FIPDes

Food Innovation & Product Design

FIPDes Day 2015
International talents in Food Innovation and Product Design

Students' Book of Executive Summaries

Erasmus Mundus

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Welcome to the FIPDes community!

Dear 2015 FIPDes Graduates

I would like to offer my sincere congratulations on your achievement – you are the third cohort to have completed a very unique academic programme in the field of food science. Thanks to the visionaries who came together over five years ago to develop a partnership of four different academic programmes that deal with the science of food – engineering, nutrition, packaging and hospitality you have had the opportunity to gain specialised academic knowledge in this field from many different perspectives.

Over 40 years ago I also graduated from a newly launched Masters programme from the University of Leeds, UK which was developed to meet the needs of the food industry at that time. However the post graduate workplace that we faced as freshly minted food science graduates was quite different to the one that you will enter today. The speed and breadth of our communication systems was so much less efficient and organisations were more likely to have a silo mentality than the cross-functional team approach of today. However despite these differences the requirements for professional success remain the same: strong knowledge and communication skills and an ability to translate your knowledge to your job. Remember your learning never ends!

So as you embark on your professional careers what are some of the challenges that you will face?

One of the biggest challenges for this century is embodied in a report from the United Nations in June 2013 that projects a population of 9.6 billion by 2050. This projection, coupled with well-documented statistics about limited resources, hunger, climate change and diet related health concerns such as obesity, will be a key challenge to develop viable solutions to feeding the future generations on this planet. Ensuring a safe, adequate and nutritious food supply that meets the dietary needs and food preferences of all individuals for an active and healthy life will be your responsibility.

Today there is also a growing lack of trust amongst consumers towards the food and agriscience community which is fueled on a daily basis by media reports. For you, the newest members of the community that will be engaged in work that feeds the consumer, I would encourage your efforts to bring a more balanced and truthful perspective to our industry and our research. There is a need to bring informed understanding to consumers and to allay fears that processed foods and emerging technologies applied to food processing put them at risk.

You have been very fortunate not only to study in a cross-functional academic programme but also to study alongside and learn from a network of individuals who come from many parts of the globe. I have the utmost confidence that you will utilise these experiences successfully in your future endeavours a nd wish you the very best for your career.

Anne Goldman

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Developing Protein Enriched Fruit Smoothie: A case study of consumer driven new food product development

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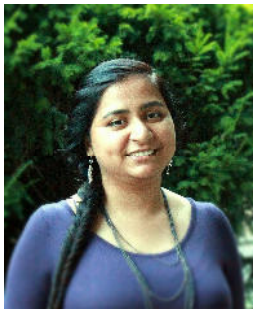

This research aims at the development of a fruit smoothie enriched in whey protein for people who are physically active or engaged in sports. The first step of the research was the development of a smoothie that tastes good and does not change upon storage. It was found that, the smoothie had phase separation, colour deterioration and flavour deterioration after few hours of production. Through this research, an unpasteurized smoothie was developed by regulating the pH, foam removal, addition of xanthan gum and use of freeze-dried raspberry powder to enhance flavour and colour of the smoothie.

In the second part of the research, a group of protein users and non-users comprising sports scholars, professors and health promotion officers at Dublin Institute of Technology were brought together to co- create the smoothie for Irish market. Consumer feedback was used at each step to enhance the recipe of the smoothie to meet their demands.

This research demonstrated the successful development of mango and berry protein smoothie. It also showed the feasibility of innovative new product development using consumer feedback.

Introduction

This smoothie is a natural product made of fruit and whey protein powder. Nutritional composition of this product is formulated to stimulate muscle recovery and feeling of satiety. It is proposed to be consumed after physical activity and in other cases of need for the increased energy. The whey protein is used due to its fast-digesting and rapid-absorbing properties over other protein sources (Boirie et al., 1997)while the proper combination of fruits is used to provide fibre and carbohydrates for the feeling of sa-

tiety(Bolton, Heaton, & Burroughs, 1981) .

In terms of physical systems smoothies can be considered comparable to a suspension with particles, cells and insoluble macromolecule aggregates suspended in a continuous phase principally made of water and soluble polysaccharides, sugars, salts and acids (Kubo, Augusto, & Cristianini, 2013)

The research was divided into two parts

Development of shelf stable smoothie

Consumer driven new food product development

Figure 1 shows consumer driven new food product development (Consumer co-creation) as an interdisciplinary approach.

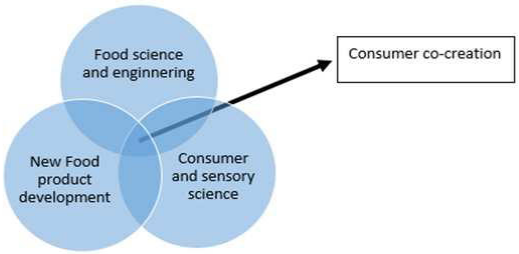

Figure 1. Consumer co-creation as an interdisciplinary approach

Research Objectives

The objectives of this research were to:

i. Develop the smoothie with reduced colour and flavour deterioration and phase separation

ii. Optimize fruit smoothie enriched in whey protein based on consumer feedback

iii. Use consumer feedback for the development of new flavour of fruit smoothie enriched in whey protein

Method

The research and experimental methodology used in this research are demonstrated in figure 2. The research was divided between AgroParisTech (France), Dublin Institute of Technology (DIT) and Teagasc (Ireland).

AgroParisTech: Development of a shelf stable smoothie (stable suspension) by altering viscosity, particle size distribution and colour stability.

DIT and Teagasc: Development of new flavours and enhancement of the smoothie based on consumer feedback.

Figure 2. Research Method Overview

Results and Discussions:

Colour

A detailed bibliographic research was carried out to understand the cause of colour deterioration in the smoothie. A possible cause might be enzymatic browning caused by enzyme Polyphenol oxidase (PPO) present abundantly in banana (Chutintrasri & Noomhorm, 2006) which is one of the ingredients of the smoothie.

Colour stability was achieved by removing foam and lowering the pH of the smoothie to inhibit the activity of enzyme PPO (Archer & Palmer, 1975; Mizobutsi et al., 2010).

Volume reduction and phase separation

Severe volume reduction in the smoothie was observed in the preliminary studies. It was hypothesized that the reduction in volume of the smoothie was due to the formation of foam due to the presence of whey protein which start collapsing after 30 minutes (Richert, 1979). Volume reduction was prevented by the removing foam from the smoothie.

Phase separation was prevented by reducing the particle size of fruits and increasing the viscosity of the continuous phase of the smoothie by addition of xanthan gum.

Consumer co-creation

A series of five focus groups was conducted at Dublin Institute of Technology (DIT). The results of each focus group are as follows:

Focus Group 1: A group of six people who are physically active and do not use whey protein supplements were asked about their lifestyle and diet. They all agreed that a balanced diet is enough to fulfil their nutritional requirements.

Focus Group 2: A group of three sports scholars who use whey protein supplements were asked about their lifestyle and diet. They all agreed that their diet is not enough to meet their nutritional requirements and they need supplements.

The groups from the first two focus groups were combined for co-creation sessions to create a perfect whey protein enriched smoothie for them.

*Focus Group 3:*The participants were asked to taste the protein smoothie and give descriptive feedback about the taste. They were also encouraged to come up with a new flavour that they would like in a product like this. Mango was the most preferred new flavour.

Focus Group 4: Participants were asked to taste mango and berry protein smoothie containing 25 grams of whey protein in each serving of 250mL.

Points of purchase (POP): They were shown pictures of POP such as health stores, gyms and convenience store and asked to pick the most preferred POP.

Packaging: Participants were asked to pick the most preferred packaging and serving size for the smoothie from the pictures shown to them.

Focus Group 5: In this session, the participants were served mango and berry protein smoothie that were developed based on their feedback in a mock up package. They were asked to serve it to themselves for a complete product experience.

Upscaling and Industrialization

The smoothie can be produced either in batch or continuous mode based on the demand in the market. The process flow diagram for upscaling of the smoothie is shown in figure3.

Conclusions

The first phase of the research at AgroParisTech dealt with detailed analysis of fruit smoothie with whey protein as a system. At the end of this research phase a shelf stable smoothie was developed by the addition of Xanthan gum(García-Ochoa, Santos, Casas, & Gómez, 2000). Colour and flavour stability was achieved by the addition of freeze dried raspberry powder(Lee, 2008).

At Dublin Institute of Technology (DIT) and Teagasc, consumer feedback gathered during focus groups was used to co-create the

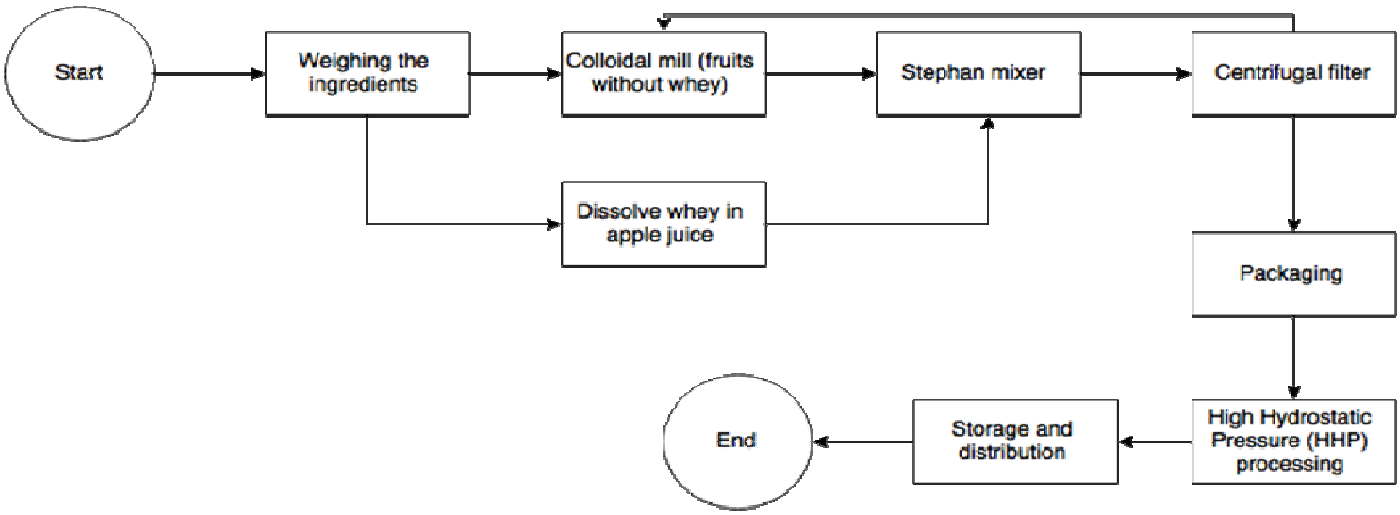

Figure 3: Process flow diagram for the industrial production of smoothie

Two flavours of smoothie: mango and berry protein smoothie containing 25 grams of whey protein in each serving of 250 ml were successfully developed at the end of consumer co -creation process.

Future Prospects

The smoothie developed in this thesis was an unpasteurized product. Further research on the use of various pasteurization methods like heat treatment, ultrasound and high hydrostatic pressure (HHP) needs to be done.

Further research has to be done on the upscaling and industrialization of the production of fruit smoothie enriched in whey protein.

This study was a successful implementation of innovative approach of consumer co-creation and market orientation to drive new food product development.

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Possible influence of processing environment on meat spoilage microbiota

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Introduction

Food microbial spoilage has been a great concern for producers, retailers and consumers. Fresh meat is a highly perishable food product due to its composition. Rich nutrient content in meat supports microbial growth, thus increase the probability of undergoing microbial spoilage [1]. Meat spoilage is an ecological phenomenon that includes the changes of the available substrates during bacteria proliferation. These climax bacteria populations, composed by a fraction of initial meat microbiota, are called ephemeral or specific spoilage microorganisms (ESO/SSO) [2, 3]. Bacteria that are present on aerobically spoiled chilled meat are *Pseudomonas spp.*, *Psychrobacterimmobilis*, *Acinetobacter spp.*, *Enterobacteriaceae*, *Brochothrixthermosphacta* and lactic acid bacteria [4].

Raw meats that are available at retail outlets come through a long chain of slaughtering and processing. Consequently, contaminations can occur in the abattoirs and butcher shops [5]. The main source of meat contamination comes from the environment [1, 6]. Therefore, understanding the role of meat processing environment in the meat spoilage process is essential.

Several studies have been conducted to assess the microbial level of environmental surfaces at meat processing plant and butchers' premises, focused more on food safety than spoilage [7, 8]. A more recent study used culture-independent high throughput sequencing method to explore the source of microbial contamination of beef steaks in meat processing line [9]. The result showed that possible microbial spoilers come from the carcass. Nevertheless, due to the method used in the study, the evolution of Operational Taxonomic Units (OTUs) relative abundance can be observed. It is considered that "the spoilage

bacteria are transferred from abattoir to the butchery environment where they start to proliferate in microenvironments where meat residues and exudates can act as substrates" [9].

Objectives

The objectives of this study were: 1) Applying traditional microbiology approach to assess the microbial load in meats and meat processing environment; 2) Using high-throughput sequencing to identify the relationship between microbial diversity, especially meat spoilage bacteria, in meats and meat processing environment; 3) Observing if there is any difference of microbial diversity between traditional butcheries and butcher counters in large-sized establishments (LEs); and 4) Observing the general hygiene of butcheries.

Materials and Methods

Sample collection

Samples were collected from butcheries (n = 20), including traditional butcheries (n = 10) and butcher counters in "grandedistribuzioneorganizzata" or large-sized establishments (LEs) (n = 10), located in Campania, Italy. Sample collection was performed twice. Meat samples collected included fresh beef cut and fresh pork cut. Surface samples collected from the premises included knife, chopping board and butcher's hand.

Microbiological analysis

Standard method pour plating was used to determine total plate count, lactic acid bacteria and *Enterobacteriaceae* by using, respectively, plate count agar (Oxoid, UK), MRS (de Man, Rogosa and Sharpe) agar (Oxoid, UK) and violet red bile glucose agar (Oxoid, UK). Standard method surface plating was used to determine *Pseudomonas spp.* and *Brochothrixthermosphacta* by using, respectively, Pseudomonas CFC agar (Oxoid, UK) and streptomycin thallous acetate agar (STA agar, Oxoid, UK) added with STAA Selective Supplement SR0151E (Oxoid, UK).

DNA extraction and sequencing

Total DNA extraction was performed using Biostic™ Bacteremia DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA). The microbial diversity was studied by pyrosequencing of the amplified V1–V3 region (520bp) of the 16S rRNA gene, following the method in previous study[10], using GS Junior Titanium Sequencing kit (454 Life Sciences, Branford, USA).

Results and Discussion

General hygiene

Process hygiene criteria of meat and products thereof are regulated in Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs [2008] OJ L338/1. The criteria were applied on the stage of carcasses after dressing but before chilling. This regulation does not apply to retail, while the samples in this study were collected from butcheries, which fall into retail category. Therefore, the result of this study could not be assessed based on the regulation. In the EU legislation, aerobic plate and *Enterobacteriaceae* counts are used as the indicators of general hygiene quality. Aerobic plate counts (APC) are frequently used to monitor the hygiene of meat production process while *Enterobacteriaceae* counts (EC) are used to assess enteric contamination.

In this study, the aerobic plate counts (APC) value of beef was ranged from 3.27 ± 1.06 to 6.45 ± 0.01 $\log_{10}$ CFU/g, with a mean value of 4.97 ± 1.02 $\log_{10}$ CFU/g. These values were considered as high, 42.5% of the samples had APC values greater than 5 $\log_{10}$ CFU/g, which shows deficient hygienic practices during processing [11] and high level of contamination. A high level of contamination in retail level might be caused by improper cleaning and sanitizing of equipment and poor employee hygiene in the store [12].

Moreover, results showed that there was no significant effect of butchery types on all bacterial counts performed for all samples in this study. These results are in accordance with two other studies in Greeceand Spain[20, 21].

Distribution of meat spoilage bacteria in the processing environment

Based on all microbiological counts which were performed in this study, chopping board had the highest average value compared to other surface samples(Figure 1). *Pseudomonas sp.* is known for its biofilm forming abilities [13]. Biofilms can form in food processing environment, including chopping board [14]. Chopping board also has the most frequent and longest contact with meatsTherefore, chopping board in butchery, in which the meat spoilage bacteria can form biofilm, might be a potential source of cross-contamination.

Microbial diversity of samples

Results showed that microbiota in fresh meat cuts and surfaces samples had high complexity, which was in accordance with other studies [9, 15-17]. In the present study, *Pseudomonas sp.*, *Brochothrix sp.* and *Psychrobacter sp.* were found abundantly in meats and surfaces samples, regardless the types of the butchery. These genera frequently occur on freshly cut and aerobic chill-storage meat [18]. However, considering of their high relative abundance in meat samples and the results of the count, this might indicate that the meat samples were highly contaminated. As mentioned above, *Pseudomonas sp.*, *Brochothrix sp.* and *Psychrobacter sp.* are responsible for meat spoilage [4].

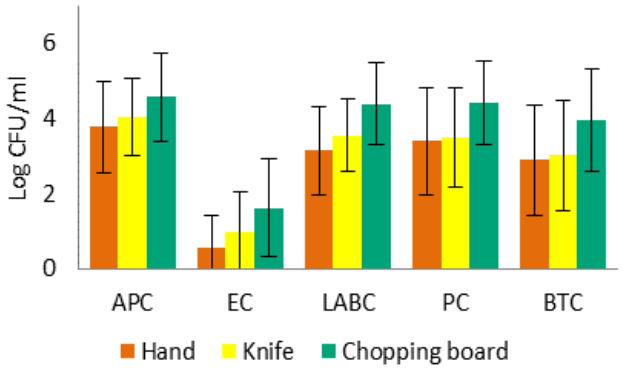

Figure 1. Microbiological counts of all surface samples. APC: Aerobic plate counts; EC: Enterobacteriaceae counts; LABC: Lactic acid bacteria counts; PC: Pseudomonas spp. counts; BTC: *Brochothrixthermosphacta counts*

As many as 38 OTUs were shared between meats and surfaces samples regardless the type of the butcheries. Among the 38 OTUs, *Brochothrix sp.*, *Pseudomonas sp.* and *Psychrobacter sp.* were found to be abundant in the samples, with average relative abundances higher than 10%. This verifies previous evidence that these bacteria might be resident microbiota in processing plants which become the final source of meat contamination [9, 19].

Conclusion

This present study showed that there was a high level of meat contamination in the observed butcheries in Campania region. There was no significant difference of microbiological counts between traditional butcheries and large-sized establishments. Compared to other surface samples, chopping board had the highest average value of all spoilage-related bacteria counts, which was similar to meat samples. This indicated that chopping board can be the potential source of cross-contamination in the butcheries' environments. *Pseudomonas sp.*, *Brochothrix sp.* and *Psychrobacter sp.*, which are responsible for meat spoilage [4], had the highest relative abundance in the samples in this study. High-throughput sequencing results in the present study also verifies the evidence from previous studies [9, 19] that these bacteria can be resident microbiota in processing plants and the final source of meat contamination. Therefore, both plate counts and high throughput sequencing results showed that meat processing environment can be an important source of meat contamination.

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Manufacture of fermented dairy products using ingredients obtained by milk cracking, in a semi-industrial scale

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Skimmed milk was treated by membrane technology to modify its native composition. The products of this process were used to manufacture fermented dairy products prototypes. The effect of these modifications, on the gel formation, fermentation, rheological and sensory properties of the products, was studied.

Confidential topic

Physiological and sensory response to fat taste in humans

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Introduction

Dietary fat is a substantial nutrient in human nutrition ensuring appropriate function of hormonal and immune system, thermal protection, functioning as a medium for fat-soluble vitamins and lastly being the most dense source of energy. However, providing the vast amounts of energy can be detrimental, contributing to excessive energy intake and body fat accumulation, therefore causing serious health-related problems (WHO, 2003). Human orosensory perception, especially during cephalic phase of eating, has a plausible role in providing the information about the consumed nutrient type, thus sending signals to different sites of the body and allowing digestive tract organs to prepare for its reception and digestion upon the nutrient type, which results in different satiating effect (Mattes, 2005). Mechanisms behind fat perception and palatability may be interconnected with the reward system. Some endocannabinoids (ECs) and N-acylethanolamines (NAEs) were demonstrated to be modulated by food liking upon mastication (before swallowing) in humans (Mennella et al., 2015 – in press). Plenty of research is focusing on the fat taste in order to assess its eligibility as a primary taste. Lack of knowledge still exist about physiological mechanisms behind the fat taste and its role in dietary choices and behavior. In this study an holistic approach considering both physiological response linked to appetite and reward system as well as the sensory description of the fat taste upon mastication (before swallowing) will be considered.

Aims

The aim of this thesis will be to evaluate human physiological response and the sensory perception to fat taste, the associated palatability and the influence on individual appe-

tite. This correlation would be a key-information to develop foods for weight management that can guarantee fat-like sensory satisfaction and energy intake in overweight/obese people, thus reducing the health risk associated to high-fat food consumption.

Materials and Methods

To this purpose specific experimental design will be pursued (Figure 1). Firstly, fat-enriched and a control pudding will be developed to be used in a human study. Recruitment of participants will be done according to individual nutritional status and eating behavior. Eligible subjects will be enrolled into the study only after signing a consent form. Before starting, the participants will be involved in defining attributes describing the foods and will be trained to be familiarized to the experimental procedures including sensory and physiological approaches. The latter will be focused on evaluation of the cephalic response to the fat stimuli measuring salivary endocannabinoids and N-acylethanolamines concentrations by a modified sham-feeding technique (MSF). Concentration of the biomarkers will be analysed using liquid chromatography and mass spectrometry (LC/MS/MS) in saliva and chewed puddings (Di Marzo, 2008).

As regards the sensory approach a specific TDS multi-sip technique will be used. (Pineau et al., 2009). Assessors will evaluate the dominance of sensations in 10 spoons of FEP and CP, creating a dynamic sensory profile of each product, distinguished by 20 second mastication of each spoon. Application of this technique is well-fitted in the sham-feeding protocol, requiring chewing but not swallowing evaluated product. Moreover, it will give an interesting insight into detailed, spoon by spoon, dominance of the fat-enriched and control pudding, revealing possible differences occurred during the time of mastication.

Results and Discussion

Endocannabinoids are key-players in human's food intake and may influence eating behavior trough homeostatic and hedonic pathways (Mahler et al, 2007, Di Patrizio 2008).

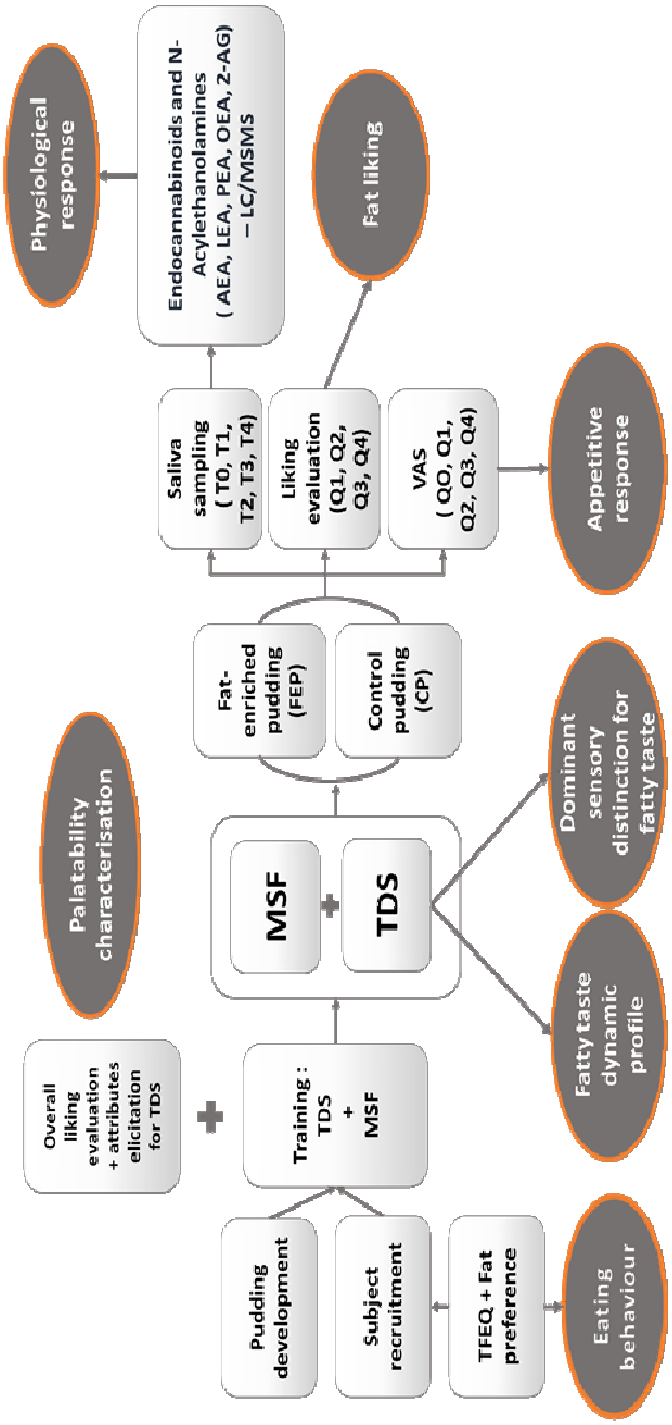

Figure 1. Study Design and Outcomes

Recent findings from the research group demonstrated that they are influenced by food palatability during the cephalic phase (Mennella et al., 2015). Fat content in the food can highly influence its palatability.

For the first time, in this study the physiological response to fat taste during chewing of a food and its influence on individual appetite

was evaluated. The main results of the study were that during 3 min of pudding mastication the levels of ECs and NAEs in the human's saliva increased. Di Patrizio et al. (2011) have focused their investigation on particular palatable nutrient – fat. The study has shown that 30 minutes of sham-feeding a lipid-based meal stimulated endocannabinoid mobilization in the rat proximal small intestine by altering enzymatic activities that control endocannabinoid metabolism. However, this effect was not observed in other peripheral organs, including tongue tissue. In the present study cephalic phase ECs and NAEs response was evaluated and detectable in human saliva after 3 min of oral exposure to the puddings.

Interestingly, FEP elicited different responses of AEA, LEA, OEA and PEA endocannabinoids compared to CP, independently of the pudding liking. No previous studies are present in the literature to compare this data. Yoshida et al (2010) has observed the presence of CB1 endocannabinoid receptors also in T1r3-expressing taste bud cells, responsible for transducing sucrose sensing. Moreover, they have observed that local CB1 activation by ECs enhances the sweet responses of isolated taste bud cells. However, in this previous study, no evidence was provided to show that sucrose activates EC signalling in the tongue or chorda tympani. As FEP has evoked a lower increase in ECs and NAEs level in our study, more investigation has to be done in the area of oral taste bud cells, like CD36, TRPC5, GPR40, and GPR120 transducing signals of fatty taste and their possible relation to CB1 receptors (Gaillard et al, 2008).

Matias et al (2012) demonstrated already that ECs and NAEs are present in saliva and may be influenced by fasting and nutritional status of subjects. These authors showed that the concentrations of 2-AG, AEA, OEA and PEA in saliva before and 1h after consumption of a meal were significantly higher in obese than in normal weight subjects. The present study showed that the response to sham-feeding of dietary fat was associated with the nutritional status of the subject. In fact, in our study, salivary AEA levels were shown to be higher for overweight subjects comparing to normal-weight subjects throughout the 20 min of experiment, upon mastication of the fat-enriched pudding (although no significant differences have been shown).

Sensory data showed that fatty taste could be distinguished in the dynamic dominance evaluation. In particular, upon MSF subjects continuously perceived a significant difference in dominance of fatty taste during chewing the fat –enriched pudding compared to the control one. This sensation had a peak at 30 sec, where fatty sensation difference in FEP comparing to CP has reached 15.2 % of difference. In the study by Bruzzone et al. (2013) after application of classical TDS evaluation on yoghurt formulation with different fat content, they similarly concluded fat's salience in the dynamic profile of this kind of product, with the special influence on its texture. In our study, apart from the fatty taste dominance during all time of the study, significantly different dominance of creaminess and compactness sensation have occurred, which have not been observed in the control pudding.

Conclusions

In this study, for the first time a two-dimensional approach aimed at assessing physiological and sensory response to fat taste in humans was developed.

All in all data led to the following conclusions:

I. Chewing a pudding (before swallowing) causes an increase of salivary ECs and NAEs in the mouth;

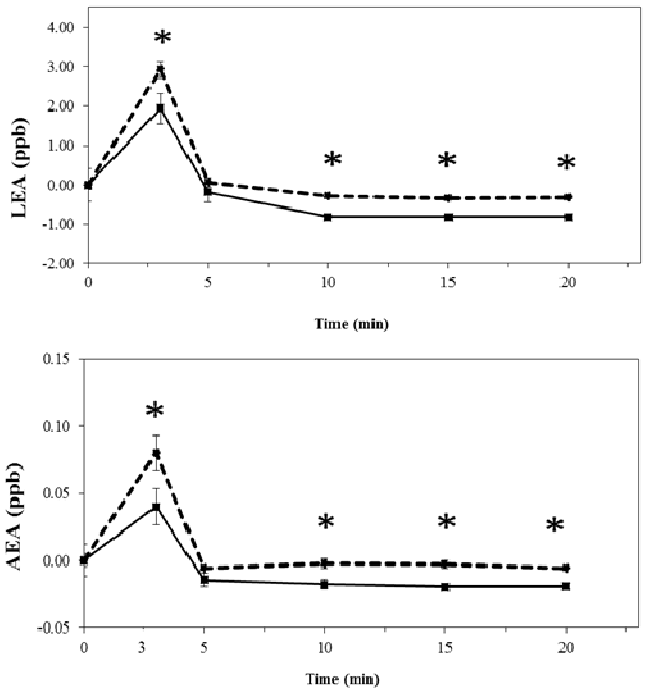

Figure 2. Salivary concentrations of AEA (anandamide) and LEA (N-lauroylethanolamine) upon FEP (dotted line) and CP (smooth line) mastication. Each point represents concentration at different time of the study (T0-baseline, TB(3) – chewed pudding concentration; T1-5 min, T2-10 min, T3- 15 min ; T4 – 20 min after sham-feeding). *p<0.05

II. The pudding with a higher fat content evokes a higher physiological response of some ECs and NAEs than equally palatable control food

III. AEA (but not LEA or PEA) response up on mastication of fat-enriched product tends to be higher in over-weight than normal-weight subjects.

IV. A tendency to increased satiety and fullness and reduced hunger was found by mastication of fat-enriched vs control product.

V. Fatty taste can be distinguished in dy namic sensory profile evaluation. Moreover it has an effect on products appearance, colour, consistency and aftertaste.

Data here presented could be used as insights into understanding of the orosensory perception of dietary fat. With the use of its dynamic sensory profile and evaluation of physiological response it evokes, this study could contribute to the design of new healthy food with the lowest fat content but preservation of its palatability and increased satiating characteristics compared to the normal food.

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Development of a healthy range of dairy products with phytonutrients

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Since ever botanical extracts were used because of their health benefits in several countries. Dairy products are also perceived as an healthy food product, this is why the association of these two ingredients could lead to very beneficial products for consumers. This project aims to bring healthier food alternative in developed countries which are fighting more and more health problems like stress, tiredness or cardiovascular diseases.

After a bibliographical research to select botanical extracts and a review of the Swiss and European legislation, the range of prototypes was developed with these extracts, according to desired organoleptic properties and the expected content of bioactive compounds in the final product, after the process, and at the end of the shelf life. The method used for the development of this range are sensory analysis and HPLC/MS. The developed products are aimed to efficiently promote good health.

Confidential topic

Functionalization of fruit juices with probiotic *Lactobacillus* strains

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Introduction

The idea of health-promoting foods is not new: Hippocrates wrote 2400 years ago "Let food be thy medicine and medicine be thy food" (Hasler 2002). Succeeding scientific researchers have continued to support this idea that diet may satisfy nutritional needs and exert a beneficial role in some diseases (Corbo et al. 2014; Otles and Cagindi 2012), leading to the concept of functional foods. EC stated that "a food product can only be considered functional if together with the basic nutritional impact it has beneficial effects on one or more functions of the human organism thus either improving the general and physical conditions or/and decreasing the risk of the evolution of diseases" (Ozen et al. 2012).
Estimated to reach USD 38 billion in 2018 (John 2015), the probiotic food and beverage category held the largest share in the functional food market as consumers continue to seek for health and wellness products and for their potential to reduce healthcare expenses (ADA 2004; Bech-Larsen and Scholderer 2007; El-Sohaimy 2012; Hawkes 2004; Henson et al. 2010; Herath et al. 2008; IFIC 2015; Malla et al. 2013; Malla et al. 2014; Ross and Amanor-Boadu 2006; Siegrist et al. 2008; Stein and Rodríguez-Cerezo 2008; West and Larue 2004)by as much as 20% ((Sun-Waterhouse 2011)).Next to Asia Pacific (main market includes Japan and China), Europe is the other major region where probiotic sales are increasing significantly, particularly Germany and France (Markets&Markets 2014; TMR 2015).

However, the probiotic market has always been dominated by dairy-based products especially yogurt(TMR 2015). Among the different functional F&B formats, fruit-based probiotic beverages are an attractive choice because of (a) consumer preference towards convenience, healthy diet and natural

ingredients; (b) naturally-occurring health-promoting components including vitamins and antioxidants; (c) possibility to meet consumer demands for container contents, size, shape, and appearance; (d) ease of distribution; (e) great opportunity to incorporate desirable nutrients and bioactive compounds; (f) have taste profiles that are pleasing to all the age groups; (g) perceived as being healthy and refreshing (Kausar et al. 2012; Prado et al. 2008; Sanguansri and Augustin 2009; Sun-Waterhouse 2011; Wootton-Beard and Ryan 2011). Despite thementioned considerations and recent food trends related to vegetarianism, milk cholesterol content, soy allergy and lactose intolerance, 100% probiotic fruit- and vegetable-based beverages choices are still limited.

Aims

This study aims to explore the potential of developing a 100% probiotic fruit juice beverage by evaluating the metabolic behavior of six strains representing all the types of lactobacilli (based on metabolism) on six commonly available commercial fruit juices. Since a minimum of 10^6 CFU/g or ml viable probiotic bacteria should be present in the food at the time of consumption in order to have the cited beneficial health effects of the probiotic (FAO 2002), the viable count of the fruit juices after overnight incubation at their optimal growth temperature of 37^0C and during storage at both chilled and ambient conditions were monitored.

As consumer are not likely to buy a healthy product with an unacceptable sensory parameters, modified flash profiling by experienced panelists of the probiotic fruit juices was utilized in lieu of consumer acceptance test, requiring at least 50 judges and a lot of time which is a limitation of this study.

Materials and Methods

Microorganisms and culture conditions

The six probiotic strains used in this research are: *L. plantarum* ATCC SD 5209 from Equilibria®, *L. reuteri* DSM 17938 from Reuterin® and, *L. plantarum* LMG-P 21021, All strains were cultivated in MRS Broth (OXOID Ltd., Basingstoke, Hampshire, England) and incubated at 37 ˚C in aerobic conditions for 24 hours to obtain overnight broth cultures. Strains were checked for purity on MRS broth added with 1.5% Agar Bacteriological no.1 (OXOID Ltd., Basingstoke, Hampshire, England), herein called MRS Agar, and incubated at 37˚C for 48 hours in aerobic conditions. For viable counts, cells of each strains in free or microencapsulated form were grown on MRS Agar. The colony counts were performed after 48 hours of incubation at 37ºC in aerobic conditions, reporting the results in CFU/ml.

Screening of the ability of probiotic strains to ferment fructose

Strain capability to ferment fructose was tested by preparing a modified MRS broth in which the glucose in regular MRS formulation was replaced with fructose. All strains were cultured in both regular and modified MRS Broth at 37˚C in aerobic conditions for 24 hours. Viable counts were expressed in terms of CFU/ml. Strains able to grow in modified MRS were then screened for ability to grow in fruit juices.

Probiotic beverage production and shelf life analysis

The six commercially available, aseptic, ambient stable fruit juices used during the initial stage of the study are 100% blueberry, apple, grapefruit, orange, pineapple and exotic juice. 0.2% of fresh overnight culture was added to fruit juice. Prior to addition to fruit juice, the overnight cultures were centrifuged at 6,500 rpm for 10 minutes. The MRS supernatant was discarded and the cells were re-suspended using fruit juice. The inoculated juices were incubated at 37˚C for 17-24 hours. The samples were then stored at both 4^0C and ambient condition for shelf life study. Bacterial enumerations were carried out routinely.

Sensory test

Ten experienced panelists were employed in the modified flash profiling of the samples which have been stored in the fridge for 2 weeks. As panelists do the napping during session 1, they also input noted sample characteristics and impressions encoded next to each sample. All the generated attributes were then pooled by the

experimenter and in the second session, judges are asked to check the global list and update their own list in a piece of paper if desired as they evaluate the samples again to make sure that only the 10 most relevant attributes are considered while giving each attribute a definition. In the third session, assessors rank the samples according to individual vocabulary list, one attribute at a time in order to the perceived level of intensity. The evaluators are provided a questionnaire where they should rank each sample on a scale based on their relative attribute intensity. The product with the lowest level of a given attribute must be placed at the beginning (left side) of scale while the highest intensity at the end (right side) of scale. This ranking evaluation is done in at least two replicates. When samples are placed in the same position, the average of positions occupied is given to these samples. Flash Profile data were then analyzed using Generalized Procrustes Analysis (GPA).

Data analysis

All analyses were carried out in duplicate and values were expressed as an average of three different experiments. Significant differences between averages at p <0.05 was determined using t-test (Microsoft Excel 2013).

Results and Discussions

Screening of the ability of probiotic strains to ferment fructose

Lactobacillus requires a fermentable carbohydrate like glucose and fructose which also serves as an energy in order to grow(de Man et al. 1960). Glucose in regular MRS formulation was replaced with fructose as this is the sugar most abundant in fruit juices (Lea 1990). Table 1 details the viable count of each strain after 24 hours at 37^{0}C in MRS and modified MRS.

The growth recorded for strains grown in both media demonstrates the utilization of both glucose and lactose in MRS and modified MRS, respectively. This agree with findings of a study on fermented pomegranate juice (Filannino et al. 2013) wherein lactobacilli were reported to utilize both forms of sugar. In general, the cultures grow better in MRS than in modified MRS with a minimum increase of at least 2.36 Log cycles. Since all strains were able to ferment fructose in the modified MRS, all of them were used in the manufacture of probiotic fruit juices.

Probiotic strain	MRS broth	Modified MRS
*L. plantarum*ATCC SD 5209	9.14	8.98
L. plantarum LMG-P 21021	9.17	8.62
L. rhamnosus DSM 16605	8.73	9.11
*L. paracasei*LMG-P 21380	9.13	8.36
L. acidophilus LMG-P 21381	8.84	8.67
*L. reuteri*DSM 17938	8.91	8.58

Mean results of three independent trials. Standard deviations were always <0.54.

Table 1. Growth of probiotic strains (log CFU/ml) in MRS and modified MRS broth after 24 hours at 37^{0}C. Viable count at time zero was standardized to about 10^6 CFU/ml.

Probiotic beverage production and storage

The cell load after overnight incubation at 37^{0}C is lowest for blueberry juice at ~7 Log CFU/ml and even registered a <5 Log CFU/ml viable count for *L. reuteri* DSM 17938. Indeed, in a study on viability of *L. reuteri*, it was found to have a strong reduction in a commercial red fruit (20% blueberry, 20% red orange, 10% pomegranate; pH 2.97; soluble solids 10.3 Bx; sugar 1.05 g/L), with a first reduction time of 0.47 day (Perricone et al. 2014). Apple, grapefruit and orange juice exhibited an ~8 Log CFU/ml growth whereas pineapple and exotic juice (contains 66% pineapple juice) displayed the highest growth at ~9 Log CFU/ml.

In this study, the loss viable count trend was worst for orange juice wherein a 70% reduction, leading to <5 Log CFU/ml in viable count has been observed after only 15 days for *L. plantarum* ATCC SD 5209 and LMG-P 21021 as well as *L. reuteri* DSM 17938 and after 26 days for *L. paracasei* LMG-P 21380. The observed loss of viability of *lactobacilli*in orange juice is in contrast to a study (Perricone et al. 2014) wherein it had cell counts of ~8 Log cycle and it took 52.52 days to a attain a decrease of 1 Log CFU/ml. Furthermore, the viable count of *L. reuteri* DSM 17938 in apple

and grapefruit juice was already <5 Log CFU/ml after only 5 days of refrigerated storage.

Meanwhile after 54 days, the rest of the strains in apple, pineapple and grapefruit juice samples maintained cell viability above 5 Log CFU/ml and all strains in blueberry juice had cell counts <5 Log CFU/ml except for *L. plantarum* LMG-P 21021 which remained at 7 Log CFU/ml. Indeed, *L. plantarum* added to a non-dairy product with a 2.8 similar pH to blueberry juice can be stored in a refrigerator for more than a month without loss of viability (Molin 2001).

Further tests on exotic juice were discontinued after the overnight growth as it mainly pineapple juice which is also being evaluated in this study.

In contrast to chilled storage, the apple, grapefruit, orange and pineapple juice samples showed fluctuating viable counts when stored at ambient conditions. Different bacteria can tolerate different temperatures, some probiotic lactobacilli can grow at mesophilic temperatures of 15 ˚C and no growth at temperatures below 20 ˚C (Doleyres and Lacroix 2005) but in general temperatures above 22ºC favor the lactobacillus species (Battcock and Azam-Ali 1998). All samples maintained viable count above ~6 log cycle until 12 days except for *L. reuteri* DSM 17938 in apple and grapefruit juice which decreased to <5 log cycle after 5 days storage at ambient conditions just like in the chilled storage. The practical implications of these results strongly agrees with previous study (Perricone et al. 2014) suggesting that a limited thermal abuse (varying patterns of subjecting sample to either 4^0C or 25^0C) could not compromise the survival of *lactobacillus* and the functionality of the probiotic fruit juices. However, since the cells are included as ingredients to probiotic food products, their concentrations must not significantly change during storage as they might alter the properties of the product and affect product shelf life (Granato et al. 2010). Because of storage at room temperature can create an overwhelming challenge for probiotic stability (Mattila-Sandholm et al. 2002) as seen in the cell count results, chilled storage is recommended.

Sensory Test

The metabolic behaviors of strains during fermentation is accompanied by changes in the sensory properties of food and is one of the major concerns in the development of fruit-juice based functional beverages (Prado et al. 2008).Considering that the judges were asked to evaluate all three fruit juices together, the global objects map show that judges were able to discriminate between the different fruit juices but not between the *Lactobacillus* strains. Looking at the relative global attributes map, more negative attributes such as acidic odor and flavor as well as pungent odor were observed in both orange and pineapple juices (Figure 1). On the other hand, the apple juices retained most of their positive original attributes with a hint of wine and alcohol odor. Because least sensory modifications were observed in the apple juices, it can be considered as the best media for the tested strains.

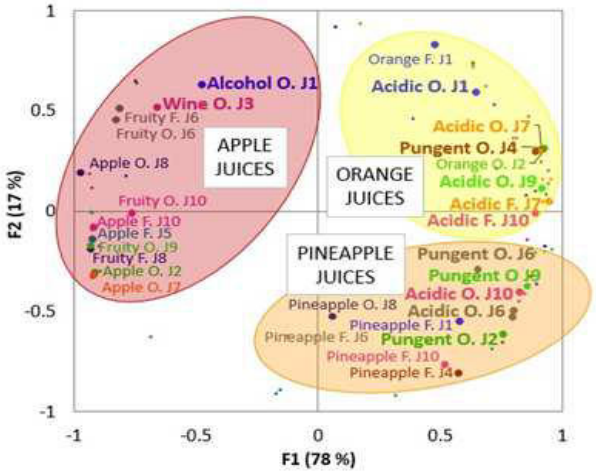

Figure 1. Global odor and flavor attributes map from the modified flash profiling involving 10 experienced panelists.

Based on the fruit juice objects map, *L. paracasei* can be considered as the best strain as the fruit juices inoculated with this strain were positioned closest to the control in apple, orange and pineapple beverages.

Conclusion

While data from this study supplement available literature regarding potential stability of probiotics in fruit juices, results also confirm the strong dependence on strain, kind of fruit juice and the need for refrigeration for enhanced probiotic stability during storage.However, as the true test of a probiotic food is its ability to survive the harsh GI conditions, further GI survival experiments are warranted. Nonetheless, with careful selection of the fruit matrix and probiotic strains, it can be concluded that fruit juices are indeed promising potential carriers for probiotics.

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A Secondary Packaging Solution Development Suitable for E-groceries through the Identification of Food Product and Logistic Requirements

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Introduction

European e-commerce for groceries has seen steady growth. The annual amount of food delivered is forecasted to grow from 133.7 to 186.3 million by 2018, an increase of 40% (Internetworldstat, 2014). The geographic target market of this research is the Netherlands, where according to Rabobank Retail Trends (2014) the e-grocery market only reach 1%, yet it will grow to be 13% by 2020 (Thiuswinkel.org, 2014). However, online grocery (e-grocery) shopping is a segment of e-commerce with difficulties all of its own. Most of these occur during the last mile, defined as the last leg of the business to consumer delivery service (Boyer et al., 2009). Issues concerning the last mile are related to the grocery business models employed. Currently there are three main e-grocery business models: ´brick and clicks´, ´ pure players´ and ´infomediaries´ that differ from each other due to the location and management of their logistic hubs (Reynolds, 2000). The use of outsourced logistics (third party logistic providers) aids e-grocery businesses with logistic activities such as receiving produce (from suppliers), stocking, picking, packing, transporting and delivering to customers and the packaging return management. This in turn induces challenges in designing packaging solutions suitable for said logistic activities. In e-commerce many single-item orders to different delivery addresses on irregular occasion represent one shift of demand compared to regular marketplace (Olsson et al. 2004). Stock (1997) suggested integrating areas such as consumer behaviour and logistics in order to improve the packaging design for e-commerce, adopting an interdisciplinary method. Secondary packaging solutions currently used for e-grocery shopping are: shopping bags, boxes and crates. But their design should be holistically considered and improved based on the chosen last mile delivery model, involving the analysis of the overall distribution network and the redesign of packaging (Aubrey & Judge, 2012). Further, e-groceries are subject to more sensitive parameters than other FMCG. For instance, time-temperature control for the assurance of quality food throughout the supply chain (Aung& Chang, 2014) and the effects of temperature changes to product quality (Tijskens & Polderdijk 1996) are of vital importance. Vibrations during transportation can also increase the risk of product damage. As proven by Colla and Lapoule (2012): “choosing an efficient logistic model and a packaging solution are critical factors for efficiently protecting food products” (Cagliano et al. 2014).

The purpose of this thesis is to develop a secondary packaging solution for home delivered food products. This solution must fulfil logistic and food product requirements for e-commerce and address difficulties relating to the last mile in grocery supply chain. The goal of this thesis is to identify the mentioned requirements. To aid the identification of said requirements and thus the development of the proposed secondary packaging solution, research questions such as “what type of food product and logistic requirements need to be considered for the development of a secondary packaging solution suitable for e-groceries?” and “which features could aid packaging solutions in meeting these requirements?” have been posed.

Methodology

Due to novelty of research towards the secondary packaging requirements in this sector, research was approached through an inductive manner, which aimed to condense qualitative data findings into summaries, and to establish links with the research questions and data. The research performed was divided into two parts: secondary and primary research. Literature used was found in the fields of e-commerce, packaging logistic and supply chain management.

Sources included articles, journals, publications and media as well as reports and newspaper form the NVC - Netherlands Packaging Centre- database.

The primary research was split into two parts: a pre-study and a case study. Both the studies aimed at gathering data on topic of Dutch e-grocery market, the packaging thereof and involved logistics.

The pre study was performed through interviews with packaging and food packaging professionals. The interviewing followed the

Empirical data from primary research	Theoretical assumptions from secondary research
- Hub and spoke network affects delivery businesses' offer and consumers' choice - High density grocery stores decreases the number of e- grocery orders - Reputation is factor that influences consumer behavior toward shopping online - Some foods require to be delivered already packed (primary packaging) and this positively affects the fulfillment - Coolant packaging is being used to solve food product removal from the cold chain - Material affects the environmental value of packaging the most - Packaging solutions suitable for e- grocery do not need to be attractive or for informative purposes - Customizing could be improved through packaging elements	- Last mile delivery is the most costly and polluting echelon - There are environmental benefits based on emission and solid waste of returnable packaging - Traditional and online food packaging has same functions - A holistic approach is needed to develop packaging solution suitable for the e-commerce

Matching Points
- Last mile delivery is the most important element of the order fulfillment process - Difficulties arise when managing the packaging return system - Difficulties arise when controlling temperature along the last mile of food supply chain - Challenges for packaging design for e- grocery is the packaging of mixed loads

Figure 1. Comparative review of findings

seven-step process of gathering data and gaining knowledge from individuals (Kvale, 1996). The case study allowed for the improvement of an existing secondary food packaging implemented by an e-grocer, deKrat.nl, Amsterdam, which provides home delivered meal foodstuffs in wooden crates. The case study aimed to illuminate decision made by the company regarding their packaging system (Yin, 2003). Tools that were used included the packaging and logistic interactions chart (Hellstöm et al., 2006) and the packaging scorecard (Olsmats& Dominic, 2003).

The unit of analysis during the primary research was the secondary packaging solutions currently used in Dutch e-grocery market (pre -study) and the wooden crate (case study). The reliability of data was possible through triangulation, which validates information gathered from the different sources. The sources of this thesis were literature, interviews with different packaging professionals, observations and packaging evaluation methods.

Results

Results that have been derived from both primary and secondary researches were compared in order to assess similarities and identify food product and logistic requirements.

Figure 1 summarizes main findings from both the researches and their conforming points.

Findings at deKrat.nl

An analysis of strengths and weaknesses of the packaging system of deKrat.nl was performed after an overview of the company itself, the product sold, the packaging used and its supply chain. The three levels of packaging within this system are: wooden crate, foldable plastic crate provided by third party logistics and EU pallet.

The pear crate was subject to improvement as it was the most used crate. The packaging's strengths included its incorporation in the company's branding strategy, as it displays the company's local and sustainable image. It is recognizable, classic, recyclable and has a low purchase cost. The most problematic areas included; volume efficiency, stackability, product protection, and packaging return management and used material.

The wooden crate used by dK showed to be unstackable when filled with produce, increasing delivery time due to many handlings, increasing inter-product damage during transportation, inducing a high percentage of transported air. This in combination with the face that the wooden crate is returned too little (30% of the entire flow) therefore susceptible to moisture and mould damage at the customers' homes revealed that there was room for improvement.

Requirements and Problem Alignment

The identified requirements were combined with the recognized problems within the case study in order to define features that the proposed solution must have to fulfil the identified food product and logistic requirements.

Product	**Logistics**
Consider the mixed load of different food goods (type, size and quantity) and/or primary packages and interactions amongst them	Consider the way groceries are delivered to consumer
Temperature control over time essential for product quality assurance	Facilitate the packaging fulfilment
Protect fragile product from mechanical damage	Reduce the number of truck freights

Proposed Solution Description

The proposed solution is an improvement of the Pear crate (500x300x200)) used by deKrat.nl in relation to the identified requirements and designated problems at deKrat.nl. This solution integrates three strategies: packaging, logistic and product. Respectively, the new solutions makes use of: two interlocking packaging separators sheets made by coloured EPS, a deposit-refund system (5 euros per crate), and a time-temperature indicator. The solution development process included three stages: planning, designing and developing of visualization models in both 2D and 3D.

The proposed improvements allow to avoid the use of the transportation packaging, thus increasing the area efficiency by 28.6 % and the volume efficiency by 31.1% (Cape Pack) of its palletisation. Finally trade-offs between the current and the new crate were analysed, showing that the increased packaging cost (from 0.30 to 5.00 euros) and the negative environmental impact of the material used (EPS)

is in balance with improved product protection and transport efficiency.

Discussion and Further Research

Logistic requirements

Most of the difficulties related to e-groceries occur during the last mile: the home delivery process. In the context of e-commerce, the definition of secondary packaging (packaging that contains many primary packaging) expands, due to the presence of mixed load in e-groceries. It is critical to facilitate the fulfilment of the mixed load in secondary packaging for food in e-commerce, done by introducing packaging components that separate different food category inside the packaging itself. Transportation and delivery issues stress the need for stackable secondary packaging solutions, which in turn improves cube utilization and avoids the use of unnecessary packaging levels. Returnable packaging is a packaging system, suitable for e-commerce, which consists of elements such as the design of reusable packaging and deposit-refund management. A deposit refund system seems to be more efficient than electronic identification for food packaging, which fails to cover the low margin revenue of e-grocery sales.

Food product requirements

Food quality assurance does not depend on the supply chain (online vs. traditional), but on the distribution network for e-groceries Food produce needs to be kept the cold, in the cold chain, to reduce the possibility of microbial product damage. Current coolant packaging solutions show to be inefficient, and can only keep food at the correct temperature for a certain amount of time. Temperature indicators ensure customer that the product has been at right temperature along the supply chain. Product protection refers also to reducing mechanical induced inter- product damage due to transportation. Separating and insulating the different food categories loaded within secondary packaging can ensure product stay protected from mechanical damage and possible moisture damage.

Features of secondary packaging suitable for e-grocery

Applying these results during the secondary packaging solution for the case study, deKrat.nl, resulted in finding suitable value-adding packaging features to address the above mentioned requirements. Separators that can be manually place in the secondary packaging facilitate eased fulfilment, as predetermined areas can be created for certain food types. Separators can increase the stackability of packaging, as it provides a larger surface area on which more crates can be stacked. This increased stackability influences volume efficiency and reduces the need for extra packaging levels such as transportation packaging. Time temperature indicators increase quality to assurance and a deposit-refund system can induce incentive for customers to return the used packaging levels. These features indirectly affect the environmental impact during transportation and packaging production.

Further research

The findings from performed research can be used as foundation for further research. A possible next step would be the implementation of the packaging solution into the supply chain of and e-grocer, and measure the packaging's performance in regards to the performance measures referred to in this thesis. Research could be done toward the primary packaging of food products, suitable for mixed loads in e-commerce. Other requirements that could be further researched are marketing requirements and direct environmental requirements related to e-groceries, as these can also influence the design, impact and functionality of a packaging.

The ever-changing trends in, and continuous evolution of e-commerce brings rise to potential study topics. Trends such as the use of omni-channel retailing in e-commerce, increased consumer need for personalized orders and the possibility of rapid globalization of start-ups, among others trends, drive the evolution of the packaging used in this sector.

References available in the full text document online.
http://www.plog.lth.se/education/fipdes/

New insights into starch hydrolysis kinetics during bread digestion:

impact of production method and contribution of salivary α-amylase

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Background and Objectives

Nutrition is a complex phenomenon for which not only the type and quantity of macro- and micro-nutrients ingested, but also the rate at which such nutrients are digested and delivered to the body are considered key factors. Specifically, it has been recognized that slowing down carbohydrates' digestion rate has the potential to improve certain metabolic conditions, promote satiety and prolong physical performance during endurance exercise [1]. Carbohydrates are a major source of energy in human nutrition [2], and starch is generally the main source of digestible carbohydrate in our diet [3], accounting for 20-50% of the total energy intake [2].

During digestion, starch undergoes extensive transformations throughout the gastrointestinal tract. Its digestive process starts in the oral cavity. As saliva is mixed with food particles, the enzymatic degradation of starch is initiated by salivary α-amylase (the main enzyme in saliva) [4]. After swallowing, gastric digestion is initiated and starch hydrolysis will proceed due to both acidic action of gastric juice, and enzymatic action of salivary α-amylase [4]. As no amylase is secreted into the stomach, only salivary amylase is considered responsible for enzymatic hydrolysis of starch up to this stage [4]. It is known that this hydrolysis can continue until the pH lowers sufficiently to inactivate salivary α-amylase [5], however, the extent to which this enzyme contributes to starch digestion is not fully understood yet [3].

The Glycemic Index (GI) is a reference tool that gives an indication of the rate at which carbohydrates are digested and absorbed. A low GI diet has been linked with a positive impact on important health markers such as

lower levels of LDL-cholesterol (low density lipoprotein-cholesterol) [6] and improved glycemic control [7]. It is then clear that in order to understand how the GI of starch-rich foods can be improved, it is necessary, on one hand, to better understand the starch digestion process and, on the other, how it can be affected by different food properties. For its rich starch content and the role it plays on our diet, bread is a good model food to study both of these elements. Additionally, bread density has been considered by some researchers the most important factor influencing the digestion rate and, thus, the GI, of this food (higher density being associated with a lower GI) [8]. However, the factors that govern this relationship are not fully understood yet [8-10].

The main goals of this work were therefore to clarify the contribution of salivary α-amylase to starch digestion and to gain a better understanding of the relationship between bread density and GI.

Materials and Methods

Three types of baguettes with different densities were studied: industrial, traditional and whole-wheat. The industrial and traditional baguettes had a similar formulation but differed in the production process what led to finished products with distinct densities (0.16±0.01 and 0.25±0.02, accordingly). The whole-wheat baguette had a distinct formulation and its production process was not known, its density was close to that of the traditional baguette (0.26±0.01). The starch content of these baguettes, as well as the proportions of digestible and resistant starches were determined and their GIs was predicted *in vitro*.

The enzymatic activity of human salivary α-amylase at pH levels between 2 and 8 was studied according to an adapted version of Bernfeld's methodology [11].

Different sets of trials were carried out *in vitro* to simulate the initial stages of digestion- oral and gastric processing.

Oral processing was simulated by artificially chewing a standard volume of baguette crumb using a domestic kitchen food chopper, and mixing it with a pre-defined volume of fresh saliva (standard bolus) or water (control bolus) (this *in vitro* procedure was previously validated by comparing the results obtained with standard *in vitro* boli and in vivo boli for each baguette). Immediately after being formed, each bolus was submitted to an independent digestion in a gastrointestinal digestion simulating system, DiDGI®, developed at the French National Institute for Agricultural Research (INRA) [12]. DIDGI® consists of consecutive compartments, designed to mimic gastric and intestinal digestion. It is controlled by a computer software (StoRM®) which allows to modify and monitor conditions such as temperature, chime stirring, pH variation kinetics and flows of digestive secretions [12]. In this study, DiDGI® was used to simulate the gastric phase only. Temperature (37 ºC), pepsin flow and chime stirring conditions were equal on all trials. The type of bolus submitted to gastric digestion and the gastric pH were selectively modified to study different elements, including the contribution of salivary amylase to starch digestion and the influence of bread structural properties on the starch hydrolysis rate. An overview of the types of trials carried out and the variable parameters is presented in Table 1.

Trials	Simulated conditions	
	Oral phase: bolus type	Gastric phase (1 hour): pH
I	Standard	pH 6 to 2 from 0 to 30 min, pH 2 from 30 to 60 min
II	Control (no amylase)	pH 6 to 2 from 0 to 30 min, pH 2 from 30 to 60 min
III	Standard	constant (pH 6)

Table 1. Overview of in vitro digestion trials carried out and variable parameters.

Results

Bread characterization: The starch content and profile, and the GI of each baguette are presented in table 2. The baguettes with similar formulation (industrial and traditional) also had similar starch contents and profiles. The whole-wheat baguette had a lower starch content than the others, and in terms of starch profile, it only differed from the industrial baguette for having a higher level of resistant

Baguette	Starch (average % of bread [w/w] ± SD, 3 rpt.)	Digestible Starch[1] (% of total starch)	Resistant Starch (average % of total starch ± SD, 3 rpt.)	Predicted GI (average ± SD, 3 rpt.)
Industrial	43,41 ± 1,31[a]	95.9	4.1 ± 0.6[a]	100 ± 3.2 [a]
Traditional	42,26 ± 4,29[a]	94.7	5.3 ± 0.6[a;b]	78.6 ± 3.7[b]
Whole-wheat	33,98 ±1,08[b]	91.4	8.6 ± 1.3[b]	86.2 ± 4.3[a;b]

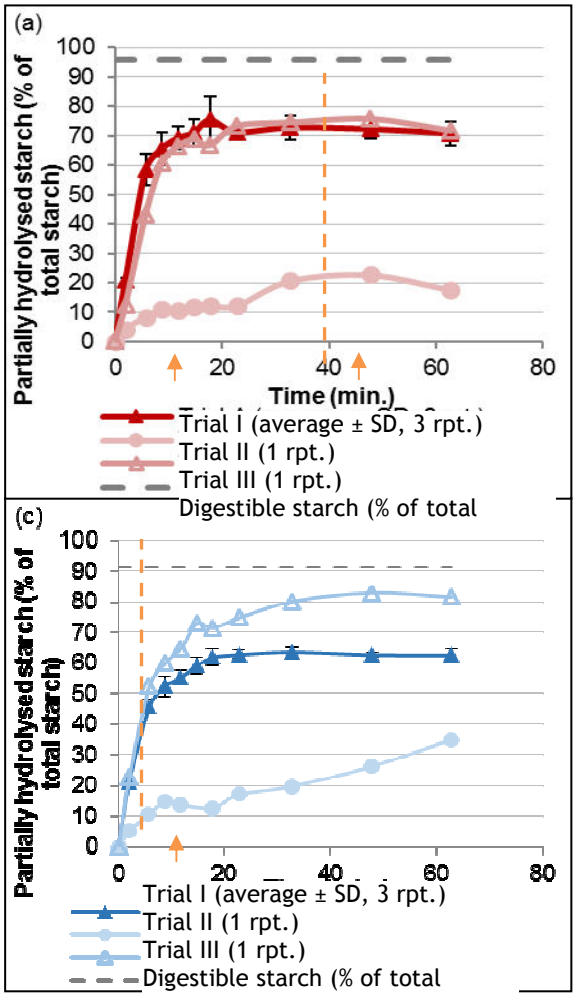
[1]Calculated as the difference between total starch and resistant starch; Different letters (a-b) within one column denote statistically significant differences (P<0.05).

Table 2 - Starch content and profile, and GI of the industrial, traditional and whole-wheat ba-

starch. The industrial baguette (lowest density) also had the highest GI and was thus used as the reference food for all GI related calculations (GI=100, Table 2). Contrarily, the traditional baguette (higher density) presented a significantly lower GI, 78.6 ± 3.7. The whole-wheat baguette had an intermediary GI which was not significantly different from the others.

Salivary α-amylase characterization: The highest enzymatic activity was observed above pH 6; 50% of maximum activity was still found at pH 4-4.5 and the enzyme became inactive at about pH 3.

Contribution of salivary α-amylase to starch digestion: The results of the *in vitro* oral-gastric dynamic digestion are presented in Figure 1 for the industrial (Figure 1a), traditional (Figure 1b) and whole-wheat (Figure 1c) baguettes. The three starch hydrolysis curves presented for each type of bread illustrate the results obtained with trials I, II and III. The results obtained with I-type trials demonstrated that salivary α-amylase continued active dur



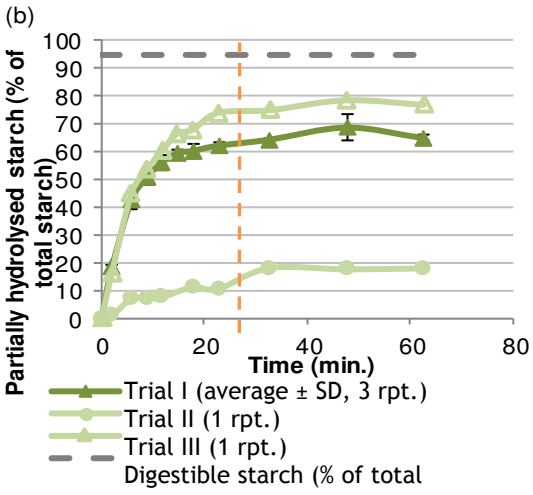


Figure 1. Comparison of starch hydrolysis rates obtained with trials A, B and C for the (a) industrial, (b) traditional and (c) whole-wheat baguettes. - - - Denotes the start of the in-vitro gastric processing. ↑ Denotes the moment when pH 3 was reached (salivary α-amylase inactivation) during I and B type

ing a simulated gastric phase of digestion. Additionally, comparing these results with those of the control study (type II trials) revealed that the enzyme was responsible for partially hydrolysing up to 50% of bread starch. To the best of our knowledge, this was the first time

the overall contribution of salivary α-amylase to starch digestion was determined. The amylolytic process followed an exponential kinetics in which the plateau was achieved within the first 20 min of digestion, before pH 3 was reached causing enzymatic inactivation.

In the presence of salivary α-amylase, the results found at constant pH (type III trials) and under acidifying conditions (type I trials) were similar for the industrial (Figure 1a) and traditional (Figure 1b) baguettes. This revealed a high effectiveness of the enzymatic hydrolysis, as similar performances were kept even under what could be considered a physiologically fast pH reduction kinetics (trial I). Furthermore, such results appear to uncover the existence of another form of starch resistance to gastric digestion as there is a percentage of digestible starch that resisted digestion up to the gastric phase. I possible origin for this phenomenon is the role played by the gluten network in reducing accessibility to starch.

Influence of bread's structure on starch hydrolysis: A comparative view of the starch hydrolysis curves obtained during type I digestion trials for the three baguettes is presented in Figure 2. As it can be observed, there is not a clear difference between the starch hydrolysis plateaus reached during the simulated digestion of the three baguettes, however during the first 15-20 minutes of digestion, hydrolysis was faster for the industrial baguette than for the traditional one. The evolution of the physical structure of bread throughout digestion is likely to be a key element governing this relationship

All digestion trials were carried out without taking into account gastric emptying. However, it is known that in vivo, gastric emptying of a solid meal starts during meal ingestion and on average, the first emptying episode can occur as early as around 7 minutes after the meal starts [13]. Therefore, by the time gastric emptying can be initiated, the results (Figure 2) show that there will probably exist more starch partially hydrolysed and solubilized in the chime upon digestion of the industrial baguette than of the traditional one.

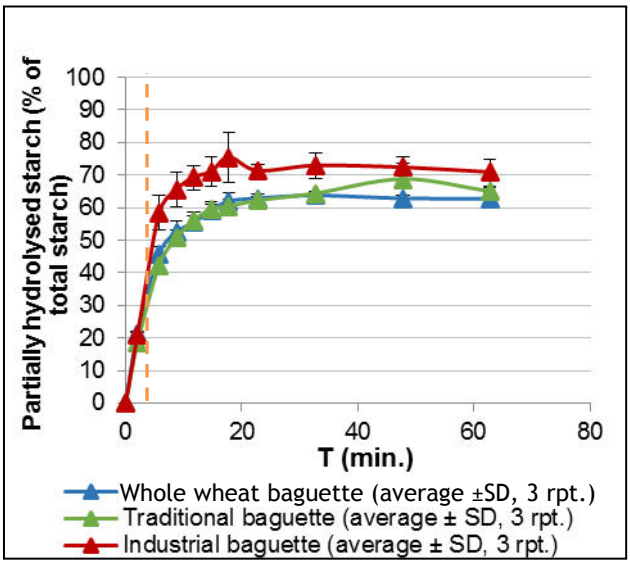


Figure 2. Starch hydrolysis during I-type digestion trials. Comparison of results for all baguettes.

- - - Denotes the start of in-vitro gastric processing.

As a result, in the case of the industrial baguette, more partially hydrolysed starch can be made available for further processing in the small intestine earlier, and therefore, higher amounts of glucose can be absorbed into the blood stream faster originating a higher glycemic response. This is in agreement with the different GIs found for these two breads (the industrial baguette having a significantly higher GI, Table 2).

Additionally, there appear to be no differences in the proportion of partially hydrolysed starch before gastric processing started for each bread (first point of the curves, Figure 2), and the rate at which salivary amylase hydrolysed starch in the beginning of the simulated gastric phases (Figure 2) was well correlated with the digestibility indicator (GI- table 2). These results highlight the significance of salivary α-amylase's activity during gastric processing for the determination of the GI.

Conclusions and Perspectives

It was shown that when formulation was kept similar, processing conditions leading to higher bread density resulted in a significantly lower GI. Such knowledge can be highly valuable in the future for the improvement of the GI of bread through simple changes in processing conditions.

Moreover, to the best of our knowledge, this was the first time it was demonstrated that salivary α-amylase plays a significant role in the digestion of bread starch during gastric phase of digestion. These findings open new opportunities to the design of innovative food ingredients or formulations with the potential to induce a decrease in the glycemic response through the incorporation of α-amylase inhibiting constituents.

Finally, the understanding of salivary α-amylase's action, allows one to ask other questions that can be relevant in terms of public health and promotion of adequate nutrition patterns. Specifically, it would certainly be interesting to verify whether the glycemic response to starch-rich meals can be decreased through an appropriate matching of starchy foods with other low pH foods or drinks as this could lead to the formulation of simple dietary advices that could be easily followed by most people.

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Sensory evaluation of complex gels by dynamic methodologies

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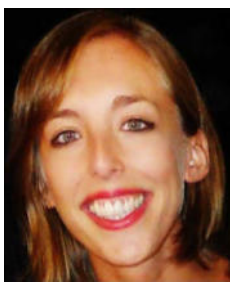

Introduction

In the last decades, the growing concern for health and quality of life has encouraged people to limit the consumption of food rich in sugar (de Oliveira Rocha & Bolini, 2015). Food industry has the challenge to reduce the amount of sugar in foodstuffs without compromising food functional and sensory quality. Sugar replacement has opened new horizons for new ingredients development to improve the nutritional status of a food product. Sweeteners have been widely used as sugar replacement, however in the last decades they have also been associated to healthy problems (Hill et al., 2014).

In this research for new ingredients, sweet proteins have become low-calorie sweeteners potentially appealing for food manufacturers and beneficial to those who need to control sugar intake, such as obese or diabetic people (Rega et al., 2015). The three best studied sweet proteins are monellin, brazellin and thaumatin, but even though their structure has been well characterized, their mechanism of action has not been elucidated yet (Picone et al., 2012).
Sweeteners together with food hydrocolloids are among the most common used ingredients in food industry, contributing to both functional and sensory properties. Food hydrocolloid applications and rheological properties are very broadly studied in order to assess all the functional features and quality control in product development and to correlate them with sensory perception (Agudelo, Varela & Fiszman, 2015).
On the other hand, it is known that when stimuli from different sensory modalities (visual, auditory and tactile) are presented simultaneously, suppressing or enhancing effects occur (Verhagen & Engelen, 2006). Color, is one of the parameters that has shown to have a strong impact on the ability

of subjects to identify food and beverages, with uncolored and/or inappropriate color items being identified correctly less frequently than appropriately colored items (Delwiche, 2014).

Aims

The first part of this research was aimed to characterize simple gels (sweetened gels) by using time intensity method. Sensory data were also correlated with mechanical curves in order to understand if there was an impact of the structure on the sweetness perception of commercial sweeteners and sweet protein.

The second part of this thesis was aimed to characterize complex gels (sweetened, acidified, flavored and colored gels) by using temporal dominance of sensation method (TDS) in order to develop food models like "jellies". Sensory data were also compared with mechanical measurements in order to understand if there was an impact of coloring agents on gels' structure therefore on sweetness perception.

Materials and Methods

1. Characterization of simples gels

The samples were prepared by using mineral water Sant'Anna.

The sweeteners used were: aspartame (granular aspartame PSL E951, Tillmanns s.p.a., Milano, Italy), saccharin sodium (BP, A.C.E.F s.p.a., Fiorenzuola d'Arda, Italy), sucrose (Eridania, Bologna, Italy), and the sweet protein MNEI. The MNEI, mutant single-chain monellin, was produced in E. Coli and purified, following the experimental protocol of Rega et al. (2015) and it was provided by the Department of Chemical Sciences of the University of Naples Federico II. As gelling agent agar powder E406 (A.C.E.F s.p.a., Fiorenzuola d'Arda, Italy) was used.

A stock solution of 1mg/mL of MNEI was prepared by dispersing the protein in water and agitating in a vortex (MS3 digital, IKA®, USA) until a complete dissolution.

Solutions of aspartame (0.21g/L), saccharin (0.1g/L), sucrose (40g/L) and MNEI (0.013g/L), were prepared (Sartorius scale, BP61, Germany).

Gel samples were prepared at three total agar concentrations – 1%, 1.5% and 2% w/w – for each sweetener.

1.1 Time Intensity (T-I) method

Subjects

Ten selected assessors (two men and eight women) participated in the time-intensity test for the evaluation of sweetness intensity in simple agar gels.

Sensory evaluation was performed in the Sensory Laboratory of the Department of Agricultural Sciences of the University of Naples Federico II.

T-I methodology

Ten trained assessors evaluated the sweetness temporal profiles of aspartame, saccharin, sucrose and MNEI in aqueous solution and in three different agar gel concentrations (1-1.5-2%). Each sample was served in a plastic cup and plate respectively, and coded with three digit random codes. First assessors put the sample in their mouths and started to score the intensity of sweetness perceived. Then, they moved it around, chewed it for 10s in the case of gels and spat it out after 5 seconds, and continued to score the sweetness intensity, for a total of 90 seconds. To record responses, the assessors moved the mouse cursor along a vertical linear anchored scale from 0 (no perceived) to 10 (highest intensity). A period of 60 seconds between two samples evaluation allowed the assessors to rinse their mouths out with water. At each session, assessors evaluated all the samples presented in a randomized order. Four replicates of t-I test were carried out.

2. Characterization of complex gels

Citric acid (citric acid Kosher, Sigma Aldrich, USA) (0.5g/L) was used as an acidifying agent.

Food red and green colorings (Estratti Liquori Affini, Napoli, Italy) (10mL/L) and spray dried wild fruits flavors (Arda Natura s.r.l., Piacenza, Italy) (0.010g/L) were also added to the mix in order to prepare complex gels similar to jellies.

Only 1 gelling percentage was chosen (1.5%).

The same sweeteners were used at the same concentration, excepting MNEI (0.039g/L).

2.1 Temporal Dominance of sensation (TDS) method

Subjects

Thirteen selected assessors (two men and eight women) participated in the time-intensity test for the evaluation of sweetness intensity in simple agar gels.
Sensory evaluation was performed in the Sensory Laboratory of the Department of Agricultural Sciences of the University of Naples Federico II.

TDS methodology

12 complex gels (3 coloring conditions for 4 sweeteners) were prepared in order to evaluate the dominant attributes over time. Samples (2 mL) were presented in a balanced and randomized order to the assessors; coded plastic plates, were used to serve them. The first session was dedicated to attribute generation; three samples were simultaneously presented to the assessors and the attributes cited by at least 50% of the assessors, in at least one sample, were selected. During the evaluation time (120 seconds), the assessors tasted the product and immediately chose the attribute considered as dominant from the attributes list provided; when the dominant perception changed, the assessors had to select the new dominant sensation. The order of attributes on the screen was randomized among the assessors to minimize the effect due to their sequence. There was a break of 60 seconds between evaluations. At each session, assessors evaluated all the samples, presented in a randomized order. Four replicates of TDS test were carried out.

3. Mechanical evaluation

Short cylindrical simple and complex gel samples (13mm in diameter and approximately 13mm in height) were submitted to uniaxial compression tests by using a dynamometer Instron 4467 (Instron Int., England) with a 100N load cell. Compression was applied at a crosshead speed of 10mm/min up to strain of 50%. All measurements were done at ambient temperature (23±1˚C).

Four replications of each gel sample were performed.

4. Data analysis

T-I and TDS methodologies

Data were collected by FIZZ software (ver. 2.45 G, Biosystemes). Trapezoidal t-I curves were built according to different parameters extracted (I_{start}, t_{Start}, I_{max}, t_{SPI}, t_{EPI}, I_{end}, t_{End}). SPSS v.16 (SPSS statistics, Chicago) was used to analyze the parameters extracted from the t-I curves.

TDS curves and difference curves were plotted according to Pineau et al., (2009). The dominance rates of the sensory attributes for each evaluated sweetener were calculated by means of FIZZ Calculation software (ver. 2.45 G, Biosystemes).

Mechanical evaluation

Mechanical data were collected by INSTRON Series IX software v.8.25.00. One-way ANOVA was performed on mechanical data. Duncan tests were performed for all samples using SPSS v.16 (SPSS statistics, Chicago).

Results and Discussion

TI results-

Average trapezoidal t-I (Fig. 4.1) curves were built according to results obtained. Standard errors were also reported for the coordinates.

Looking at the curves (Fig. 4.1) it is possible to see that in solution MNEI presented a significantly different sweetness profile over time compared to the others, regarding in particular the I_{max} and t_{End}. Indeed, generally (Fig.4.1) sweetness intensity decreased at increasing gelling percentage, especially in the case of the MNEI protein, for which sweetness perception was negatively affected by the structure. Saccharin showed less variance

compare to the other sweeteners, while sucrose and aspartame followed a more similar profile. Generally for all the sweeteners no big differences were found between 1.5 and 2% of gelling agent.

As far as dynamic temporal profiles of the investigated sweeteners, results were quite different from the previous study (Di Monaco et al., 2014). MNEI sweetness in aqueous solution showed a different profile from aspartame; in fact, differences were found in terms of parameters extracted (I_{max} and t_{End}) of their t-I curves. Regarding the other sweeteners, MNEI differed from saccharin and sucrose at the beginning of sweetness perception (t_{Start}), and from saccharin also at the end (t_{End}), as well as in the maximum intensity (I_{max}). So the sensation conferred by MNEI generally started to decrease earlier than the other sweeteners, at different times, and it was extinguished completely, underlining a different mechanism of interaction between the protein and sweet taste receptors as has been reported by different studies (Spadaccini et al., 2003; De Simone et al., 2006;), Assadi-Porter et al., 2010).
According to Asakura et al. (2015), sweet proteins, such as MNEI and thaumatin, exhibited stronger interactions with the taste buds showing a long-lasting taste perception; however, these results were not as those expected although the concentration used was 3 times lower (13mg/L) than the one used by those researchers (40mg/L).
Moreover, the volume of the tested solutions was much lower than that used in the previous studies (Di Monaco et al., 2014). The lower volume was chosen in order to use the same volume of the gel samples and to compare the results. The volume of each gel sample was around 2mL, so 2mL of solution were tested instead of 10mL as it appeared in previous works. This large difference in volume could be related to the decreasing of sweetness perception therefore to the dynamic sensory profile; however there are not studies in literature focused on this specific topic. Kamarunas et al. (2015) suggested a fourfold increase in liquid volume for older adults to perceive an approximate perception of volume compared with younger healthy adults. These findings regarding the effect of liquid volume on perception could also explain our results regarding MNEI. Probably, when the sweet taste is given by a molecular used at very small concentration, also the volume of solution in which it is dissolved could affect the sweetness perception over time and could explain the difference between the present work and the previous one (Di Monaco et al., 2014).
The main objective of this first part was to study the effect of gelling percentage on sweetness perception imparted by different sweeteners and, the dynamic temporal profiles of the sweetened gels showed an impact of texture on sweetness perception as it was expected, especially in the case of MNEI.
The results showed that different gelling percentages affected sweetness perception more than sweeteners affected gelling structure; only small differences were observed at low agar concentration (1%). In the case of MNEI,

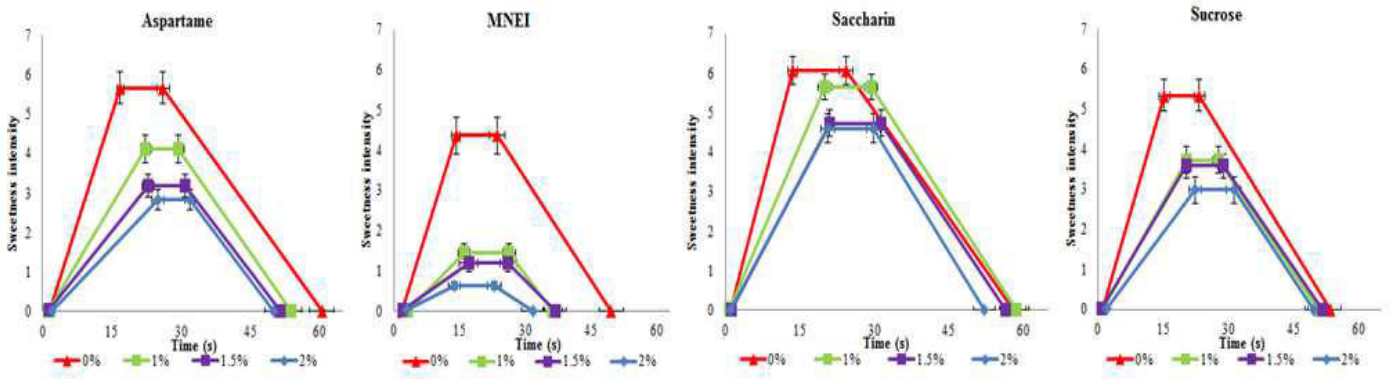

Figure 4.1. Average trapezoidal t-I curves for all sweetened samples.

there are not data supporting or explaining these results; only studies carried out with thaumatin were found in literature (Ohashi et al., 1991) showing the interaction between that sweet protein and hydrocolloids, and the effect of hydrocolloids such as carrageenan on the reduction of sweetness intensity.

TDS results

The second objective of this study was to characterize complex sweetener gels by using TDS method and to correlate the data with mechanical measurements in order to better understand if there was an impact of the coloring agent and sweeteners on the structure. In this work, only agar gel at 1.5% concentration was used due to the desired tough texture (Banerjee & Bhattacharya, 2011) and also regarding the previous results that showed less effect of sweetener on the mechanical properties of the gels.

For the characterization of complex gels, MNEI concentration was increased by three times in order to obtain gels that really tasted sweet. Moreover, the pH of MNEI stock solution, as well as the other sweeteners stock solutions, was modified (pH=3) in order to put the sweet protein in its best conditions according to Rega et al. (2015); however TDS curves did not show sweet attribute as dominant for this sweetener in colored gels but only for uncolored gels. These results could suggest a possible interaction among MNEI and coloring agents' therefore further studies should be performed in order to understand what kind of interaction took place.

In contrast to MNEI, sweet attribute was dominant for the other sweetened gels. However, also an interaction of coloring agent was observed for aspartame colored gels that showed less dominance rate for this attribute than that uncolored gel.

Most of attributes characterized as dominant were related to gel texture. This could be explained by the fact that the pH of agar gels was decreased (pH<6) by affecting the strength of the gel and by giving a more brittle consistency.

On the other hand, mechanical measurements showed an impact of coloring agent on texture gels. Red coloring agent affected fracture stress values of MNEI and saccharin gels, while green coloring agent did not. However, both coloring agents, red and green, increased the fracture strain of sucrose.

Conclusions

Several interesting results were obtained from this research, especially regarding the MNEI sweet protein.

Dynamic sensory methodologies were useful tools to evaluate sweetness perception over time and to obtain information on investigated sweeteners, in particular MNEI. Differences regarding sweeteners in solutions and gels were reported by time-intensity method which showed a decrease of sweetness intensity perception when sweeteners were added in a solid matrix. TDS method proved to be a very interesting sensory approach, providing information about the complexity of MNEI evaluation. However, the visual effect of color on taste/flavor perception was not reported.

The main interesting results of this work were related to MNEI. Previous studies (Di Monaco et al., 2014; Rega et al., 2015) proved that this sweet protein can be used in beverages; however the present work showed an effect of more complex systems like gels on sweetness perception, suggesting an interaction of the protein with the hydrocolloid. Therefore, further studies should be performed in order to better understand this interaction by using different gelling agents. Moreover, MNEI was the sweetener most affected by the evaluation conditions, such as the decreasing of solution volume, so, also in this case further studies should be done in order to determine the effect of volume on sweetness perception.

Results showed that MNEI protein appears to be very sensitive to different conditions like, agar interaction or coloring agent interaction; thus, it could be suggested the use of new technologies to add the protein to the food, like encapsulation, in order to protect it and its sweetness.

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Creating a new food category - Inputs from stakeholders in the Swedish market

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Introduction

The food industry faces a large number of challenges. For instance, market saturation, high competitiveness and a continuous challenge to meet heterogeneous requirements of different stakeholders around the food business. For that, companies need to understand the dynamic of its industry and built strategies to face those challenges and go beyond. Innovation could be used as a strategic change process that could lead to business growth and customer satisfaction. There are a couple of studies related to food innovation in the Swedish market. One previous study suggests that creating a new category could be considered one criterion for success to achieve innovation.

A category is considered a business unit and the creation of it involves a whole supporting system from food companies and suppliers, up to retailers. Creating a new category could not just be launching a new product but creating new market shares, new experiences and new solutions for the consumers, where the aims could be from indulgence and convenience to social and environmental impact.

Throughout this study, the author explored elements for the positioning of a new food category on the market based on insights obtained from some stakeholders within the Swedish food business.

Objective

The study was driven by the following research question:

How is a new food category successfully created?

To answer to this, three objectives were established:

1. Obtain insights about the meaning and relevance of a new category from different stakeholders.

2. Discuss the evolution of a successful positioning of a food category.

3. Discuss the role/contribution of the selected stakeholders.

Likewise, the purpose of the study was to identify the drivers and challenges in the creation of a new food category .

Methodology

An exploratory study was carried out with an overall qualitative approach, considering a mix-method following the same research question. The mix-method included literature survey, 15 semi-structured interviews considering two case studies: *Oatly (concept assortment)*and *Santa Maria (Tex-Mex assortment).* Thesewere selected as a result of the feedback of the interviewees and own research.The unit of analysis to be studied was the positioning of a new category.

The collected data was processedby triangulation of the multi-sources of evidence, and data analysis was performed by categorization (thematic analysis) and a cross-case analysis. Findings were made to build up the answer to the research question. Finally a set of conclusions and recommendations for future research was done.

Results and Discussion

Definitions and insights among stakeholders

A common interest towards the approach and purpose of the study among stakeholders was identified. Some overlapping concepts between a [new] category creation and new product introductions occurred. Overall insights were obtained: (1) *Among Retailers*: New introductions of products with a common element across different existing categories. A new category must not necessarily be a new product range but it can be the new way of delivery and usage.(2)*Among market research and academia*: Definition based on retailing management, as it is the store that defines how and where the products should be allocated on the shelf according to a category management approach, which attempts to maximize profits within a category. On the other hand, consumers could define what is a new category as they are the ones who decide what is included. (3) For *food manufacturers*: The creation of a new category could be a way of positioning their product at the market place (4) *For packaging suppliers*: More focus on market segments, however this could be an opportunity as a starting point of creating new market opportunities from their side.

Evolution of a successful positioning of a food category- considering drivers and challenges

A total of 50 drivers and 30 challenges to create a new food category were identified. *Communication* and *following market trends* were the two drivers that all the stakeholders mentioned. Three common drivers only mentioned by the two case studies (*Santa Maria* and *Oatly*): *upgrade, entrepreneurial culture and assertiveness*. Other key drivers such as *motivation to grow, alternative of use, uniqueness, targeting the untargeted and visibility*.

Regarding challenges, *market penetration* and *packaging opportunities* were the most mentioned among stakeholders.

From a retailer's perspective, when accepting a product, it can be the starting point of a category. Thus, to be in constant tracking of customers' behaviour is key. For manufacturers, to place products and concepts that could reach beyond the existing demand. Finally for packaging industries, it is important to work in collaboration with manufacturers to address the growth opportunities in the marketplace.

Furthermore, creating new market opportunities is not a static process but a dynamic one. This is a clear example of what happened to *Santa Maria* and what *Oatly* is facing at the moment.*Figure I* shows a brief summary of

the elements to consider when creating a category. This is based on insights obtained from the case studies and from the interviews with stakeholders.

Finally, it is important to keep in mind that the creation of a new category takes time and it is built step by step.

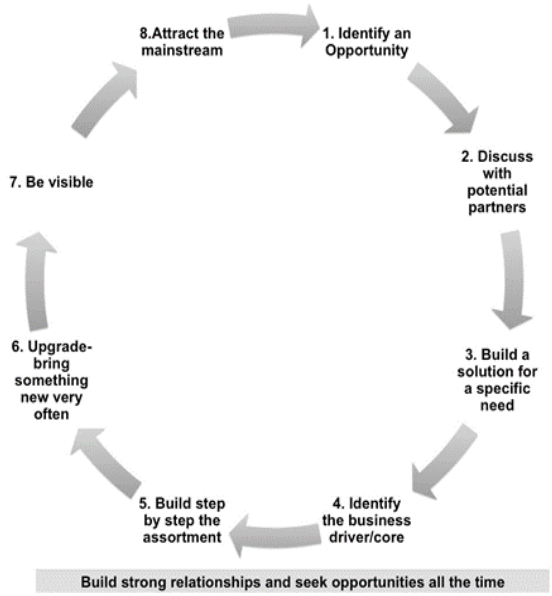

Figure I. Elements to consider when creating a category in a nutshell- *Based on insights from case studies and some stakeholders*

Role/contribution of the selected stakeholders

Each stakeholder plays an important role in the creation of a food category. Retailers and marketing research bodies are mainly oriented in the interaction between the consumer and the value chain. On the other hand, packaging and food manufacturers more oriented towards the connection of the product and its elements. Academia mostly takes role of receptor rather than developer, which means that provides service upon request, but in some cases, such as in *Oatly*, it can be the starting point of a product development with a business approach. Moreover, maintaining strong relationships between stakeholders is a key driver. For instance, retailer and food manufacturer could start a new category.

Conclusions

The topic of creating a new category in the food market is mainly a starting point of discussion of how a business unit in the food domain behaves and can bring new ideas and ways to address future challenges.

The creation of a new category is not a one-moment event but a process that needs evolution.*Figure II* represents an overview of how a new food category could be created.

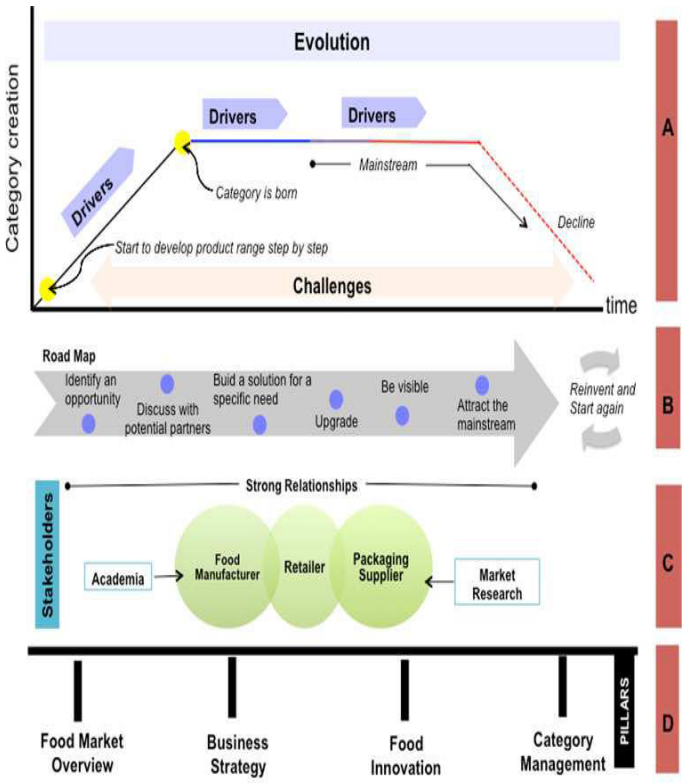

Figure 2. Overview of how a new food category could be created

A. General life cycle curve of a [food] category where drivers and challenges exist throughout all the cycle. The first section of the curve corresponds to the start up of a potential category and this could be with the introduction of one innovative product. Once a group of products share common targets and/or characteristics, a category is born.Then, the category starts to grow until it becomes mainstream and its time frame will be shaped according to

market needs.

B. Suggested road map of the creation of a categoryfollowing a chronological progress. The road map can be reinvented by constantly seeking new opportunities in the market place.

C. Corresponds to the role of stakeholders and the interaction among them. One key driver is to maintain strong relationships. Retailer and food manufacturer could start a new category. The marketing research body is mainly monitoring opportunities and giving the service and information to the units that develops the product and service. On the other hand, packaging suppliers play a role in the development of alternative products as a solution provider of added-value elements to differentiate a product that could help to start a new category. Lastly, academia plays a triple role, one as an educator of principles, also as a research body of new knowledge and as a collaborator with the industry.

D. Having a solid knowledge of the market behaviour from different angles (retailer, manufacturer and supplier) and to be up to date about the dynamic of categories can bring new opportunities. Likewise, considering a strategy following innovation principles could help to develop alternative and unique food solutions.

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The value of rib design in food packaging: from packaging company and consumer perspectives

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Introduction

Packaging and packaging design play an important role in food supply chains. Nowadays, with more competition, companies need to carefully consider their packaging designs and innovate continuously designs to enhance their competitiveness. Tetra Pak is not an exceptional case. Recently, the Tetra Pak Material Treatment team has started to get interested in rib designs and wants to apply these designs on Tetra Pak carton containers.

Ribbing is a popular design feature that has been applied for a long time to many food packages. However, a comprehensive understanding of the functions of ribs on food packaging has not been fully achieved yet. Lacking of this understanding brings Tetra Pak difficulty in evaluating whether their carton containers can inherit the rib design with its functions or not. Besides, missing the consideration of rib's drawbacks may lead to risks for the company when the ribs are applied on their carton containers. Therefore, there is a research demand of exploring all advantages and disadvantages of the ribs on plastic bottles, metal cans, and other food packaging. Based on the findings of this research, the company will be able to evaluate if their carton containers can inherit the rib's benefits and overcome the disadvantages of the rib design.

In addition, the team is interested in understanding the general perception of Swedish consumers toward the rib design as well as which rib designs on food packaging are favored by Swedish consumers. Therefore, there is a demand of researching Swedish consumers to know their perception toward the rib design and their most favorite rib design on Tetra Pak carton containers. Based on that, the team can choose the right design of ribs to apply for ensuring a

high success ratio of a new Tetra Pak container on the Swedish market.

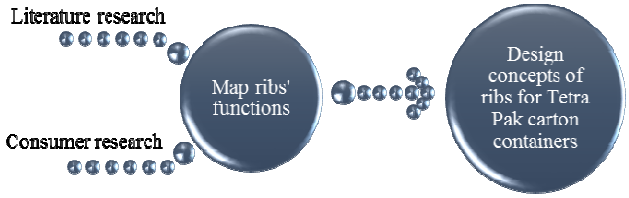
Objective

The main purposes of the master thesis is to:

- Map all functions of ribs on food packaging based on the evaluation in all aspects of a packaging system, from technical, economic, environmental aspects to marketing and consumer convenience.
- Propose rib design concepts for Tetra Pak carton containers.

Methodology

The research process carried out in this thesis is designed by combining quantitative and qualitative research. In particular, it consists of a literature research on food packaging with ribs, and two consumer studies which include face-to-face surveys in parallel with observation on 100 Swedish consumers. The collected empirical data is analyzed and compared by a statistical method - Chi-square test. Finally, a mind-map model is used to construct all ribs' functions on food packaging based on findings of the consumer and the literature research. The map of the ribs' functions and the in-depth understanding of the consumer perception create a basis for selecting the utmost potential rib designs to propose for Tetra Pak carton containers.



Results

Results from literature research

According to the literature research, the technical functions of rib designs on food packaging include (1) preventing deformation caused by the sterilization process and external pressures, (2) increasing top load strength, (3) enhancing resistance to bending, (4) reducing packaging materials and weight, (5) protecting coating layers from cracks, (6) assisting a sterilization process, (7) creating thermal insulation, and (8) maintaining the appearance of product packaging. Besides, rib designs help to minimize costs thanks to less packaging materials, high production and transportation efficiency, and less damaged products. Rib designs support reducing negative environmental impacts, such as reducing natural resource usage, energy consumption, and emissions caused by the production and the recycling processes. In terms of marketing and consumer convenience, ribs' applications provide better aesthetic appearance for packaging. However, if ribs are not designed in an appropriate way, they can hinder label and stacking processes as well as require high investment cost.

Results from consumer studies

Regarding metal cans, ribs do not provide any convenience functions for holding. Furthermore, the ribs reduce the readability of product information on cans' bodies.

Regarding plastic bottles, rib designs make plastic bottles more appealing and more convenient during handling and use. In terms of aesthetic appearance, the consumers prefer wavy ribs in vertical direction. With large bottles, the consumers prefer deep ribs over shallow ribs regarding the convenience.

Regarding drivers for purchasing decisions of the consumers in both groups, the most persuasive criterion that the consumers are willing to pay more for is environmental friendliness. Convenience and aesthetic appearance are important issues to appeal consumers but it is not clear whether the consumers are ready to pay more for them or not.

Comparison between two consumer studies

Regarding the attributes of the two researched groups in the two studies, the groups are different from the age ranges of consumers and the ratios of working areas. The consumers of Group 1 are mainly from 31 to 65 years old while the consumers of Group 2 are mainly from 20 to 40 years old or from 51 to 65 years old. Although there is a difference in the age ranges between the two groups, the largest age range of both groups is 31-40 years old. In addition, the variety of working areas in Group 2 is wider than the one in Group 1.

These differences can lead to the difference in the perception of the two groups. Therefore, a statistical method (Chi-square test) is used to check the correlation of the two groups and their perception.

According to the Chi-square tests, there is no significant difference between both groups in the perception and preference toward rib designs on metal cans and plastic bottles. Moreover, there is no significant difference between the two groups toward purchasing decisions. Similar tests between two genders showed the independence between the consumer perception and gender. Therefore, the two groups can be gathered into one big group to evaluate the general perception, preference, and drivers for purchasing decision based on the proportion of consumers' choices.

Discussions

According to 100 consumers' responses, it is hard to conclude that the ribs provide any convenience (handle-ability) for cans in the consumer perception. Indeed, the observation showed that the consumers did not recognize the difference between a non-ribbed can and a ribbed can until the ribs were mentioned. The result can be explained by the fact that metal cans are firm, thus the little added rigidity from ribs' structure is hard to be recognized by consumers. Meanwhile, the metal cans used in these studies are 274ml cans, thus the consumers can hold them easily even for the non-ribbed can. However, it is certainly that the ribbed can is harder to read product information than the non-ribbed can because the ribs create an uneven surface on the can body.

Regarding plastic bottles, the results show that the wavy shape and vertical direction are the most preferred designs of ribs on plastic bottles by the Swedish consumers. Indeed, the observation revealed that some of the consumers who chose the wavy ribs preferred the embossed rectangular shape ribs when visualizing in the beginning. However, when these consumers handled the two bottles, they felt the wavy ribs made the bottle tighter fit their hands. Thus they changed their preference to the wavy ribs. In reality, some consumers are not detail-oriented people or in a hurry. Thus, they may select packages based on the aesthetic appearance rather than take time to "test" packages before buying them. In contrast, if consumers are detailed-oriented or have more time to consider, they may chose packages based on convenience, as what is found in this research. Therefore, in order to attract consumers and create the loyalty to the product brand, packaging companies need to include both attributes - aesthetic appearance and convenience in their design, especially when packaging companies focus on on-the-go products or large containers. In terms of convenience, the deep ribs support the consumers in grabbing heavy bottles easier. According to observation, the female consumers in both groups felt difficulties when handling the large bottle without ribs because they had quite small hands. Most of female consumers commented that the deep ribs helped to lock their hands and prevent the bottle from falling down. Thus, the deep rib design is applicable for large containers and helps packaging companies reach consumers who are women and children.

Regarding drivers for purchasing decisions, 46% of the Swedish consumers taking part in this research are willing to pay more for the aesthetic appearance. Besides, 57% of the consumers are willing to pay more for the convenience function. Indeed, the two largest subgroups in the group who are ready to pay more for the convenience function are the 31-40 year old group and the 51-65 year old group. It can be explained by the fact that normally 31-40 year old people have children and handle large bottles (family size packages) often. Thus together with permanent income, they want to buy convenient packaging for their children to handle for reducing risk of spilling product. For 51-65 year old people, they may not have strong hands to hold large containers and they might have a good financial situation. Therefore, they are ready to pay a little bit more (1kr) for the convenience function. Based on this result, if Tetra Pak targets the 31-40 year old consumers or the 51-65 year old consumers, the company should focus on the convenience function in packaging design. Regarding the environmental performance, 82% of the consumers are ready to pay more for it. It can be explained by the level of

education of the consumers in these studies. The researched Swedish consumers are mainly employees in the packaging industry and young students who are living in Lund – a big city in Sweden. As a result, they may have a higher level of education and more recognition toward environmental issues than general consumers. Besides, there is a possibility that some consumers pretend to pay but do not do so in reality. This result indicates that if the company targets urban population, it should insist on the communication of the environmental benefits of the new designs to create a competitive advantage.

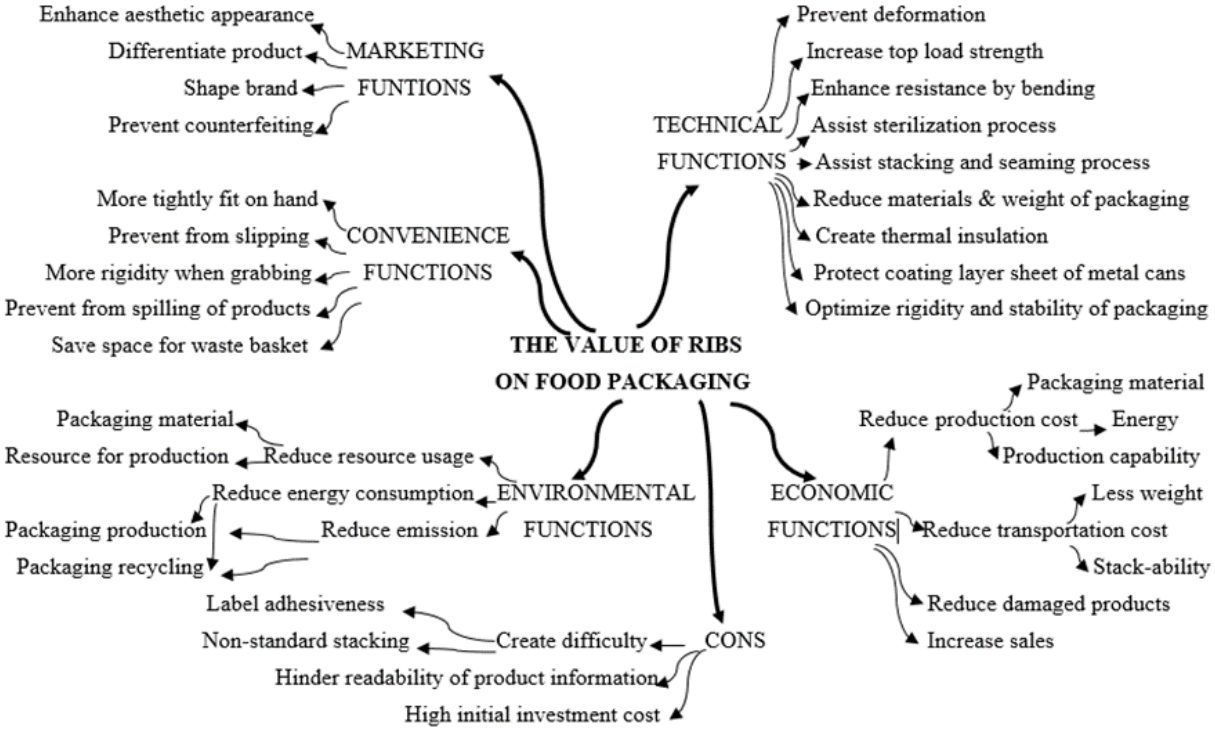
Conclusion

Rib designs applied to food packaging have both advantages and disadvantages but the former surpasses the latter. Originated from the demand of preventing deformation, ribs are applied to food packaging and lead to other advantages.

Regarding technical functions, rib designs help to increase top load strength, enhance resistance to bending, reduce packaging materials, and protect coating layers of metal sheets used to make cans from cracks, corrosion, and leakage. For carton materials, rib designs can create a thermal insulation function and optimize product stability. Regarding economic functions, rib designs help to minimize costs from using less packaging materials, increasing production capacity and transportation efficiency, and from less damaged products. Moreover, appealing rib designs also help to increase sales. In addition, rib designs can support to decrease negative environmental impacts by reducing natural resource usage, energy consumption, and emissions caused by production and recycling processes of packaging. However, rib designs carry some disadvantages related to label adhesiveness, non-standard stacking and high investment cost.

In terms of marketing, rib designs can enhance aesthetic appearance of packaging, differentiate products, shape brands, and prevent counterfeiting. In the perception of researched Swedish consumers in these studies, rib designs support convenience by making packaging more tightly fit on their hands and more rigid when grabbing, especially for large packages. In addition, consumers are willing to pay more for packaging that provides increased convenience or has less negative environmental impacts. However, rib designs may obstruct readability of product information.

The thorough understanding of ribs' functions and the consumers' perception provides a ba-



sis for proposing ten design concepts of ribs for Tetra Pak carton containers. In addition, insights into what drives consumers' purchasing decision help Tetra Pak to develop carton containers with ribs that approach successfully their target consumers and at the same time surmount one main ribs' disadvantage.

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Preparation and Characterization of Antimicrobial Packaging Films from Cricket Chitosan Enriched with Schisandra Chinensis Extract

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Profile in a nutshell:

Vita graduated as summa cum laude from BSc in Molecular Biology and Biochemistry (Charles University, Czech Republic) and has 4 years research experience in organic chemistry and plant biotechnology in Czech Republic and Japan prior to FIPDes.

During FIPDes, she gained valuable insight in food and packaging R&D, which led her not only to create novel active packaging polymer in South Korea, but also to co-found a waste-combatting & award-winning startup called FoPo, for which she will be working full time after graduation.

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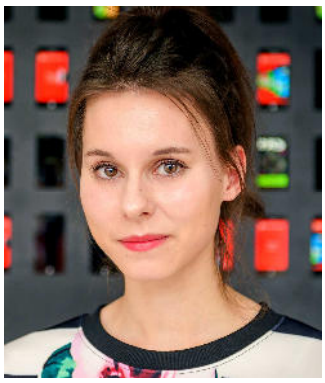

Introduction

Renewable, biodegradable and active packaging is currently considered to be a very important part of the research and development in packaging industry. Considering the negative environmental impact of oil-derived plastics as well as food losses due to microbiological spoilage or oxidative quality deterioration, packaging produced and disposed of in a sustainable way which can also prolong food shelf-life is certainly desirable.

Therefore, innovative bioplastics derived from microbial, plant or animal sources have been explored as replacement for conventional plastics. Currently, the main deterrent to mass use of bioplastics is their high production cost and in many cases inferior mechanical and physicochemical properties. These challenges can however be overcome with further research leading to production optimization and to exploration of blends and coatings which could improve the ability of the polymers to fulfill necessary functions.

One of such biopolymers currently being explored as potential source for manufacturing bioplastics is chitosan, which is partially deacetylated form of chitin, one of the most abundant biopolymers on earth. It is currently harvested from crustacean exoskeletons, namely crabs and shrimps, giving thus added value to formerly a waste product. It is a very interesting and versatile material, as it can form films when solubilized in mild acids, homogenized and casted. It has also inherent antioxidant and antibacterial properties.

However, with aquaculture experiencing issues with sustainability, many researchers

have started looking for alternative sources of chitin and chitosan. Insect-derived chitosan appears particularly promising, as it was shown to be very similar to crustacean chitosan, displayed antioxidant and antimicrobial properties and it can be harvested as a by-product of insect protein purification.

Chitosan packaging films have fairly good water vapor permeability and oil leakage resistance, oxygen barrier properties and high potential to have very favorable life cycle assessment and reduced price once production is optimized. Their current disadvantage is low resistance to water solubility and pH and difficulty in processing. In terms of active packaging potential, chitosan films have antibacterial and antioxidant properties. Antibacterial properties of chitosan and chitosan films appear to be dependent on many factors, such as molecular weight, deacetylation degree, pH, film preparation method, film properties, food matrix present and the characteristic of the microbial strain.

Antibacterial and antioxidant properties of chitosan films can be further improved by incorporation of natural extracts, many of which appear to be very compatible with chitosan matrix. These substances can have positive or negative impact on the physicochemical and mechanical properties of chitosan films, for which reason different herbal substances are currently being explored.

Two promising plants with antioxidant and antibacterial properties are *Schisandra chinensis* and *Bupleurum falcatum*. These extracts were shown to inhibit a wide spectrum of bacteria, moreover, *S. chinensis* leaves, which are currently discarded as a waste product, have superior antioxidant and antibacterial properties in comparison with the fruits of the plant.

Therefore, the aim of this study was to evaluate the possibility to purify chitosan from new insect with potential as a food protein source (*Gryllus bimaculatus*), assess its ability to form films and enrich those films with antibacterial and antioxidant extracts from the two above mentioned Korean herbs. As far as the author is aware, insect chitosan has never been used to cast films, and *S. chinensis* and*B. falcatum* extracts have never been used in active packaging films either.

Methodology

Reagents were of analytical grade or higher, crickets were obtained from an insect farm and plant material from herbal market and supermarket.

Methodology was based on relevant previous studies whenever available, and when necessary adjusted to fit the scale of chitosan production, which in turn influenced the maximum area of films that could be produced. All adjustments of methodology were explained in detail and taken into consideration during evaluation of results.

All experiments except for chitosan purification and antimicrobial compound extraction were done at least in triplicate with appropriate controls. Statistical analysis was performed by Excel, difference between means was assessed by ANOVA (one-way analysis of variance) with $P < 0.05$ being the threshold for significance.

Results and Discussions

Cricket chitin was successfully purified by a procedure consisting of previously described deproteinization and demineralization method, supplemented with newly discovered decolorization method using substance X. Cricket chitosan was then purified by a novel method optimized by Kyo-Sung Chae. The large scale purification showed that difficulties arise from the scale of the process, mainly due to difficult protein removal. Therefore, the deacetylation degree was relatively lower than the usual deacetylation degree of shrimp chitosan. Molecular weight was estimated from previous experiment conducted by Chae Kyo Sung, and expected to be lower than 7 kDa, which is much lower than commercial shrimp low molecular weight chitosan.

S. chinensis fruit ethanol extract and *B. falcatum* root ethanol and methanol extracts were prepared. The methanol extract was subsequently fractionated into hexane, ethyl acetate, ethyl ether and water fraction. However, the only fractions which were water-

soluble and could therefore be used in the following experiments were the two ethanol extracts and *B. falcatum* root methanol extract water fraction. Total polyphenols of these three fractions were measured. *S. chinensis* fruit ethanol extract contained the highest level of polyphenols, which was comparable to other studies. *B. falcatum* root extracts were found to be less rich in polyphenols, however no similar studies were conducted previously. The high level of polyphenols in fruits of *S. chinensis* was considered to be also indicative of the possible even higher level of polyphenols in leaves. As total polyphenols have been shown to correlate well with antioxidant activity, the results were considered to indicate high possibility that the higher polyphenol content is indicative of higher antioxidant activity, and as protection from oxidation is a desirable feature of active packaging, the two ethanol extracts with higher levels of polyphenols were selected for further testing.

S. chinensis fruit ethanol extract and *B. falcatum* root ethanol extracts were then standardized by dilution with distilled water to contain equal levels of polyphenols and their minimum inhibitory concentration was tested against two Gram-positive (*B. cereus* and *L. monocytogenes*) and two Gram-negative (*E. coli* and *V. parahaemolyticus*) strains by disc-diffusion method. *V. parahaemolyticus* failed to grow, which was likely caused by adverse incubation conditions. *E. coli* was not susceptible to any extract at any concentration tested (up to 0.750 mg total polyphenols/ml), however *L. monocytogenes* was inhibited by *S. chinensis* extract at 0.750 mg total polyphenols/ml and *B. cereus* by 0.500 mg total polyphenols/ml. While higher susceptibility of Gram-positive strains to *S. chinensis* extract was expected, literature review suggested that Gram-negative strains should have been inhibited, albeit at higher concentration. The lack of antibacterial activity of *B. falcatum* extract was surprising, as ethyl ether and ethyl acetate fractions of ethanol extract were reported in literature to possess very strong antibacterial activity against both Gram-positive and Gram-negative strains. It appears that the antimicrobial compounds were not extracted in sufficient quantity by ethanol extraction. Based on these results, *S. chinensis* fruit extract was considered to be a more suitable candidate for incorporation in chitosan films, also as *S. chinensis* leaves were shown previously to possess even stronger antimicrobial activity than fruits. It was decided that *S. chinensis* fruit extract would be incorporated in the films at 0.750 mg total polyphenols/ml film-forming solution, which was equivalent to approximately 4% (w/w) of total solids.

Subsequently, four types of films were casted: film prepared from cricket chitosan, film prepared from shrimp chitosan, film prepared from cricket chitosan enriched with *S. chinensis* extract and film prepared from shrimp chitosan enriched with *S. chinensis* extract. In all cases, peelable films were formed, which proved that cricket chitosan has film-forming properties. During the experiment, it was observed that cricket chitosan film-forming solutions had higher viscosity, possibly due to lower deacetylation degree, and that solutions containing *S. chinensis* extract had higher wetting properties, likely due to interaction with the chitosan hydroxyl and amino groups.

Shrimp chitosan film without extract did not exhibit any antibacterial activity, cricket chitosan film without extract inhibited *B. cereus* after prolonged incubation, but not other strains. This results is most likely caused by the ability of very low molecular weight chitosan to diffuse from the film, while higher molecular weight shrimp chitosan is tightly bound within the film matrix. Films infused with *S. chinensis* extract inhibited both Gram-positive strains, with *L. monocytogenes* seeming sensitive only to *S. chinensis* extract and *B. cereus* sensitive to the extract and also cricket chitosan. Compared to other antibacterial chitosan films prepared, *S. chinensis* extract at 4% concentration performs better than certain essential oil which have been used for film enrichment, but in some cases is inferior.

As for physicochemical properties, cricket and shrimp chitosan films were comparable in terms of thickness, density, moisture content, water vapor permeability, but differed in water solubility and swelling degree, possibly due to difference in deacetylation degree. Color was

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. For a 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 German respondents are interested in sustainability and preferred the natural 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 general 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 in 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.

Conclusion

The present study showed that cricket chitosan can be purified and used to prepare thin films enriched with *S. chinensis* extract suitable for use as packaging polymers. Shrimp chitosan was also compatible with the extract. The prepared films enriched with *S. chinensis* extract manifested antibacterial activity against Gram-positive bacteria and pure cricket chitosan film also inhibited *B. cereus*. Concerning physicochemical properties, cricket and shrimp chitosan films were comparable in terms of thickness, density, moisture content, water vapor permeability, but cricket chitosan had lower water solubility and swelling degree, which means cricket chitosan gels might be more resistant when used for packaging of food with higher moisture content. Color was also different, possibly due to different color of the chitosan powder used to prepare the gels. Addition of *S. chinensis* extract led to increase in thickness, density, water solubility, swelling degree, and moisture content. On the other hand, water vapor permeability was decreased. Upon incorporation of the extract, color also changed into red-brown. *S. chinensis* extract therefore improved barrier properties against water vapor, however it also made the films less resistant to solubilization in water. The darkened color might possibly influence light absorbance properties, but that could not be assessed in the present study. Antioxidant activity of the films was not measured, but it can be expected to correlate with the total polyphenol content in the films enriched with extract.

Additionally, novel decolorization method of highly pigmented cricket chitin was discovered in the process of chitosan purification, which is relatively safe, economical and does not require harsh conditions. Moreover, behavior of the film-forming solution suggested that rheological properties of chitosan solutions might be dependent strongly on deacetylation degree.

In summary, it was shown that cricket chitosan is equivalent or superior (in the case of water solubility as well as antibacterial activity against *B. cereus*) to shrimp chitosan as source for chitosan packaging films and that addition of *S. chinensis* fruit extract improves water vapor barrier properties and antimicrobial properties, however the films become less resistant to water.

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Influence of some food additives on the growth of probiotic strains

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The growth of 6 strains of probiotic bacteria was assessed in liquid media containing different substances: sweeteners, organic acids, colorants and flavourings. The growth of probiotic bacteria was affected in presence of some compounds especially vanillin. The protection of *Lactobacillus reuteri* was assessed by microencapsulation in alginate (M) and in chitosan-alginate (CM) matrix using vibrating technology with the main purpose to improve its tolerance to vanillin at 1% concentration. The results indicate that the chitosan- alginate microcapsules have a better protection against from vanillin. These results encourage microencapsulation as a tool for the protection of probiotic bacteria.

Introduction

Nowadays, the health-food market is increasing every year. In this context, foods which claim to have a health-promoting or disease-preventing property beyond the basic function of supplying nutrients are called functional foods. Accordingly, probiotics are been added more and more into foods, being probiotics the most common ingredient of functional food. Probiotics are defined as live microorganisms which, when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2001).

The inclusion of probiotics into foods and beverages is an attractive option for companies interested in new and healthy products. However, the delivery vehicle is likely to influence probiotic functionality in many ways, including inducing changes in the cell composition and physiological status of the probiotic (Sanders & Marco, 2010). When considering the contributions of food format to probiotic functionality, a key consideration is the presence of the different ingredients that may inhibit, increase or de-

crease probiotic efficacy. Studies are rarely focused in the interactions among potential functional components in probiotic foods. The food matrix has the potential to affect probiotic survival, physiology, and potentially efficacy; but, little is known on how the food matrix and product formulation impacts probiotic functionality. Unfortunately, after exposure to some ingredients, the number of live microorganisms that could reach eventually the gut is too low to exert their action, making cell protection necessary.

Microencapsulation is the most promising technique applied to enhance bacteria viability. Microencapsulation, simply stated, is a means of packaging, separating, and storing materials in microscopic capsules for later release under controlled conditions. Microcapsules are small particles with diameters ranging from a few micrometres to a few millimetres. Depending on the microencapsulation technique used, different types of particles can be obtained. The vibrating technology is one of the most widely used methods for the production of microcapsules by extrusion. Where in the control and application of varying vibrational/ oscillation frequency with defined amplitude causes a break in the laminar jet produced when a liquid is extruded through a nozzle at certain flow rates (Whelehan & Marison, 2011).

Aims

The aims of this research is to study the effect that some additives (sweeteners, organic acids, colors and flavors) have over specific probiotic strains used in common food matrixes, and to evaluate the protection effect achieved by matrix microcapsules (using alginate and chitosan as polymer matrices) of the probiotics using vibrating technology.

Materials and methods

Probiotic strains and culture conditions

Six strains of probiotic bacteria (*L. plantarum* ATCC: SD 5209, L. *plantarum* LMG P-21021, *L. rhamnosus* DSM 16605, *L. paracasei* LMG P-21380, *L. acidophilus* LMG P-21381 and *L.reuteri* DSM 17938) were cultivated in MRS Broth (OXOID Ltd.,) and incubated at 37 ˚C in aerobic conditions for 24 hours to obtain overnight broth cultures. For viable counts, the bacteria were grown on MRS supplemented with 1.5% of Agar Bacteriological (OXOID Ltd.,) known as MRS agar. The colony counts were performed after 48 hours of incubation at 37ºC in aerobic conditions reporting the results in CFU/ml.

Evaluation of the effect of additives on Lactobacillus strains

The effect of additives was evaluated in terms of their potential inhibitory or lethal action on cells growth. Testing media was prepared by supplementing MRS broth with each additive respecting the concentration. Aspartame (0.03, 0.1 and 0.2%), xylitol (1%), D-xylose (1 and 10%); sorbic acid, tartaric acid, malic acid and citric acid (1%); ascorbic acid (0.05 and 1%), curcumin (1, 5 and 10%), cinnamon (0.2, 0.5 and 1%), ginger (1, 3, 5 and 10%) and vanillin (0.2, 0.5, 1, 1.2, 1.4 and 1.6%). Those media were further inoculated at 0.2% of the overnight broth cultures and incubated at optimal growth conditions. Cell growth of the strains in the presence of each additive was assessed by turbidity observation and cells load in the microscope at 100X.

Microencapsulation of L. reuteri DSM 17938

Microencapsulation of bacterial cells was carried out by using the encapsulator B-395 Pro equipped with an 80 mm nozzle and a syringe pump (BÜCHI Labortechnik, Flawil, Switzerland). The overnight cell culture of a defined volume of *L. reuteri* DSM 17938 was centrifuged at 6500 rpm for 10 minutes. The cell pellet was washed in an equal volume of sterile Ringer solution (Oxoid), centrifuged again at the same conditions and finally suspended in an equal volume respect to the initial culture broth of a 1.7% alginate (Sigma, product no. A2033) solution. To obtain a matrix alginate microcapsule (M), a syringe was loaded with 50ml of the alginate cell suspension and place on the encapsulator. The suspension was then extruded applying a vibration frequency of 2500 Hz, electrode voltage of 950 V and a flow rate of 3.9 ml/min. Alginate droplets containing bacterial cells were hardened in a 0.5 M solution of calcium chloride solution (in ratio 4:1 cell-alginate suspension) for about 20 min in constant stirring to obtain

monodisperse microcapsules. The suspension of capsules in calcium chloride was collected in sterile bottle and left at room temperature for 30 minutes for sedimentation of the microcapsules. The supernatant was removed and the capsules were finally suspended in a volume of Ringer solution equal to the volume of the initial cell suspension, to restore the initial concentration. To coat the alginate microcapsules with chitosan, the microcapsules previously prepared with the alginate solution were added to the chitosan solution 1:10 (w/v) and stirred for 15 min. After sedimentation the supernatant was removed and re- suspend in Ringer solution to restore the same volume.

Evaluation of the effect of additives on microencapsulated L. reuteri cells

Additives showing inhibitory or lethal effect on free *L. reuteri* cells were tested against microencapsulated *L. reuteri* cells in order to assess the possible protective effect of the capsules. Testing media was inoculated at different levels, 0.2 and 10%, with microencapsulated cells. Bacterial viability was evaluated at 0, 24 h (at optimal conditions) and 7days (at 4ºC) after the inoculum to assess the resistance of the bacteria to the compounds within time. Control samples (without the assessed compound), were prepared at the same inoculation and time conditions

Statistical Analysis

Analyses were carried out in duplicate, and cell counts were expressed as the mean of three independent experiments. Standard deviations and standard error were calculated. A One-way ANOVA, Tukey method and T-test analysis were performed using SPSS software (IBM SPSS Statistics for Microsoft Windows v.22.0) to ascertain significant differences between the averages; significance was declared at $p \leq 0.05$.

Results and discussion

Additive effect in the growth of Lactobacillus strains

Results showed that among the substances tested, the sweeteners, organic acids, and colourings did not significantly interfere with the growth of probiotic bacteria strains used in this study, at all concentrations tested. The results suggest that the sweeteners had no inhibition influence on the probiotic bacteria, in accordance with previous studies in model systems and food matrices such as dairy-based processed foods (Esmerino et al., 2013). {Vinderola, 2002, Influence of compounds associated with fermented dairy products on the growth of lactic acid starter and probiotic bacteria} Confirming the potential of sweeteners for use in low-calorie functional foods. Similarly with Vinderola et al., (2002), colorants used mainly for fermented milks as curcumin did not affect the growth of the probiotic bacteria here evaluated even though a slight inhibition was observed in the growth of *L. reuteri* DSM 17938, compared with the other probiotic strains.

There is very little information regarding the role of organic acids in the survival of probiotic bacteria. However, taking into account the fact that these organic acids are commonly used preservatives with antimicrobial properties, it was expected that they would have a negative effect on cell survival. Nevertheless, both citric and ascorbic acid did not have an inhibitory effect, as similarly reported in other studies (Nuealkaekul & Charalampopoulos, 2011).

Ginger and cinnamon have been added to food since ancient times, not only as flavouring agents, but also as folk medicine and food preservatives. Antimicrobial properties of ginger and cinnamon against pathogen bacteria have been reported. Yet, there are also studies that have evidence that ginger has a supporting effect in the probiotic growth and activity (Singh & Kaur, 2011), using ginger-based beverages could be used as carriers of probiotic bacteria and thus serve as health drinks for consumers. Our results support the consideration that ginger and cinnamon can be used in probiotic products even at a high concentration.

On the other hand, vanilla in particular has extensive applications in functional products that include yogurts and fermented milks, so its influence in the viability of probiotic bacteria must be further studied. Until now, Vinderola et al., (2002) have found that vanilla

flavour only affected the growth of few strains tested *(S. thermophilus* and *L. delbrueckii subsp. bulgaricus).* Additionally, our study results show that vanilla has a growth inhibition on all the strains evaluated after 1% concentration. The minimal inhibitory concentration was defined at 1%.

Additive effect against microencapsulated strain

After the inoculation of the microcapsules in the vanilla medium (using the defined MIC at 1% concentration), microcapsules were evaluated by optical microscopy at 0h and 24h. At 0 h, alginate microcapsules have a perfect spherical shape with homogenous cellular distribution, however after 24 h the microcapsules had modifications in the wall of the microcapsule showing a damaged microcapsule and releasing the cells of *L. reuteri* from the matrix with alginate (figure 1). A similar behaviour was seen for the alginate-chitosan microcapsules.

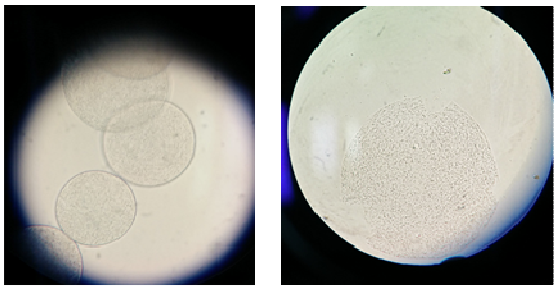

Figure 1. Alginate matrix microcapsule (M) of Lactobacillus reuteri DSM 17938 in a 1%vanillin media immediately after incubation (left) and after 24 (right). Optic microscope image in bright field.

The results of the cell count in vanillin medium are shown in figure 2. The bacterial load of free (FC) and microencapsulated *Lactobacillus reuteri* cells (M and CM) remained un-changed immediately after 24h. It is important to consider, that during the first 24h the bacteria were in optimal conditions (anaerobic at 37ºC). Nevertheless a considerate reduction of Log cycles occurred after 7days in contact with vanillin. In contrast, the control sample (*L. reuteri* in storage in all conditions for 7 days without vanillin) showed very small reduction, enough to consider it insignificant. Deducing that the reduction of cells after the 7 days was due to the presence of vanillin and not to the storage condition.

Additionally, cell survival percentages between periods of time show that alginate microcapsules (M) have the lowest survival rate for both inoculum conditions after 7 days. The protective effect of microcapsules coated with chitosan (CM) on the survival of *Lactobacillus reuteri* cells is still evident at a condition of 10% inoculum, obtaining 9 % of survival compared to 8.4% for free cells after 7 days in 4ºC storage. These results lead to say that CM microcapsules have a better effect in the protection of *L. reuteri* accordingly to other authors (Lee et al,. 2004; Malmo et al., 2013), that reported the enhancing effect of chitosan coating on the protection of alginate microencapsulated bacterial cells probably protecting further from the permeation of vanillin.

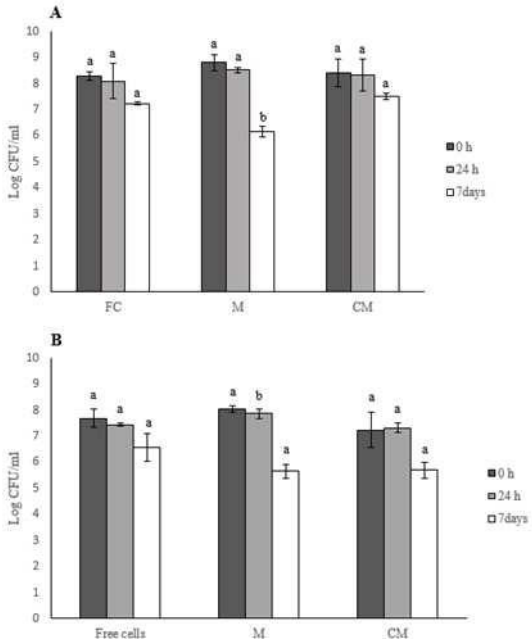

Figure 2. Viable counts and survival percentage of free and microencapsulated Lactobacillus reuteri DSM 17938 in a 1% vanilla medium at 10% inoculum (A) and 0.2% inoculum (B). (FC: free cells; M: alginate microcapsules; CM: alginate-chitosan microcapsules). The error bars represent standard deviations Different letters labelling bar graph of the same category of time, indicate that mean values are significantly different (p≤0.05) as determined by One way ANOVA and Tukey test.

Conclusions

The *Lactobacillus strains* evaluated are resistant to food additives assessed. Some of the compounds used were not inhibitory at the concentration used for industrial manufacturing, while for others, strain dependent effects were observed. Microencapsulation by vibrating technology only showed effect of protection with a matrix alginate-chitosan microcapsule for the combination of additive and strain. These results encourage the microencapsulation as a tool for the protection of probiotic bacteria where different types of microcapsules should be assessed.

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Quantification and Mechanistic Understanding of Spore Damage Repair

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Profile in a Nutshell

- MSc in Food Innovation and Product Design;
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Master Thesis tutor :

Dr. Tjakko Abee

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Bacillus cereus is spore-forming bacterium, which is ubiquitous present in environment, including soil, sediments, dust and plants. *B. cereus* is an agent of food spoilage and foodborne disease that can be found in many types of food and food ingredients [1]. *B. cereus* can form heat resistant spores under adverse environmental condition, such as nutrients shortage and desiccation. Spore-forming bacteria cause problems for food industry since different treatments aiming to eliminate bacteria cannot always kill spores.

Wet heat treatments are mostly used in food industry for inactivate or control bacteria and/or spores. Thermal process of spore suspension may activate, inactivate, sublethally injure or have no effect on spores [2]. The major targets for wet heat are considered to be proteins and enzymes [3]. However, proteins and enzymes have different denaturation temperatures and it is not clear which specific enzyme(s) or protein(s) are critical targets. Cazemier et al suggested those are likely proteins functional in both germination and outgrowth system that are damaged simultaneously [4].

During outgrowth of wet heat damaged spores, processes are operative to repair damage and enable vegetative growth [5][6]. Previous study in the lab had revealed that several genes were specifically upregulated during germination and outgrowth of heat treated *B. cereus* spores. Over expression of certain gene may suggest its involvement in *B. cereus* spores heat damage repair. Most of the identified genes are poorly studied and their functions in spore heat damage repair remain unclear. Deletion mutants of

those genes were constructed to study their behavior.

The aim of this study was to characterize germination and outgrowth capacity of heat treated spores among *B. cereus* ATCC 14579 and its deletion mutants. Furthermore, to identify whether deletion of selected genes affect recovery of heat damaged spores.

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Carboxylated Cellulose Nanocrystals Extraction from Kraft Pulp Using Ammonium Persulfate as Low Cost Source & Sustainable Method for High Quality Flexible Packaging Bio-coating

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Profile in a nutshell:

Gerald gives an entrepreneurial twist and social cause to food innovation by being a co-founder of FoPo Food Powder, CocoVia Probiotic drink and 2 other food businesses in the Philippines

He has experiences in developing new products, from conceptualization to creating a business plan on selling it to end consumers, after taking up BS Management of Applied Chemistry

He is the Philippine youth advisor in South East Asian Youth Environment Network (under UNEP), and he continues his passion for the environment in by developing eco-friendly plastics in his thesis

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Master Thesis tutor : Dr. Luciano Piergiovanni, Erika Mascheroni, Riccardo Rampazzo, Dr. Ivan Maximov

One of the leading challenges presented in 21st century for packaging industry is to address the growing environmental problems related to non-renewable flexible packaging. This leads to new growing interest in bio-based materials, among them cellulose nanocrystals (CNCs), which have already shown good performance in improving anti-fog and oxygen & water vapor barrier properties when applied to flexible film. A fast and low-cost CNC extraction was explored in this research by using unbleached Kraft pulp as the cellulosic source and treatment with ammonium persulfate as sustainable method for extraction. Presence of CNCs and its properties were verified and investigated using fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), and x-ray diffraction (XRD). CNCs were then used to coat PET plastic film and were subjected to contact angle measurement, oxygen permeability, transparence, and haze for comparison. Tests have shown excellent barrier and optical properties, comparable to cotton linter CNC coating extracted using acid hydrolysis, even with lower amount of CNC and thinner coating used by Kraft pulp. Making CNC bio-coating more affordable can reduce the amount of plastic usage in production leading to reduction of total weight, which can provide economic benefit to producers and environmental benefit through reduced energy use during transport.

Introduction

One of the leading challenges presented in 21st century for packaging industry is

to address the growing environmental problems related to non-renewable flexible packaging. This leads to new growing interest in bio-based materials, particularly cellulose nanocrystals (CNCs) (see figure 1), which have already shown good performance in improving anti-fog and oxygen & water vapor barrier properties when applied to flexible film. Current existing extraction methods include use of acids, enzymes and oxidizers, by mechanical means, or combinations of these to isolate CNCs from the cellulosic material.

A fast and low-cost CNC extraction was explored in this research by using unbleached Kraft pulp as the cellulosic source and treatment with ammonium persulfate (APS) as sustainable method for extraction. APS extraction has recently been attracting attention due to its properties being ideal for CNC extraction, such as low long-term toxicity, high water solubility, and low cost compared to its sodium and potassium counterparts, as well as to other previous harsh extractive agents. Presence of CNCs and its properties were verified and investigated using fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), and x -ray diffraction (XRD). CNCs were then used to coat PET plastic film and were subjected to contact angle measurement, oxygen permeability, transparence, and haze for comparison. PET film was chosen since it is one of the most common type of plastic being used in food packaging.

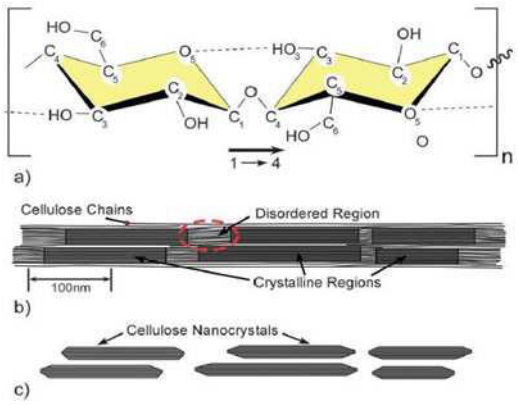

Figure 1. Simplified illustration of (a) cellulose molecule repeat unit, showing the 1-4 linkage & intramolecular hydrogen bonds (dotted line), (b) cellulose microfibril showing crystalline & disordered regions, and (c) cellulose nanocrystals after extraction, due to acid hydrolysis of the amorphous (disordered) region (Moon et al., 2011)

Materials and methods

Characterization of kraft pulp material was performed together with InnovHub – Paper Division, who assisted in performing the experiments according to standards being used in the paper industry. For the CNC extraction, swelling preparation was initially performed, where 10g dry weight of Kraft pulp was placed in 1000 ml beaker, dilutedin 1-layer of distilled water, and stirred using magnetic stirrer. Heater temperature was increased to 70˚C for 30 minutes, then cooled down in a cold water bath to reach the room temperature of 25˚C. 340.5 g of ammonium persulfate (APS) was added to the cooled Kraft pulp solution to reach 1.5M APS, and was then stirred for another 30 minutes to allow the powder to dissolve completely.

APS Extraction of Kraft Pulp

This extraction method was based on patent with publication number EP 2513149 A1 filed by Leung, et al. It made use of 1.5M APS and was heated for 16 h at 70˚C with high stirring speed for the cellulose nanocrystals extraction to occur completely. The sample was then removed from the heater, and was centrifuged using deionized water at 15,000 RPM for 20 minutes to concentrate the cellulose. It was centrifuged several times until it increased the pH level from around 0.2 to 3 (approximately 6 times). pH correction was then performed to the Kraft CNC solution, increasing it to pH 8 to avoid aggregation of the crystals in acidic environment. It was then subjected to ultrasonicator (UP400S 400W, hielscher Co., Germany) at 0.7 cycles of 20 minutes at 70% output to distribute CNCs evenly in the suspension. The solution was vacuum filtered using Whatman glass microfiber filter (grade GF/F, 0.7 µm) to remove fibers that did not react fully with APS treatment, and other big cellulose agglomerates and large contaminants that might have been introduced during the process.The Kraft CNC suspension was subjected to lyophilizationby using a freeze drying machine (LIO-

PET film (25x20 cm^2) for 20 rounds of rolling on one side of the plastic for approximately 3 minutes,improving adhesion of the nanocrystals on the surface of PET film. Automatic film applicator (ref 1137, Sheen Instruments, Kingston, UK) was used to apply the Kraft CNC solution evenly on top of the PET.

Two samples were created: sample 1 having applied only 1-layer of Kraft CNC, while sample 2 was made by directly applying another round of coating using the automatic film applicator immediately after drying the first layer. It was then dried using the blower and air-dried for 24 hours.

Results and Discussion

TEM was used to identify physical properties of Kraft CNCs extracted in nanoscale level.

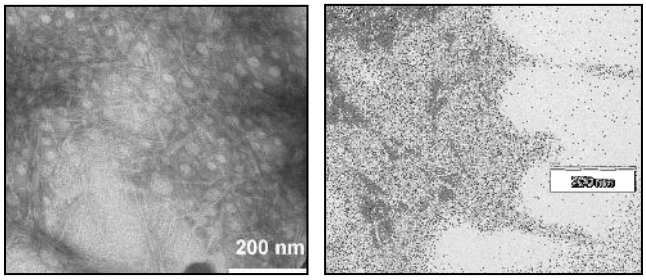

Figure 2. TEM image of Kraft CNCs (left) and cotton CNC (right) at 92,000 x magnification

Upon observing the image, it must be noted that the CNCs obtained have two distinct shapes: spherical and rod-like (see figure 2). APS concentration can influence the shape of CNC, as shown by the experiment involving different concentrations applied to a lyocell fiber matrix. It yielded a mixture of rod-like and spherical CNCs for 0.5M APS, but produced 100% spherical CNCs at 1M APS. On the other hand, acid hydrolysis extraction of Kraft pulp have yielded only rod-like crystal structure.

Raw material	kappa number	α-cellulose %	β-cellulose %	γ-cellulose %	lignin %	Ash
Kraft pulp	35.48	86.8	0.37	13.57	7.87	0

Table 1. Characterization of Kraft pulp raw material

The Kraft pulp sample obtained was carefully characterized to identify its kappa number, lignin, and α, β, γ cellulose contents (see table 1). Having a kappa number of 35.48 for the sample acquired is around the kappa number range of 30-35 for Kraft pulp that underwent conventional cooking. Having the lignin content of 7.87% shows that the Kraft pulp is subjected to an alkalinity of 20-25% in a span of 60-90 min. The high α cellulose in the resulting characterization experiment shows that previous processes have caused low degradation to the cellulose.

Sample	Thickness (nm)
Uncoated PET	0
Kraft 1-layer coating	132.90
Kraft 2-layer coating	411.39
Cotton layer coating	660.00

Table 2. Coating thickness comparison of kraft 1-layer, kraft 2-layer and cotton

The thickness values in Table 2 show that the cotton CNC coated PET film has the highest thickness, which can be explained by the total amount of CNC used in the solution.

The wettability of different samples shows that PET coated with cotton CNC has the best anti-fog property due to its very low contact angle measurement, allowing the water to spread to the solid surface. It is closely followed by Kraft 2-layers, with Kraft 1-layer exhibiting the lowest wettability. The increased amount of carboxylated CNCs in 2-layer compared to 1-layer have improved its hydrophilic interaction with polar water, thereby lowering the contact angle.

Cotton APS managed to have a high transparency, with its transparency value being close to the bare film. Kraft 1-layer and 2-layer have lower transparency values, even though both of them are thinner than cotton APS (see table 2), and lower percentage of CNCs applied in the coating (2.5% vs 7%). This can be due to the fact that in comparison to cotton linters, which has been bleached and contains >99% cellulose, the unbleached Kraft pulp as source is relatively unpure, hence ion impurities

might have influenced the slight decrease in transparency.

To further verify the optical property of the samples, a sample logo with a subtitle of font 6, and website URL with font 11, were used. PET coated films still do have the same level of readability for both font sizes as compared to the bare film. This shows that in application to production, using Kraft CNC coating (both 1-layer and 2-layer at 2.5%) has almost negligible influence to transparency.

Oxygen Transmission Rate (O2TR (cc m-2 24h-1) 23°C)				
%RH	Kraft 1-layer	Kraft 2-layer	Cotton	Bare*
0				74.95
30	0.10	0.10	0.10	
40	6.72	3.94	4.2	
50	15.428	7.78	8.30	82

Table 3. Oxygen transmission rate values of Kraft 1-layer, Kraft 2-layer, cotton and bare PET

Given the relatively thinner size of both Kraft single and double layers as compared to cotton as seen on table 2 (132.90 nm and 411.39 nm vs 660.00 nm, respectively), and using less amount of CNC in the solution (2.5% vs 7%), the result has shown that Kraft CNC has exhibited a good potential as bio-coating source to improve oxygen barrier properties for PET film (15.43 O_2TR for Kraft 1-layer and 7.78 O_2TR for Kraft 2-layer vs 82 O_2TR for bare under measurements at 50% RH) (see table 3).

Conclusions

The experiment has exhibited that high quality CNCs can be extracted from unbleached Kraft pulp, an unpure cellulose material source, and can be utilized as a high quality bio-coating for PET to improve its packaging properties.

Ammonium persulfate has proven to be an efficient extracting agent for unbleached Kraft pulp. Kraft pulp preparation, which includes swelling using distilled water at elevated temperature (70°C) for 30 minutes, cooling down, and mixing it with APS at room temperature for 30 minutes, were shown to be important steps to execute before proceeding with APS activation via heating. Kraft CNC was successfully applied on PET, and different parameters related to packaging were performed. Oxygen permeability is an important property particularly for food packaging due to its potential effects on quality and shelf life. Kraft 2-layer showed positive results in terms of oxygen permeability rate at 30% and 40% RH. This is comparable to CNC coatings extracted using acid hydrolysis and acquired from cotton linters, which contains > 99% alpha-cellulose. Optical properties were also tested, given the transparent flexible packaging's importance to better market the products on the shelves by showing the actual product to consumers via see-through packaging, while keeping its protective barrier properties. Both Kraft 1-layer and 2-layer showed hydrophilic contact angles when in contact with water, denoting a good wettability and anti-fog property. However, more tests must be performed to verify its response when used in actual packaging. It must be highlighted that these results are based on 2.5% CNC re-dilution with distilled water after freeze drying using Kraft CNC extracted from the experiment, compared to 7% re-dilution of cotton CNC from acid hydrolysis. This re-diluted solution was used for coating application on PET film. This shows that Kraft CNC needed a lower amount of CNC concentration to achieve similar improvements observed from cotton CNC.

Utilizing unbleached and semi-processed Kraft pulp as cheaper material to extract CNC, in comparison to the heavily-processed cotton linters, was proven to be possible. Given the results, it can therefore be concluded that CNC bio-coating sourced from unbleached Kraft pulp provided a high quality bio-coating for PET, improving its optical and permeability properties. It has also provided a better alternative for a low cost extracting process at a shorter time (against acid hydrolysis, which includes dialysis step that lasts for 3-4 days). Application of this type of bio-coating can lead to reduction of total packaging weight by utilizing thinner and lighter plastics since its barrier properties were already improved. This can eventually provide economic benefit to producers and environmental benefit such as reduced energy use during transport.

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Investigating Product Behavior During Storage in Packaging Materials - A Study on Freshly Baked Croissants

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Profile in a nutshell:

- Graduated with bachelors in Diary Technology from NDRI, Karnal, India.
- Four years of work experience in food research and development with an affiliate of ConAgra Foods, US in India.
- Lantmännen has recently awarded Priyanka with Jacob Bennets fund for the implementation and contribution of master thesis work.

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Very short shelf life of 'freshly' baked croissants is a growing concern for food retailers. It is affecting the economics of business, causing 10 to 40% in-store food wastage, and puts natural resources under severe stress. The factors affecting the shelf life are loss of crispness of the crust, and increased firmness of the crumb. This is largely due to migration of water from crumb to crust during storage period. Using appropriate packaging material the rate of moisture migration can be slowed down. Currently *paper/polyethylene laminate*, *polyethylene terephthalate* and *polypropylene* materials are used to pack croissants. However, the product shelf life is not more than 12 hrs. The study concluded that using monolayer *oriented-polypropylene* film with perforations (WVTR of 10-14 g m^{-2} day^{-1}) and multilayer *oriented- polyethylene terephthalate* film with or without *ethylene vinyl alcohol* polymer (WVTR of 9.2-9.5 g m^{-2} day^{-1}) the moisture migration phenomenon in croissants is slowed down. Further, the crispness of packed croissants was lost at 6 h storage, however softness of crumb is preserved up to 24 h.

Introduction

'Freshly' baked and unpacked croissants sold in the supermarkets today have a very short shelf life, of approximately 4 to 6 hrs. The consequence of short shelf life is increased food wastage in the stores that can range from 10 to 40%. This not only affects the economics of businesses but also puts natural resources under severe stress. For example, in the UK some 800,000 tons of bakery products are purchased each year and never eaten, mainly due to short life span of the product. Furthermore bakery

products wastage has an ironic touch to it as discarded product is still suitable for human consumption. Identifying means to reduce wastage due to short shelf life of baked product is of key interest to the industry as well as for sustainable consumption.

The factors affecting the shelf life of baked croissants are loss of crispness of the crust, and increased firmness of the crumb. This is largely due to migration of water from crumb to crust during storage period. The migration of water can be slowed down by, for example, making changes in the formulation of product. However, this is often time consuming and an expensive process. Use of protective coatings such as, polysaccharides, fats, etc. is another alternative to slow down moisture migration. Yet another and a practical alternative is to use appropriate packaging material. Currently *paper/polyethylene laminate*, *polyethylene terephthalate* and *polypropylene* materials are used to pack croissants. However, the shelf life is not more than 12 hrs. Moreover, there is a lack of empirical evidence on how croissants change its properties during storage in packaging materials. In the absence of detailed empirical knowledge, it is not possible to find the most optimal packaging material. This thesis aims at bridging this gap in the knowledge following an empirical approach. It combines both quantitative and qualitative methods for analyzing product behavior.

Objective

The posed research question for study is could the migration of water in croissants be slowed down by use of packaging materials with varying water vapor barrier properties. At the same time, it is important that the croissants keep characteristic eating quality, i.e. crispness in the crust and softness of crumb up to 48 hrs. **The study should be able to offer empirical evidence for making decisions on which material is best to extend the shelf life of croissants up to 48 hrs as demanded by the food retailers in the UK.**

Method

In the empirical studies conducted in this work, first, a market survey was done including visits to In-store bakeries in the UK market to identify various packaging materials and define the needs. Ten commercially available packaging materials were selected for investigation. The selected packaging materials were described as monolayer film, multilayer flexible film and paper-base multilayer flexible film. The water vapor transmission rates of materials were in the range from 0 to 20 g m^{-2} day^{-1} (38 ˚C, 90% RH), cf. Figure 1. Second, to understand the moisture migration phenomenon in unpacked and packed croissants quantitative measures such as croissant's *moisture content* and *water activity* value were measured at 1 h, 6 h, 12 h, 24 h and 48 hrs after baking. Qualitative measures such as *texture analysis* and *sensory evaluation* were also performed to evaluate crispness of crust and firmness of crumb. The measurements for the materials were compared for differences with significance following the *analysis of variance*

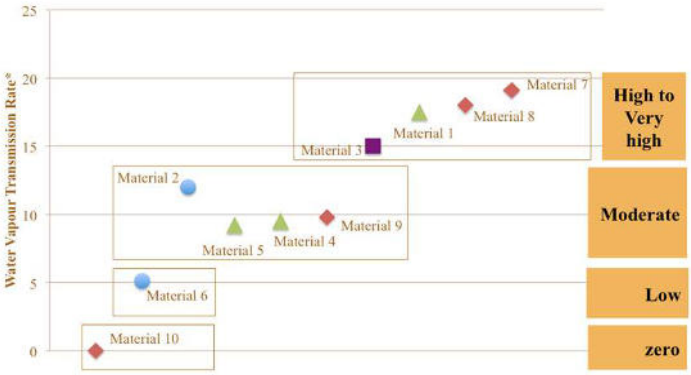

Figure 1. Water Vapor Transmission Rate of ten packaging materials used in the study. (g m^{-2} day^{-1}) at 38 ˚C, 90% RH. *Source of WVTR values: Material 1- Literature; Material 2- assumed twice the value for Material 6; Material 3- Literature; Material 4, 5, 6, 7, 9- Provided by supplier; Material 8- Literature; Material 10- by definition.

tests.

Results and Discussions

It was found in the study that when croissants are packed the rate of moisture migration from crumb to the crust and subsequently to the surrounding atmosphere is slowed down. The slowed moisture migration is further influenced by the composition of packaging material and its water vapor barrier property. The product behavior during storage was acceptable in monolayer film and multilayer flexible

film compared to the paper based multilayer flexible film. The organoleptic shelf life of croissants is limited to 12 h when packed in materials currently used in the UK, i.e. Material 1 and Material 8. Thus, materials with very-high water vapor transmission rate (WVTR) of 18-20 g m^{-2} day^{1} are not suitable packaging material for shelf life extension of croissants. Further, packaging materials with very low WVTR are also not suitable for croissants packaging.

Conclusion and Recommendations for future work

The study concludes that an optimal material for croissants packaging provides a controlled rate of moisture transfer in the product and the surroundings. The product crispness is lost at 6 h storage when it is packed, however, softness of crumb is preserved up to 24 h. The three suitable materials identified are: monolayer *oriented-polypropylene* film with perforations (WVTR of 10-14 g m^{-2} day^{1}) and multilayer *oriented- polyethylene terephthalate* film with or without *ethylene vinyl alcohol* polymer (WVTR of 9.2-9.5 g m^{-2} day^{1}). Using these materials the moisture migration phenomenon in croissants can be slowed down.

This study shows that approach to problems concerning moisture migration in the product requires understanding of the differences in the water activity within the product and its surrounding atmosphere. When selecting an optimal packaging material for a new product or a product with a formulation change the first step could be to obtain the product moisture sorption isotherm. This isotherm will give the critical values of moisture content and water activity. These critical values should be then tested for organoleptic acceptability using sensory evaluation methods.

As for further study, it can be an objective and future work to evaluate product behavior in customized monolayer-packaging material with optimal number of holes and hole size. It is recommended to include packaging design as a factor in investigation and consideration to get consumer feedback or a larger group of subjects for conducting product sensorial evaluation.

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Innovative technology in Camembert-type cheese making process

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Profile in a Nutshell

- MSc in Food Innovation and Product Design
- BSc in Agricultural Science, specialization: Dairy science and technology, Alexandria University (Egypt)
- International internships experience in agriculture and food field

Master Thesis hosting lab:

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Master Thesis tutor :

Catherine BEAL, INRA Grignon

In camembert cheese technology, industries faces phage risks, economical and time consumption problems related to some steps within the production process. A new production diagram has been developed presenting possible solutions. Chr. Hansen has been working on the development of a new starter culture that is able to cope with thesechanges.

The development of this new starter culture include adding different adjunct cultures with different dosages and preview their effect on acidification kinetics, cheese biochemical composition, metabolites production and overall cheese final profile comparing it to the standard one.

Pilot production was carried out in Chr. Hansen soft-cheese pilot plant followed by cheese analysis during the production and along ripening.

Gas Chromatography was done to detect mainly diacetyl, acetaldehyde and ethanol production along with other volatile compounds. High performance liquid chromatography was carried out to measure organic acids and compounds. Biochemical analysis was performed as well to know the moisture fat free basis and mineralization percentage in the cheese (MFFB% and Ca/SNF%).

The experimental cheese analysis results varied dependently on the different adjunct culture used. This allows us to preview some of the chosen adjunct culture impact on camembert cheese technology for a better selection in the new culture combination.

Confidential topic

Food packaging trends for the refrigerator - The perspective of packaging professionals

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Profile in a nutshell
With an interest in human nutrition, healthy foods and innovation, Elisabete seeks learning experiences which will bring positive results to the lives of consumers.

- Food business developer at *VentureLab*, Lund (Sweden) of healthy cake alternatives;
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Introduction

Primary food packaging is the packaging closest to the consumer serving functions of protection, convenience/utility and communication, amongst others (Pousette et al., 2014). Despite these core functions having existed for decades, the way they are offered can change as a consequence of many drivers, namely new ways of shopping, busier lifestyles, fast communications and/or environmental pressures (Ryynänen and Rusko, 2015).

Every day the consumer interacts with the primary food packaging and the refrigerator. As the food industry innovates, new packaging has to be accommodated in the consumers' home, in the refrigerator, where it continues to protect and preserve the food it contains. For Electrolux, which is "*one of the global leaders in household appliances*" and a manufacturer of refrigerators, anticipating this interaction is part of their mission, where development emerges as a response to consumer needs. Therefore, the main problem this research addressed, was to understand how the primary food packaging of chilled and frozen foods will, in the near future, influence the development of new refrigerators.

The main goal of the study was to explore the trends of the primary packaging of chilled and frozen foods, in order to analyse how they are likely to impact the design and development of future refrigerators.

This exploratory study considered three research questions: (1) how will packaging for chilled and frozen foods evolve in the near future; (2) what are the current drivers of these packaging changes and the implications for the future of packaging; and (3) how might all of these factors affect the design and development of future refrigerators? These questions were further explored

into external characteristics of packaging (such as formats, materials, functions and dimensions) and drivers for change, such as consumers.

The research delimitations included a time frame, for the study of the trends, of five to ten years, in other words, trends for 2020-2025, as well as the study of two European countries: the United Kingdom (UK) and Sweden. In addition, the type of food products analysed were the ones targeted to adults.

Methodology

A qualitative research was considered as the best approach to this understudied subject. The research was exploratory, connecting both the food packaging and the refrigerator and, in addition, focused on the future, on insights not published by the companies. This motivated the need for conducting in-depth semi-structured interviews, in order to have direct contact with packaging experts and gain access to personal and professional insights.

The data collection and organization of the interviews followed the seven stages presented by Kvale and Brinkmann (2009): (1) thematic conceptualization, (2) design, (3) interview, (4) transcription, (5) analysis, (6) verification and (7) reporting. For this study, thematic analysis was the chosen method to explore the collected data and also identify and describe the common themes shared during the interviews.

Observation was another form of primary data gathering by collection and analysis of packaging characteristics of four food product categories: milk, yogurt, chilled fruit juice and frozen ready meals, in the United Kingdom and in Sweden. The data was collected via online observation of e-stores and grouped in categories, such as type of material, format, volume and/or weight of the product. Then, their frequency was analyzed and results considered. The selection of these categories was based upon the fact that the interviewed experts where from these areas of food production.

Secondary data was also collected by conducting a literature review on primary food packaging and relevant keywords. The purpose, was not only to aid in the investigation of the appropriate topics for the interviews, but also to complement, and later contrast, the published content within academia to what is developed in an "*industrial*" setting.

Results and Discussion

From observation and literature research was noticeable that the current packaging in UK and Swedish stores lacks standardization. The analysis of the four products revealed that milk and yogurt packaging where the most different between the countries. While in the UK, the majority of 1L milk products are packaged in plastic, with a curved shape, in Sweden the same volume is packaged in paperboard, in a rectangular shape. In both countries, alternatives to dairy are packaged in paperboard, with a rectangular shape. In relation to yogurt, most products in the UK, are packaged in plastic single pots of 110 to 450g, while in Sweden most yogurts are packaged in a 1L paperboard gable top and in Tetra Top.

Chilled fruit juice is usually packaged in a 1L paperboard bottle in both countries, although in the UK, this product can also be frequently found in plastic bottles. Frozen ready meals are mainly packaged in carton, in a rectangular shape, in both countries.

The disparities between formats and sizes affects the refrigerator design because it challenges the personalization and adaption of the appliance to the packaging it contains. Nonetheless, that seems to be the necessity, as consumer trends point towards the growth of individualized and customized products which are also convenient, healthy and sustainable.

Convenience was mentioned as one of the main trends for the future, both in the literature research and by the interviewees. In the literature research, convenience for a consumer means packaging that saves time and makes their life easier. In general, consumers look for easy openings and closures, multipacks and portion control. Which is in accordance with the perspective of the interviewees. It was said how, in the future, products will allow consumption at different occasions, such as "on-the-go" eating and weekend indul-

gence. Simultaneous, this trend relates with a consumer that will be looking for products that fit their needs, and their needs only.

Product personalization is very likely to increase in the future. For some interviewees technology will be the facilitator of that customization by for example the incorporation of printed technology in the packaging. This will allow interactive labels to be used to send messages targeted specifically towards the consumer interests.

Another scenario is likely to become possible with the growth of online shopping. As consumers shop online for products, a database can be recorded allowing each time a better customization of the needed amount.

Health is another trend following concerns of diseases such as obesity and diabetes, but also concerns with appearance and general well-being. Future markets will also have to consider this trend when developing new products. As Teck Kim et al. (2014) mentioned, the rising of organic products will push the market towards antimicrobial or antioxidant activity-enhancing packaging, to increase health benefits and ensure safety. At the same time, packaging will also serve the function of providing portion control with servings that fit the needs of the consumers and "on-the-go" lifestyle (Teck Kim et al., 2014).

Food packaging should also be environmentally friendly (Han, 2014) as consumers become more aware of what is sustainable and how to choose packaging that is recyclable, renewable or biodegradable (Teck Kim et al., 2014). A perspective which packaging experts agreed upon as for them, consumer perception regarding packaging has to be addressed in the future in order to shift from "unnecessary" and "wasteful" into something positive.

An approach to this concern can be portion control by providing smaller packages. This would increase the packaging material used, but decrease the amount of food wasted, and at the same time, would offer the convenience of not having to store an opened package.

The current adult consumer is ageing and will become a senior contributing to the proportion of the over sixty population. Today, those represent 23% of the European population, but due to reduction in fertility and increased longevity, this population segment will grow to 34% by 2050 (UnitedNations, 2014). A projection which is important to consider due to concerns on natural resource reduction and the need of suitable packaging for the aged consumer (Duizer et al., 2009). Nevertheless, the packaging and food industry is not addressing this age group in a particular way. For the experts, the advantages of offering a convenient package go beyond a specific target benefiting all consumers.

Considering the vision for the "*future of packaging*" by authors Gerding et al. (1996) and Louis (1999), and comparing it with the available packaging today, it was noticeable that packaging has been evolving at a slower rate than what was expected. A possible explanation relates to the conservative attitude consumers have towards packaging innovation, and to the fact that they are conformists. This combination of characteristics means that the majority of consumers follow each other's decisions and do not want to see dramatic changes in their usual food products.

In five to ten years, packaging will suffer some changes, although very incremental. In terms of materials, the interviewees mentioned the rise of bioplastics and the decrease of glass as it becomes a material mainly used for premium products, especially wines. A possible alternative for the most common beverages packaged in glass will be flexible pouches, even though the current consumer perceives this format as being used in low quality products. Other formats and shapes are not likely to surge due to cost and production line limitations.

The biggest change might be in the packaging dimensions. The trend is towards smaller packages, multi-packs and packages with several compartments which will allow more variety and a wide range of flavors to be available. Bulkier packages will also increase, although not as much as the smaller ones. The intent is to satisfy the family households with 2L or 3L products. In the future, it is possible that the

most frequent milk volume of today (1L) will slowly fade away to give place to bigger milk packages of 1,5 and 2L, but also smaller packages of half a liter (especially in Sweden).

Active and intelligent packaging will be present in the future, mostly in the form of time-temperature indicators and thermochromic ink. However, packages with radio frequency identification (RFID) tags, edible packaging and packaging with nanotechnology are not likely to become mainstream by 2025 because of its high cost, legislation process and consumer perception.

Despite the current packaging development process not consider the refrigerator, in the future, the appliance might have to be contemplated as the need for improving the refrigerator "communication" with the consumer increases and packaging might have to be the mediator. Also, consumer trends demand more personalization and convenience in a market shifting from products to services. What this means is that in five to ten years the consumer is very likely to shop for a service of an organized personal kitchen instead of only a refrigerator. The consumer would not buy a refrigerator like we know it today, but a core structure, a backbone for the shelves and compartments that fit best the products he or she will purchase online. Enabled by the online database, where the different packages are registered, a service of adaptation can evolve throughout the years by accommodating the lifestyle changes a consumer experiences.

Conclusions and Further Research

Food packaging and refrigerators are both in our lives, evolving to a set of consumer demands. Convenience is one of the most important drivers of packaging changes, with health, sustainability and personalization becoming increasingly more relevant in a future where technology and online shopping will set a new pace to developments.

Primary food packaging is expected to become smaller, but also bulkier, satisfying the need for portion control and new occasions. Usage of bioplastics, pouches, active and intelligent packaging are likely to increase while the use of glass and less sustainable materials is expected to decrease.

In the future, the refrigerator development is likely to respond to personalization by exploring a new feature: detachable compartments. Besides consumer trends, the motivation is also the shift of business models from products to services. However, such evolution will happen gradually in a process that requires collaboration across the supply chain.

In order to start this process, further research should consider the in-depth study of the most frequent type of products stored in the refrigerator. As an option, the establishment of partnerships between a refrigerator manufacturer and a food company / retailer should be considered. Furthermore, future studies should focus on the consumer perception of packaging in relation to the refrigerator and on the perspective of packaging professionals influencing the products in the consumer refrigerator.

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Retail and Consumer Perceptions on Paper Packaging for Flour in Indonesia - With Insights on Perceptions of Environmental Sustainability

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Profile in a Nutshell

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Introduction

Indonesia is the world's fourth populous country with around 250 million inhabitants. To fulfil the needs of all these people the amount of packaged goods produced are continuously increasing. On the other hand, a higher interest in the environment has lead to increased considerations of the environmental impact of the packaging that we collectively produce. Currently the Indonesian market is dominated by plastic packaging. Paper packaging obtained from sustainable sources could be a potential alternative that is more environmentally friendly.

In 2014, Yessica Ariesta worked on a study with support from the Swedish paper manufacturer, BillerudKorsnäs, to investigate the potential of using paper as a packaging for dry products in Indonesia. Using a systems approach, the supply chain in Indonesia was observed and analysed with focus on understanding the dry food manufacturer's role in the system. In continuation of Ariesta's research, the retail and consumers were the focus in the present research.

Objective

The purposes of this research are detailed in these four points:

I. Further deepen the knowledge about retailers and consumers perception of current packaging of flour in Indonesia, and the potential of replacing them with paper packaging.

II. Provide information that could be used as the basis for creating a business strategy to enter the Indonesian food industries market.

III. Provide information that could be used as basis for direction of future technical developments in designing a pack-

age.

IV. Obtain an understanding of how environmental sustainability and awareness drives purchase behaviours of retail and consumers in Indonesia.

Method

The research was designed using exploratory case study approach. The methodology was carried out in three main stages. An initial overview of the topic was obtained through secondary research of literature review. A preliminary understanding of how Indonesian consumers used flour was then obtained through an online survey. The results of this survey then provided a point of reference in the creation of the semi-structured interview questions. Primary research was conducted in Indonesia through semi-structured interviews with consumers and modern market retailers, through market visits to modern retail stores. A mixed method technique was employed, using a combination of semi-structured interviews and observational techniques.

Results and Discussion

The results of the online survey indicated that 77% of the respondents within the target group currently kept a stock of flour at home for cooking purposes. The storage location was primarily in the cupboard, in containers or bound with a rubber band. The main problem encountered with flour that was cited in the survey are insects, rancid smell, messy and leaking package.

The primary requirement from the product and packaging for the consumer was a clear indication of protein content. This is important for the consumer as it influences the results of cooked finished products. Food safety is regarded as a must-have quality attribute in which well-trusted brands are perceived to indeed deliver safety.

Type of Research	Target group	Method	Objective
Secondary literature review	-	Research based on published references	Obtain a theoretical frame of reference
Preliminary quantitative research	Indonesian consumers	Online survey	Obtain initial understanding of the flour consumer in Indonesia
Market analysis	Retail stores in Indonesia	Store visit	Visual understanding of flour displays in stores.
Qualitative analysis of retail	Merchandiser/ Buyer at Retail	Semi structured Interview	Why and how decisions are made on type and placement of products.
	Key personnel on retail floor	Semi structured interview and observations	Understanding how the product is handled from delivery to display at retail.
Qualitative analysis of consumer	Consumers (Use flour in cooking)	Semi structured interview and observations	Understanding of how the product is brought home stored and used.

Table 1. Methodology Summary

The requirements for retailers are displayed in the following figure.

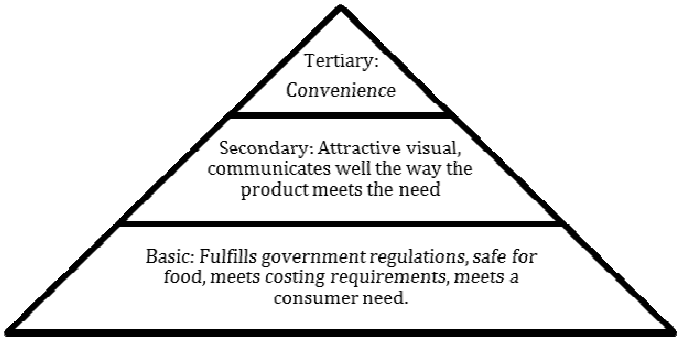

Figure 1: Hierarchal function of packaging for modern market retail

Environmental attributes are recognized as a beneficial attribute however will not inflict disappointment when not fulfilled, thus fall into the Kano's category of attractive attributes.

The existing plastic packaging was evaluated and compared with the alternative paper packaging.

Consumers had positive perceptions towards the paper packaging. In reference to the LOHAS (lifestyle of health and sustainability) consumer segmentation proposed by French and Showers in 2008, the interviews tended to show similar attributes to the Naturalites segment, as their key concern in purchase of products was the impact to health.

	Current Plastic Packaging		**Alternative Paper Packaging**	
	Strengths	**Weaknesses**	**Strengths**	**Weaknesses**
Consumer	Attractive visual Good material quality (thick)	Lack of reseal Messy Spilling content Negative association with plastic	Environmental benefit Unique Attractive Neat Easy to use and store	Lack of strength Unsuitable for humid climate Risk of leaks or punctures
Retailer	Durable Attractive visuals	Leakages still occur	Unique Environmental benefit Nice, premium look Improved display efficiency	Lack of durability Unsure with profitability Unsuitable for humid climate

Table 2. Perceived strengths and weaknesses of current and alternative packaging by consumers and retailers

Retailers, on the other hand, had major concerns with the costings, and durability of a paper package. The highly manual supply chain in Indonesia was seen as a large threat, as manual handling occurs during almost all stages of the retailer's logistics including picking for delivery to individual stores, receiving and storage at stores, shelving and also consumer handling in the store.

Both consumers and retailers can relate to the importance of environmental sustainability, however environmental sustainability is not a key consideration during purchase or evaluation of products. Environmental product attributes are thus an *attractive* product attribute such as defined by Kano in the theory of attractive quality in which the attribute provides satisfaction when achieved but does not cause dissatisfaction when not fulfilled.

Conclusion

Consumers associate paper packaging with novelty, uniqueness, premiumness, exclusivity, and high quality. Retailers perceive paper packaging as unique and premium as well; however, they have major concerns about the strength and durability of the package. Furthermore, an important consideration for retailers is costs. While consumers expressed a willingness to pay more for a product that is safer for themselves and for the environment, retailers were sceptic that such a package would still be profitable. Finally, a major concern is the preservation of food quality due to the humid climate of Indonesia.

As the underlying claim is that paper is more environmentally friendly than plastic, a strong foundation in research is required to support the claim. Future research also includes technical tests on strength and durability, ensure food quality through barrier and material research, and considerations of reasonable costings. Lastly, the ways of communicating environmental attributes to Indonesian consumers can be further investigated.

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FerulicAcid (FeAc) by Ion Exchange Resin from Hemicellulose Hydrolyzate

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Introduction

Extraction and recovery of ferulic acid from a hydrolysate obtained after an enzymatic hydrolysis of a by-product lignocellosic biomass (wheat bran and straw) was investigated to get ferulic acid.

Ferulic acid (FA) belongs to the family of hydroxycinnamic acids. It is a phenolic acid that is found abundantly in the hemicellulose of plant cell walls. Extraction of FA from agricultural crop residues (biomass) has contribution for developing sustainable world as it use renewable by products to produce high valuable products like Ferulic acid (Zhao et al., 2011).

Ferulic Acid can be used in ingredients of many drugs, functional foods and nutraceuticals (Ou and Kwok, 2004). The hemicellulose hydrolysate is obtained from lignocellulosic biomass by enzymatic hydrolysis using hemicellulases cocktail (Benaceur, 2013). After the hydrolysis the purification and extraction of FA from the hydrolysate is the main objective of the research.

The hydrolysate contains not only FA but also other components like Sugars, Proteins, and Anions like Chlorides, Sulfates. Nitrates, Phosphates …etc which comes from the raw materials, wheat bran and wheat straw, and the growth media during the hydrolysis and this make the extraction and purification challenging.

Weak anionic resin has been chosen based on its cost-effectiveness, need lesser quantity of chemical for regeneration which has a plus on contributing on green world and also by its availability and applicability in the industry (Moldes et al., 2003). The extracted FA will be used as an additive micro molecule for the production of biodegradable plastics (Benaceur, 2013).

General Objective

The general objective of the research is to extract, purify, and recover FeAc to maximum potential from the hydrolysate through eco-friendly purification techniques and optimizing the process for better yield, saturation, regeneration and concentration.

Materials and Methods

Hemicellulose hydrolysate is obtained from lignocellulosic biomass by enzymatic hydrolysis using hemicellulases cocktail in a different research work (Enzymatic deconstruction of wheat bran xylans by a thermophylic bacterium, Thermobacillus xylanilyticus, to produce valuable molecules at UMR FARE, Reims).

Ion exchange is the process through which ions in solutions are transferred to a solid matrix which in turn releases ions of different types but with the same polarity. The ions in solutions are replaced by different ions originally present in the solid.

FeAc has a high affinity for the free base form. Weak anionic resin under free base form was chosen for its advantage of the weak bond formed during the saturation and can easily be regenerated by lesser quantity during the regeneration using NaOH (Konrad, 1990).

The sorption in ion exchange resins can be ascribed to two different phenomena, adsorption and ionic exchange, which can happen simultaneously (Moldes et al., 2003). The two basic steps on weak resin anion exchange are saturation and regeneration. Weak anionic resin under free base form is able to fix weak acids under their protonated form: in the saturation step, the proton of the acid reacts with the doublet form of an ion-pair (it is not properly an ion-exchange reaction, even though it is commonly called ion exchange). This reaction is intended to fix FeAc in the place of free lone pare of electron on the free base form. Once equilibrium of the resin is reached on saturation step, the outlet of the column becomes in equilibrium with the feed and no more FeAc can be fixed. Then column has to be washed with demineralized water to remove interstitial feed solution before the saturation step is undertaken.

A preliminary acidification process was needed because the actual hydrolysate were in a neutral pH at 7 which is a favorable pH for the enzymatic hydrolysis. This pH is not convenient for the anionic resin under free base form. Acidification was done using Cation resin under H^+ form in a batch mode. A second preliminary step, centrifugation, was also introduced because acidification causes protein precipitation in the hydrolysate and would lead to plugging of the anionic column.

Identification of FeAc and sugar were carried out by high performance liquid chromatography (HPLC). A capillary zone electrophoresis system was used for complete separation of chloride, nitrate, sulfate, phosphate, and carbonate at the end of the separation process.

Results and Discussion

From these result, it was noticed that the hydrolysate contained much more Cl than FeAc. These anions come from the raw materials (bran and straw). It was also confirmed that the hydrolysate contains sugars and proteins in addition to the anions and the phenolic compounds.

During the preliminary treatment (acidification and centrifugation) it was managed to separate the proteins before the hydrolysate pass to the column. This is confirmed by the higher turbidity of all batch hydrolysates decreased effectively (< 10NTU).

Three of the experiments were done on the same condition acidification by cationic resin and separation by anionic resin under free base form. FeAc was able to be fixed on the resin effectively in the saturation step even though it was not able to reach complete saturation due to the availability of the hydrolysate in a lesser volume (<115BV).

From the mass balance result, 93mg of FeAc were sent to the column and 100% of it were fixed on the saturation step. There was no FeAc detected until the end of the saturation process. On the other hand Cl were also fixed and the resin become saturated quietly faster

(17BV).

Sugars were effectively excluded in the process.

During the regeneration step, 80mg (>75% of fixed "ferulate") were recovered at a higher concentration (>10g/L) with less than 2.5BV of regenerant NaOH. But at the same time Chlorides were also recovered in a higher concentration (>20g/L) (Liu et al., 2004). Even though it was possible to separate the proteins, sugars and recover FeAc with Cl, as the main objective is to get only FeAc at the end, it was needed to find another options to full fill the objective of the study.

Two methods were proposed. Eliminating the Cl before the ion exchange by electro-dialysis and direct injection of the hydrolysate to the anion exchange under Cl form without acidification.

Hydrolysate were passed through electrodialysis before the acidification and centrifugation process. Electro-dialysis were done for 40 minutes. The current decreased rapidly from 3.9A to 1.0A during the first 5 min of the process and slowly decreased until the end of 40 minutes giving final current 0.2A. There were also rapid decrease of conductivity of the hydrolysate during the first 5 minutes from 6.1mS/cm to 2.00 mS/cm and slower decrease to 0.5 mS/cm until the end of the 40minute period. The pH of the hydrolysate was decreased from 6.2 to 3.75 uniformly. On the other hand the pH and conductivity of the concentrate (NaCl) increased from 3.4 to 6.7 and 8.5 to 13.0mS/cm respectively. Similarly to the hydrolysate the increase were rapid during the first 5minutes of the process and gets slower and uniform for the rest of the minutes. These confirmed that the transfer of the ion was rapid in the first 5 minutes and then get slowed after.

After the electrodialysis the concentrate and diluate were subjected to analysis of FeAc and Cl content. From these analysis it was confirmed that there were a total transfer of Cl ion from the hydrolysate to the concentrate but the transfer was not only for Cl it was also for FeAc. At the end of electrolysis process 100% of Cl were removed from the hydrolysate.

From all of the pretreatments made for this experiment (ED, acidification and centrifugation), 60% of the original FeAc were lost. Finally these hydrolysate were injected to the anionic resin under free base form and only FeAc were recovered during the regeneration process. From the mass balance 13.8mg of FeAc was injected and 100% of it was fixed during the saturation process and 98% of it (13.5mg of FeAc) recovered at the end of regeneration process by using <3.00BV of regenerant. In this process Cl were eliminated and pure FeAc were recovered.

Acidification with cationic resin and separation with anionic resin under free base form worked well to exclude sugars and proteins but it had problem of Chlorides. So removing the chlorides before the separation by electrodialysis were effective even though the electrodialysis step should be optimized in order to decrease the loss of FeAc.

Separation with anionic resin under Cl form didn't work well thought the result should be confirmed by repetitions.

Conclusion

Lignocellolosic biomass can be a good source for obtaining FeAc. This research work strengthens the position of using agricultural and industrial by products as effective as possible.

The hydrolysate obtained from the lignocellosic biomass by enzymatic hydrolysis were characterized for its composition and the complexity of the hydrolysate were considered for choosing the right method to recover FeAc from the hydrolysate. Weak anionic resin were chosen as a favorable ion exchange type based on the kinetics, affinity to phenolic acids, capacity and susceptibility to regeneration.

The hydrolysate were pretreated before the ion exchange. The pretreatments were acidifi-

cation to reduce the pH from 7.00 to 3.00 and centrifugation to remove the proteins.

100% of the injected FeAc were fixed on the resin during the saturation step and it was able to recover greater than 75% of fixed FeAc in the regeneration step by less than 2.5BV of regenerant by using weak anionic resin under free base form. It was possible to extract and recover concentrated FeAC (>10g/L) as a salt. Sugars were eliminated effectively. However, it was not possible to separate the chloride from FeAc in the regeneration. Another preliminary process were added in order to remove the chlorides before separation process.

Electrodialysis was chosen based on the hydrolysate complexity and the intended component to be removed, Chlorides.

In conclusion, weak anionic resin under free base form with preliminary treatments of electrodialysis, acidification and centrifugation presents a favorable results for FeAc recovery from the hydrolysate obtained from a lignocellulose biomass by enzymatic hydrolysis.

Prospective

I. Electrodialysis step should be optimized by reducing the residence time to less than 10 minutes during the process so that the loss of FeAc in the process will be minimized.

II. In this experiment FeAc was obtained as salt “Ferulat”, in order to get FeAc as an acid using bipolar membrane after the separation.

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Qualitative improvement of crèmes desserts with the use of soy through process and formulation

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Soymilk appears to be a good candidate for cow's milk replacement in dairy desserts to prevent cow's milk allergy or intolerance and for veganism consumption. Current range of products made with soy has been improved by optimization of the composition in raw materials and the process on pilot and industrial scale. Methodologies have been developed and implemented to characterize the modifications and determine possible improvements.

Confidential topic

Consumer Co-Creation In New Food Product Development: the Case of High Protein Smoothies

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Consumer co-creation in NPD is the practice of collaborative product development between companies and consumers. Thus, co-creation allows consumers to take an active and central role as participants in the NPD process. Consumer co-creation represents an attractive approach for companies for a variety of reasons. In particular, ideas generated through co-creation will more closely mirror consumer needs.

For the purpose of this study, a group of protein users and non-users comprising sports scholars, professors and health promotion officers at Dublin Institute of Technology (Dublin, Ireland) were brought together to co-create the protein smoothie for Irish market.

This research demonstrated the feasibility of innovative NPD using consumer feedback.

Introduction

It has been clearly recognized that successful NPD depends on a deep understanding of consumer needs and product development efforts that meet those needs (Hauser, Tellis, & Griffin, 2006). However, this process is often rather difficult because these needs are often complex and may not always be identified through traditional marketing research methods (Churchill, von Hippel, & Sonnack, 2009; O'hern & Rindfleisch, 2010; Von Hippel, 2005). The inability to adequately assess and fulfill consumer needs is often a key reason for new product failure (Ogawa & Piller, 2006). However, by involving consumers more actively in the NPD process, new product ideas can be generated, which are more likely to be valued by consumers, thereby increasing the likelihood of new product success.

In addition, involving consumers in the NPD process canimprove product quality, reduce

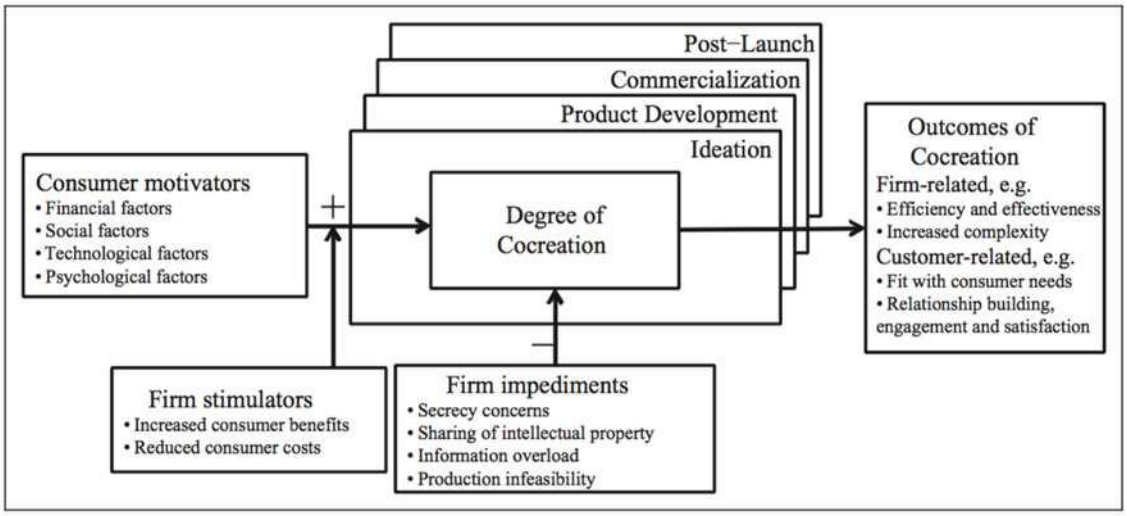

Figure 1. Conceptual framework of consumer co-creation (Hoyer, Chandy, Dorotic, Krafft, & Singh, 2010)

risk, and increase market acceptance (Business Wire 2001).Consumers are able and willing to provide ideas for new goods or services that may fulfill needs that have not yet been met by the market or might improve on existing offerings (Ernst, Hoyer, Krafft, & Soll, 2010).

Aims of the study

- Find a market gap for the new functional beverage
- Target consumers for the co-creation sessions
- Recruit screened consumers and retain them for 5 sessions
- Develop the product prototype in between the sessions 2 and 3
- Improve the product prototype with each coming session
- Innovate the co-creation method with engaging tactics
- Product attributes to be varied based on consumer input:
    - Flavor
    - Protein content
    - Packaging type
    - Unit size
    - Claims
    - Label design
- Design online survey based on obtained qualitative data
- Verify qualitative data obtained during co-creation with quantitative method: the conjoint analysis.

Materials and Methods

Collection of consumer feedback was achieved through series of five focus groups. The participants were recruited through an online sign-up form sent to the sports scholars at DIT. Respondents were divided by their use of protein supplements to “protein users” and “non-users”.

The series of five focus groups were divided in two parts:

I. Idea validation (concept testing)

II. Consumer co-creation

Overall approach of the research is shown in figure 2.

To verify and further explore the qualitative data obtained from focus groups, the online survey targeted to consumers residing in Ireland during the past 1 year was designed using the industry standard Survey Monkey platform ("Survey Monkey Online Survey Platform," 2015). The survey was sent online, via email and social networks (Wright, 2005). Complete survey contains 70 questions. Conjoint analysis was used to assess the importance of 3

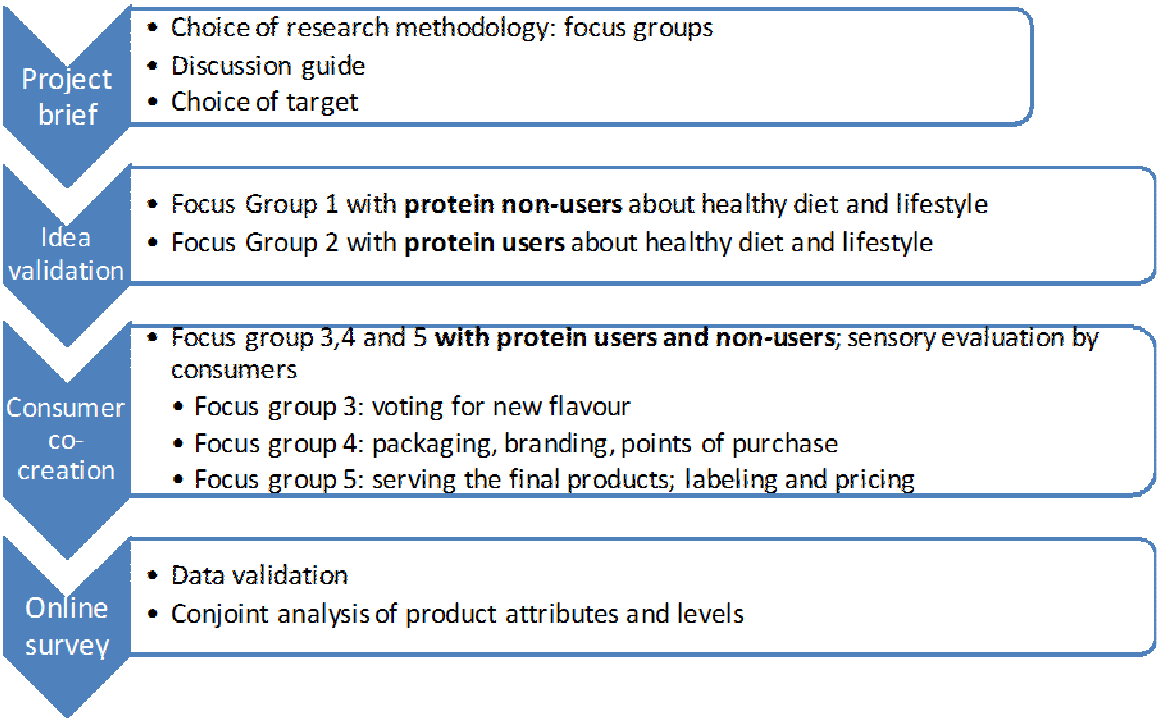

Figure 2. Summary of the consumer-centered research

product attributes: protein content, price and claim (statement) as seen in the Figure 3.

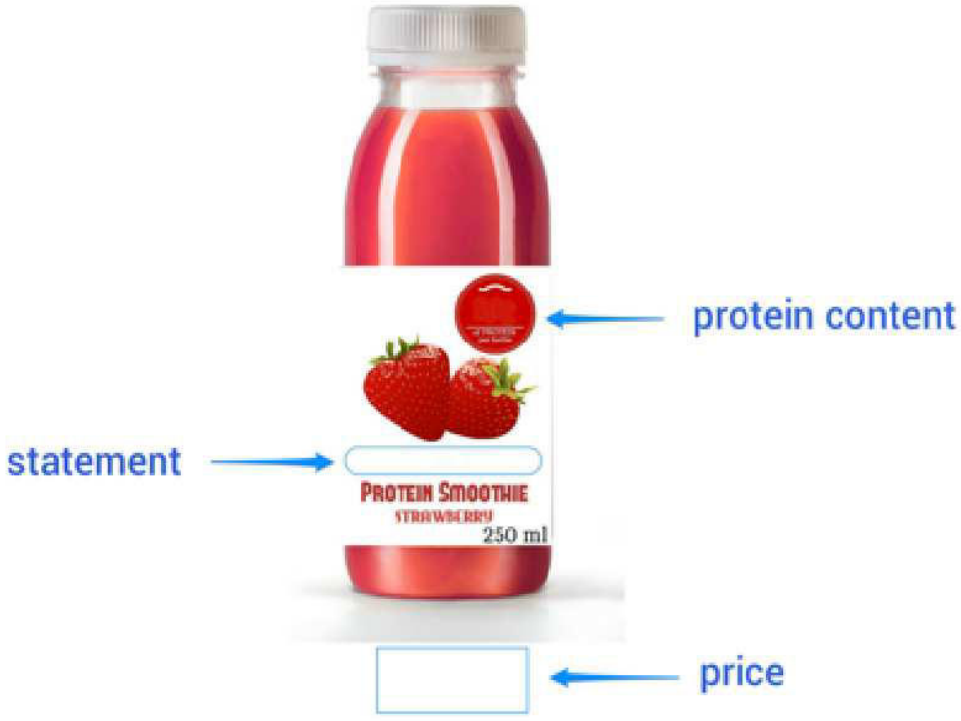

Figure 3 Varied product attributes

Results and Discussion

Focus Groups and Consumer co-creation

Idea Validation: In a series of two focus groups, two separate groups of consumers who do not use protein and who use protein were asked about their lifestyle and their opinion about supplements. The major findings are listed in Figure 4.

The demographics of the focus groups are listed in Figure 5.

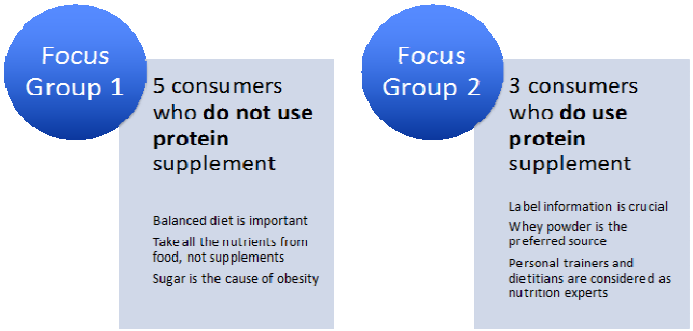

Figure 4 Major findings of idea validation stage

Profile	FG1	FG2	FG3	FG4	FG5
Group Size	5	3	10	9	8
Gender					
Male	1	3	7	9	8
Female	4	0	3	0	0
Age					
21 and under	2	2	6	7	6
22 to 34	3	1	4	2	2
Use of protein supplements					
Yes	0	2	3	5	5
No	3	0	5	2	2
Sometimes	2	1	2	2	1

Figure 5 Groups demographics

Two final prototypes of mango and berry protein smoothie, containing 25 grams of protein per 250ml were developed using consumer feedback during three co-creation sessions that followed the idea validation stage. The timeline is shown in Figure 6 and final product prototypes are shown in figure 7.

Activity	Apr-15							
	23	24	25	26	27	28	29	30
Focus Group 3								
Development of Mango Protein smoothie								
Focus Group 4								
Development of final prototype of Berry and Mango Protein smoothie								
Focus Group 5								

Figure 6 Timeline of consumer co-creation sessions

Figure 7. Final product prototype

	Combination	% Willingness
Card 1	30 gram-3.49-all natural	37.50
Card 2	20 gram-2.99-no claim	20.83
Card 3	30 gram-2.49-no claim	75.00
Card 4	20 gram-3.49-2 of your 5 a day	20.83
Card 5	25 gram-3.49-no claim	16.67
Card 6	30 gram-2.99-2 of your 5 a day	54.17
Card 7	25 gram-2.99-all natural	41.67
Card 8	25 gram-2.49-2 of your 5 a day	79.17
Card 9	20 gram-2.49-all natural	66.67

Figure 8. Orthogonal design and consumer willingness to buy the smoothie (green-highest, orange-lowest)

Online survey – Conjoint Analysis

At the time of writing this report, total of 39 respondents had started the survey. The integral part of the survey (conjoint analysis) was responded by 25 participants. More responses are needed to make the results statistically significant (Green, Krieger, & Wind, 2001). Based on collected results, the most preferred option for purchase is smoothie which contains 25g of protein, with claim "2 of your 5-a-day" sold at the price of 2.49eur per 250ml unit size.

Conclusions and Recommendations

- If screened properly, lead users and consumers can be a creative resource in the NPD process
- Focus group research leaves space for method innovations with high engagement of participants
- Co-creation of food products presents a challenge due to food safety reasons
- Quantitative data collection methods are more efficient but qualitative data is richer
- Further research is needed to determine the market success of co-created food product

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Effect of high-pressure enzymatic hydrolysis on functional properties of a soybean by-product

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The disposal of by-products is a big environmental problem for the plant food processing industries. However, the high proportion of insoluble dietary fiber in soybean residue limits further processing. No studies are present in the literature evaluating the effect of HPEH on soybean pulp. In this report the effect of the process of fiber composition, antioxidant activity and several physiochemical properties such as swelling capacity, oil-holding capacity, water-holding capacity, and solubility, was tested. HPEH does significantly increase the soluble dietary fiber content, enabling easier incorporation and further processing; it also released antioxidants in the soybean pulp and increased the capacity to retain glucose thus increasing respectively the antioxidant potential and the hypoglycemic effect of the ingredient.

Introduction

Approximately 1.2 kg of soybean residue is produced from 1 kg of soybeans during tofu processing, resulting in huge volumes of soybean residue. [1]. As the production of soymilk and soymilk derivatives continue to increase, the disposal of soybean residue could become an increasing problem. [2]

Soybean residue is a possible source of functional compounds, both for technical and nutritional properties. [3] Soybean residue has a high fiber content, approximately 60% (dry weight), while the high fiber content of the soybean residue makes the residue an interesting product with health promoting effects. At the same time, the high fiber content is one of the constrains of using soybean residue in a wide type of food products [2].

An interest has been raised for this material in recent years, with a dried soybean residue powder being incorporated into various products such as hamburgers, nougat,

cookies, and bread. However, not a lot has been done with manipulation of the material; the residue is often simply oven-dried and grinded before incorporation.

Aims

The aim of the thesis was to evaluate whether high-pressure enzymatic hydrolysis (HPEH) is a suitable technology to change the fiber ratio of soluble versus insoluble dietary fiber of soybean byproduct thus increasing the nutritional and technological value of this product.

No studies were present in the literature evaluating the effect of HPEH on soybean pulp.

The aim was achieved through application of high-pressure enzymatic hydrolysis to a dietary fiber-rich lyophilized soybean residue and chemical characterization of dietary fiber composition of the resulting product. Moreover the effect of the process on antioxidant activity and several physiochemical properties such as swelling capacity, oil-holding capacity, water-holding capacity, and solubility, was tested.

Materials and Methods

The soybean residue was provided by Kongseal Social Enterprise (Incheon, South Korea) from tofu production. All reagents and chemicals used were of analytical grade.

High-pressure enzymatic hydrolysis

The wet soybean residue was lyophilized using a Vacuum Tray Freeze Dryer (Il Sin Bio Base Co. Ltd, South Korea). The dry soybean residue was mixed with water (1:15 w/w) and 0.1% (approximately 100 units/g of soybean residue) of Ultraflo Max (Novozymes, Bagsvaerd, Denmark) was added for the enzymatic hydrolysis [4], and then inactivated by heating the samples at 95°C for 5 min. The enzymatic conditions were: pressure at 300 MPa and temperature 60°C [3] for 30 minutes. Dwell time was measured from when isobaric and isothermal conditions were reached. The resulting hydrolyzed product was lyophilized and ground into a fine powder and sifted through a 200 mesh sieve. A scheme of the process is reported in Fig 1.

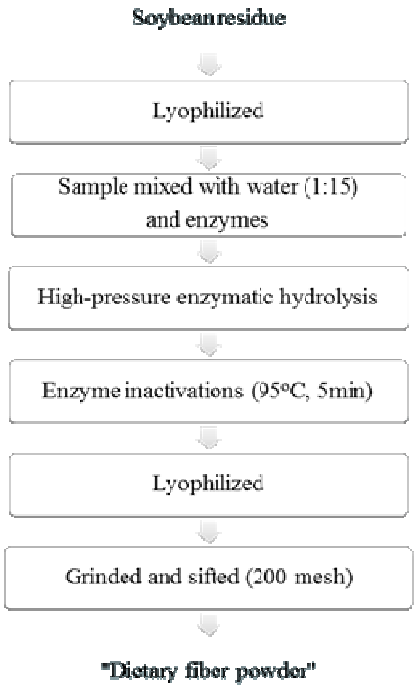

Figure 1. Scheme of the high-pressure enzymatic hydrolysis process

Fiber Composition

Soluble, insoluble and total dietary fiber was determined though sequential enzymatic digestion by heat-stable α-amylase, protease and amyloglucosidase.

The insoluble dietary fiber was filtered, and the residue was washed with warm distilled water. Combined solution of filtrate and water washings are precipitated with 95% ethanol (EtOH) for soluble dietary fiber determination, the precipitate was filtered and dried. Both SDF and IDF residues are corrected for protein, ash and blank, for the final calculation of SDF and IDF values.

Particle size

Particle size measured using a laser diffraction particle size analyzer (Mastersizer 2000, Malvern Instruments, Worcestershire, United Kingdom).

Water-binding capacity

Measured after rehydration in distilled water, centrifuged and dried overnight at 110°C. [5]

Water- and oil-holding capacity

Measured after rehydration in distilled water or commercial oil, and centrifugation, residue weight recorded. Capacity was calculated as g of water or oil per g of dry sample. [6]

Swelling capacity

The bed volume was determined by swelling of the sample in distilled water. Results expressed as ml of swollen sample per g of dry initial sample. [5]

Solubility

The supernatant from the water-holding capacity test was dried at 40°C overnight, and weight.

DPPH antioxidant activity assay

Antiradical activity 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) was used to measure the antioxidant activity in terms of radical scavenging ability of the samples [7]

The soluble antioxidants were extracted and then centrifuged using IEC CL30R Thermo Scientific (Waltham, USA).

The supernatant was transferred with a pipet to eppendorf tubes and centrifuged (MICRO CL21R, Thermo Sientific, Waltham, USA) again at 14800 rmp for 10 min at 5°C.

Stock solution of DPPH in methanol was diluted 1:20 to obtain absorption value of 0.9 ±0.02 at 517 nm. 200 µL of extract was mixed with 1mL of diluted DHHP reagent, wrapped in aluminum foil, and allowed to react for 10 min. The sample was centrifuged immediately before transferring into a plastic cuvette and measuring absorbance at 517 nm by an UV-VIS spectrophotometer (T92+, PG Instruments, Leicestershire England).

Antioxidant activity was calculated and corrected for blank. The results expressed as micromoles of Trolox equivalent per 100 g of sample.

QUENCHER

Is a rapid procedure for measurement of antioxidant capacity directly on the solid sample by mixing them with 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), free radicals, followed by a subsequent spectrometric measurement [8]

Stock solution of DPPH and methanol was diluted 1:20 in methanol:water (50:50, w/w) to obtain absorption value of 0.9 ±0.02 at 517 nm.

The insoluble samples were freeze-dried (Heto Lyolab 3000, Thermo Scientific, Waltham, USA) and transferred into 15 mL tubes. Then 1 mL of DPPH solution was added and the tubes were placed in an orbital shaker in darkness for 2h until centrifugation at 4000 rmp for 4 min to obtain an optically clear supernatant for the spectrophotometrical absorbance measurements at 517 nm.

Glucose dialysis retardation index (GDRI)

Glucose solution, was added to sample before agitation under continuous shaking for 1h at room temperature. The mixture was transferred to previously hydrated dialysis bags 12-14 000 MWCO, (Spectrum Laboratories, Inc. Rancho Dominguez, USA) and the samples were placed in a thermostatic water bath at 37°C and dialyzed against 400 mL of distilled water under continuous stirring for 1 h. At 15, 30, 45 and 60 min, a sample of 2 mL was collected, and the glucose concentration was quantified spectrophotometrically using Glucose (GO) Assay kit from Sigma Aldrich (Saint Louis, USA) for quantitative enzymatic determination of glucose.

Under the same conditions, bags containing only glucose solution were used as control. The results were corrected against a baseline glucose assay with water.

Statistical analysis

Experimental data were analyzed using one-way ANOVA (Analysis of variance), all statistical analysis was performed using SPSS software. Duncan's multiple range test was used to determine whether mean values were significantly different ($P<0.05$). All results expressed as mean value ± standard deviations.

Results and Discussion

The soluble dietary fiber fraction increased with the high pressure enzymatic hydrolysis

process till SDF 30.20±0.3 g/100 g. High hydrostatic pressure treatment had reportedly a total SDF conversion of just under 20g/100g, at pressure: 400 MPa and 60°C [3]. Enzymatic treatment with Ultraflo L® resulted in 10.7±3.3 g/100 g SDF [4]. Compared with these results the new high-pressure enzymatic process applied of soybean pulp is more effective and produces more soluble dietary fiber, making the HPEH as effective as the combined effects of the high hydrostatic pressure and the enzymatic treatment.

The grinding decreased the mean particle size. This reduction could be enough to make the particle small enough to not cause negative textural perception, as this and mouthfeel is critical for acceptability of a food product [9].

The HPEH increases the water-holding capacity of the sample compared to the non- treated sample at pH 8. However, the water-binding analysis shows an increase for the non-treated sample as well. The samples can hold more water due to the smaller particle size (bigger surface area) and soluble fraction. However, size reduction of particles can also result in fewer and smaller macrospores and it might be a reason why the water-binding samples gave different results.

The oil-holding and swelling capacity is lower for the grinded samples and especially the treated sample. This might be related to the fact that the smaller particle size makes the sample less bulky, and the method used in this report could be effected by this.

The solubility is greatly increased for the HPEH sample and it was coherent with the increased amount of soluble dietary fiber. See fig 2.

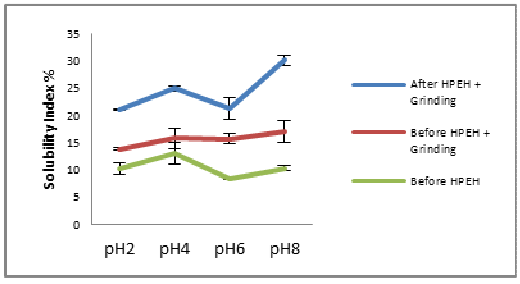

Figure 2. Solubility at different pH

The hydrolysis releases antioxidants from the matrix as seen in Fig. 3, displaying almost twice the antioxidant activity compared to the untreated sample. HPEH uses moist heath so the formation of glucosides is favored [10]. This might explain the unchanged insoluble antioxidant activity of the material.

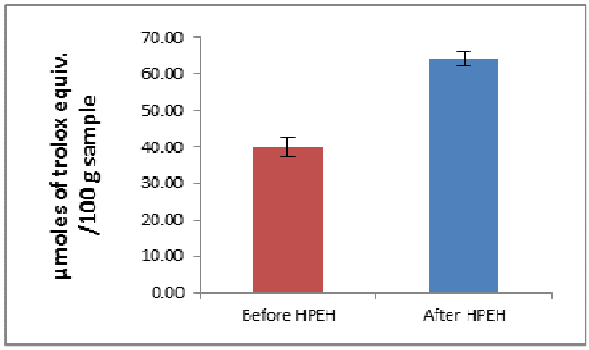

Figure 3. Soluble antioxidants in treated and untreated grinded samples expressed as µmoles of trolox equivalent per 100 g sample.

The hydrolysis probably released polyphenols of soluble antioxidants. This could be beneficial for the gut microbiota and functional properties, and this coupled with the increased amount of soluble dietary fiber increase the value of the produced powder for further application in food products.

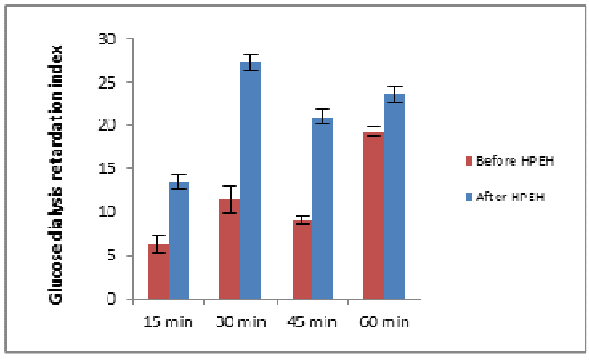

Figure 4. Glucose dialysis retardation index (GDRI)

The retardation of glucose for the treated sample was higher than the untreated sample, as seen in fig 4. GDRI seems to be related to soluble dietary fiber, but also the internal structure and surface properties. GDRI can be used to predict the effect the fiber has on the delay of glucose absorption in the gastrointestinal tract. [11].

Conclusions

High pressure enzymatic hydrolysis does significantly increase the soluble dietary fiber content in the soybean pulp, enabling easier incorporation and further processing.

The high pressure enzymatic hydrolysis also changes the physiochemical properties of the soybean by-product.

HPEH is a suitable technology to change the fiber composition of a soybean by-product, with a soluble dietary fiber content of the combined value of high-pressure and enzymatic treatment on its own.

Moreover this work also showed that HPEH modified the antioxidant capacity of the ingredient by increasing the soluble antioxidant activity and several physiochemical properties; by decreasing swelling capacity and oil-holding capacity while increasing water-holding capacity at pH 8 and solubility.

The results suggest that the treated soybean residue could be used as a possible functional ingredient to increase the soluble dietary fiber and the antioxidant potential as well as to reduce glycemic index of added products.

The data indicated that the high pressure enzymatic hydrolysis is a very useful process to increase the technological application of the soybean residue and its nutritional value. Future studies should evaluate the incorporation of the new ingredient in different food products and to assess its effect on sensory properties of the new foods.

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Slow Digestible Starch Study in Korean Samguang Rice by Different Milled Methods

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Introduction

Slowly digestible starch (SDS) refers to starch material with a moderated rate, hydrolyzed to glucose, during its transit through the human small intestine. (Vandeputte & Delcour, 2004) It is considered as healthy resource of food, offers a range of health benefits for the treatment and prevention of several diseases, such as obesity, cardiovascular diseases and non-insulin diabetes. Changes to the chemical structures of starch molecules or changes that make the molecules or granules more crystalline tend to reduce the rate of starch digestion. Starch retrogradation, as an unavoidable phenomenon that gelatinized starch changes from an amorphous state to a crystalline one, could be used to reduce the digestibility of starch and it was suited for preparing SDS and resistant starch (RS). Meanwhile, rice, as a major cereal crop and the staple food source for half of the world population, is always considered as a high GI food that has high digestibility. (Ahmed, Al-Jassar, & Thomas, 2015)So that this thesis mainly focused rice starch retrogradation study and its slow digestibility through studying the effects of milling methods, moisture content, and particle sizes. To explore the potential usage of the sample rice to have slow digestible ability.(Lehmann & Robin, 2007; Shin et al., 2004).

Methodology

Impact of milling methods and particle sizes on the retrogradation properties and forming slow digestible starch of Korean Samguang rice flour were investigated. In this work, a single and dual-retrogradation treatment were applied on wet milled sample (NWRP, 75μm), dry milled sample with respectively particle size 49,100 and 142μm (DMRP49, DMRP100, DMRP 142) to prepare slowly digestible starch (SDS) products from the Samguang (variety) rice powder. Flow chart as follow.

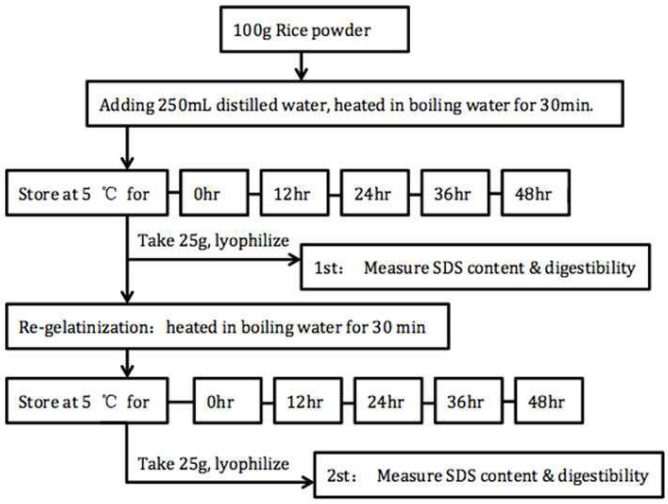

Figure 1. Sample preparation: single & dual retrogradation process

Starch proportion analysis was conducted through imitating the human intestine environment with two enzymes: α-Amylase & Amyloglucosidase. (Tian et al., 2013)Reducing sugar was measure at 20min& 120min; slow digestible starch was calculated base on the glucose change. And digestibility was using similar method, glucose content was measure every 10min from 0min to 150min to draw the digestibility curve. And calculated by the formulas as follow:

$$RDS(\%) = [(G_{20} - FG) \times 0.9/TS] \times 100$$

$$SDS(\%) = [(G_{120} - G_{20}) \times 0.9/TS] \times 100$$

$$RS(\%) = [TS - (RDS + SDS)]/TS$$

G_{20}: Glucose content after enzyme hydrolization for 20min (mg);

FG: Free glucose content before enzyme process (mg);

G_{120}: Glucose content after enzyme hydrolization for 120min (mg);

TS: Total starch content in sample (mg);

Morphology study was undertaken by scanning electron microscope, and melting temperature range, and enthalpy were measured by differential scanning calorimeter.

Results and Discussion

Multivariate analysis was applied by software SPSS .

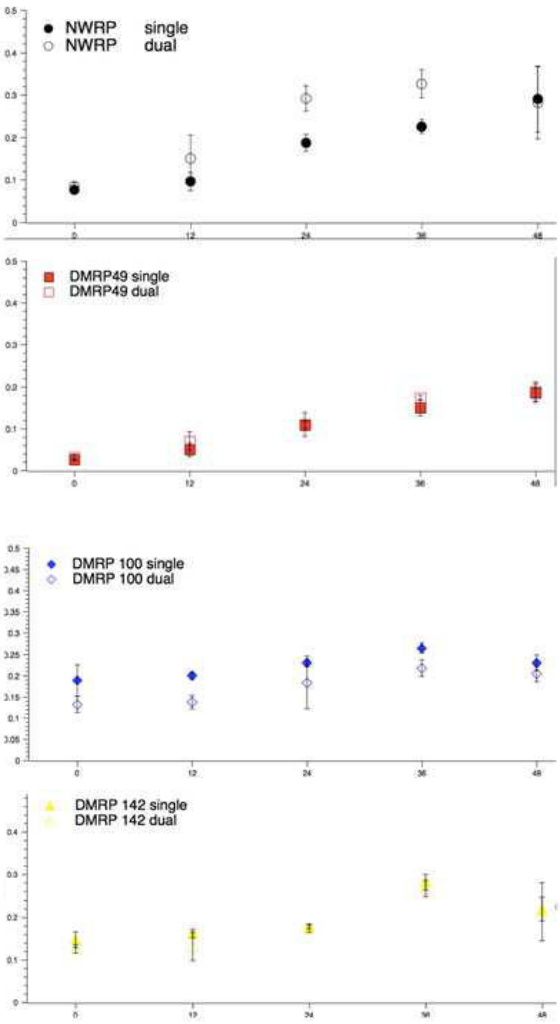

Figure 2. Scatter diagram of SDS content of 4 different samples with different milling method and particle sizes in single& dual retrogradation (x axis: retrogradation time (h), y axis: SDS content)

SDS contents change with retrogradation of all samples were presented in Figure 2, and similar trends were observed that in Samguang rice.

RDS content analysis: for different treatment: there's no significant difference among different retrogradation time. At beginning of retrogradation, the difference caused by different

samples was significant, however when retrogradation started for 12h, the behaviors of 4 samples are similar. From 24h till 48h, the differences between 4 samples are significant. However for different samples, the results are significant. Those two results might indicate that, for the same variety of rice, due to different milling method may have different digestible and physicochemical properties, but were not affected by heating methods.

SDS content analysis: Treatment impact was different, since different heating methods changed the structure of samples, so in the beginning (20min), glucose contents of different retrograded samples were significantly different. This directly reflected by RDS content. However, SDS content, which reflects the enzyme hydrolysis speed, when there's no significance found between two heating method, showed that after retrogradation, the digestibility of samples, either conducted by single or dual retrogradation, are similar.

Despite the difference caused by single& dual retrogradation, an extraordinary attention was put on DMRP 49, as its smallest particle size, which stands out from other samples at most time (0h, 12h, 24h,and 36h). Meanwhile sample NWRP shows significant difference among other samples at 0h and 48h as well. In all situation, sample DMRP100 and DMRP142 behave as one group. These results may indicate that in the beginning, rice powder properties differ from each other mainly due to particle sizes, and then their properties would become similar due to heat treatment.

In conclude, for sample DMRP49, dual retrogradation could generate more SDS, while for DMRP100 and DMRP142, single retrogradation was dominant for generating SDS.

In the morphology study, wet milled rice powder has bigger cavities than dry milled samples, mainly the more SDS content, the bigger cavities. For NWRP 48h, different heat treatment doesn't affect the SDS generation as well as morphology. For DMRP 49, very opposite surface morphologies were observed at 36h and 48h. So dual retrogradation at 36h and single retrogradation at 48h had bigger cavities, which positively correlated with higher SDS content.

Conclusions and Perspectives

Comprehensive results showed that there was significant difference between single and dual retrogradation that changed the structure of samples in the beginning 20min of hydrolysis, which presented as RDS content. However, in

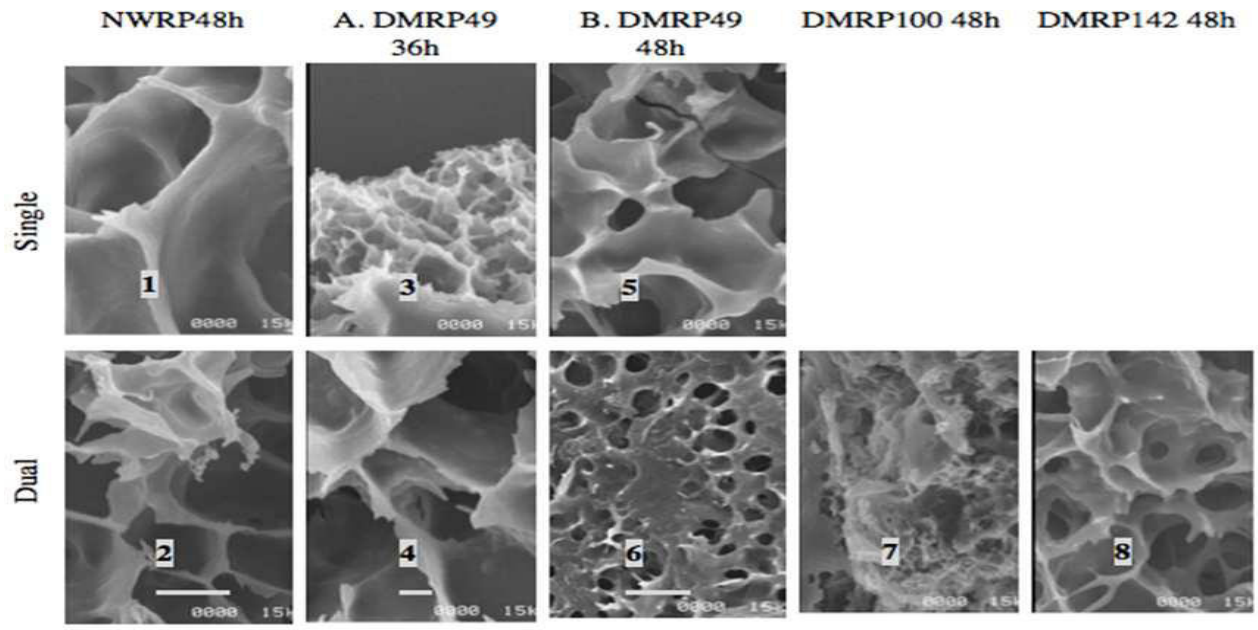

Figure 3. Scanning electron micrographs of retrograded samples

long term, the rate of digestibility within 120min, which presented as SDS content, didn't affected by the retrogradation method. And also samples were different from each other due to different particle sizes.

NWRP showed the extraordinary potential of generating more SDS; comparatively 3 DMRP samples had difficulty to form SDS. Among dry milled samples, DMRP4 showed the weakest ability to generate SDS. The difference between different milling methods may cause by heat absorption and air pressure during process, which compress the amorphous zone. And the difference between different particle sizes might correlate with chain length of amylopectin, damage starch content, water content, and surface area.

The innovation of this work is studying the impacts of milling methods and particle sizes on retrogradation properties of rice starch, which is relatively new in this area. And the result gave more supports on the theory part of the future application of retrogradation.

For future study of this area, more detective methods can be used, like spectroscopic methods including NMR, IR and Raman spectroscopy to explore more about mechanism. And the other macromolecules can be combine together with starch to form a V-type crystalline structure, which on one hand could increase SDS content, on the other hand could increase the stability of the system.

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