperature and the ionic environment. They are hydrated at the temperature around 70 °C changing into the random coil in the solution. By cooling down the temperature to 20-40 °C carrageenan chains transform into helical forms, where they form a reversible gels (Michon C, Cuvelier G, Launay B, & Parker A, 1996)

In previous studies, (Matignon, Barey, et al., 2014), pointed out that although carrageenan is adsorbed by raw and swollen granules, but there is no penetration of carrageenan inside starch granules. And when authors observed carrageenan chains inside starch granules, they speculated that carrageenan is trapped inside starch granules in folded form. Also, concentration of carrageenan remaining in continuous phase depends on affinity of carrageenan chains with starch granules surface. Further, they showed that presences of carrageenan in continuous phase of a starch paste/gel have an impact on the swollen starch granules' sizes and surface states. Starch granule diameter increased when carrageenan chains were presented in solution, especially low-molecular-weight carrageenan. mentioned behavior of earlier, this carrageenan did not differ in raw or swollen starch. Adsorption level depends on charge of carrageenan (lower the charge, higher adsorption percentage) but also on the molecular weight (smaller weight, higher adsorption percentage).

These researchers speculated that the interactions between starch and carrageenan could be due to carrageenan/endogenous starch proteins (Matignon, Barey, et al., 2014).

#### **Materials**

Two fixed percentages of these components, typical of dairy desserts, were chosen: 0.15 wt% for carrageenan and 2 wt% for starch. Two carrageenan samples iota (Ci), kappa (Ck) and two maize starches, waxy Adipate and waxy hydroxypropyl (HP) were used. The mixtures were made by pasting starch in aqueous solutions containing, or not, carrageenan.

#### **Methods**

In this project, Confocal Laser Scanning Microscope (CLSM) TCS SP2 AOBS (Leica, Germany equipped with: UV - VIS sources / Motorized focal plan with a 65 objective was used.

Mixture preparations followed the procedures outlined by the above researchers (Matignon, Barey, et al., 2014; Matignon, Moulin, et al., 2014) (Fig. 1).

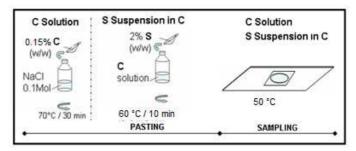


Figure 1 : Mixture preparation of carrageenan and starch for CLSM observations, C solution: Carrageenan solution and S suspension: Starch suspension

#### **Staining**

Setting up the multiple staining, carrageenan was labeled with classic staining method called RITC labeling, (Rhodamine B IsoThyoCianate), Starch granules labeled with APTS (8-Amino-1,3,6-PyreneTrisulfonic Acid) and starch endogenous proteins labeled with

CBQCA (3-(4- CarboxyBenzoyl) Quinoline -2-CarboxAldehyde). This was done under supervision of lab technicians in AgroPariTech.

Because of low PH that may cause possible disruption of starch granules, an improvement of starch labeling with APTS was done where the PH was adjusted to 7. This proved to save the starch granules intact.

Another factor of uncertainty was due to the excitations wavelength of components under the laser ray by CBQCA Labels, therefore the excitation wavelength was separated to limit their superimposed emission spectra (Matignon, Moulin, et al., 2014).

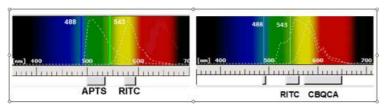


Figure 2: Excitation and emission wavelength of different fluorescent dyes. Grey rectangles give a wavelength domain in which is performed a measurement of emission signal.

# **Results and Discussions**

# Method confirmation, (starch staining with CBQCA - raw and swelled)

The suspension was created in a solution of 0.1 M NaCl to create an ionized environment as dairy products. The concentration of suspension was 2% of starch. Subsequently, suspension was measured under CLSM. Starch granules and their endogenous starch proteins

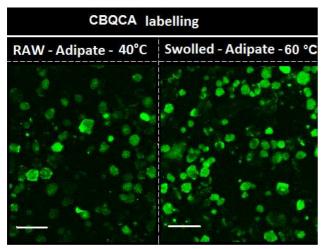
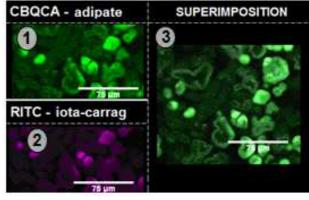


Figure 3 : CLSM observation of Adipate in NaCl solution, labeled with CBQCA staining

were observed in correct wavelength (green fluoresce) as in figure 3. The entire starch granules were stained. The labels showed that endogenous proteins were not homogeneously spread, causing high and low intensity in the labeling. All starch endogenous proteins were stained and visible in the picture. However, heat treatment was not completed and granules were not adequately swollen.

# Adipate starch / and lota carrageenan microstructure observation (Double labeling)

Adipate was labelled with CBQCA and swelled in the Ci solution. The created suspension containing starch and carrageenan were prepared and brought the following results. In this condition, it was observed that carrageenan interact with starch after swelling and carrageenan seen all around starch granules. The labeled carrageenan seemed to be adsorbed onto the surface of swollen starch granules and their chains are localized where endogenous starch proteins are (Fig.4).



- Staining of the endogenous proteinpasted of adipate starch granules,
- Staining by absorbed labeled Ci
- 3. Colocalization (white) of two staining.

Figure 4: Adipate starch labeled with CBQCA staining pasted in iota-carrageenan solution and the colocalization of two staining.

In this case, the iota solution was clear before adding starch. Pictures show that carrgeenan were adsorbed on the starch granules as expected and the result is shown in Fig. 4.

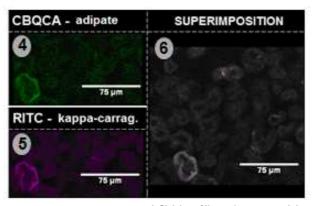
Carrageenan are located mainly where the endogenious protein are. Some of the granular

are not swollen completely due to low tempreture at 60 °C.

Adipate starch / and kappa carrageenan microstructure observation (Double labeling)

Adipate was labelled with CBQCA and swelled in the Ck solution. In this case, the kappa solution was cloudy and unclear to start with. This might have effected the clarity of pictures (figure 5). Lacking clarity of images can be partly expalined by the variation in laser intensity of the microscope or, unsuitable excitation wavelength for the component.

However, the pictures show bigger granuale of adipate in compare with iota carregeenan. Everything being the same, Kappa Carrageenan behaved similarly as iota carrgeenan in adsorbing around the starch granules.



- Staining of the endogenous protein pasted adipate starch granules,
- Staining by absorbed labeled Ck,
- 6. Colocalization (white) of two staining.

Figure 5 : Adipate starch labelled with CBQCA staining pasted in kappa-carrageenan solution and the colocalization of two staining.

# Conclusion and perspective

In these two variations, the carrageenan/starch mixed suspension, a shell of carrageenan was observed on the starch granules, which indicates an adsorption of carrageenans. This result could explain the 'protective role' of carrageenan on starch granules (Tischer, Noseda, de Freitas, Sierakowski, & Duarte, 2006; Tye, 1988).

CLSM do not allow a quantitative comparison, however, it can confirm the interaction between Adipate starch and the two types of carrageenan. Starch swells differently in the presence of iota-carrageenan compared to kappa-carrageenan resulting in bigger granules in presence of the later one.

The bigger granules results can be an important factor in choosing either of these carrageenans in production of different products and their desired texture and consistency. The different carrageenan also can help to create different methods of production for similar products.

Further study on the effect of thermomechanical treatment for these two carrageenan types is recommended.

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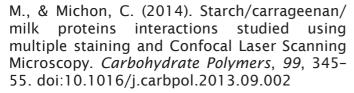
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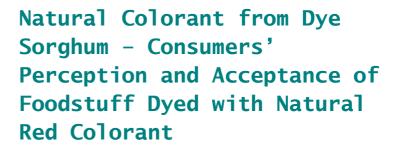
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Consumers have shown interest everything that is natural, including food colorants. Colouring food has been part of human's diet for a long time, finding natural colorants in fruits, vegetables, spices and herbs. With the development of chemically synthesized food colorants, the concerns about safety and their usage have increased, despite being easy to produce, stable, intensive and cheap. However, finding natural dyes with the characteristics as the artificial ones seems to be a difficult task, since colorants derived from nature are easily altered by physical acts such as light, temperature, and oxygen.

Different studies within this field suggest to investigate natural sources that contain deoxyanthocyanins as they are more stable than normal anthocyanins. A plant where deoxyanthocyanidins have been found in is Dye Sorghum, a variety of the sorghum crop which is originated in drier parts in Africa. Dye sorghum [Sorghum bicolor (L.) Moench] is grown for the red pigments that are concentrated in the leaf sheaths. The dye sorahum piaments contain deoxyanthocyanidins (apigeninidin luteolinidin) in a high and qualitative amount that inhabit health-promoting properties, as these are higher than other fruits and vegetables, in particular in antioxidant activity (Jacob et al. 2013) and cytotoxic on human cancer cells (Kayodé et al. 2012).

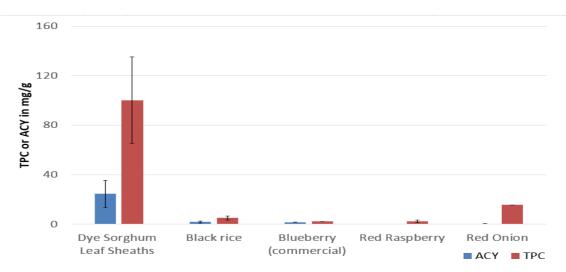


Figure 1. Total Phenolic Compound and Anthocyanin in Dye Sorghum compared to other fruits and vegetables (adapted from Kayodé et al., 2011)

Dye Sorghum is currently used in Africa for goat-skin leather, mats, textiles, strips of palm leaves and grasses used in basketry, but also to colour cheese and lick stones for cattle (Kayodé et al. 2011), and is yet to be studied as a food colorant in different food products.

# **Aims & Objective**

This study concentrates on the possibility of Dye Sorghum as a food colorant in Europe and its acceptance by consumers. After introducing this colorant into a food product, consumers are asked to participate in a consumer test evaluating their acceptance and perception of a product containing Dye Sorghum as food colorant. Moreover, this study aims to examine to what extent consumers can be influenced by information given to them.

#### **Materials and Methods**

#### **Product Elaboration**

Allura Red AC (E 129) powder (obtained from Sigma-Aldrich, Vienna, Austria) was diluted in a ratio of 100 mg/kg. 12.5 g of Dye Sorghum leaf sheaths were extracted during magnetic agitation at laboratory level in 1000 mL of boiled potable water for 20 minutes. Residues were removed and the extract was filtered and sterilised using a 0.22 micron pore sized syringe filter unit (pore size 0.22 µm, diameter 0.33 mm, sterile, disposable; obtained from Sigma-Aldrich (Vienna, Austria)).

# **Colour Measurements**

The colour of the liquid colorant was determined determined using a Hunter laboratory colorimeter (ColorFlex EZ, Hunter Associates Laboratory Inc., Reston, VA) with an illuminant of D65 and standard observer of 10°. Colour measures are given based in the CIE L\* a\* b\* colour space.

#### **Consumer Tests**

Consumer tests were carried out in two different cities; Wageningen in the Netherlands and Osnabrueck in Germany. Wageningen and Osnabrueck were chosen as the target cities for the carrying out of the consumer tests due to their similarities in education and demographic aspects. Consumer tests were carried out in two different cities.

For the examination of the influence of information, two different questionnaires were prepared. The socio-demographic questionnaire consisted of questions about nationality, age, occupation, knowledge of food industry and food colorants, and an interest in sustainability. Data was then analysed in order to compare the two countries, determine the effect of information, as well as investigate the relevance and connection of attitudes and knowledge.

| Group 1 ("Long Questionnaire")                    |                                 | Group 2 ("Short Questionnaire")                           |                                 |  |
|---|---------------------------------|---|---------------------------------|--|
| Information Test                                  |                                 | Information   | Test                            |  |
| Introduction - no information about test products | Likeability test                | Introduction and detailed information about test products | Likeability test                |  |
| Little information about test products            | Paired preference<br>test       |   | Socio-demographic questionnaire |  |
| Detailed information about test products          | Paired preference<br>test       |   |                                 |  |
| Information about price (buying price)            | Willingness (yes/no)            |   |                                 |  |
|   | Socio-demographic questionnaire |   |                                 |  |

Table 1. Overview of questionnaires and information included in consumer test

# **Results and Discussion**

# **Product Colour**

Colour measurements of the pure Dye Sorghum extracted resulted in a too high colour difference between the extract and the Allura Red AC dilution. A 1:4 dilution of extract in water resulted in the following colour parameters.

|                                  | Colour Parameters |      |      |      |      |      |      |
|----------------------------------|-------------------|------|------|------|------|------|------|
| Product                          | L*                | SD   | a*   | SD   | b*   | SD   | ΔΕ   |
|                                  | 23.               | 0.30 | 47.2 | 0.02 | 39.1 | 0.18 |      |
| Allura Red AC (Dilution 0.01 %)  | 55                | 3    | 1    | 2    | 8    | 7    |      |
| ,                                | 7.6               | 0.07 | 34.3 | 0.02 | 12.9 | 0.11 | 33.2 |
| Dye Sorghum Extract              | 4                 | 1    | 5    | 1    | 3    | 3    | 8    |
| Dye Sorghum Extract (Dilution 25 | 24.               | 0.17 | 42.7 | 0.12 | 38.0 | 0.24 |      |
| %)                               | 27                | 0    | 4    | 0    | 0    | 7    | 4.68 |

Table 2. Colour measurements and colour difference between artificial and natural colorant

# **Consumer Test**

Final results of questionnaires are displayed in the table below. In the short questionnaire where detailed information about the products were given beforehand the likeability test, 55 % of the Dutch and 53 % of the German respondents preferred the natural colorant. When no information was given before, 54 % of the Dutch and 32 % of the German

respondents preferred the natural colorant; both numbers were increased by giving information, resulting in 93 % of the Dutch and 76 % of the German respondents to prefer the product containing Dye Sorghum after provided with detailed information.

|          |        |  | No          | General     | Detailed    | Willingness |
|----------|--------|--|-------------|-------------|-------------|-------------|
|          |        | Product Attributes   | information | information | information | to pay more |
| Long Q.  | Dutch  | Brightness and<br>Transparency were<br>significantly better<br>rated for artificial. | 54%         | 87%         | 93%         | 89%         |
|          | German | All artificial product attributes were significantly rated higher.                   | 32%         | 69%         | 76%         | 69%         |
| Short Q. | Dutch  | Brightness and<br>Transparency were<br>significantly better<br>rated for artificial. |             |             | 55%         | 52%         |
| short Q. | German | All artificial product attributes were significantly rated higher.                   |             |             | 53%         | 69%         |

Table 3. Results of consumer test; acceptance of natural colorant

The results of the data analysis reveal that there are connections between the answers consumers gave and their attributes and knowledge. According to the "Elaboration Likelihood Model" (Solomon et al. 2006), consumers will either use the peripheral or the central route when they process information. permanent attitude change and acceptance of the product, consumers follow the "central" route to persuasion. In case of this study, 54 % of the Dutch and 34 % of the respondents interested German are preferred the natural sustainability and colorant and have a high involvement with the product. All respondents have a high ability of process the information given (Attention). The first message given including

information, increased the acceptance of product attributes to 90 and 72 % respectively, and final detailed information persuaded 97.5 and 75 % of Dutch and German consumers. The message communicated informed consumers about the origins of the food colorants and increased the belief sustainability and consequence of purchasing the natural product, as rural agriculture in Africa will be supported. In total, after detailed information was provided, 70 % of the German and 92 % of the Dutch respondents would be willing to pay more for the natural product.

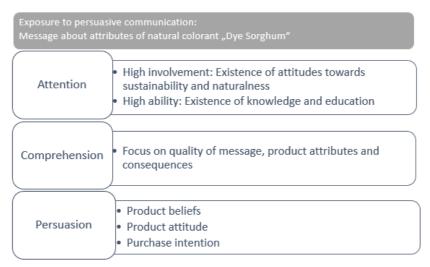


Figure 2. Steps taken in the "central" route in the Elaboration Likelihood Model" a simplified scheme (information based on Solomon et al., 2006)



The high involvement of consumers in a product dyed naturally with Dye Sorghum as well as the general preference for fair prices to farmers and the preservation of biodiversity gives a great opportunity to introduce this colorant on the European market. The artificially dyed product containing Allura Red AC was ranked higher in attributes (brightness, transparency, redness, overall colour) when no information about the products was given; this colorant is known and accepted. When information was given to consumers, step-bystep, the involvement was deepened and consumers were open to accept the natural colorant from Dye Sorghum. However, when detailed information was given before product the likeability answering test, attributes from the artificial colorant were rated higher. Therefore, a communication strategy has to be developed in which consumers will be informed with the details and differences of food colorants to ensure that consumers will have a temporary attitude change and acceptance of the natural colorant Dye Sorghum.

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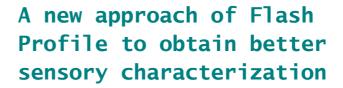
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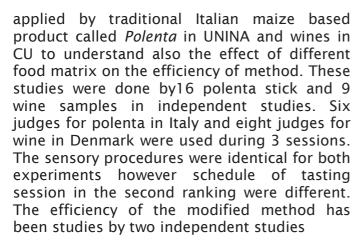


In order to increase the development of individual vocabularies while shorten the easiness of sample ranking the increase the interpretability of the results. modifications have been applied to FP. Napping was applied in the first seesion of Flash Profile and restriction in generated attribute number was brought to new method. New version was applied to two independent studules done by *Polenta* sticks and white wine. Finally a consensus was found for the most of the evaluated attributes in the polenta study and high concentration of flavors were discriminated from each other In the wine study, however low concentration were not significantly discriminated.

# Introduction

The Flash Profile (Siefferman, 2000) is one of the new methods for acquiring rapid sensory evaluation of products. It is descriptive sensory analysis method, which gives freedom to individual to create on his vocabulary to describe sensory differences among products. Thus the method needs less time for subjects to reach a consensus vocabulary and training on the use of intensity scales. However, lack of concensus between judges and the high number of generated attributes can blur the FP results and make difficult to interpret. Therefore a new approach of Flash Profile was brought to improve semantic interpretation of the method in the characterization of food products.

By means of this project, the possibility of use of improved method was explored by two different kinds of studies. This was done to test modified version with more than one kind of samples. One of the studies was done in the University of Naples (UNINA) and the other was in University of Copenhagen (CU). The method has been



#### **Aims**

The new approach was brough to Flash Profile to improve its semantic interpretation. There was one arrangement in the first session and one in the intersession. Firstly, Napping was integrated to Flash Profile in the first session to help judges to focus on differences among products. The consideration was that judges created more discriminative attributes as they moved from Napping to Flash Profile

By means of restriction in attributes number, the burden on analyser to interpret dicriminative attributes aimed to decrease. Two studies were done to asses the effect of a modification on the interpretation of results.

#### Materials and methods

16 polenta sticks prepared by different formulation, breading, and storage type and cooking method have been prepared for 6 judges in polenta study in the University of Naples. In wine study, 6 wine samples with 3 different flavor compounds in two different concentrations were served to 8 judges. Also pure wine samples and 2 replications were also provided to see judge consistency and differentiation ability.

Judges were practiced subsequent three sessions. Firstly they performed Napping by placing samples on the map according to similarities and differences between samples. Similar products were placed closer while they were further as the differences increase. During napping they also practiced ultra-flash profiling by commenting on product characterization. After this session, a global list of attributes from all judges generated in

the ultra-flash profile was prepared. In the second session, this global list was distributed to judges to generate their own final list. At the end of this session they have decided on most discriminative attributes according to their criteria. In the third session, samples were served simultaneously and judges ranked the samples according to their final attribute list.

Data were analyzed by GPA by means of XLSTAT. Diversity of generated attributes and their relations to sample characterization have been discussed through report and consensus maps for both studies were presented.

#### **Results and discussion**

In Flash Profile, each judge generated 4-10 attributes for a total of different attributes were 30 Judge used relatively more attributes in Napping. The range of the attributes numbers in Napping was from 5 to 13 and the number of different attributes used in napping was 31 for Polenta study. Most frequently used attributes in Napping were external crunchiness, oiliness (similar to greasiness), creaminess, softness, yellow color, graininess. In Flash Profile, crunchiness and greasiness were the most frequent attributes and all judges have agreement on discriminating importance of these attributes. Creaminess and fried odor have the second position in regarding to frequency of use. Creaminess was used 5 times and fried odor was selected by 4 judges out of 6. Thus, judges were consistent mainly about discriminant attributes in Napping and Flash Profile. The difference was that some discriminative attributes in Napping were eliminated by judges in FP which can help to obtain more clear results. In wine study, same phenomena appeared. Eight judges generated 5 to 10 attributes number of different attributes were 34. Judges were also consistent about most discriminative attributes and less discriminative ones were eliminated. The number of attributes was relatively high in wine due to complexity of samples.

From the GPA performed on the average data of Polenta, it was observed that first two factor were accounting for 60%, 14% and total

74 % of the total variation among the samples. Judges were able to discriminate between oven cooked samples and fried samples because all of the oven cooking style were on the right side and fried products located on the left side. Also, judges were able to discriminate between breading type in oven cooked samples. Breading type I was located on the first guarter where breading type II stayed on the fourth quarter. There was not a clear separation between fresh prepared samples from frozen samples in oven cooking style. That showed judge cannot discriminate between fresh and frozen samples in oven cooking style. In fried samples, judges were able to separate samples according to storage condition and breading type.

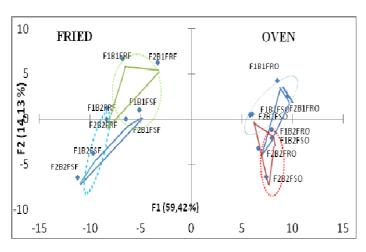


Figure 3.6. Consensus configuration of samples from GPA

Flash profile (Figure 3.6) as well as Napping (Figure 3.15) could give a cluster according to cooking type. Flash Profile also showed the discrimination according to breading type and storage conditions. However Napping do not provide discrimination in respect to breading types and storage conditions.

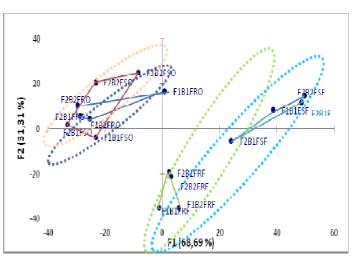


Figure 3.15 Configuration of samples by judge in Napping

From the GPA performed on the data from the first evaluation session, it appeared that there were 2 factors accounting for 58 % of the total variation between the products from the whole set of samples. Factor 1 and 2 accounted for 35%, 23% of the total variation respectively. The GPA plot of F1 versus F2 showed that some identical attributes had a similar meaning for the different judges. According to Figure 1, all of the strong samples were separated well from concentration. Among strong samples, benzaldehvda located on the second quadrant, twophenylethanol were opposite side of benzaldeyhda as being at the first quadrant and lastly isoamyl strong was in the third quadrant. This figure proved that judges were able to discriminate among all strong samples. Regarding to concentration solutions, only isoamyl was separated from other samples, judges could not distinguish between benzaldehyad and two phenyl ethanol in low concentrations

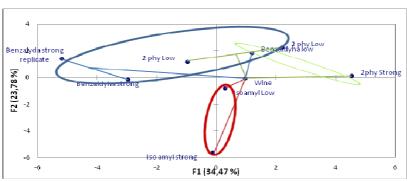


Figure 4.6: Configuration of consensus by GPA on average data



Napping data was compared with Flash Profile. It was seen that Flash Profile data were significantly better than Napping. In both of the studies, samples were better discriminated by Flash Profile.

Also polenta samples gave more interpretable result for analyzer. The reason can be summarized that the matrix of the samples has a significant effect on method performance. Also the level of differences and similarities among samples is an important consideration in Flash Profile

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# Satiating effect of a new food ingredient

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Weight gain, obesity and other dietary problems are a public health issue and a growing threat to health in countries all over the world; both conditions represent the fifth leading risk for global deaths (WHO, 2013).

Obesity is a chronic disease prevalent in both developed and developing countries, affecting children and adults as well. Being overweight is a serious risk factor for many diseases such as Type 2 diabetes, coronary heart diseases, hypertension and certain forms of cancer (Seidell, 2006; Visscher & Seidell, 2001). Obesity has additional negative effects, such as psychological sufferina because of potential stigmatization and discrimination (Puhl & Heuer, 2009).

In order to attenuate the increasing number of cases in overweight and obesity, considerable advances have been made. Nevertheless, obesity prevalence continues to increase sharply, and the challenge has never been greater; much of the scientific evidence on the regulation of appetite comes from animal studies and the specific mechanisms underlying food intake and biomarkers of action in humans are incompletely understood.

Most work on satiety has been in the nutrition and sensory sciences and this gives numerous opportunities for new satiety enhanced foods, attempts to reduce food intake at any particular eating occasion (satiation) and across eating occasions (satiety) have taken a number of different routes. for example changing food composition to develop stronger physiological satiation/satiety signals, building on smart external stimuli at the moment of purchase/consumption improving the palatability of satiety enhanced foods.

Positioning of satiety enhancing food products is a challenge and the EU regulation (EC) N° 1924/2006 on nutrition and health claims requires convincing scientific evidence for any claim. Scientifically, the key question is how to select most promising ingredients and to demonstrate the required evidence for their effect (Blundell, 2010). Several satiety enhancing foods may be successful in the short term, but little evidence currently long term effectiveness. supports their Therefore, longer term studies that examine satiety process both in-vivo and in-vitro are necessary together with more attention for biomarkers to identify and measure the working mechanisms of new satiety enhancing foods and ingredients.

In the context of the new food product development it has been speculated that bitter tastants trigger the release of GI peptides, including PYY and GLP-1 into the bloodstream in both human and rodent, thus they may be implicated in fundamental mechanisms of caloric intake regulation and may participate in the pathogenesis of common metabolic disorders.

Understanding a complex process as satiety, involving physiological, psychological and social processes is extremely challenging; future research will need to bridge the gap between different types of satiety research to get supported understanding of the mechanisms in real life. Nevertheless it is clear that fundamental changes are needed to bring to the overweight epidemic to an end.

#### **Aims**

The objective of this study is to evaluate the satiating effect of a new food ingredient, a microencapsulated bitter compound and to identify targets for developing new food products that may reduce energy intake.

#### **Materials and Methods**

**Foods:** two vanilla puddings having the same structure and composition but one included the microencapsulated bitter ingredient.

**Subjects:** Both sex participants were selected among students of Agricultural and Food Science Department of University of Naples "Federico II". They were negative for presence

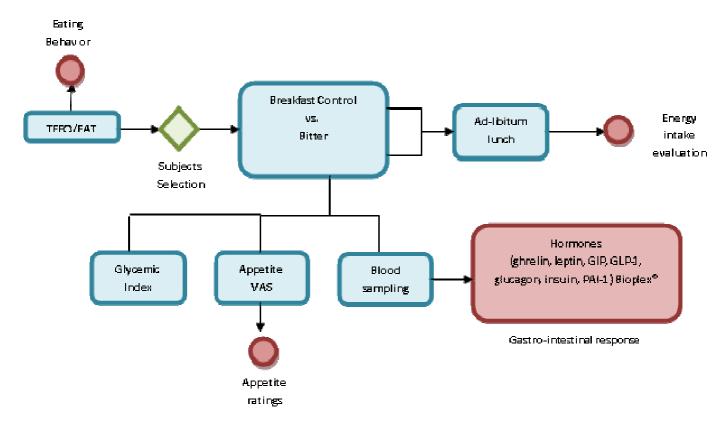
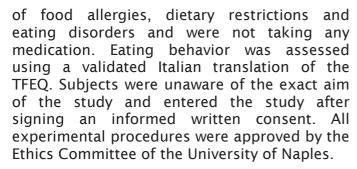


Fig 1 Study design and outcomes



**Study design:** The satiating effect was evaluated in parallel to the effects on feelings, appetite cues and energy intakes. The study design and specific outcomes are schematized in Fig 01.

All experimental measurements took place early in the morning with fasting subjects.

Appetite sensations: Once arrived to the laboratory subjects rated their feelings and sensations over 100 mm visual analogue scales (VAS). In regards to feelings, the questionnaire comprised several questions regarding how cordial, satisfied, relaxed, sleepy, energetic, alert the subjects felt. In order to assess appetite, the terminology developed by Rogers and Blundell (1979) was used, subjects were asked to indicate on the scale their hunger, fullness, satiety, thirst and power to eat. After completing the VAS, subjects were submitted to a first blood drawing (baseline). Then participants were presented with breakfast and instructed to eat it completely within 15 minutes. Successive blood samples were taken at 30, 60, 120 and 180 minutes after breakfast. At 1200 h subjects were invited to have an ad libitum lunch. They were called individually and left free to choose their lunch based on their desire to eat. Subjects were asked to consume the test meal until they felt "comfortably full". During the meal test, subjects were sited separately and they were not allowed to see or talk to each other. The water consumption in the time interval between breakfast and lunch was measured and food intakes at lunch were calculated as the difference in weight of the dishes before and after lunch.

After the ad libitum lunch, participants left the research facilities, but they were asked to fill out a food diary until 0900h of the day after. Energy intake at ad libitum lunch at the

following hours of the treatment day was calculated based on the individual consumption and nutritional composition of each food.

**Biochemical analyses:** Hormones levels (amylin, ghrelin, GIP, GLP-1, glucagon, insulin, leptin, PP and PY) were measured by a multiplexed assay using Luminex Technology.

#### **Results and discussion**

Appetite and Feelings; data indicated that the evaluated pudding was not sufficient to modify subjects' feelings and appetite ratings, in comparison to a palatable pudding (control) since no statistical differences of VAS scores depending on time and treatments were found.

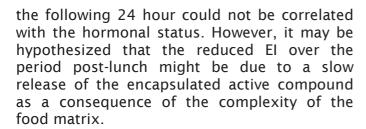
**Energy intake**; Total energy intake (EI) at ad libitum lunch was slightly higher for control than bitter taste pudding after consumption however there was no significant difference between treatments (p > 0.05).

The energy intakes over the rest of the day following the consumption of each pudding and the contribution of ad libitum lunch and of meals consumed over the post-lunch time until the morning after were recorded and a trend of reduction of energy intake was found with two-tailed T-test under equal variances assumption.

Glycemia; Blood glucose peaked at 30 min after consumption of both puddings. Sixty minutes after consumption of the breakfast glycemia returned to baseline values remaining the same from baseline until 120 min. A slight decrease was reported at minute 180. However, there were no significant differences among both treatments (p > 0.05).

Gastro intestinal hormones; Similar responses of GI hormones following the ingestion of control and bitter puddings were recorded. In our study, no statistical significance was found for the hormonal AUC values, except for a slight trend to decrease glucagon secretion between treatment groups (p-value = 0.05) after consuming the bitter compound.

As hormone response was monitored only in the short-term (over 3 h post-breakfast), the relevant trend to reduce energy intake over



# **Conclusions**

Protectina from false consumers and misleading claims is an important objective of the current EU legislation on nutrition and health claims. Taken together, the data reported in this study demonstrated that a vanillin pudding that contained microencapsulated bitter ingredient doesn't have a significant difference in comparison to a control pudding in terms of feelings, appetite ratings or energy intake in the shortterm. However an interesting trend to reduce energy intake over the next 24-hour was observed, which we believe deserves further research in order to evidence its long term satiety-enhancing effectiveness.

The work is still ongoing and further trials with more subjects are needed. Nevertheless, conclusive data will shade lights on relations between bitter stimuli, feelings, sensations and gastro-intestinal hormone release, thus offering a great insight for developing high palatable and rewarding foods with an increased satiety effect.

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# The effect of diet on human gut microbiota

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Gut microbiota plays a critical role in functions that sustain health and it is a positive asset in the host defenses (1). It can be considered as an essential organ, which provides the host with enhanced metabolic capabilities, protection against pathogens, education of the immune system, and modulation of gastrointestinal development (2).

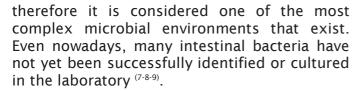
Several studies have begun to map out associations of environmental factors such as long-term dietary choices on the fecal microbiota. This thesis project, analyzed the microbial diversity of fecal samples of 100 healthy individuals who followed a longterm omnivore, ovo-lacto-vegetarian or vegan diet, from four different Italian cities. We investigated whether dietary habits can have an impact in shaping the gut microbiota and whether they can alter its composition. creating potential a predisposition to disease.

Increased understanding of interactions between gut microbiota, diet and the host will open up new possibilities of producing new ingredients for nutritionally optimized foods, which promote consumer health through microbial activities in the gut (3).

#### Introduction

Intestinal microbiota is defined as an open ecosystem that includes a variety of metabolically active microbial populations that exist in a temporary space-region and play an important role in the host's health. These bacteria can be considered together as a metabolic organ which also has the properties of being adaptable and rapidly renewable (4-5-6).

The gut microbiota is complex both in number of species and in their interactions;



The gut microbiota can be modified by various external factors such as diet, medication, micro-climate, temperature or stress, and factors specific to the individual such as age and location in the intestinal tract. Nevertheless, in a healthy individual, intestinal microbiota is kept in a relative balance during life and until advanced age, when major changes occur in its composition (10-11).

The relationships among the intestinal flora, the diet and human health have been widely studied. It is not completely understood though, how the different environments and wide range of diets that modern humans around the world experience, have affected the microbial ecology of the human gut (12). However, dietary habits are considered one of the main factors contributing to the diversity of human gut microbiota (2). Further research is essential to obtain an overall comprehension of the effect of diet on human intestinal microbiota. We believe that its role in human health deserves further study.

# **Aim**

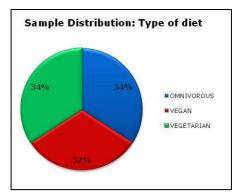
The aim of the present work is to assess whether dietary habits can have an impact in shaping the human fecal microbiome, and if they can alter its composition.

Several studies have begun to map out associations between diet and the bacteria and viruses of the human gut. Therefore, it can be said that gut microbial composition depends on different dietary habits just as health depends on microbial metabolism. However, the association of microbiota with different diets in human population has not yet been shown (13).

# Materials and methods

In order to carry out this study, one hundred (100) healthy adults volunteers were enrolled. All participants were 18 to 55 years old, and respected a normal body mass index (BMI > 18 (22  $\pm$  2.3). The individuals followed three

different types of long-term diets, habitual omnivore (n°=34), ovo-lacto-vegetarian (n°=34) or vegan (n°=32) diet. The samples analyzed in the study were provided by four different institutions in Italy. (Figure n° 1)



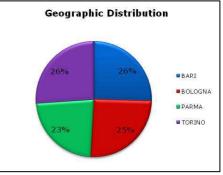


Fig. 01: Sample Distribution

The total DNA extraction from the fecal samples was carried out by using the Power Soil DNA isolation kit (MO BIO Laboratories, Inc. Carlsbad, CA). The microbial diversity was assessed by pyrosequencing of the amplified (520 bp) V1-V3 region of the 16S rRNA gene using a 454 GS Junior platform (454 Life Sciences, Roche Diagnostics, Italy) and library preparation conditions previously described

The methodology used in this study is 16S pyrosequencing. The rRNA amplicons from bacterial DNA directly extracted from the fecal samples were sequenced, and the sequences compared to reference databases to identify the operational taxonomic units (OTUs). The number of sequence reads identified as the same OTU was calculated, and a quantitative estimation of the occurrence of each OTU in the samples analyzed was then given (15).

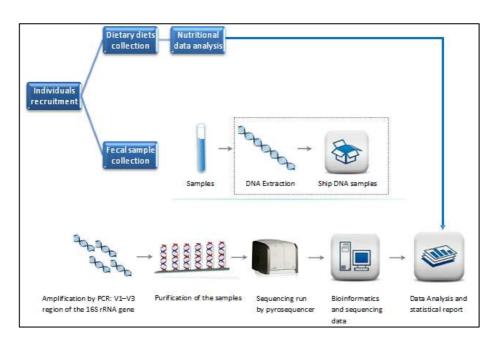


Fig. 02: Study design & Outcomes

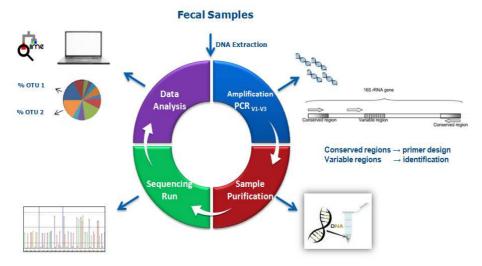


Fig. 03:Overview of the Method

The study design and specific outcomes were schematized in Figure n°2 and an overview of the method used is summarized in Figure n° 3.

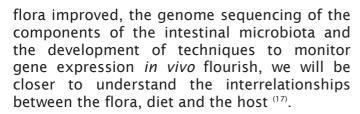
#### **Conclusions**

The complex communities of microorganisms that colonize the human gastrointestinal tract play an important role in human health (16).

The metabolic activity of the intestinal flora has influence over the synthesis, digestion and absorption of nutrients and elimination of toxic or anti-nutrients in the diet (17). The composition of the diet also exerts an

important influence on the intestinal ecology (17), and it can influence the microbiota.

The objective of this study was to assess whether dietary habits can have an impact in shaping the human fecal microbiome, and if they can alter its composition. As our understanding of the gut microbiota continues to grow, the development of techniques for analysis of changes in the composition of the



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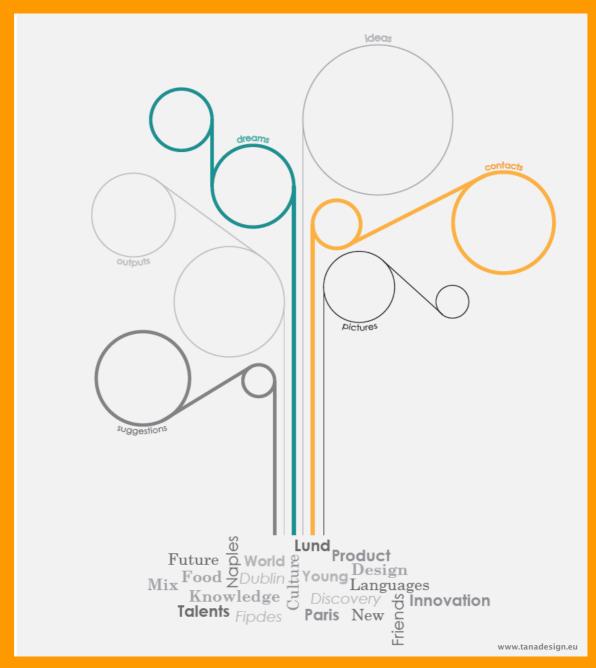
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