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TRANSFERTS DANS LES SYSTEMES EMBALLAGE/ALIMENTS: STRUCTURATION A FACON DE MATERIAUX MULTICOUCHES POUR L'EMBALLAGE SOUS ATMOSPHERE MODIFIEE DES PRODUITS FRAIS

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TABLE OF CONTENTS

ABBREVIATIONS

LIST OF ABBREVIATIONS

1-MCP	1-methylcyclopropene
AB	Amido black
$Ba(OH)_2$	Barium hydroxide
$BaCl_2$	Barium chloride
$BaCO_3$	Barium carbonate
Ca	Caseinates
CO_2	Carbon dioxide
CTIFL	Fruits and vegetable interprofessional technical center
Cw	Coating weight
CZ	Corn zein
DNA	Desoxyribonucleic acid
DPHEA	2,2-diphenyl-2-hydroxyethanoic acid
DSC(m)	Differential scanning calorimetry (modulated)
DTE	Dithioerythiol
E	Young modulus
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
EtOH	Ethylene
FID	Flame ionization detector
FITC	Fluorescein isothiocyanate
FTIR	Fourier transformed infrared spectroscopy
GC	Gas chromatograph
Ge	Gelatin
GRAS	Generally recognized as safe
HCl	Hydrochloric acid
HDPE	High density polyethylene
He	Helium
IAM	Iodoacetamine
IATE	Research unit: Agropolymer engineering and emerging technologies
ICS	Charles Sadron institute
IR	Infrared
Ki	Inhibitory constant
Km	Michaelis constant
LbL	Layer-by-layer
LDPE	Low density polyethylene
MCT	Mercury cadmium telluride detector
MF	Fish Myofibrillar protein
MIC	Minimal inhibitory concentration
MMT	Montmorillonite
MW	Molecular weight
Na	Sodium
NaCl	Sodium chloride
NHS	N-hydroxysuccinimide

O_2	Oxygen
OPP	Oriented polypropylene
P_x	Permeability to gas x
PDA	Potato dextrose agar
PDDA	Poly(diallyldimethylammonium chloride)
PE	Polyethylene
PEI	Polyethylimine
PET	Polyethylene terephthalate
Pe_x	Permeation to gas x
PHA	Polyhydroxyalkanoates
PHBV	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate
PMMA	Poly(methyl-methacrylate)
PP	Polypropylene
PSS	poly(sodium-4-styrene sulfonate)
Q_{10}	Increase of respiratory rate for a 10°C increase of temperature
RDA	Requirement driven approach
RH	Relative humidity
RQ	Respiratory quotient
RR_{O2}	Respiratory rate
S	Permselectivity
SB	% Strain at break
SDS	Sodium dodecyl sulfate
SE-HPLC	(Size exclusion) High performance liquid chromatography
SEM	Scanning electron microscopy
Si wafer	Silicon wafer
SP	Support paper
SPC	Soy protein concentrate
SPI	Soy protein isolate
TCD	Thermal conductivity detector
TEM	Transmission electron microscopy
Tg	Glass transition temperature
TM	Tailor-made
TS	Tensile strength
UMR SQPOV	Research unit: Security and quality of vegetal products
UV	Ultraviolet
WG	Wheat gluten
WGcomp	Composite layer containing gluten and fibers
WG-Film	Self-supported wheat gluten film
WG-Paper (WGP)	Paper coated with wheat gluten
WGr	Heat reticulated wheat gluten
WGr-Paper (WGrP)	Paper coated with heat reticulated wheat gluten
WPC	Whey protein concentrate
WPI	Whey protein isolate
WV	Water vapor

WVP	Water vapor permeability
XPS	X-ray photoelectron spectroscopy

INTRODUCTION

CONTEXTE SOCIO-ÉCONOMIQUE ET TECHNIQUE

L'emballage des aliments occupe aujourd'hui une place prépondérante dans les procédés de transformation/conditionnement notamment grâce à une demande accrue pour des produits alimentaires pratiques (à emporter et préparer), de plus longue conservation (réduction de la fréquence d'achat) et présentant de meilleure qualités organoleptique et nutritionnelles (réduction de l'usage d'additifs ou de procédés de conservation dénaturant). L'emballage joue aussi un rôle clé dans la réduction des pertes en denrées alimentaires tout au long de la chaine de distribution, qui représentaient en Europe, 20% de fruits et légumes et 14% des produits carnés en 2007 [Gustavsson et al. 2011]. Le rôle de l'emballage a longtemps été limité à une simple protection physique, garantissant une quantité nominale de produit et limitant l'impact des chocs mécaniques et les contaminations microbiologiques extérieures. Aujourd'hui, l'emballage peut même interagir avec l'aliment comme le prévoit la réglementation cadre UE 1935/2004 concernant les matériaux et objets destinés à entrer en contact avec les denrées alimentaires. De nouvelles fonctions sont apparues comme celles des emballages actifs *«conçus de manière délibérée pour contenir des constituants actifs destinés à être libérés dans les denrées alimentaires ou à absorber des substances provenant de celles-ci»*. Entre temps, dans les années 70, la technologie d'emballage sous atmosphère modifiée (EAM, plus connue par les consommateurs sous le nom d'atmosphère protectrice) a vu le jour et s'est largement répandues pendant une quinzaine d'années, notamment pour les produits alimentaires dit de 4ème gamme. Elle consiste à remplacer l'air contenu dans l'emballage par un mélange approprié de gaz (différentes combinaisons d'oxygène, dioxyde de carbone, et azote) qui doit être le plus proche possible de l'atmosphère optimale recommandée, elle-même spécifique de chaque denrée et qui permet d'allonger sa durée de vie. Réalisée au moyen d'un balayage gazeux en association obligatoire avec un matériau d'emballage hautement barrière aux gaz, cette technologie s'est avérée assez coûteuse, nécessitant le recours à des matériaux multicouches sophistiqués et non recyclables. Son utilisation se limite aujourd'hui à certains aliments à forte valeur ajoutée (comme les viandes ou les charcuteries coupées). La mise en œuvre de systèmes actifs de type sachet ou films absorbeurs d'oxygène et/ou émetteur de CO_2 ont pris le relai dans les années 90 et se sont largement répandus sans pour autant résoudre entièrement les problèmes de coût et d'impact environnemental.

Pour les produits respirant (vivants), tels que les fruits et légumes, l'atmosphère peut être modifiée par le simple jeu des échanges gazeux au travers de l'emballage qui s'équilibrent avec l'activité respiratoire de l'aliment (consommation d'oxygène et production de dioxyde de carbone) (Figure I.1), générant ainsi une atmosphère modifiée d'équilibre à l'intérieur de l'emballage.

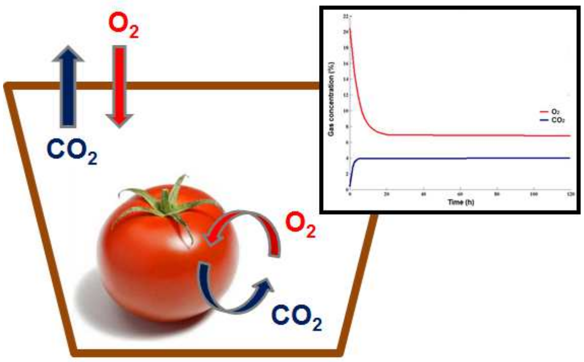


Figure I.1. Principe de l'emballage EAM passif et évolution de l'atmosphère (%O_2 et %CO_2 à l'intérieur de l'emballage

Chaque type de fruits et légumes est unique et présente donc des besoins spécifiques en termes d'atmosphère optimale ou de protection contre les dommages physiques ou les développements microbiens. Si la technologie de l'EAM passif est éprouvée, son succès dans la filière fruits et légumes demeure limité car il est difficile aujourd'hui d'atteindre l'atmosphère optimale recommandée à cause de deux freins majeurs que rencontrent les transformateurs/conditionneurs :

- l'absence d'approche et d'outils d'aide à la décision permettant de déterminer au cas par cas les propriétés de transferts des emballages en réponse aux besoins des produits respirant pour générer l'atmosphère recommandée pour une conservation optimale du produit.
- L'absence de matériaux d'emballage conçus et dimensionnés « à façon » pour présenter une gamme de propriétés de transferts couvrant la large gamme de besoins des produits respirant.

Trouver un emballage adapté au produit repose aujourd'hui majoritairement sur une approche de type essai-erreur (aussi appelée « pack and pray ») basée sur l'expérience et qui se révèle extrêmement couteuse en temps et en ressources. De plus les matériaux synthétiques aujourd'hui utilisés dans l'industrie de l'emballage alimentaire (polypropylène, polyéthylène basse et haute densité, polyéthylène téréphtalate,…) sont trop barrière à l'oxygène pour la grande majorité des produits respirant, entraînant une anoxie certaine et une altération accélérée des produits par apparition de déviations métaboliques. Ils sont donc micro ou macro-perforés ce qui permet d'étendre leur perméance à l'oxygène mais leur fait perdre leur caractère permsélectif (S, ratio of the CO_2 perméation on the O_2 perméation). De tels matériaux ne permettent pas d'atteindre des atmosphères à la fois pauvres en oxygène et en dioxyde de carbone, pourtant recommandée pour la plupart des végétaux.

En conclusion, les fruits et légumes frais sont la plupart du temps emballés dans des matériaux plastiques surdimensionnés, issus de ressources non renouvelables, qui sont ensuite perforés afin d'obtenir un matériau qui n'assurera pas la conservation optimale du produit mais exprimera juste des fonctions basiques comme le regroupement ou la protection physique. Alors que la consommation de fruits et légumes frais est aujourd'hui fortement encouragée pour ses effets indubitablement positifs sur la santé, leur mode de distribution et de conservation ne permet pas d'assurer facilement une consommation journalière de produits de bonne qualité.

ENVIRONNEMENT ET CONTEXTE SCIENTIFIQUE

Des activités de recherche sont actuellement développées dans différents centres de recherche européens pour concevoir des matériaux présentant des perméabilités aux gaz (oxygène, gaz carbonique, éthylène etc.), mais aussi à la vapeur d'eau, adaptés à l'emballage des aliments, notamment frais. L'UMR IATE s'implique depuis une dizaine d'année sur le développement de matériaux biodégradables composites et/ou nano-composites, pouvant être associés à des systèmes actifs de type absorbeurs d'O_2 ou émetteurs de CO_2, permettant de créer naturellement une atmosphère intra-emballage favorable à la conservation/évolution du produit (EAM passif ou actif). Un outil numérique de simulation (Transféromatic) permettant de prédire les concentrations gazeuses dans l'espace de tête de l'emballage qui résulte d'un équilibre entre transferts de gaz via l'emballage et la consommation/production par le produit a également été développé. Par la suite un outil similaire adapté aux EAM actifs a rendu possible le dimensionnement d'un emballage (polyéthylène basse densité micro-perforé + absorbeur d'O_2) permettant d'améliorer considérablement la conservation des endives avec maintien de la blancheur des feuilles et retard de rosissement du chicon et du développement microbien par rapport aux emballages macro-perforés actuellement utilisés [Charles et al. 2008]. Sur le plan des matériaux, les faibles teneurs en O_2 et en CO_2 nécessaires pour préserver les qualités organoleptiques et nutritionnelles des champignons de Paris, ont pu être atteintes grâce au développement de films à base de gluten, suffisamment perméables à l'O_2 et permsélectifs vis-à-vis de l'O_2 et du CO_2 [Guillaume et al. 2010a]

L'objectif de cette thèse est de contribuer au renforcement de l'approche de type intégrative et des outils d'aide à la conception de nouveaux emballages, développés au sein de IATE, en restant focalisés sur les matériaux bio-sourcés et biodégradables, conçus à partir d'agro-polymères, et sur leurs spécificités en termes de propriétés de transfert.

OBJECTIFS SCIENTIFIQUES ET TECHNIQUES

C'est dans ce contexte que mes travaux de thèse ont été conduits, dans le cadre du projet ANR TAILORPACK "Emballages biodégradables adaptés à la conservation des fruits et légumes" et financé par l'Agence Nationale pour la Recherche (ANR-07-PNRA-029). Coordonné par l'UMR Ingénierie des Agropolymères et Technologies Emergentes (IATE), le projet a été mené en partenariat avec le Centre Technique des Fruit et Légumes (CTIFL) de Lanxade et de Saint Rémy de Provence, l'UMR Sécurité et Qualité des Produits d'Origine Végétale (SQPOV) d'Avignon, l'équipe Multicouches de polyélectrolytes de l'Institut Charles Sadron (ICS) de Strasbourg, un fournisseur de matières premières industrielles d'origine végétale ADIVEC et la papeterie Gascogne Paper.

Les objectifs appliqués étaient de :

(i) développer un outil d'aide à la décision convivial prenant en compte les besoins des produits dans le but de concevoir des matériaux à façon, sur la base de propriétés de transfert ciblées ;

(ii) dépasser les limitations des matériaux synthétiques conventionnels, en associant des matrices végétales : des protéines de gluten de blé pour leurs propriétés de transfert intéressantes et des supports papiers pour assurer un renfort mécanique.

D'un point de vue scientifique, il s'agissait de :

(i) mettre en place une approche basée sur l'ingénierie reverse permettant de prendre en compte les besoins des produits végétaux dès le départ et de les transcrire en propriétés de transfert requises (cibles) de l'emballage pour concevoir des matériaux à façon ;

(ii) comprendre et mettre en œuvre des moyens de modulation (via la structuration, composition, mise en forme,…) des propriétés de transfert des matériaux protéiques (à base de gluten de blé) afin de réaliser les matériaux d'emballage répondant aux propriétés définies grâce à la nouvelle démarche de conception.

PLAN DE TRAVAIL

L'organisation du manuscrit est présentée dans la figure I.2 et repose sur 2 axes d'études. En ce qui concerne l'approche, un état de l'art recense l'évolution des modèles de simulations qu'ils soient spécifiques à des phénomènes précis (transferts de matières, respiration des produits, etc…) ou globaux (modèles couplés) et les améliorations qui pourraient leur être apportées. Par la suite, l'utilisation d'un outil de simulation en ligne (développé dans le cadre du projet) pour traduire les besoins du produit en propriétés de transfert, est étudiée dans un premier chapitre au travers de simulations/prédictions pour une gamme de fruits et légumes cibles. Enfin l'approche globale mise en place pour concevoir un matériau par ingénierie reverse est détaillée (5 étapes) et validée à travers le développement d'un emballage optimal pour la conservation des fraises dans le chapitre 3.

Concernant le volet matériaux, les propriétés relatives à l'emballage (de transfert et mécaniques) des matériaux protéiques et les possibilités de modulation de ces propriétés au travers de diverses stratégies de structuration, de formulation ou de procédé sont d'abord discutées dans l'état de l'art. Le premier chapitre démontre l'intérêt des protéines de gluten de blé comme matière première pour créer des matériaux couvrant une large gamme de perméation à l'oxygène (Pe_{O2}) et permsélectivités. L'étude de la relation structure-propriétés de transfert dans des matériaux à base de protéines de gluten de blé est présentée dans le chapitre 2 et pour 3 cas distincts de modulation de leurs propriétés de transfert:

- *Matériaux multichouches micro-structurés*, papiers enduits de gluten. Cette partie est consacrée à l'étude de l'impact de la structure interne d'un support fibreux destiné à l'enduction sur la structure du matériau enduit résultant et ses propriétés de transfert aux gaz (et de surface).
- *Matériaux multicouches nano-structurés*, assemblage couche par couche [nano-argiles/gluten] sur base papier. Cet article montre la possibilité de transposer la technique de construction couche par couche (déposition alternée de

polyélectrolytes de charge opposée) d'un support modèle synthétique régulier à un support « naturel », irrégulier et poreux tel que le papier, tout en utilisant des agropolymères à la place des polymères synthétique pour les dites couches. Il présente aussi comment ces couches (nature et nombre) et le support choisi pour le dépôt influencent les propriétés de transfert de ces matériaux.

- *Matériaux multicouches micro-structurés modifiés à l'échelle moléculaire*, papiers enduits de gluten et traités au CO_2. L'impact de la sorption de CO_2 sur les propriétés de transfert des matériaux résultants est ici discuté.

En dernier lieu, le chapitre 3 aborde la structuration d'un matériau actif pour la rétention/libération d'agent antimicrobien volatile pour la conception d'EAM dit « actif ».

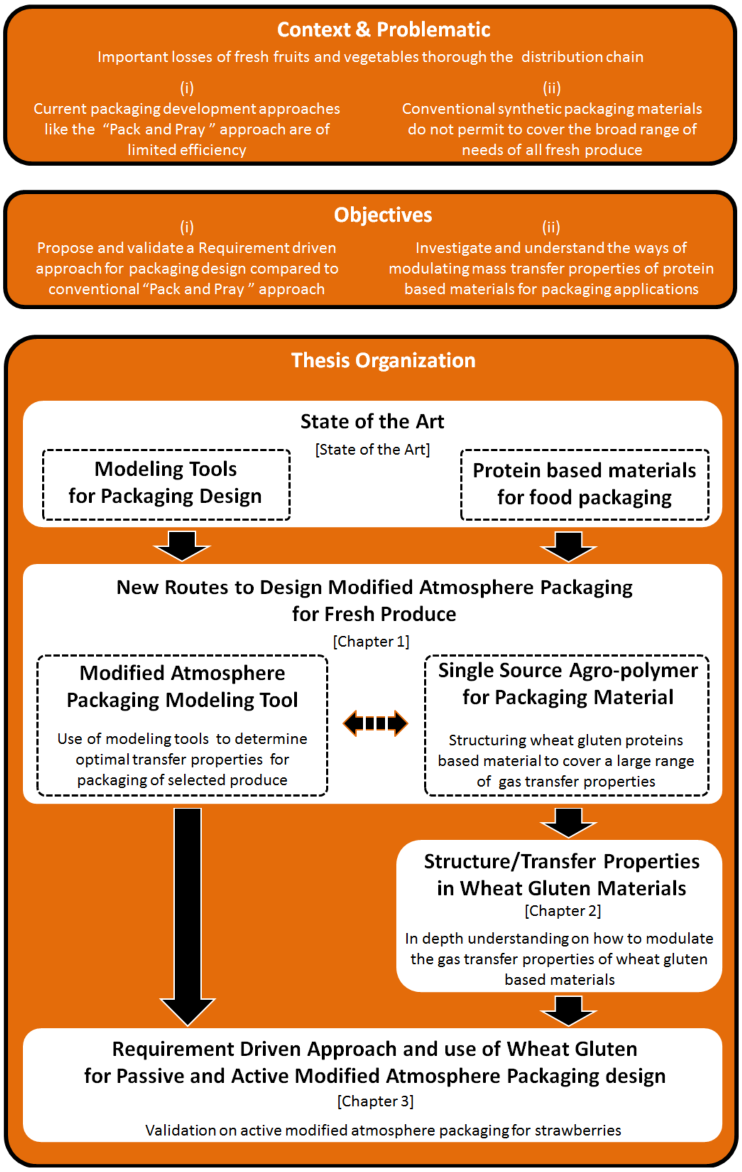
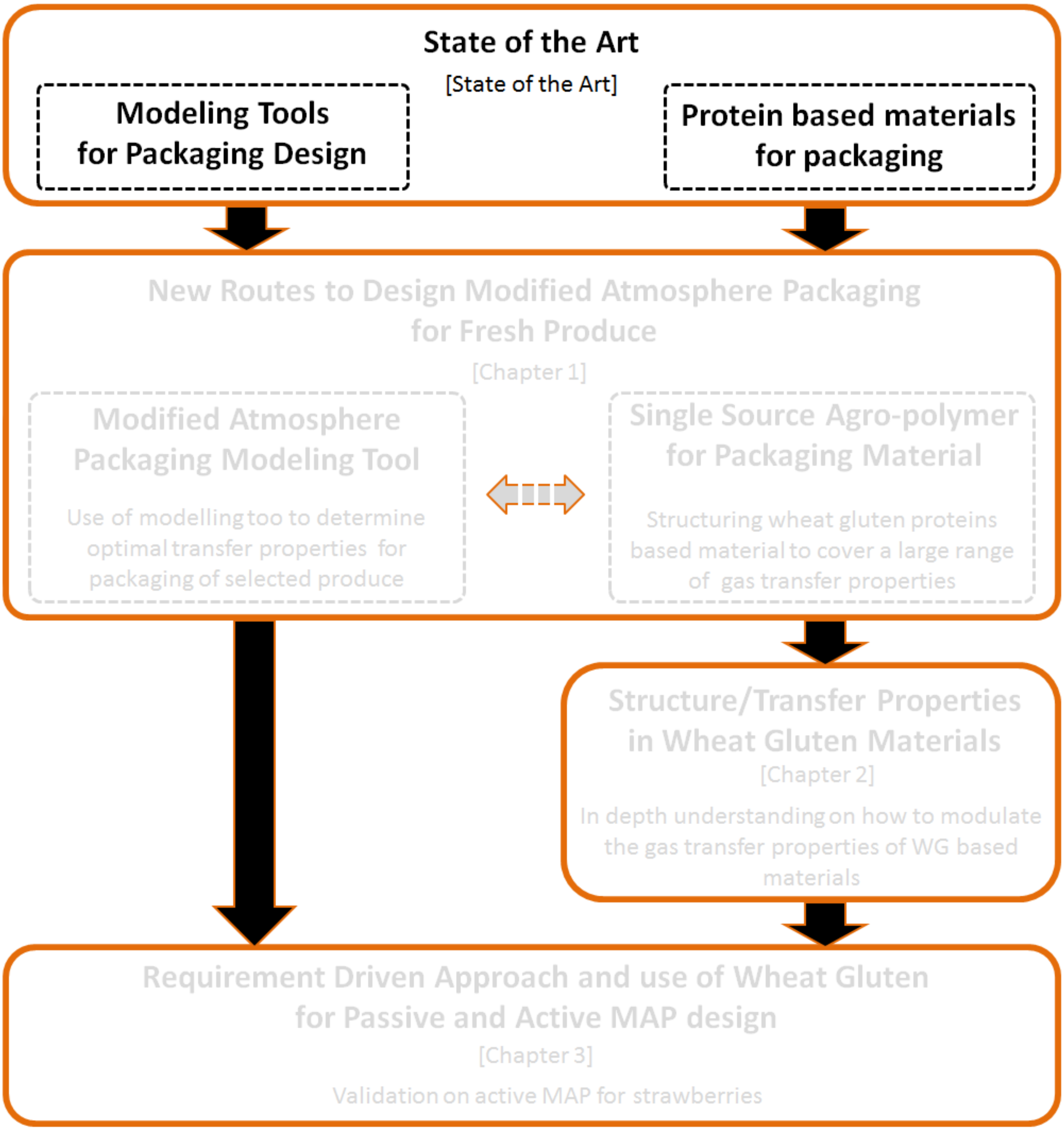


Figure 2. Contexte, objectif et organisation de la thèse

STATE OF THE ART



This first part consists in an analysis of the state of the art dealing with the possible application of requirement driven approach (RDA) to design packaging using modified atmosphere packaging (MAP) modeling tools and the pros & cons in using proteins as packaging materials, both for MAP of respiring produce.

This review work will allow definition of the critical points to investigate for improvement of packaging design for fresh and respiring produce and so set the scientific goals of the present thesis.

Part 1

Toward requirement driven approaches to design food packaging: focus on fresh and respiring products

INTRODUCTION

Over the last decades, conventional “trial and error” approaches, time and cost consuming, were phased out in favor of integrated and requirement driven approaches based on modeling tools and reverse engineering to develop decision-making tools in various fields of research and development (from oceanography to aerospace science) and used for different purposes: reduction of costs, increasing efficiency of a process, etc. However, this is not a generalized trend and some sectors just start to consider this powerful methodology. One of them is the food packaging’s sector and more precisely packaging of fresh fruits and vegetables, as a whole or fresh-cut. In 2011, nearly half of the fresh fruits and vegetable production was lost before consumption and in western countries, up to 30% of the losses happened during steps of the distribution chain where most of the produce are packed [Gustavsson et al. 2011]. Such figures really raise the question on how to increase the efficiency of their packaging.

Fresh fruits and vegetables are living (respiring) and fragile products with short shelf life. In addition to common food degradation phenomena, physico-chemical (dehydration and oxidation) and microbiological, they also undergo physiological degradations that inevitably lower their quality. One way to improve their storage is to use chilled temperature, and this is currently applied to fresh-cut produce [Manolopoulou et al. 1998; Vargas et al. 2006], but it is costly, difficult to manage and most of whole produce is distributed at ambient temperature. The other route is to change the atmosphere surrounding the produce in order to reach optimal atmosphere of storage, specific to each produce [Kader et al. 1989; Barron et al. 2002; Charles et al. 2008; Sandhya 2010]. Such atmosphere can be reached in controlled atmosphere rooms or modified atmosphere packaging (MAP). In this case, it relies on the natural interplay between the produce physiology (consumption or production of gases and vapors) and the transfer properties of the packaging material (gases and vapors permeation rates). MAP can also be considered as active: in addition to the afore-mentioned interplay, inert gas mixture can be flushed during conditioning or active systems (to release or absorb gases and/or vapors) can be added to the packaging as object or within the packaging material.

Whatever the technology used, success of MAP relies in quickly reach the optimal atmosphere recommended, then, designing and processing of MAP should rely on the smart use of materials tailored to the application. Then it should take into account, since the very beginning, the produce requirements and translate them into expected mass transfer properties. To allow and promote such a requirement driven approach, it is necessary to combine biological science to characterize the physiology of the target produce, material science to characterize mass transfer properties of the packaging, and mathematical science to perform simulation on modified atmosphere and properly dimension MAP. The aim of this work is to provide an overview on existing literature on MAP modeling that could be used in requirement driven approach as a first step of reverse engineering.

KEY ELEMENTS FOR UNDERSTANDING MAP OF FRESH PRODUCE

Physiology of fresh fruits and vegetables

Once harvested, fresh fruits and vegetables have to draw on their own reserves to maintain their cellular integrity. During respiration, stored carbohydrates are broken down into glucose, which is oxidized into CO_2, water and energy (adenosine triphosphate, ATP) according to several enzymatic steps but limited by the activity of the cytochrome oxidase.

$$C_6H_{12}O_6(Glucose*) + 6O_2 \rightarrow 6CO_2 + 6H_2O + 36ATP(energy*)$$

As soon as these substrates become unavailable, other carbonated resources that are essential (constitutive protein or membrane lipids) are consumed, leading to the death of the produce. Thus the potential shelf life of fruits and vegetables is closely related to their respiration rate, expressed as the quantity of O_2 consumed (or in a minor extend CO_2 released) per time and per mass of produce: the lower the respiration rate, the longer the potential shelf life [Marcellin 1975; Paull 1994]. Respiration is specific to each produce, in term of specie and variety, but may differ depending on its ripening stage, mainly for climacteric ones. Difference between climacterics and non-climacterics relies on their ability to synthesize the growth hormone ethylene in an autocatalytic way or not, respectively [Robertson 2006]. Minimal processing (peeling, slicing, etc.) causes a stress that induces an increase in respiration activity from 2 to 8 fold [Brecht 1995], and stimulate ethylene production from 5 to 20 fold [Pech et al. 1994]. In association with aerobic respiration, moisture vapor is produced and natural dehydration occurs, caused by diffusion of moisture vapor from the high concentration compartment in fresh product to the low concentration in the surrounding environment.

Optimal storage conditions

Aside lowering storage temperature that is known to reduce physiological reactions, another factor affecting the produce physiology is gas composition. Usually, lowering the O_2 level is really effective in reducing respiration, but anoxia (a switch to anaerobic catabolism and growth of anaerobic flora that produce undesirable off-flavors and off-odors) should be avoided [Nguyenthe et al. 1994; Varoquaux et al. 2005]. High CO_2 levels (more than 10%) might also reduce the respiration and ripening of several commodities [Mathooko 1996; Varoquaux et al. 2005] and can limit or inhibit the production of ethylene [Rothan et al. 1994; Rothan et al. 1997]. High CO_2 levels induce a bacteriostatic effect on aerobic flora but might lead to the development of anaerobic microorganisms. Moreover, injuries might occur when a fresh product is exposed to a level of CO_2 above its tolerance limit as for example formation of brown spots or yellowing that are common visual degradations caused by a high CO_2 content [Zagory et al. 1988; Lopez Briones et al. 1992]. Together with CO_2, 1-methylcyclopropene (1-MCP) is also considered as a competitive inhibitor of ethylene action and can be used to delay ethylene production and the respiration crisis [Fan et al. 2000; Hershkovitz et al. 2005]. Since the optimal combination of O_2 and CO_2 greatly depends on the respiratory activity of the product (values range from less than 35 up to 300 mg O_2 kg^{-1} h^{-1}) and its sensitivity to CO_2, there is no unique atmosphere composition that could be applied to all fresh commodities. Critical concentrations of O_2 and CO_2 exist for a lot of fruit or vegetable [Kader et al. 1998; Bishop et al. 2008].

Mass transport in MAP of fresh produce

As long ago as the 1930s, controlled atmospheres were used in storage rooms during shipment and transportation to preserve the freshness of fruits allowing the maintenance of low O_2 and moderate to high CO_2 levels. That is why MAP historically refers to a ratio of O_2 and CO_2 in the headspace of packaging. It can be and is more and more extended to other gases (e.g. argon and even xenon) or vapors (water vapor, ethylene, aroma compounds, etc.). In passive MAP, gas and vapor exchanges occur between the produce and its surroundings as well as through the packaging material as indicated in Figure S.1. Initially the headspace composition is air and then, after a transient period, it reaches a steady state when gas and vapor permeation through the material balances gas and vapor consumption and production from the produce. This steady state atmosphere must be as close as possible to the optimal recommended atmosphere otherwise it might be detrimental to the quality of the commodity [Floros et al. 2005].

Thus it is essential to carefully select a film with suitable gas and vapor permeabilities. Otherwise, the produce can be exposed to unsuitable gas compositions during the transient period, thus preventing the positive effects of the steady state atmosphere. It should be stressed that most synthetic polymer films exhibit too low a permeability to gases and vapors and most often need to be perforated (micro-holes) to allow sufficient gas exchange. Using active MAP, gas can be flushed (inert gas mixture) to quickly reach a desired ratio of O_2 and CO_2 and suppress the transient period of passive MAP so that the initial headspace composition is different from normal air. As shown in Figure S.1, active MAP can also be achieved by the release or absorption of gases or vapors from or by active agents in combination with gas/vapor permeation. This opens up new possibilities for packaging such as the controlled release of antimicrobial agents in case of temperature abuse and/or the removal of unsuitable substances such as ethylene when produced.

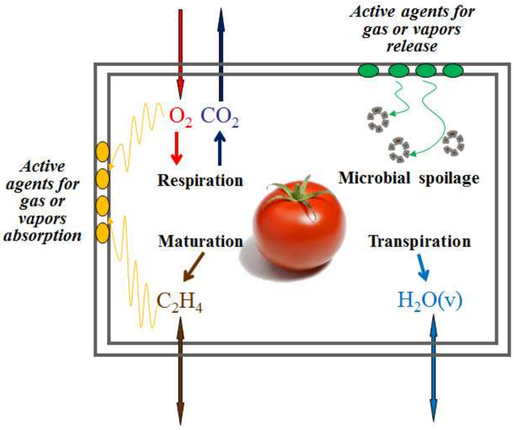


Figure S.1. Mass transport phenomena in passive and active MAP in relation to produce physiology.

(adapted from Guillaume et al.2011 : Modified atmosphere packaging of fruits and vegetables : modeling approach [Guillaume et al. 2011])

MODELING FOOD PHYSIOLOGY

Respiration

Fresh produce respiration models based on the Michaelis-Menten equations are the most common and the most fitting ones (Table S.1). They consider aerobic respiration as a single limiting enzymatic reaction (based on the activity of the cytochrome oxidase) in which the substrate is oxygen and express the respiratory rate as a function of the O_2 partial pressure and the Michaelis constant for O_2 consumption (apparent dissociation for enzyme/substrate complex). Basic Michaelis-Menten model has been first used by Chevillote et al. in 1973 [Chevillote 1973]. It has since been improved by taking into account the potential inhibitory effect of carbon dioxide content on oxygen consumption by the produce through the 4 common types of Michaelis-Menten inhibitions (competitive, uncompetitive, non-competitive and the combination of competitive and uncompetitive) as detailed in previous well documented reviews on the subject [Peppelenbos et al. 1996b; Fonseca et al. 2002]. The non-competitive inhibition is preferred among the other equations due to its simplicity of use and good fit with most existing data concerning common products [Fishman et al. 1995; Peppelenbos et al. 1996b], nevertheless uncompetitive inhibition might be more accurate in some cases [Fonseca et al. 2002]. The CO_2 production rate might be easily calculated as the produce of the O_2 consumption rate time the respiratory quotient (RQ, which is the ratio of CO_2 production on O_2 consumption) [Peppelenbos et al. 1996a]. The respiratory quotient is close to unit when sugars are catabolize (see respiration reaction: 6 moles of O_2 for 6 moles of CO_2) otherwise it is higher or lower; but in this case, catabolism of essential molecule occurs and the produce is in its end life.

Other approaches exist to model the respiration of fresh fruits and vegetable but they remain marginal in comparison to the Michaelis-Menten one. Linear [Henig et al. 1975; Fishman et al. 1996], polynomial [Yang et al. 1988; Gong et al. 1994], exponential [Cameron et al. 1989; Edmond et al. 1991; Beaudry et al. 1992; Beaudry 1993; Smyth et al. 1998; Del Nobile et al. 2006] models have been tried along with Langmuir adsorption based ones where the controlling mechanism was the adsorption of one molecule of O_2 at an active site of the cytochrome oxidase complex [Makino et al. 1996b; Makino et al. 1996a]. Several combinations of the model previously evocated (MM-exponential, exponential-polynomial, etc…) have also been considered and tested but did not give very good simulation/experiment adequacy [Talasila 1992; Talasila et al. 1992].

Transpiration

Basic models for fresh produce transpiration consider the moisture transfer through the skin as a function of biophysical and thermophysical parameters such as diffusivity, surface cellular structure, skin thickness or geometry (Table S.1). But they refer to parameters that are difficult to assess. More advanced models, as the ones used by Kang and Dong in 1998 [Kang et al. 1998] and Song et al. in 2002 [Song et al. 2002], are based on mass and heat transfers and take into account the respiration of the produce to predict the rate of vaporization. These models have been validated on apple and blueberry in fixed or controlled relative humidity (RH) and temperature conditions.

Production of other volatile compounds

There are very few model established on the production of ethylene and volatile compound by fresh produce (Table S.1). Grotte et al. in 2006 established a polynomial/logarithm model to predict ethylene production of apricot as a function of the amount of degree-days received after blooming [Grotte et al. 2006].

MODELING MASS TRANSPORT THROUGH PACKAGING MATERIAL

Gas transfer through dense and homogenous films is driven by solution-diffusion mechanisms. Such mechanisms, where gases first dissolve into the matrix at face of the sample exposed to higher gas content, then diffuse across the matrix to the face of the sample exposed to lower gas content and finally desorb from that face, are commonly modeled using the first Fick’s law (Table S.1). In this case, the driven flux is the difference in gas content between the 2 faces of the matrix and the resistance coefficient is the permeability towards the considered gas. This permeability can be decomposed into two terms: the diffusivity and the solubility, diffusivity being mostly dependent of the gas molecules sizes while solubility is more affected by the affinity between the gas and the matrix [Matteucci et al. 2006; Matteucci et al. 2008].

Composite material

When considering composite films (at macro or micro scale), the models become more complex as the properties of each components (matrix/filler or each layers) must be taken into account (Table S.1). In case of multilayer films, the overall permeability of the composite can be expressed as the association in parallel of the permeabilities of each layer (balanced by their respective thicknesses). And in case of matrix/filler structure, the same relation remains valid, except that the permeabilities of each component are not balanced by their respective thicknesses but by their respective quantities [Guillaume et al. 2011]. However it must be noted that these two models assume perfect adhesion between the layers or the different phases of the composites and so cannot predict the potential effects of any defects such as for example partial peeling in the case of multilayer composites.

Nano-composite material

The case of nano-composites is even more complex as the model should take into account the shape and the organization/orientation of the fillers inside the matrix in addition to the charge added (Table S.1). The first models, like the Bruggeman's model and its simplifications considered only spherical fillers [Matteucci et al. 2008]. Aris in 1986 [Aris 1986] and Cussler et al. in 1988 [Cussler et al. 1988] adapted it to flakes shape fillers assumed to be oriented perpendicularly to the gas flux and took into account the aspect ratio of the filler. Finally, Sorentino et al. in 2006 [Sorrentino et al. 2006] improved it by taking into account the orientation of the fillers in regard of the one of the gas flux. This last model was proven accurate and fitting on polycaprolactone, polyurethane and polypropylene matrix filled with modified montmorillonites nanoclays [Sorrentino et al. 2006] but required determination of parameters very difficult to assess such as the volume and diffusion coefficient at the interface region between the matrix and the nano-charges.

Micro-perforated material

Finally, for synthetic films, the case of micro and macro perforation has widely been assessed (Table S.1). In case of perforated structure, the gas transfer can be considered to happen solely through the perforations (gas transfer is 10^6 times greater through perforations (air) than through low density polyethylene (LDPE) for example [Mannapperuma et al. 1989]) and so the first Fick's law does not directly apply. To overcome this problem several authors have tried to use the Stephan-Maxwell flow equation but such model, despite being accurate was not versatile due to its high complexity [Guillaume et al. 2011]. Other authors have tried to resort to Fick's law modifications and obtained interesting results [Heiss 1954; Edmond et al. 1991; Fishman et al. 1996; Fonseca et al. 1996; Chung et al. 2003]. But the most efficient and versatile models to date are an improved model by Fonseca et al. (2000) [Fonseca et al. 2000] based on the one of Edmond et al. (1991) [Edmond et al. 1991] for a single perforation, an evolved model based on Fick's law established by Techavises at al. in 2006 [Techavises et al. 2008] (able to model transfer of O_2, CO_2, N_2 and water vapor) and an empirical model built by Gonzalez et al. in 2008 [Gonzalez et al. 2008].

MODELING MASS TRANSPORT IN ACTIVE PACKAGING

Release of active agents

Aside of the classical O_2, CO_2 and water vapor (WV) transfer, it is also possible to simulate the retention and the release of more complicated compounds like aroma compounds and/or antimicrobial volatile agents (Table S.1). In such case, the compound is usually already embedded inside the polymeric matrix and will be released from it into the packaging internal atmosphere. The concentration of the compound inside the matrix varies with the time and the position, following the second Fick's law. At the interface between the intra packaging atmosphere and the matrix, the equilibrium is assumed to be reached instantaneously, so the rate of substance transfer from the matrix to the atmosphere is equal to the rate which this substance reaches the interface. And such rate is driven by the internal diffusion through the matrix. In 2008, Chalier et al. [Chalier et al. 2008] successfully used this model to predict the kinetic release of carvacrol from a soy protein matrix in various conditions of temperature and humidity. Humidity was found to be the triggering effect of the release as increasing it above 80% lowered the glass transition temperature (Tg) of the soy protein isolate (SPI) matrix and allowed carvacrol diffusion towards the interface. There also is another approach, more adapted to release into a liquid phase and mostly used for drug delivery application [Reinhard et al. 1991; Cypes et al. 2003] that is based on a simplified derivation of the second Fick's law. This model has been validated by Lin et al. in 2007 [Lin et al. 2007] for release of aspirin from a Poly(methyl-methacrylate)/silica composite when the release remained inferior to 60% of the initial load.

Scavenging system

Oxygen and/or carbon dioxide scavengers can be introduced in the packaging, under the form of sachet or embedded in the packaging material, to reduce the transient period and so reach the optimal atmosphere composition for preservation faster. In case of sachets it is possible to model the kinetic of O_2 or CO_2 adsorption by a first order equation involving an exponential function (Table S.1). This model can be completed by a Arrhenius type equation to take into account the influence of temperature [Charles et al. 2004]. It should be noted that he initial content in packaging was proved to have only few to no impact on the adsorption kinetic [Tewari et al. 2002; Charles et al. 2004] and that some O_2 scavenger on the market can also cause parasitic adsorption of CO_2 (no parasitic adsorption of O_2 by CO_2 scavenger has been reported yet) [Charles et al. 2004].

DEPENDENCE TO ENVIRONMENTAL PARAMETERS

Temperature dependence

As many physical or biological parameters, the respiration (and consequently transpiration) rate of fresh produce and the gas and vapor transfers through polymeric films are impacted by the temperature (Table S.1). This effect can be described by a simple Arrhenius law with the energy of activation for oxygen consumption or gas permeation respectively; or by the Q_{10} relation that is the ratio of the respiratory rate or gas permeation at a given temperature to that at this temperature plus 10°C [Guillaume et al. 2011]. It should be noted that Q_{10} is commonly used by physiologist, whereas Arrhenius relation and the energies of activation are more commonly used in the field of material science. Also, in the peculiar case of perforated films, the influence of the temperature on film permeation is considered negligible [Silva 1995; Fonseca et al. 2000].

Relative humidity dependence

Transfer properties of polymeric films may also dependent of the relative humidity, especially for agro-materials such as protein-based one that often undergo lowering of their glass transition temperature (Tg) and swelling with the increase of the RH. However there is currently no global model to simulate the impact of the RH on the transfer properties of all polymers. There were some attempts focused on one type of polymer and one gas, as for example the study conducted on the influence of moisture content on the ethylene transfer through wheat gluten (WG) films by Mujica-Paz in 2005 [Mujica Paz et al. 2005]. But the polynomial model proposed was not adaptable to other materials or conditions.

Table S.1. Models that could be used for MAP prediction (gas exchanges through packaging material and produce physiology)

Phenomenon	***Model***	**Equation**	**Details**	**Limitations**	**Ref**
		Produce physiology			
Respiration **(O_2 consumption)**	*Michaelis Menten*	$R_{O2} = \frac{R_{maxO2} \times P_{O2}}{Km_{appO2} + P_{O2}}$	R_{O2}: respiration rate; $Rmax_{O2}$: maximum R_{O2}; Km_{appO2}: apparent Michaelis constant; P_{O2}: oxygen partial pressure	For simple case with no inhibition only	[Chevillote 1973]
	Michaelis Menten, competitive inhibition	$R_{O2} = \frac{R_{maxO2} \times P_{O2}}{Km_{appO2} \times \left(1 + \frac{P_{CO2}}{Ki_{CO2}}\right) + P_{O2}}$	R_{O2}: respiration rate; $Rmax_{O2}$: maximum R_{O2}; Km_{appO2}: apparent Michaelis constant; P_{O2}, P_{CO2}: O_2 & CO_2 partial pressures; KiC_{O2}: inhibitor-enzyme constant	-	[Peppelenbos et al. 1996b; Fonseca et al. 2002]
	Michaelis Menten, uncompetitive inhibition	$R_{O2} = \frac{R_{maxO2} \times P_{O2}}{Km_{appO2} + P_{O2} \times \left(1 + \frac{P_{CO2}}{K'i_{CO2}}\right)}$	R_{O2}: respiration rate; $Rmax_{O2}$: maximum R_{O2}; Km_{appO2}: apparent Michaelis constant; P_{O2}, P_{CO2}: O_2 & CO_2 partial pressures; $Ki'C_{O2}$: inhibitor-enzyme-substrate constant	-	[Peppelenbos et al. 1996b; Fonseca et al. 2002]
	Michaelis Menten, non - competitive inhibition	$R_{O2} = \frac{R_{maxO2} \times P_{O2}}{(Km_{appO2} + P_{O2}) \times \left(1 + \frac{P_{CO2}}{K''i_{CO2}}\right)}$	R_{O2}: respiration rate; $Rmax_{O2}$: maximum R_{O2}; Km_{appO2}: apparent Michaelis constant; P_{O2}, P_{CO2}: O_2 & CO_2 partial pressures; $Ki''C_{O2}$: both inhibitor-enzyme-substrate and inhibitor-enzyme constant	-	[Peppelenbos et al. 1996b; Fonseca et al. 2002]
	Michaelis Menten, competitive and uncompetitive inhibition	$R_{O2} = \frac{R_{maxO2} \times P_{O2}}{Km_{appO2} \times \left(1 + \frac{P_{CO2}}{Ki_{CO2}}\right) + P_{O2} \times \left(1 + \frac{P_{CO2}}{K'i_{CO2}}\right)}$	R_{O2}: respiration rate; $Rmax_{O2}$: maximum R_{O2}; Km_{appO2}: apparent Michaelis constant; P_{O2}, P_{CO2}: O_2 & CO_2 partial pressures; KiC_{O2}: inhibitor-enzyme constant; $Ki'C_{O2}$: inhibitor-enzyme-substrate constant	-	[Peppelenbos et al. 1996b; Fonseca et al. 2002]
	Linear (example)	$R_{O2} = x \times y_{O2}$	R_{O2}: respiration rate; y_{O2}: quantity of O_2 in %; x: slope in $ml.kg^{-1}.h^{-1}.\%^{-1}$	No verification of fit adequacy	[Henig et al. 1975]

	Polynomial (example)	$R_{O2} = a + \left(b^2 - c \times (d - y_{O2})\right)^e$	y_{O2}: quantity of O_2 in %; a, b, c, d & e: constants determined empirically	Constants valid only in determined conditions	[Gong et al. 1994]
	Exponential (example)	$R_{O2} = a + \left(b \times exp(-c \times t)\right)$	R_{O2}: respiration rate; a, b & c: constants determined empirically t: time	Constants valid only in determined conditions	[Smyth et al. 1998]
Respiration (CO_2 production)	-	$R_{CO2} = RQ \times R_{O2}$	R_{CO2}: CO_2 production rate; R_{O2}: respiration rate; RQ: respiratory quotient	-	[Peppelenbos et al. 1996a]
Transpiration	-	$m = \dfrac{Q_i - \left(w \times C_s \times \frac{dT}{dt}\right)}{\lambda}$ with: $Qi = w \times \left(\frac{2186}{6}\right) \times \left(\frac{R_{O2}+R_{CO2}}{2}\right) \times \alpha$	m: vaporization rate; Q_i: internal heat; C_s: specific heat; w: weight of produce; T: temperature λ: latent heat vaporization; R_{CO2}: CO_2 production rate; R_{O2}: respiration rate; α:ratio of energy dissipated as heat	-	[Kang et al. 1998; Song et al. 2002]
Emission of ethylene	*Polynomial & logarithm*	$ln(ethylene\ production) = a \times DD + b$	DD: sum of degree-day; a & b: constants determined empirically	-	[Grotte et al. 2006]
Transfers in passive packaging					
Gas (O_2/CO_2/C_2H_4) and vapor exchanges through simple material	*1st Fick's Law*	$J_A = P_A \times s \times \frac{P_2 - P_1}{e}$ with $P_A = D_A \times S_A$	J_A: steady state flux of gas A; P_A: coefficient of permeability for gas A; P_1 & P_2: gas partial pressure across film; e: thickness; s: surface; D_A: diffusion coefficient for gas A; S_A: solubility coefficient for gas A	-	[Matteucci et al. 2006; Matteucci et al. 2008]
Gas and vapor exchanges through multilayered composite material	*Additivity of permeability coefficients*	$\frac{e_T}{P_T} = \sum_{i=1}^{n} \frac{e_i}{P_i}$	P_T: global coefficient of permeability; e_T: global material thickness; P_i: coefficient of permeability of layer I; e_i: thickness of layer I; n: number of layers	-	[Guillaume et al. 2011]
Gas and vapor exchanges through in-bulk composite material	*Bruggerman's model*	$\frac{(P_C/P_M) - (P_D/P_M)}{1 - (P_D/P_M)} \times \left(\frac{P_C}{P_M}\right)^{1/3} = 1 - \Phi_D$	P_C, P_M, P_D: coefficient of permeability for composite, matrix and disperse phase; Φ_D: disperse phase volume fraction	Spherical fillers, non-applicable to nano-composites	[Matteucci et al. 2008]

Gas and vapor exchanges through nano-composite material	*Cussler's model*	$\frac{D_c}{D_0} = \left(1 + \frac{\alpha^2 \times \Phi_D^2}{1 - \Phi_D}\right)^{-1}$	D_C & D_0: diffusion coefficient with and without nano-charge; α: nano-charge aspect ratio; Φ_D: disperse phase volume fraction	For impermeable nano-charges oriented perpendicularly to the gas flux only. Effect of matrix/charge interface neglected	[Cussler et al. 1988]
	Improved Cussler's model	$\frac{D_c}{D_0} = \frac{(1 + \beta \times \Phi_D)}{\left((1 - \Phi_D) + \Phi_D \times \left(\frac{L + 2e}{L \times \sin\theta + 2e \times \cos\theta}\right)^2\right)}$ with: $\beta = \left(\frac{V_s \times D_S}{V_f \times D_0} - \frac{V_s + V_f}{V_f}\right)$	D_C & D_0: diffusion coefficient with and without nano-charge; β: fitting parameter β: fitting parameter; L & e: length and thickness of nano-charge; θ: angle of pellet orientation; V_s & D_s: volume and diffusion coefficient at the interface; V_f: volume of unmixed matrix Φ_D: disperse phase volume fraction	V_s and D_s are very hard to calculate and depend of many parameters. The model has been validated for $\beta = -1$ only	[Sorrentino et al. 2006]
Gas and vapor exchanges through perforated material	*Empirical*	$TR_A = a \times S_h^b$	TR_A: transmission rate of gas O_2 or CO_2; a & b: empirically determined constants; S_h: perforation surface	Only validated for O_2 and CO_2	[Gonzalez et al. 2008]
	Empirical	$y_a = y_a^e + (y_a^i - y_a^e) \times \exp\left(-\frac{K}{V} \times t\right)$ with: $K_{O2} = a \times D^b \times L^c$ $K_{CO2} = \beta \times a \times D^b \times L^c$	y_a: O_2 or CO_2 volumetric concentration at time t; y_a^e: volumetric concentration in the surrounding atmosphere; y_a^i: initial volumetric concentration; K: mass transfer coefficient; V: package volume; t: time D: tube diameter; L: tube length; a, b & c: empirically determined constants	Only validated for O_2 and CO_2	[Fonseca et al. 2000]
	Fickian model	$\frac{dV_i(t)}{dt} = n_p \times D_i \times (p_i^{out} - P_i^{in})$	$V_i(t)$: volume of gas i inside the package at time t; n_p: number of perforation; D_i: effective permeability of one perforation to gas I; p_i^{out} & P_i^{in}: partial pressure of i outside and inside the package		[Techavises et al. 2008]

Transfers in active packaging					
Active compound release from material	*Simplified Fickian mode*	$M_t/M_0 = k \times t^{0.5}$ with: $k = 4\sqrt{\frac{D}{\pi e^2}}$	(Mt/Mo): fraction of compound released; k: constant specific to compound; D: diffusion coefficient of the compound in the material; e: thickness of the material	Valid only for release of less than 60% of the initial load	[Chalier et al. 2008]
	Fickian model	$-D \times \frac{\partial C}{\partial x} = k \times (C_{L,t} - C_{L,\infty})$ where: t>0 and x=L and material surface/atmosphere concentrations equilibrium is assumed to be reached instantaneously	x: distance; t: time; L: package thickness; C: package concentration in active compound; $C_{L,t}$: concentration at material surface $C_{L,\infty}$: concentration required at the surface to maintain equilibrium with the concentration in the package atmosphere	-	[Guillaume et al. 2011]
Absorption by scavengers (O_2, CO_2, WV)	*First order equation*	$N_{A\,t} = N_{A\,0} \times (1 - \exp(-kt))$ where the temperature dependence of k can be expressed as: $k = X \times exp\left(\frac{E_a}{RT}\right)$	$N_{A\,t}$: adsorption capacity at time t; $N_{A\,0}$: maximal adsorption capacity; k: adsorption rate constant; X: temperature dependent factor; E_A: energy of activation; R: gas constant; T: absolute temperature	-	[Charles et al. 2004]
Impact of environmental parameters					
Impact of temperature on produce respiration	*Arrhenius*	$R_{O2} = R_{O2\,0} \times \exp\left(-\frac{E_a}{RT}\right)$	R_{O2}: respiration rate; $R_{O2\,0}$: temperature dependent factor; E_a: energy of activation; R: gas constant; T: absolute temperature	-	[Guillaume et al. 2011]
	Q_{10} relation	$Q_{10} = \left(\frac{R_{O2\,1}}{R_{O2\,2}}\right)^{\frac{10}{(T_2 - T_1)}}$	Q_{10}: ratio of respiration rate at T and T+10; $R_{O2\,1}$ & $R_{O2\,2}$: respiration rates; T_1 & T_2: temperatures	-	[Guillaume et al. 2011]

COUPLING MATHEMATICAL MODELS

All the mathematical models presented before are of great interest and importance to design MAP for fresh produce. But to be efficient they need to be combined to allow simulation of the headspace atmosphere evolution or prediction of the optimal transfer properties of the packaging to reach the optimal atmosphere for conservation.

MAP simulation models ("mass balance")

There are several MAP modeling tools based on models often referred as "mass balance models" that take into account at least both the effect of produce respiration and the effect of packaging transfer properties on headspace atmosphere composition [Jacxsens et al. 2000a; Jacxsens et al. 2000b; Paul et al. 2002; Del Nobile et al. 2007; Techavises et al. 2008]. Some even take into account the presence of scavengers as the example model from Charles et al. 2003 detailed in Figure S.2, for modeling of O_2 and CO_2 content inside an active packaging (O_2 scavengers) for chicory [Charles et al. 2003]. However, most of these models only allow simulation of the atmosphere composition evolution (not prediction) and so cannot be used directly for reverse engineering and packaging conception by RDA. Furthermore, none of them consider complex material such as (nano)composite and they are not always easy to use or to understand for all the stakeholders of the fresh fruits and vegetable industry and doing all calculations manually can take a large amount of time.

(Eq 1 - O_2)

$$\frac{dnO_2}{dt} = \underset{\substack{\textit{First Fick's Law}\\ \text{Gas transfer through packaging}}}{\left[\frac{PeO_2 \times S}{e} \times (pO_2ext - pO_2pkg)\right]} - \underset{\substack{\textit{Michaelis-Menten}\\ \text{Produce respiration}}}{\left[RR_{O2} \times m\right]} - \underset{\substack{\textit{First order equation}\\ \text{O}_2\text{ absorbtion by scavengers}}}{\left[(N_{O2})_{to} \times (1 - exp(-kt))\right]}$$

(Eq 2 - CO_2)

$$\frac{dnCO_2}{dt} = -\underset{\substack{\textit{First Fick's Law}\\ \text{Gas transfer through packaging}}}{\left[\frac{PeO_2 \times S}{e} \times (pCO_2ext - pCO_2pkg)\right]} + \underset{\substack{\textit{Michaelis-Menten}\\ \text{Produce respiration}}}{\left[RR_{O2} \times m \times RQ\right]}$$

$$with{:}\ RR_{O2} = \underset{\substack{\textit{Michaelis-Menten}\\ \textit{(non-competitive inhibition)}}}{\frac{RR_{O2max} \times pO_2pkg}{(Km_{O2app} + pO_2pkg) \times \left(1 + \frac{pCO_2pkg}{Ki_{CO2}}\right)}}$$

Figure S.2 – Mathematical model for simulation of the evolution of headspace composition inside an active MAP

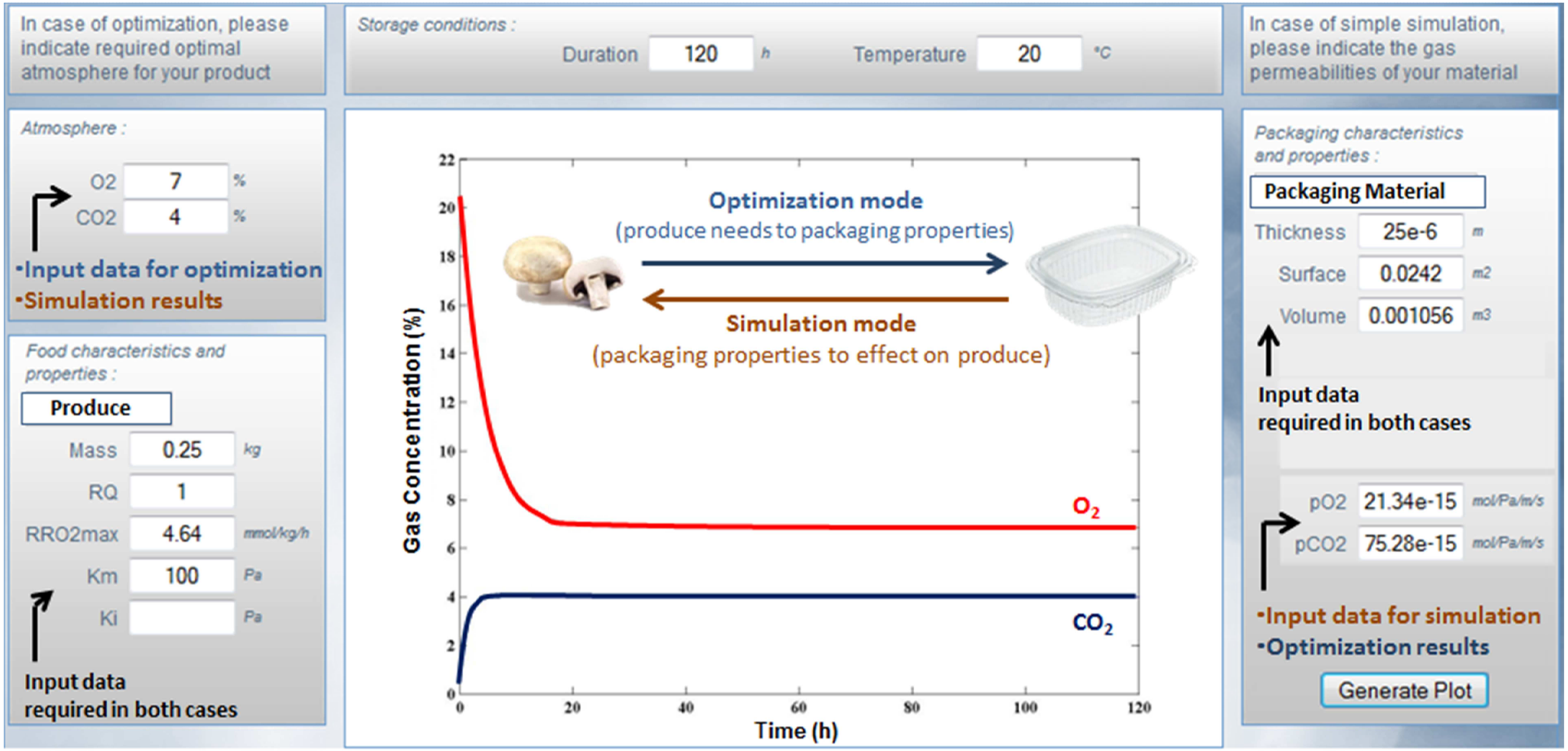
MAP simulation tools (simulation and prediction)

So with the aim of developing and promoting reverse engineering and the requirement driven approach for MAP design, user friendly MAP prediction software such as Tailorpack (www.tailorpack.com) or PackinMap [Mahajan et al. 2007] (www.packinmap.com), have been built. These simulation tools are able to predict the evolution of the intra-packaging atmosphere by modeling: the respiration of the produce, the permeability of the packaging (dense film with tailorpack and microperforated film with packinmap), and the influence of the temperature (on produce and packaging). In addition, by using numerical loops and a fitting algorithm, they are also able to predict the optimal oxygen and carbon dioxide permeabilities properties a packaging should feature for optimal preservation of a selected produce. Usually three types of input data are required to run such tools: respiratory parameters of the produce, targeted, basic dimensions of the packaging, and optimal atmosphere composition (in prediction mode) or gas transfer properties of the packaging material (in simulation mode).

The Tailorpack (Figure S.3, Table S.2) modeling tool is a free to use web-application that does not include a database on produce and packaging material. The advantage is that you can enter precise data about your produce physiology (from bibliography or personal measurements), but the obvious drawback is that personal databases on both produce and packaging materials are required as the application will give you the optimal permeabilities the packaging material should feature, but will not suggest any commercial film that feature such properties to the user.

The PackinMap tool is a commercial web application that is based on the same principle than the Tailorpack one but coupled with databases on fresh produce respiration, fresh produce optimal atmosphere for preservation and packaging materials transfer properties [Sousa-Gallagher et al. 2013]. These databases allow the software to suggest an actual material to use for packaging in addition of the optimal transfer properties result and relieved the user of any particular preliminary bibliographical researches on the produce. In addition, the PackinMap application can suggest to micro-perforate a particular film when appropriate.

With such tools, it is already possible for any stakeholder of the fresh fruits and vegetable industry to both easily simulate the evolution of the intra-packaging atmosphere composition (simulation mode) or easily calculate the required gas transfer properties (P_{O2}, P_{CO2}, S) of a packaging material for optimal conservation (prediction mode) and then select the appropriate material for optimal preservation.



In case of optimization, please indicate required optimal atmosphere for your product

Storage conditions :

Duration 120 h

Temperature 20 °C

In case of simple simulation, please indicate the gas permeabilities of your material

Atmosphere :

O2 7 %

CO2 4 %

•Input data for optimization

•Simulation results

Food characteristics and properties :

Produce

Mass 0.25 kg

RQ 1

RRO2max 4.64 mmol/kg/h

Km 100 Pa

Ki Pa

Input data required in both cases

Packaging characteristics and properties :

Packaging Material

Thickness 25e-6 m

Surface 0.0242 m2

Volume 0.001056 m3

Input data required in both cases

pO2 21.34e-15 mol/Pa/m/s

pCO2 75.28e-15 mol/Pa/m/s

•Input data for simulation

•Optimization results

Figure S.3 – Interface of the Tailorpack web application for MAP modeling

Table S.2. Example of the use of the Tailorpack MAP modeling tool, input data and results

Produce	**Input Data**											**Results**		**Is there an existing conventional plastic that fits the requirements?**
	Produce characteristics				**Packaging Dimensions**					**Optimal Atmosphere**		**Optimal Packaging Permeability**		
	RR_{O2max}	**RQ**	**Km_{appO2}**	**Ki_{CO2}**	**Type**	**Surface**	**Thickness**	**Volume**	**Mass**	**O_2**	**CO_2**	**O_2**	**CO_2**	
	($mmol.kg^{-1}.h^{-1}$)		**(kPa)**	**(kPa)**		**(m^2)**	**(µm)**	**(m^3)**	**(g)**	**(%)**		**(10^{-16} $mol.Pa^{-1}.m^{-1}.s^{-1}$)**		
Chicory	1.400	0.74	9.26	1515	Sachet	0.2140	50	0.00200	500	1	5	2.8	12.2	Yes (LDPE)
Apricot	1.000	0.78	4.50	-	Light Wood Punnet*+Wrap	0.0756	50	0.00100	500	3	2	17.9	137.5	No

*Considered as totally permeable to gases, only wrap regulates gas transfer

PERSPECTIVES

Current MAP modeling tools are quite new and still need to be improved before being considered as decision-making tool. First of all, they are only available for the whole produce and do not take into account processing steps encountered in fresh cut produce (washing, peeling, slicing, etc.). The increasing demand in such ready to eat food would drive further researches on the influence of such processing steps on the physiology of fruits and vegetables, leading to more literature and data. Databases management is also a bottleneck to overcome. At this time, when they exist, they are restricted to few produce and very few materials (since modeling tool consider at this time material as dense film or micro-perforated ones). They do not refer to complex (nano)composite material that can be used to overcome some weakness of neat films. In addition, cost, availability of raw material, potential contaminants, processability, mechanical properties, environmental impact, and so one, are various elements that need to be considered when designing packaging and should be part of future powerful decision-making tools and databases. This would allow to select and rank a bunch of fitting existing materials not only on the main permeability criteria but also on additional secondary criteria such as the price or a special feature (cost, transparency, color, biodegradability, etc). And, if no material matches the defined criteria well enough, one can imagine a tool that would propose the optimal formulation and structuration of a new material to match them. Of course, such powerful tool would require further improvements in the accuracy and versatility of the current models for structure/transfer permeability relationship in composite materials (especially the nanostructured ones).

There is still no modeling application that includes the water vapor and ethylene transfers, probably mostly due to the difficulty to model such physiological behavior. And there is also no application for Active MAP that takes into account either the presence of scavenger or the incorporation of active agent inside the packaging material for a release in conditions of use/retailing. These two last improvements could be envisaged, given the establishment of complete database about the scavenger formulas and the antimicrobial compound diffusivity inside (at least) common polymeric matrixes respectively. Then other improvement would be to take into account the variability of the fresh produce in terms of respiratory characteristics. The PackinMap software includes an option that allows the user to define the % incertitude and so obtain minimal and maximal P_{O2} and P_{CO2} optimal values instead of single ones, thanks to a monte-carlo simulation. While interesting and allowing fast calculation this method does not take into account real variability of the produce related input data. Recently, Guillard et al. (2011) [Guillard et al. 2011] proposed another approach to implement the impact of variability into a model similar to the one used by the Tailorpack application. This approach was based on interval analysis instead than on probability and gave a better compromise between accuracy of the results and simulation time according to the authors.

Part 2 (Review)

Multi-scale structuration of protein based materials for MAP of fresh produce

Thibaut Cagnon, Carole Guillaume, Valérie Guillard and Nathalie Gontard

Trends in Food Science – Submitted

INTRODUCTION

Over the last decades, research and development programs on eco-friendly materials, so-called “bio-plastics”, have boomed as an alternative to plastic based materials in order to lower environmental impact such as reducing the consumption of fossil fuel resources and plastic wastes accumulation, saving non-renewable energy and decreasing greenhouse gas emission. This route does not constitute a total substitution of plastics, that are today ubiquitous, but a replacement in specific applications where the balance environmental impact/efficiency is high such as food packaging. It has been demonstrated in UK that 11.9 million tons of short-lived food and their packaging were discarded within a year of manufacture, and packaging wastes contributed 6 million tons of carbon dioxide to the atmosphere (report by the Waste and Recycling Action Programme, 2010). Besides environmental purpose, bio-plastics should exhibit original properties far from conventional materials that should better meet the requirements of foodstuff than synthetic polymers, and most of all short lived commodities as living or respiring ones.

Bio-plastics classification relies on the origin of polymers, renewable or fossil, the synthesis of polymers, nature-made or chemical, and/or their end life, i.e. recyclable, biodegradable, or compostable [Averous 2004; Guilbert et al. 2011]. Among them, extractible agropolymers, as polysaccharides and proteins, appear of great interest because in addition to good film forming ability [Gennadios 1994; Cuq et al. 1998; Gontard et al. 2011], they benefit from low green-house gas emissions and energy use since they do not require deconstruction/reconstruction steps, and exhibit a remarkable biodegradability and non-ecotoxicity [Domenek et al. 2004]. Due to the heterogeneity of their constitutive amino-acids and their consequent folding pattern, proteins offer larger chemical functionalities and network structures than other polymers and that leads to unique mass transfer properties that depend on physical, chemical and environmental parameters (including greaseproof character [Trezza et al. 1994; Gallstedt et al. 2005] or the possibility to be used as a vector for volatile compounds [Ben Arfa et al. 2007a; Chalier et al. 2008; Mascheroni et al. 2010; Mascheroni et al. 2011; Balaguer et al. 2012]). One of the most influent parameter is the relative humidity (RH). In low relative humidity conditions, protein based material are considered as barrier material [Gontard et al. 1996b; Guilbert et al. 1997; Cuq et al. 1998] but when RH is increased (higher than 60%), they show high oxygen and carbon dioxide permeabilities that permit O_2 and CO_2 exchanges [Gontard et al. 1996b] favorable to modified atmosphere packaging of fresh fruits and vegetables, as it was previously demonstrated on mushroom [Barron et al. 2002; Guillaume et al. 2010b]. Such a behavior is very promising for modified atmosphere packaging of fresh produce that relies on the natural interplay between the produce respiration (oxygen consumption and carbon dioxide production) and gases permeation through the packaging material in order to slow down the produce respiration and so delay physiological and biochemical changes, and thus degradation rate [Kader 1987; Kader et al. 1989; Solomos et al. 1989]. Each fresh produce features its own MAP requirement for optimal preservation and so requires a specific packaging but all roughly need an environment poor in both O_2 and CO_2 [Kader et al. 1989], which is not easily reach with most of the conventional synthetic plastics, even when they are micro-perforated [Exama et al. 1993; Al-Ati et al. 2003].

Despite interesting mass transfer properties, development of protein-based material for food packaging is still limited due to their poor mechanical properties (too brittle at low relative humidity or too sticky at high relative humidity). However this limitation can be overcome through various reinforcement strategies at different scales, acting on physical parameters (e.g. temperature and pressure) or chemical ones (e.g. fillers, plasticizers, pH, other polymers). The aim of this section is to review how different structuration, shaping and formulation strategies can improve mechanical properties of protein-based and how these strategies affect their mass transfer properties, focusing on potential MAP applications for fresh produce. First part of this work is dedicated to the original properties of self-supported protein based materials adapted to MAP applications in comparison to other polymer from fossil or renewable resources. Second and third parts deal with the different structuring strategies at micro- and nano-scales, respectively, to produce either mono- or multi-layer(s) material. The third part is based on the more recent literature and/or prospective strategies.

SELF-SUPPORTED PROTEIN BASED MATERIALS: GENERAL CONCERNS FOR MAP APPLICATIONS

Properties required for MAP applications

There are two types of mandatory material properties for MAP applications: mechanical and mass transfer properties. Regarding the first category, the materials must present sufficient rigidity (Young modulus, E) and resistance (Tensile strength, TS), and/or sufficient elasticity (Strain at Break, SB) depending on the application foreseen (punnet/tray or film/sachet, respectively). Regarding mass transfer properties, the materials must present adequate oxygen, carbon dioxide and water vapor permeation values for optimal preservation of the produce considered. It should be noted that while water vapor transfer in protein based materials have been extensively studied, less attention has been paid to oxygen transfer and very few to carbon dioxide transfer as can be seen on Table S.3 (page 82). Then the permselectivity ratio (CO_2 permeation / O_2 permeation), important for MAP, is not always easily available. This raised the need of future complete studies on transfer properties for MAP application, looking at water vapor, oxygen and carbon dioxide permeabilities altogether.

One particularity of MAP is that this technique often involves high relative humidity inside the packaging, especially in case of fresh and respiring produce packaging. So as protein materials are known to exhibit different properties according to the relative humidity [Gontard et al. 1992; Gontard et al. 1996b; Cuq et al. 1997a; Cuq et al. 1997c; Pouplin et al. 1999], it is important to look for mechanical and transfer properties at the proper relative humidities, usually over 85%RH, when looking for such applications. However, most studies on protein materials are conducted either at the very low relative humidity of 0% RH (e.g. ASTM D 3985-95 for oxygen permeability measurement) or at the moderate one of 50% RH (e.g. ASTM D638-10 and ASTM D3039 for mechanical testing or SCAN-P 53:84 for air permeation measurement). Then, in the following sections, protein based material properties are discussed whatever the RH considered but studies on material properties at high RH should be conducted in the future to be relevant with MAP application of fresh produce.

Material processing from proteins

Among all the proteins available in nature, only those widely available from food industry by-products (from cereal, milk or fish / meat products transformation) are studied for material processing, either from plant or animal resources. Among plant sourced proteins, wheat gluten (WG) and soy proteins are the most studied. Wheat gluten proteins are produced by removing most of the starch and other soluble substances from the wheat flour under extensive water washing. They consist in proteins [Gallstedt et al. 2011] of various molecular weights (MW): glutenin polymers between 150000g.mol^{-1} and more than 5 millions g.mol^{-1}, monomeric gliadins between 17000 and 70000 g.mol^{-1}, and then small proteins (albumin and globulin) with less than 17000 g.mol^{-1} [Bietz et al. 1972; Lasztity 1986; Morel et al. 2000b; Lagrain et al. 2010]. Soy proteins can be divided in two groups, the isolate (SPI) and concentrate (SPC). The isolate (SPI) contains at least 90% of proteins and is produced by a mild alkali or aqueous extraction followed by isoelectric precipitation. The concentrate (SPC) contains only between 65 and 72% of proteins and is produced from aqueous liquid extraction or acidic leaching. Both SPI and SPC are complex mixtures of proteins with widely different properties depending on their molecular weight; the two main fraction are two globulins: the β-conglycinin (MW: 160000 g.mol^{-1}) and the glycinin (MW: 340000 g.mol^{-1}) [Kunte et al. 1997; Cho et al. 2004]. The corn zein (CZ) is also of interest (despite being less studied) and is mainly constituted of monomers and disulfide linked oligomers.

Regarding animal sourced proteins, whey protein (WP), gelatin (Ge), caseinates (Ca) – especially the sodium caseinates (NaCa) –, and fish myofibrillar (MF) proteins are the most common for film shaping. Whey proteins are byproduct from cheese-making that can be obtained by ultrafiltration and spray drying either under the form of concentrate (WPC, 25-80% protein) or isolate (WPI, >90%protein) [Ramos et al. ; Cuq et al. 1998; Krochta 2002; Gallstedt 2011]. They are mainly constituted of β-lactoglobulin (MW: 18400 g.mol^{-1}), α-lactalbumin (MW: 14200 g.mol^{-1}), bovine serum albumin (MW: 66500 g.mol^{-1}). Gelatin can be sourced from fishes or mammals and is mostly used in the drug delivery field to build “softgel” capsules for encapsulation [Gallstedt 2011]. Caseins (mainly α-casein with MW of 23000 g.mol^{-1}, β-casein with MW of 24000 g.mol^{-1} and κ-casein with MW of 19000 g.mol^{-1}), as WP, originate from milk and are mostly used as edible coatings or as drug delivery systems like gelatin [Elzoghby et al. 2011]. Finally, fish myofibrillar proteins (mainly myosin with MW of 470000 and actin with MW of 42000) are obtained by purification and concentration of fish muscle proteins (they constitute 50% of the muscle).

All proteins exhibit good film forming properties due to their ability to form a cohesive network with different kind of interactions depending on the nature of their constitutive amino-acids (hydrogen-bonds, disulphide bonds, isopeptic bonds, hydrophobic interactions, etc). The final structure of their network and the type, number and strength of its bonds is dependent on the film forming process considered. Indeed, as conventional synthetic polymers, proteins can be processed through dry (extrusion, molding, pressing) or wet (casting, coating) processes. Due to the high temperatures involved material build through dry process often present a denser and more reticulated network than the one build through wet process. So the impact of high temperatures, first during process and then as post treatment, on the properties of the material will be further discussed later in this review. In addition to these process considerations, it is important to note that almost all self-supported protein based films require a minimal amount of plasticizer in their formulation to maintain their film form at standard conditions of temperature and humidity (implying that there is no such thing as real neat protein material). As plasticizer can impact the protein network structure by loosening it and favoring chains mobility, the impact of various type and contents of plasticizer will also be discussed later on.

Mechanical properties of self-supported protein based materials

Protein Origin. When looking at the mechanical properties of each protein at moderate RH (around 50%) it appears that Ca caseinates films seem to present the highest rigidity with E value of about 750MPa [Monedero et al. 2010], followed by SPI with 500 MPa [Cao et al. 2007b], WPI films with 160MPa [Shaw et al. 2002a] and WG with only 10MPa [Tunc et al. 2007], all for casted films with content of plasticizer between 10 and 25% (Figure S.4). Mechanical properties of Ca and Ge films are greatly impacted by the protein type and origin respectively. β-casein films present the best mechanical properties by far with TS up to 10000 times and 2 times higher, and SB up to 6.5 times and 14 times higher than α_{s1}-casein films and caseinate films respectively (note: testing conditions were different) [Motoki et al. 1987; Banerjee et al. 1995; Mauer et al. 2000]. And, pork gelatin based films exhibit better overall mechanical properties than fish and beef based one (with TS up to 10MPa and SB up to 90.55%) [Wang et al. 2007; Nur Hanani et al. 2012].

Shaping process. As stated, the properties of all protein based materials were conditioned by the processing mode chosen. For example, thermomoulded, compression molded and thermopressed WG films present better mechanical properties than casted ones and may even be suitable for punnet/tray applications [Gallstedt et al. 2004; Gallstedt et al. 2005]. And slightly better mechanical properties are obtained for CZ self-supported films processed by extrusion blowing than by solvent casting [Luecha et al. 2010]. This could be attributed to a tighter and more reticulated gluten network in the materials processed at high pressure and/or temperature [Cuq et al. 2000].

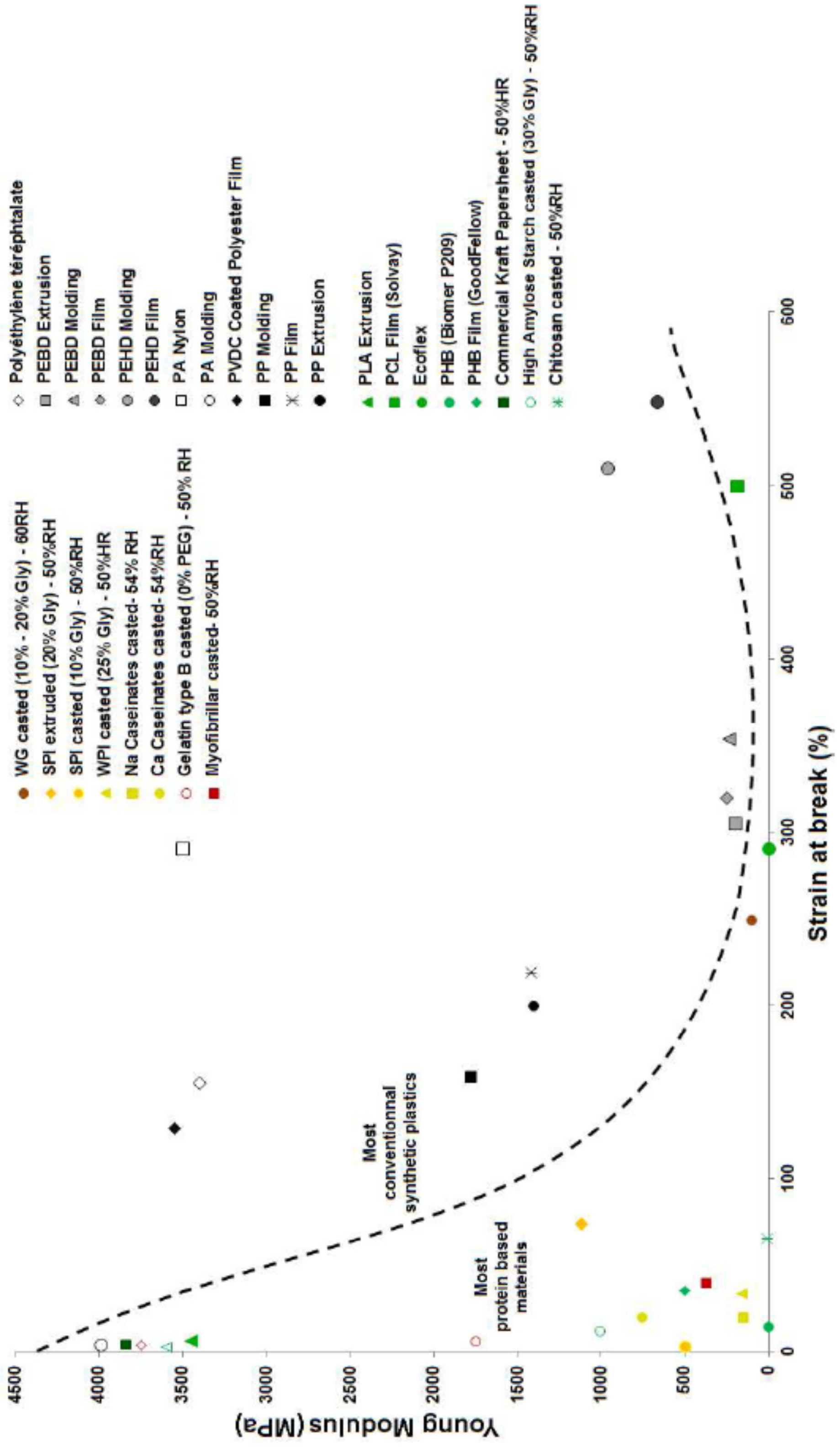


Figure S.4. Mechanical properties of protein based materials compared to conventional plastics and other agro-based materials for packaging

Relative humidity. The surrounding RH can also greatly affect their mechanical properties. At low to moderate RH (<50%), all neat protein based material presenting no or low plasticizer content are under glassy state, extremely brittle with moderate to high E and TS and dramatically low SB for plastics and so cannot be handled or further processed (cut, folded, etc…). Oppositely at very high relative humidity (>90%) they are under rubbery state and are easily deformable (high SB). But they exhibit very low rigidity (E) to the point of some undergoing deformation under their own weight [Gontard et al. 1996a; Cuq et al. 1998; Zhang et al. 2001; Lens et al. 2003; Gallstedt et al. 2005]. For example, E of casted WG films can be reduced from 1000MPa to 10MPa when increasing RH from 20% to 70% (Figure S.5).

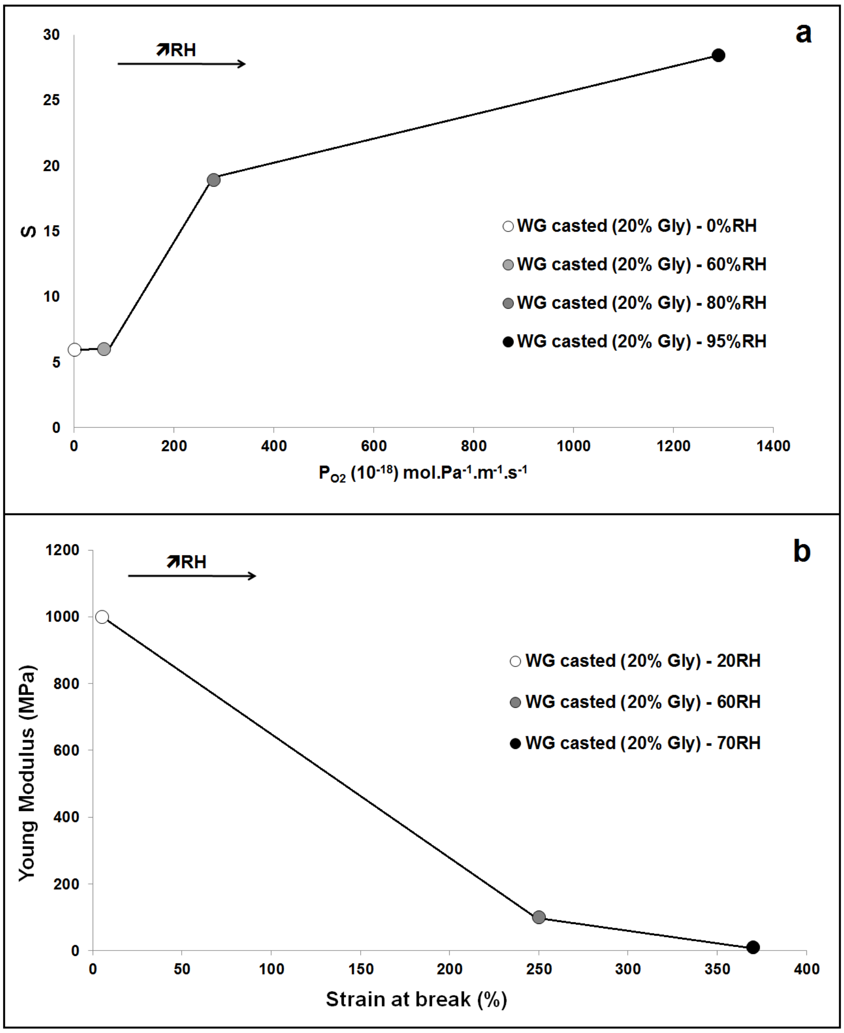


Figure S.5. Impact of relative humidity on transfer (a) and mechanical (b) properties of wheat gluten films

Mass transfer properties of self-supported protein based materials

Protein Origin. Just as for mechanical properties, each type of protein presents particular transfer properties. For instance, at moderate RH, casted gelatin films [Kolodziejska et al. 2007] present very low water vapor permeability (WVP) (only up to 0.43x10^{-10}mol.Pa^{-1}.m^{-1}.s^{-1}) whereas WG [Lee et al. 2005] and SPI [Rhim et al. 2002] films present a moderate one (up to 4.16x10^{-10}mol.Pa^{-1}.m^{-1}.s^{-1} and 1.58x10^{-10}mol.Pa^{-1}.m^{-1}.s^{-1}, respectively) and WPI [Shaw et al. 2002a] a high one (up to 17.75x10^{-10}mol.Pa^{-1}.m^{-1}.s^{-1}) in comparison, due to their more hydrophilic nature [Guerrero et al. ; McHugh et al. 1994; Rhim et al. 1998; Cho et al. 2004; Oses et al. 2009a; Cho et al. 2010]. It should be noted that comparing the P_{O2} and permselectivities of the proteins is complicated (Figure S.6) due to lack of data on the later parameter. The transfer properties of protein based materials are also very dependent of the protein concentration as SPC and WPC films displayed poorer gas barrier than their counterpart SPI and WPI films, respectively. Finally, Ge and Ca are dependent of their origin. For example, fish gelatin films exhibits a 2 time lower P_{O2} than the one of mammal gelatin films (for protein content of 8%) [Nur Hanani et al. 2012] and a slightly lower WVP (probably due to lower proline and hydroxyproline (amino-acids) contents [Avena-Bustillos et al. 2006; Chiou et al. 2008; Karim et al. 2009]).

Shaping process. The shaping process also impact the transfer properties, as the films built through wet process (e.g. casting) are less permeable to gas and vapor than their dry processed (e.g. thermomoulding) counterparts. For example, extruded CZ films present a WVP 1.5 times lower than casted CZ films. Furthermore, for a given shaping process the temperature had an influence on the transfer properties as WG films thermoformed at 80°C exhibited a WVP of 6.25x10^{-11} mol.Pa^{-1}.m^{-1}.s^{-1} against 4.47x10^{-11} mol.Pa^{-1}.m^{-1}.s^{-1} for a WG film thermoformed at 120°C [Angellier-Coussy et al. 2011]. These observations can both be related to the network structure of the materials as casting of protein films build a loose and open network while thermo-molding creates either highly intricate polymeric network (due to the formation of additional covalent bonds like iso-peptide bonds) or a cross-linked network during heat treatment (especially at temperatures of above 120° C) which reduce gas and vapour transmission [Micard et al. 2001; Hernandez-Munoz et al. 2004; Mangavel et al. 2004].

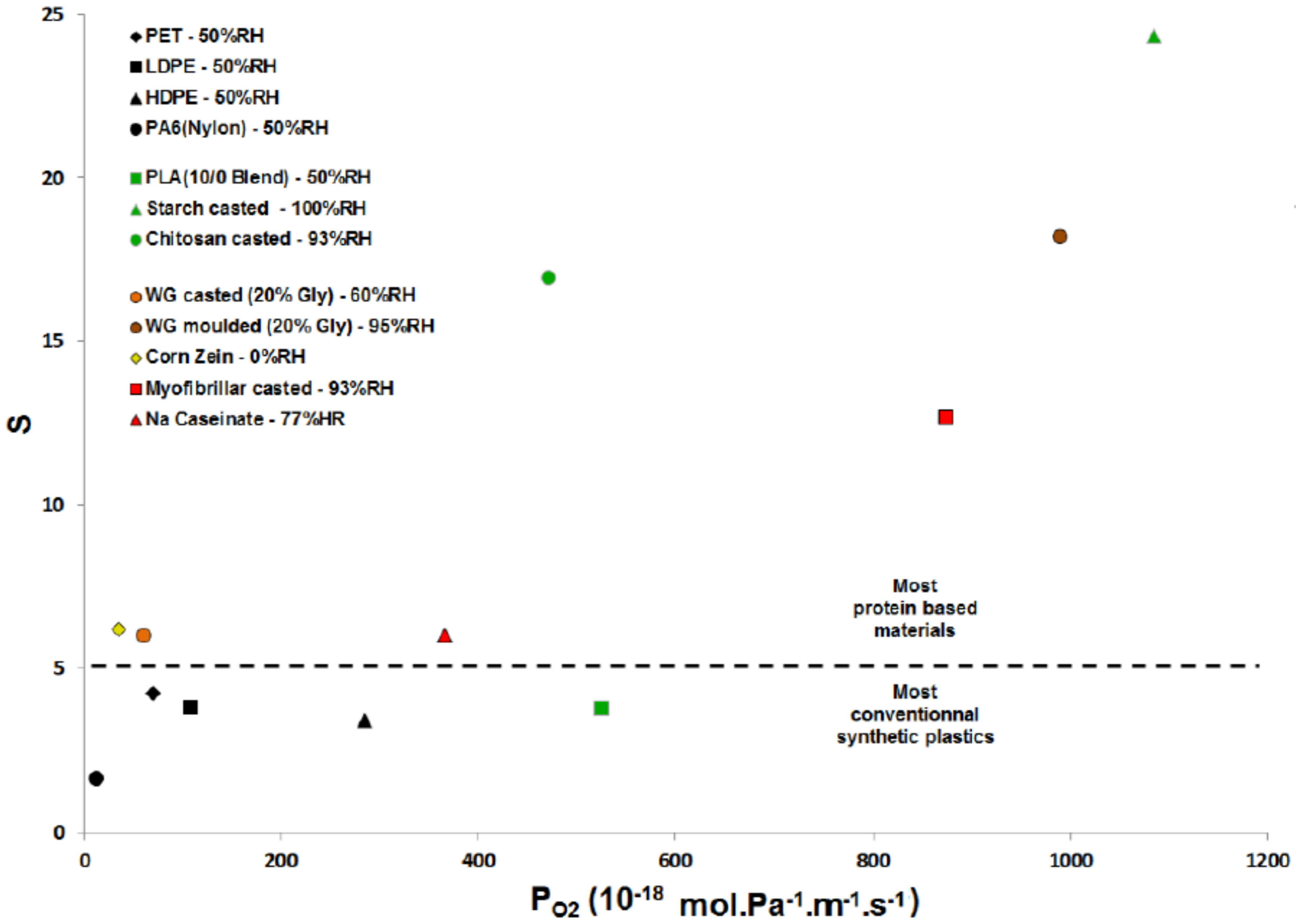


Figure S.6 Transfer properties of protein based materials compared to conventional plastics and other agro-based materials for packaging

Relative humidity. Transfer properties of protein based material are also very dependent of the RH. This dependency can be attributed to the plasticizing effect of water that decreases the glass transition temperature (Tg) of the films [Gontard et al. 1996a; Cuq et al. 1997c; Pouplin et al. 1999] (by increasing the disorder, free volume and mobility of the macromolecular chains) and so increase the gas and vapor transfers through the materials. Not all protein based materials are equally affected by RH changes. WG films are clearly the most sensitive (Figure S.5) as Gontard et al. in 1996 [Gontard et al. 1996b] reported a P_{O2} and P_{CO2} of 1290 x10^{-18} mol.Pa^{-1}.m^{-1}.s^{-1} and 37000x10^{-18} mol.Pa^{-1}.m^{-1}.s^{-1} for measurements at 95%RH, values which are respectively 1040 and 4960 times higher than the one measured at 0% Rh . It should be noted that the selectivity of such films was also greatly affected as it rose from 6 to almost 28. This was partially attributed to the higher CO_2 solubility in water than that of O_2 and mainly to the interaction between CO_2 (combined or dissolved form) and binding sites (covalent, electrostatic, hydrogen, or hydrophobic bonding with amino-acids) of the WG protein only unveiled at high relative humidity (due to high chain mobility) [Pochat-Bohatier et al. 2006]. WVP of cast WG films, was also affected by RH, as Roy et al. in 2000 [Roy et al. 2000] reported a 10000 increase of WVP for a 70%RH increase. CZ [Aydt et al. 1991] and MF [Guilbert et al. 1996] based films present similar RH dependence regarding their P_{O2} but their P_{CO2} (and so their S) seems to be less affected by RH changes than the one of WG. The WVP of WP [McHugh et al. 1994], SP [Brandenburg et al. 1993], Ge and NaCa [Fabra et al. 2012] and the P_{O2} of NaCa [Fabra et al. 2012] appear to be less sensitive to RH.

Comparison to other agro and synthetic plastics for packaging

Protein based materials can be compared to other agro-based materials which are of interest for food packaging related applications such as cellulosic materials (highly structured polysaccharides), polysaccharides, lipidic materials and naturally engineered, by enzymatic or bacterial process, agro-polymers (e.g. Polyhydroxyalkanoate, PHA or Poly(3-hydroxybutyrate-co-3-hydroxyvalerate, PHBV). The cellulosic materials derivated from wood are widely used for food packaging in form of sachets or cardboard punnet due to their interesting mechanical properties in terms of rigidity and/or tear resistance depending on both their composition (ratio cellulose/lignin) and shaping process. However, these materials are not of interest for MAP due to their porous and so non-gas-barrier character (with P_{O2} of 10^{-12}mol.Pa^{-1}.m^{-1}.s^{-1} or higher). Polysaccharide based materials (from starch, starch derivatives, alginate, chitosan or gums [Kester et al. 1986; Krochta et al. 1997; Ryu et al. 2002;

Khoshgozaran-Abras et al. 2012]) exhibit good film forming properties (through hydrogen and hydrophilic bonding) and acceptable rigidity for packaging application [Bahrami et al. 2003; Gallstedt et al. 2005] (unlike most neat protein films at high RH). However, most of them present limited elasticity at moderate humidity (like the proteins) with 1-25% strain at beak at 50%RH) [Ryu et al. 2002; Escalante et al. 2012] and also low moisture barrier properties [Jang et al. 2011], which might be drawbacks for packaging applications of fresh produce (possible condensation and then microbial growth). As for proteins they exhibit low oxygen permeability at moderate RH [Escalante et al. 2012] and are very sensible to relative humidity with selectivity up to 17 and 24 (against less than 5, at 0%RH) for chitosan and starch based films respectively [Allen et al. 1963; Gontard et al. 1996b]. Only red algae and agar based films seemed to exhibit a different behavior regarding mechanical properties with a higher elasticity and a lower rigidity [Jang et al. 2011; Rhim 2011]. Lipids films (beeswax, carnauba wax, fatty acids, shellac resin, triglycerides, etc…) [Baldwin et al. 1997; Rhim et al. 1999; Morillon et al. 2002] are only used for coating purposes (directly on the produce) as they usually do not form cohesive stand-alone films and exhibit dramatically low rigidity (even lower than plasticized protein films at high RH). Regarding transfer properties they exhibit interesting water vapor barrier properties [Morillon et al. 2002; Monedero et al. 2009] but are highly permeable to oxygen compared to protein based materials. The naturally engineered (via metabolic transformation of carbon sources by microorganisms) agro-polymers [Madison et al. 1999; Verlinden et al. 2007; Lu et al. 2009], such as PHA or PHBV, are material presenting far higher rigidity than the protein based ones. They indeed present mechanical and transfer properties often similar to petroleum derived polymers (such as polypropylene, PP or polyethylene, PE) but combined to the advantage of biodegradability [Lee et al. 1999]. However, their use for MAP applications for fresh produce would be limited by their too high barrier properties and high price [Choi et al. 1999; Philip et al. 2007]. Finally, chemically engineered agro-polymers such as the well-known polylactic acid (PLA) usually exhibit rigidity and gas transfer properties (P_{O2} & S) close to the one of conventional synthetic plastics [Komatsuka et al. 2008] but a very poor strain at break compared to proteins (around 2%). They also present a brittle behavior similar to the one of most proteins at low RH and are more moisture sensitive than conventional plastics [Bledzki et al. 2010; Jollands et al. 2010; Nampoothiri et al. 2010].

Protein based material can finally be compared to conventional synthetic plastics (PE, PP, OPP, PET…) widely used in the food packaging field (Figure S.4 & Figure S.6). Contrary to most protein based materials, these conventional synthetic plastics exhibit limited to no sensibility to relative humidity. Whatever the humidity, they feature lower oxygen and carbon dioxide permeability than the materials based on proteins. Furthermore, in any RH conditions, they exhibit limited selectivity (lower than 6) [Bakker 1986; Exama et al. 1993; Poyet 1993; Fishman et al. 1995; Al-Ati et al. 2003; Okuji et al. 2006]. Thus most synthetic films are not adapted to gas transfer requirements of most fresh produce because they can induce anoxia damages [Kader et al. 1989; Exama et al. 1993], but they can be adapted to muscle food packaging (but not optimal) [McMillin 2008]. The ones also featuring very low WVP are optimal for dry food conservation (e.g. biscuits). It is always possible to enhance their P_{O2} by micro or macro perforation. But doing so would fix their permselectivity at a value of 1 and so prevent them to create atmosphere content both low in O_2 and CO_2 (which are recommended for most fresh produce) [Exama et al. 1993; Guillaume et al. 2011]. Regarding mechanical properties, the conventional plastic commonly used for food packaging (such as PE, PP, PET, OPP) obviously fit well mechanical requirements for fresh fruits and vegetable packaging as they can feature either high enough rigidity for physical protection of the produce against shock or sufficient strain at break for wrapping application depending of their formulation.

So, given their interesting and wide transfer properties proteins appear to be the raw materials that present the best hope for replacement of synthetic for MAP applications. Still, it is necessary to find ways to improve their poor mechanical properties (either too brittle or not rigid enough). This could be achieved by many structuration, shaping process and formulation modulation strategies as detailed in Figure S.7. Protein based materials can be strengthen by chemically modifying the proteins during formulation or after shaping, introducing fillers in the protein matrix or being structured as composites (either monolayered polymer blends or multilayered) to benefit from higher mechanical properties of other agro-polymers. And all these strategies can be applied either for micro or nano-scale structuration of the materials. In the following parts, the effect of these modulation strategies on the mechanical properties of protein based materials will be discussed along with their concomitant effect on the transfer properties and the data will be summarized in Table S.3 (page 82).

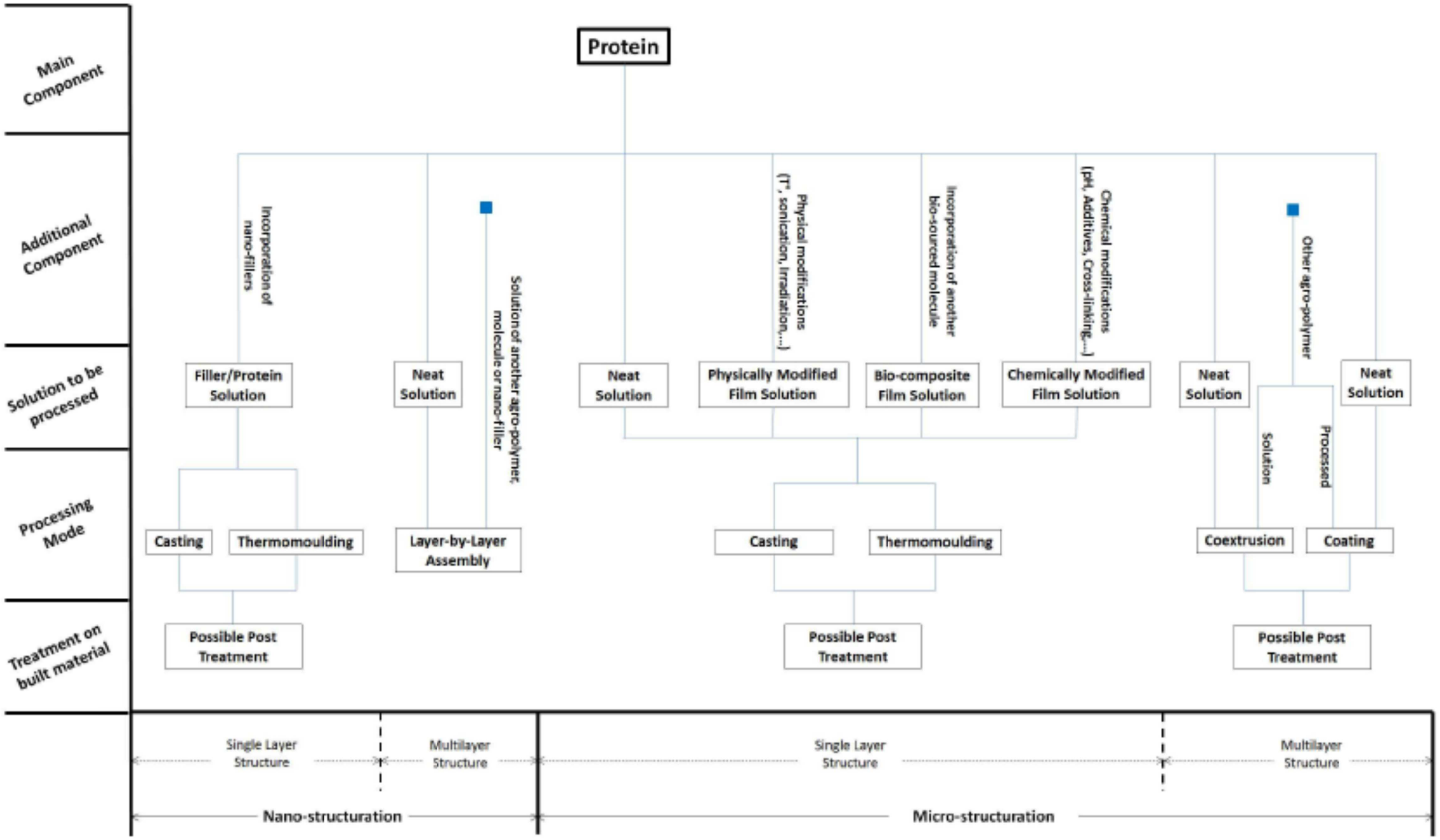


Figure S.7. Strategies for modulation mechanical properties of protein based materials

MICRO-STRUCTURING PROTEIN BASED MATERIALS: MODULATION OF THEIR MECHANICAL AND MASS TRANSFER PROPERTIES

Monolayer Structure

The most intuitive and common ways to modulate the mechanical properties of materials is to play on the chemistry during formulation or post-processing treatments, while keeping the micro-scaling process of self-supported materials.

Chemical modification during formulation.

- ***pH***

pH is an important factor for processing protein by casting. First, it must be set far enough of the considered protein isoelectric point to allow good dispersion and obtain homogeneous casting solutions (e.g out of pH 4-5 for SPI and gelatin and out of pH 6-7 for WG) [Gennadios et al. 1993]. Second, it has been demonstrated to greatly affect the final mechanical properties of the protein based materials. Some protein based materials feature better properties at basic pH than at acidic one, like SP which exhibit best mechanical properties (especially E and TS) at basic pH (pH 10) due to optimal unfolding of the protein at this pH [Brandenburg et al. 1993; Cao et al. 2007b]. Others, like WG materials, exhibit higher strain at break and puncture strength when prepared in acidic conditions whereas they feature higher young modulus and tensile strength when prepared in basic ones [Gontard et al. 1992; Kayserilioglu et al. 2003]. And finally, some like myofribillar fish protein materials present better overall mechanical properties (E and SB) at acidic pH (E:370 for 300MPa, SB 40 for 30% and no significant difference for TS) whereas others like SPI films seems to present both better mechanical (puncture strength and deformation) and also moisture barrier properties when prepared at basic pH (pH 9) than in acidic condition (pH 3) [Were et al. 1999].

- ***Fractionalization***

Isolating a precise fraction of protein can affect the final properties of the material. For example, films made from different SPI fractions (<100kDA, 100KDa-300kDa, >300kDa) isolated by ultrafiltration presented different mechanical properties [Cho et al. 2004]. The 2 lightest fractions gave films with lower strength than standard SPI films whereas the heaviest faction brought a slight increase of tensile strength (+0.2MPa). All films made from fractionated SP featured lower %strain at break than stand SP films. However, fractionalization does not seem to affect the gas transfer properties as the WVP was similar for all SPI films whatever the fraction isolated [Cho et al. 2004]. On the contrary, isolating either the glutenin or the gliadin from wheat gluten had an effect on both the transfer and mechanical properties. Hernandez-Munoz et al. in 2004 [Hernandez-Munoz et al. 2004] showed that glutenin rich films (heaviest fraction) exhibited higher tensile strength but lower elongation at break than gliadin rich films (lightest fraction). Glutenin rich films were also less permeable to water vapor than gliadin rich ones [Hernandez-Munoz et al. 2004]. Finally, films produced from different caseins subunits presented different WVP values with 3.13 x10^{-11} mol.Pa^{-1}.m^{-1}.s^{-1} for β-casein films against values between 1.26 x10^{-10} mol.Pa^{-1}.m^{-1}.s^{-1} and 2.24 x10^{-10} mol.Pa^{-1}.m^{-1}.s^{-1} for caseinate films [Banerjee et al. 1995; Mauer et al. 2000].

- ***Crosslinking***

It is possible to strengthen and increase the density of the protein network by increasing the number of intermolecular bonds through cross-linking. This can be achieved by heating (as for thermoprocessed materials) but also by incorporation of various molecules in the formulation such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS) [Tropini et al. 2004], genipin [Gonzalez et al. 2011], aldehydes, or even natural tanins [Cao et al. 2007a; Zhang et al. 2009].

Addition of genepin to SPI film forming solution increased the TS of the final material from 3.2 to 4.6 MPa, reduced its WVP of 30% and improves its resistance to biodegradability on the soil (20 more days necessary for total degradation) [Gonzalez et al. 2011]. Synthetic chemical crosslinking agents such as formaldehyde or EDC are often more efficient than natural ones like dialdehyde starch (which only improved the water resistance and slightly affected the mechanical properties of SPI films) or tannin acid (which did not affect the WVP of Gelatin films) due to their limited MW and so higher mobility which allows them reach the reactive amino groups of the protein matrix more easily [Rhim et al. 1998; Cao et al. 2007a]. In any case, it should be noted that an over addition of crosslinking agents can have adverse results and deteriorate both the mechanical and barrier properties of the film [Tropini et al. 2004].

Among other modifications that can be chemically induced in soy protein materials, denaturation of the proteins by alkaline chemicals can lead to an increased rigidity (while maintaining other mechanical and transfer properties) [Brandenburg et al. 1993] and arylation of soy protein based material by 2,2-diphenyl-2-hydroxyethanoic acid (DPHEA) to an increase of E and a higher resistance to water (but also result in a decrease of TS and SB) [Kumar et al. 2009].

- ***Plasticizers***

Oppositely to cross-linking that tightens the protein networks, it is possible to loosen them by incorporation of plasticizers in the formulation. Substances like water, glycerol, sorbitol or some sugars can bring this plasticizing effect by enhancing molecular chains mobility inside the network. Their efficiency depends on their molecular weight and their interaction with the protein matrix (mostly through H bonds) [Gontard et al. 1993; Pouplin et al. 1999; Irissin-Mangata et al. 2001; Ghanbarzadeh et al. 2006].

After water [Gontard et al. 1993; Gontard et al. 1996a; Cuq et al. 1997b], glycerol is the most used and studied plasticizer of protein based materials. It is well known that adding glycerol to WG [Gontard et al. 1993; Mastromatteo et al. 2008], SPI [Cao et al. 2007b], CZ [Ryu et al. 2002], WPI [Shaw et al. 2002b; Shaw et al. 2002a; Barreto et al. 2003] or caseinates [Barreto et al. 2003] film forming solutions or powder mix increases the maximum strain at break but decrease the tensile strength and the young modulus of the final materials. For example, addition of 0.4g of glycerol to a SPI film forming solution can divide its TS and E by five or more while multiplying its SB by 5 [Cao et al. 2007b]. Glycerol also affected the gas transfer properties of the protein films. P_{O2}, P_{CO2} or WVP of WG [Gontard et al. 1993] or WPI [Shaw et al. 2002a] films increased with addition of glycerol.

Sorbitol is another quite common plasticizer that brings similar changes to protein films than glycerol. For example it reduces the Tg and the brittleness of milk protein based films by the same proportion than glycerol while also increasing the elongation at break of the materials [Barreto et al. 2003].

Sugar molecules such as glucose, fructose or galactose can also have a plasticizing effect on CZ films [Ghanbarzadeh et al. 2006]. All sugar molecules had the same effect on the Tg of the CZ films, which is probably due to their similar molecular weight. However the type of sugar (fructose, galactose or glucose) had a strong effect on the mechanical properties as addition of galactose allowed creation of films exhibiting higher TS, SB and E than those containing glucose or fructose. It can be noted that contratry to sorbitol or glycerol addition, increasing the sugar plasticizer content (dose doubled from 0.5g/g of CZ to 1g/g of CZ in the work of Ghanbarzadeh et al. in 2006 [Ghanbarzadeh et al. 2006]) did not significantly affect the mechanical properties of the final material.

Plasticizing effect can also be achieved by chemical modification of the proteins. SDS can be added to SPI film forming solution (up to 40%) to multiply the strain at break by 4 and divide the tensile strength by nearly 2 through a prevention of hydrophobic bonds creation by occupation of hydrophobic sites, resulting in a weaker structure [Rhim et al. 2002]. However use of such molecule decreases the WVP of the material (and not increases it as common plasticizers would do) probably because of the large hydrophobic portions of SDS molecules [Rhim et al. 2002].

Finally addition of salts also plasticize protein matrixes as it was observed on SPI supplemented with NaCl that increase the water content of the protein film and thus plasticize it (increased SB) [Cao et al. 2007b].

- ***Other compounds***

Hydrophobic additives such as fatty acids (hexanoic, oleic, palmitic etc…) or lipids can also be added to protein matrixes to modify their properties. They act as plasticizing agents but, in some case, may exhibit antagonist effects. For example, Beeswax addition into protein matrixes increase their P_{O2} and P_{CO2} like a plasticizer [Fabra et al. 2012] but reduced their strain at break, increased the strength and shift the fracture behavior from plastic to brittle as a cross-linking agent would do [Song et al. 2008; Monedero et al. 2009]. On the contrary, Oleic acid, as castor oil [Song et al. 2008], has a standard plasticizing effect on the mechanical properties of the protein films with increase in the strain at break and decrease in strength and rigidity [Pommet et al. 2003; Monedero et al. 2009]; and it also acts as a plasticizer regarding gas transfer properties by promoting molecular mobility inside the matrix and thus increasing the P_{O2} and P_{CO2} of the materials [Fabra et al. 2012]. It can be noted that the effect on transfer properties was more important than the one observed for beeswax addition, probably due to the solid state of beeswax at room temperature which limited the improvement of molecular mobility in the matrix [Fabra et al. 2012].

Despite these differences, all hydrophobic compounds shared the same effect on materials WVP. Addition of oleic acid to WG [Pommet et al. 2003], caseinate [Banerjee et al. 1995; Monedero et al. 2010; Fabra et al. 2012] and SPI films [Monedero et al. 2009; Monedero et al. 2010], addition of soya oil to WPI [Shaw et al. 2002a] and addition of beeswax to caseinate [Monedero et al. 2010; Fabra et al. 2012] and SPI [Monedero et al. 2009; Monedero et al. 2010] were found to significantly reduce the WVP of casted films compared to films containing equivalent amount of glycerol due to their hydrophobic character. Oleic acid and beeswax can even be combined to obtain higher reduction of WVP of caseinate films (10 times reduction instead of 2 with compounds used individually) thanks to combination of hydrophobic wax and surfactant oleic acid [Fabra et al. 2012]. On the contrary, Modereo et al. in 2009 [Monedero et al. 2009] did not notice any synergy in terms of WVP reduction but explanations were not given. Finally it should be noted that addition of oleic acid and beeswax combined did not manage to sufficiently improve the mechanical properties of SPI casted film to make them a suitable replacement for synthetic plastics according to the same authors.

It can be noted that other compounds also present non-conventional effects on the protein matrixes. For example cysteine (at pH 7) [Were et al. 1999] added to SPI films increased the tensile/puncture strength while decreasing strain at break or puncture elongation but left the transfer properties unchanged.

Agro-polymers composites

- ***Agro-polymers blend***

To create better mechanical properties or more adequate gas transfer properties, proteins can be co-processed with other protein(s) or other agro-polymer(s) as in bulk homogeneous composites.

Increasing amount of WG in SPI film formulation at neutral or slightly basic pH improved mechanical properties of SP films in terms of puncture strength and tensile strength but reduced the puncture deformation until the 3:1 SPI:WG ratio. Regarding transfer properties there was no significant trends for WG addition: all tested ratio induced films with reduced WVP compared to SPI films (up to 4.5 five time for a 2:1 ratio at neutral pH), and with increased P_{O2} (up to 4 times for 2:1 ratio) except for 3:1 ratio (which remained unchanged). Authors attributed this behavior to a lower content of hydrogen bonding in the WG/SPI composites than in the SPI films even if they could not explain the behavior of film obtained with the 3:1 ratio [Were et al. 1999].

Combination of gelatin with WPI [Wang et al. 2010] or SPI [Cao et al. 2007b] was also tested. Regarding the WPI films, gelatin was found to act somewhat like a plasticizer regarding both mechanical and transfer properties. Increasing the gelatin content decreases the TS of the WPI film while increasing its elongation and brought higher WVP and oxygen permeability [Wang et al. 2010]. It should be noted that it was possible to enhance each properties of this WPI/Gelatin composite by addition of various amount of sodium alginate, a substance showing high compatibility with gelatin. By means of simulation, Wang et al. in 2010 [Wang et al. 2010] stated that the best compromise film formulation for material overall properties (mechanical and barrier) was 8g of WPI, 12g of gelatin and 5g of sodium alginate. But when looking for maximal mechanical or barrier properties individually other combinations (WPI/gelatin/sodium alginate) could be considered. For example TS max of 10.77MPa was obtained with 20/8/12g and P_{O2} min of 3. 85x10^{-18} mol.Pa^{-1}.m^{-1}.s^{-1} with 10/16/14g) [Wang et al. 2010]. Regarding the SPI/gelatin composite, the main interest was to improve the mechanical properties of SPI films to obtain an interesting material at lower cost than pure gelatin film. It was also found than a SPI:Gelatin ratio of 4:6 prepared at pH 8 and elevated temperature (>50°C) exhibited far better mechanical properties than SPI films (around 3 folds for tensile strength and elastic modulus and 1.5 folds for elongation) [Cao et al. 2007b]. Finally, casein/SPI (containing oleic acid and beeswax) composites have also been studied. 1:1 SPI:caseinate films featured better tensile strength and Young modulus, and also lower WVP than their pure SPI or caseinate equivalents [Monedero et al. 2010].

Combination of protein based and other agro-polymers has also been tried. Gelatin/starch composites have been studied by Al-Hassan et al. in 2012 [Al-Hassan et al. 2012], and increasing the content of starch did not have any significant impact on the WVP which remained around 4.63x10^{-12} mol.Pa^{-1}.m^{-1}.s^{-1} (which was two time higher than the one of pure starch film in the same measurement conditions). It should also be noted that gelatin / starch composites with higher gelatin content featured lower rigidity and tensile strength but higher strain at break.

Addition of dispersed fibers or cellulosic compounds can also be of interest. Incorporation of methylcellulose in casted and heat treated WG films containing glycerol significantly increased their tensile strength, young modulus and strain at break due to the fact that cellulose derivates consist in long straight chains of high molecular weight that exhibit better mechanical properties than gluten protein chains. However, addition of methylcellulose in thermomoulded WG films had a clear negative impact on the mechanical properties of the final films due to the poor plasticization effect of glycerol to methylcellulose in dry conditions [Zuo et al. 2009]. Addition of methylcelluose had less impact on the transfer properties. At low RH, it was found to increase the WVP of the final film but at high RH no clear impact was found as the WVP only varied between 1x10^{-11} and 1.5x10^{-11} mol.Pa^{-1}.m^{-1}.s^{-1} [Zuo et al. 2009]. Addition of Xylan Birchwood to WG films also allows modulation of mechanical properties but to a lesser extent. Incorporation of high contents of xylan (over 40%) was found to increase the Young modulus (and slightly the tensile strength) of the final material but also to highly reduce its strain at break (more than 5 times). And, as with methylcellulose incorporation, no clear effect on the WVP was noticed. It should be noted that not all xylan are compatible with WG matrixes as the author evidenced that incorporation of grass or corncob xylan significantly reduced strength and rigidity of the gluten films [Kayserilioglu et al. 2003].

Gums can also be of interest as they can act like a plasticizer for the mechanical properties while maintaining the water vapor barrier properties of the protein films. Oses et al. in 2009 showed that a 50/50 WPI/Mesquite gum composite exhibited lower TS and Young modulus but higher strain at break than a control SPI film (2Mpa against 12.1MPa, 23.9 MPa against 421.3 MPa and 70.7% against 4.4%, respectively); but the composite featured the same WVP than the control WPI film: 3.08x10^{-11} mol.Pa^{-1}.m^{-1}.s^{-1} which was due to the hydrophilic nature and the similar WVP of both components [Oses et al. 2009a].

- ***Protein embedding***

Organized fiber networks can also be used to embed proteins and create composites. In most case such structure increased the rigidity and the strength of the final composite but decreased its strain at break, as it's the case for common matrix/fibers composites. For example, Mastromatteo et al. in 2008 [Mastromatteo et al. 2008] observed an increase of the Young modulus up to +400MPa but also a significant SB decrease for incorporation of spelt bran into a WG matrix. In some case incorporation of a fiber network can increase all mechanical properties as for the incorporation of ramie fiber inside SPI films. If the orientation of the fibers is correctly chosen (depending of the future mechanical solicitations) and the amount of fibers sufficient (30% at least), all mechanical properties (E, TS and SB) of the SPI films will be enhanced [Kumar et al. 2009].

Regarding gas transfer properties, organized fibers network/protein composites usually presents higher gas transfer properties than the protein-only materials due to possible presence of interfacial defects between the fibers and the matrix that creates preferential path for gas diffusion.

Post treatments

- ***Temperature***

Aside its impact during processing, temperature can also affect the protein materials properties through thermal treatment applied on the already processed films. For almost all proteins (SP [Rhim et al. 2000; Sabato et al. 2001; Kim et al. 2002; Cao et al. 2007b], WG and its different fractions [Hernandez-Munoz et al. 2004], Gelatin [Cao et al. 2007b], or other animal proteins [Garcia et al. 2005]), exposition to high temperatures (above 80°C) lead to materials presenting higher strength and rigidity (e.g. TS of gliadin film multiplied by 10 to reach 16MPa after heat treatment [Hernandez-Munoz et al. 2004]) and lower strain at break and water vapor permeability (e.g. WVP of gliadin film divided by 3 [Hernandez-Munoz et al. 2004]). Such changes are mainly attributed to a disruption in the protein network by heating, resulting in the exposition of previously buried chemical groups (sulfhydryl and hydrophobic in case of SP and WG) that can react and induce a cross-linking that strengthen the intermolecular network [Kim et al. 2002; Hernandez-Munoz et al. 2004]. The opposite behavior – increased heat leading to weakening of the material – exists but was seldom observed. As an example WG films prepared in basic conditions (pH 11) and dried at 80°C presented higher SB (2 times) and lower TS and E (3 times) than the ones dried at room temperature. This may be attributed to the pH conditions because in the same study no clear effect of the temperature was observed for quite similar film prepared in acidic conditions pH (pH 4) [Kayserilioglu et al. 2003].

- ***Irradiation***

Gamma irradiation (up to 50 kGy) on processed protein based materials is known to provoke cross linking and so strengthening of the films and reduction of its permeability to gases [Lee et al. 2005] [Lee 2005, WG irradiation].

- ***Other treatments***

Other post treatments such as plasma or corona treatments or calendering could be applied to protein based material but have never been investigated to our knowledge.

Multilayer structure

Another solution to reinforce the mechanical properties of protein based material is to process multilayered composites.

Coating. The most common protein based multilayered materials are protein coated fibrous supports (papers and cardboards) [Trezza et al. 1994; Han et al. 1999; Gallstedt et al. 2004; Gallstedt et al. 2005; Ben Arfa et al. 2007a; Chalier et al. 2007b; Gastaldi et al. 2007; Andersson 2008; Guillaume et al. 2010a; Khwaldia et al. 2010; Mascheroni et al. 2011; Cagnon et al. 2012], but coating synthetic supports [Hong et al. 2006; Lee et al. 2008] were also considered. Interestingly, coating on fibrous or synthetic supports allow use of plasticizer free proteins, bringing quite different transfer properties compared to self-supported equivalents.

Among bio-based supports for coating, paper and cardboard are among the most studied and the most interesting for fresh fruits and vegetable packaging because they were found to exhibit gas transfer properties well suited for such produce once protein coated [Guillaume et al. 2010b; Gouble et al. 2011a] and are rigid enough to serve as sachet or lids for punnet. Due to their rough and porous character, there are 2 important criteria to meet in order obtain good transfer properties (i.e. close to the protein film ones) for fibrous support / protein composites: a sufficient amount of protein deposed (coating weight > 10g.m^{-2}) and a homogeneous deposition (absence of defects on the coated layer and regular thickness) [Gallstedt et al. 2005; Guillaume et al. 2010a]. A recent study by Guillaume et al. in 2010 [Guillaume et al. 2010a] also highlighted an additional criteria: the penetration of the protein layer inside the support paper. In case of high coating weight (Cw>20g.m^{-2}), important penetration of the protein inside the paper should be recommended as it create a thick interaction zone just below the paper surface that help to mechanically protect the apparent protein layer from cracks and so to maintain interesting barrier properties. Paper coating by spreading the protein solution onto the sheet surface has already been performed with a wide variety of protein including WG [Gallstedt et al. 2005; Chalier et al. 2007b; Gastaldi et al. 2007; Guillaume et al. 2010a; Mascheroni et al. 2011; Cagnon et al. 2012], WPI and WPC [Han et al. 1999; Han et al. 2001; Gallstedt et al. 2005], CZ [Trezza et al. 1994] , SPI [Ben Arfa et al. 2007a] and the protein coated papers usually feature lower barrier properties and lower permselectivity than their respective self-supported protein based materials. For example, at high relative humidity, WG coated papers have been found to exhibit permselectivity values from 2.03 [Guillaume et al. 2010a] to 12 [Cagnon et al. 2012] whereas WG self-supported film have been found to exhibit values up to 28 [Gontard et al. 1996b]. Aside of the minimal 10g.m^{-2} limit, the coating weight deposited is of importance as increasing it gives to the final coated material gas transfer properties that are closer to the one of its respective protein based material. In 2005, Gallstedt [Gallstedt et al. 2005] evidenced that, for WPI, WPC and WG coated papers, the higher the coating weight, the more "neat protein like" the transfer properties (e.g. lower air permeation for higher coating weight, Cw). Indeed air permeation of WG coated paper was 100 time lower for a coating weight of 27 g.m^{-2} than for one of 14 g.m^{-2}, and air permeation of WPC coated paper was 10 time lower for a coating weight of 20 g.m^{-2} than for one of 10 g.m^{-2}). Finally, the type and the structure of paper support were also found to have a great influence on the coated material properties. In

2010, Guillaume et al. reported a 2 times higher selectivity for WG coating on untreated paper than on treated paper (pre-coated with starch and calcium carbonate [Guillaume et al. 2010a].

The mechanical strength of the cellulosic support / protein coating composites is obviously more dependent of the nature of the support than of the coated protein. However, the type and the quantity of protein deposited (coating weight) was also of importance. Generally, the more protein, the lower the rigidity and the strength but the higher the strain at break. But WG coating seemed to less affect the Young modulus of the composite (5400MPa, similar to uncoated paper sheet) than WPI (4300MPa) for similar coating (Cw: 14 $g.m^{-2}$) performed on the same support [Gallstedt et al. 2005].

Coating on cellulosic support can also be performed by compression-molding [Gallstedt et al. 2005; Cho et al. 2011]. In such case, Gallstedt et al. in 2005 [Gallstedt et al. 2005] have demonstrated that the lower the pressure during the process, the thicker and the more homogeneous the apparent protein (WG) layer on top of the paper and hence, the lower the oxygen permeability (P_{O2}: $2.99x10^{-18}$ $mol.Pa^{-1}.m^{-1}.s^{-1}$ against $5.18x10^{-18}$ $mol.Pa^{-1}.m^{-1}.s^{-1}$ for materials processed at 350 and 900bar respectively and tested at 0%RH and 23°C). Regarding mechanical properties, compression molded WG/Papersheet composites can exhibit Young modulus up to 10 times higher than WG films but feature a strain at break down to 10 times lower. The authors also evidenced that, as for coating by spreading, the type of sheet used in the molded composite is important as using paperboard instead of laboratory papersheet created composites exhibiting lower Young modulus and strain at break both, due to the inferior mechanical properties of the paperboard [Gallstedt et al. 2005].

Proteins can also be coated on conventional plastic such as PP [Hong et al. 2006; Lee et al. 2008; Tihminlioglu et al. 2010], OPP [Hong et al. 2004], PVC [Hong et al. 2004] or PE [Hong et al. 2006]. However such composites will not be easily biodegradable and will only be destined to high barrier packaging applications (using the highly barrier character of proteins at low RH) and so not to respiring produce packaging applications as presence of a synthetic layer would greatly limit their gas permeation.

Layered protein composites. Two proteins or a protein and another bio-based material can also be processed together to create multilayered composites and obtain intermediate mechanical and transfer properties between the two compounds. As an example of protein/protein composite, Cho et al. in 2010 [Cho et al. 2010] combined the smoothness and excellent O_2 barrier properties at low to moderate RH of SPI films to the higher mechanical strength and better sealability of CZ films to create an interesting edible and safe (in regard of food/packaging interactions) alternative to conventional plastic individual pouches for Chinese noodle oil preservation. Pol et al. in 2002 also worked on SPI/CZ laminates composites to obtain materials with improved O_2 and WVP barrier properties [Pol et al. 2002]. As an example of protein/other agro-polymer composite structure, protein/starch layered composites were experimented by Ryu et al. in 2002 through a study on the properties of CZ coated amylose starch films [Ryu et al. 2002]. The WVP of such composites was higher than the one of self-supported CZ films but remained low enough for the composite material to be considered as a possible replacement for synthetic cheese packaging according to the authors. And at the same time, the tensile strength of the composite was higher than the one of CZ films and the SB was only slightly inferior.

NANO-STRUCTURING PROTEIN BASED MATERIALS: MODULATION OF THEIR MECHANICAL AND MASS TRANSFER PROPERTIES

Monolayer Structure

In bulk incorporation of nano-fillers have been more and more studied in the past decade for enhancement of mechanical and barrier properties of all kind of polymers, from synthetic plastics [Gloaguen ; Dufresne et al. 1997; Manchado et al. 2005; Pereira de Abreu et al. 2007; Choudalakis et al. 2009; De Azeredo 2009; Ferreira et al. 2011] to protein based ones [Guilherme et al. ; Miao et al. ; Tang et al. ; Lu et al. 2004; Tunc et al. 2007; De Azeredo 2009; Kumar et al. 2010; Luecha et al. 2010; Yuan et al. 2010; Hemsri et al. 2011; Souza et al. 2012]. There is also several studies centered on the construction and use of nano-composites for food packaging applications [Tang et al. ; Avella et al. 2005; Brody 2006; Pereira de Abreu et al. 2007; De Azeredo 2009; Silvestre et al. 2011; Cagnon et al. 2012], mostly with the aim of increasing oxygen barrier properties and mechanical strength of the materials [Silvestre et al. 2011]. In order to successfully do so it is important that the nanofillers are well dispersed, and orientated [Gloaguen ; Bharadwaj et al. 2002; Sinha Ray et al. 2003; Silvestre et al. 2011].

Nano-clays. Nanoclays are the most common nano-fillers as they are available in large quantities and at moderate price. The clays can be natural or chemically modified to improve their properties or their adhesion with the matrix [Bharadwaj et al. 2002; Jang et al. 2011;

Rhim 2011]. Nanoclays have been initially used to reinforce the mechanical properties of various materials including the protein based ones [Ranade et al. 2003; Pereira de Abreu et al. 2007; Tunc et al. 2007; Chivrac et al. 2009]. But interesting effects on improvement of the gas barrier properties were also observed. There are 3 key factors to successfully modulate (reduce) the gas transfer properties of materials with clays: a good interaction between the matrix and the protein (polymer) to avoid defects that would create preferential path for gas transfer; a good exfoliation and a good (and mono-directional) orientation of the clay platelets to create a tortuosity effect that will increase the gas molecule path through the composite and so reduce gas transfer [De Azeredo 2009] (models by Nielsen in 1967 [Nielsen 1967] then Beall in 2000 [Beall 2000]). Achieving such requirements will also ensure improvement of the materials TS and Young modulus (usually at the expense of its strain at break). The most commonly used nano-clays are the montmorillonites either under their natural form or modified for better clay/matrix compatibility.

Addition of nanoclays has a significant impact on the mechanical properties of all protein based materials by increasing their tensile strength and young modulus at the expense of their strain at break (WG [Tunc et al. 2007; Yuan et al. 2010; Cho et al. 2011], CZ [Luecha et al. 2010], SPI [Kumar et al. 2010]); same effect had been witnessed on other biomaterials (starch [Tang et al. ; Avella et al. 2005; Souza et al. 2012], agar [Rhim 2011], red algae [Jang et al. 2011], chitosan [Vermogen et al. 2005; Wang et al. 2005; Li et al. 2011] and PLA [Petersson et al. 2006; Jollands et al. 2010]). However, nanoclays does not always have the expected effect on the gas transfer properties. If water vapor permeability of protein based materials was found to be reduced by nanoclays incorporation in many case thanks to the water absorption properties of the clays [Tunc et al. 2007; Luecha et al. 2010], the gas transfer properties were not always significantly reduced [Tunc et al. 2007]. This was attributed to an imperfect exfoliation and orientation of the clays inside the protein (gluten) matrix which prevented the tortuosity effect. Finally, it should be noted that almost all studies involving nanoclays reinforcements (of proteins or other polymers) were directed towards maximizing the barrier properties either with no special application targeted or for packaging of food that require high barrier properties toward WVP (dry foods) or towards P_{O2}/P_{CO2} (such as meats or beverages) [Pereira de Abreu et al. 2007; McMillin 2008; Lagaron 2011; Silvestre et al. 2011].

Nano-fibers (cellulose whiskers and microfibrils). There are a lot of different nano-fibers that can be used as filler for composite materials from highly crystalline cellulose whiskers to the micro-fibrilated cellulose, they differ in preparation method, properties and targeted applications [Hubbe 2008; Siqueira et al. 2010].

Cellulose whiskers are highly crystalline nano-structures that come from agricultural by-product. They are mostly used as mechanical reinforcement as they exhibit very high E and TS (up to 250GPa and 10 GPa respectively) [Helbert et al. 1996; Mathew et al. 2002; Hubbe 2008; Siqueira et al. 2010] and are also known to increase the thermal stability and the Tg of the polymeric matrix [Helbert et al. 1996]. As for the nanoclays, filler/matrix compatibility, aspect ratio of the filler, dispersion and orientation are of major importance for properties enhancement. There are only few studies on their effect on transfer properties but they are supposed to somehow reduce permeability to gases and aroma compound via creation of a maze-like structure (tortuosity effect) just as the nanoclays. However they are not always very efficient as evidenced on conventional or bio-based plastics [Hubbe 2008; Sanchez-Garcia et al. 2008]. Added to gelatin, they were found to increase the TS (up to + 25MPa) and the young modulus (up to +160MPa) [George et al. 2012] but also storage modulus and the hardness of the film [Chang et al. 2012] and to enhance the cohesion of the matrix for charges up to 4%. The nano-whiskers of bacterial cellulose were also found to delay rehydration of the composite films [Chang et al. 2012] and to reduce their WVP up to minus 20% [George et al. 2012] by hydrogen bonding between the nano-whiskers and the matrix, and by the supposed establishment of a percolated network of nano-whiskers [Chang et al. 2012; George et al. 2012]. Same properties modulations were observed for cellulose whiskers addition to starch based materials [Angles et al. 2000; Angles et al. 2001; Lima et al. 2004].

Cellulose microfibrils are longer and more flexible particles than the straight and crystalline whiskers [Hubbe 2008; Siqueira et al. 2010]. They also present a higher surface area and a web like structure [Montanari et al. 2005], and despite their name they feature nano-size diameter (and micrometer length). Their elastic modulus is lower than the one of whiskers with value up to 150GPa [Iwamoto et al. 2009] leading to expect lower rigidity and tensile strength improvement if used as reinforcing filler[Iwamoto].

Other nano-fillers. Less common nano-fillers have also been studied to modulate the properties of protein based materials. In 2004, Lu et al. [Lu et al. 2004] studied the impact of chitin whiskers incorporation into SPI films. These nano-fillers were prepared by chitin acid hydrolysis and feature a nanometer scale diameter and length both [Lu et al. 2004; De Azeredo 2009]. They evidenced a great improvement of both mechanical properties (TS and

Young modulus) and water resistance of the material after chitin whiskers addition but did not investigate the effect of these fillers on transfer properties. It can be noted that similar properties improvements were observed for chitin whiskers incorporation into other kind of agro-based materials such as chitosan films [Sriupayo et al. 2005; Li et al. 2011].

Incorporation of nano-silica has also been tested. When introduced in a WG matrix, they were found to increase all mechanical properties in regard of the neat WG film even if they had a clearer impact on strength than on elongation (with highest properties for 5 to 7% silica). As for the nano-clay, the type of nano-silica had an impact on the matrix/nano-filler compatibility and so on the properties of the composite [Hemsri et al. 2011]. The nano-silica were also found to act as compatibilizers between starch and polyvinyl-alcohol through C-O-Si links if introduced in small amount (2.5% being the optimal charge) [Tang et al. 2008; Xiong et al. 2008], thus improving the mechanical properties of the composite but also, and more interestingly, its water vapor barrier properties (reduction of WVP by 60% for the composite containing 2.5% of SiO_2 compared to the one devoid of it). So they may also be of use to modulate the transfer properties of protein/protein or protein/bio-based polymer.

Finally, it is also possible to use carbon nanotube to reinforce polymers. Lot of study are focused on composites featuring ultra-high rigidity and tensile strength based on synthetic polymers and carbon nanotube [Lau et al. 2002; Manchado et al. 2005; Zeng et al. 2006; Kim et al. 2008]. Incorporation of carbon nanotubes on biopolymers such as PLA [Brody 2006], PCL [Sanchez-Garcia et al. 2010] or starch [Fama et al.] has also been studied to enhance mechanical properties or reduce oxygen permeability of their respective neat materials. However, to our knowledge, there are currently no studies available on reinforcement of protein based material by carbon nanotube or use of carbon nanotubes to modulate the properties of food packaging materials. This is likely due to the high price of the carbon nanotube compared to the cost of the food to be packed.

Overall it appeared that incorporation of nano-scale fillers into protein matrixes is of great interest for both mechanical properties enhancement and reduction of WVP but improvements of the exfoliation and orientation of the nano-fillers inside protein matrixes are still needed to achieve tortuosity effect in such composite materials and so significantly reduced their permeability to oxygen and carbon dioxide.

Multilayer Structure (LbL assembly)

Layer-by-layer assembly is a nano-structuring technique based on alternate deposition of mono-molecular layers of chemical substances of opposite electronic charge onto a substrate. This technique was first performed and described by Decher and co-workers [Decher et al. 1991; Decher et al. 1992; Decher 1997; Decher et al. 1998] and actively investigated since [Lvov et al. 1995; Izquierdo et al. 2005; Ariga et al. 2006; Jang et al. 2008; Priolo et al. 2010; de Villiers et al. 2012; Hong et al. 2012]. There are mainly 3 ways to perform the assembly: dipping, spray assisted LbL and spin assisted LbL. Dipping is the oldest while spraying is the fastest technique. LbL can allow construction of very thin and resistant films and precise modulation of the mechanical, gas transfer [Jang et al. 2008; Priolo et al. 2010; Holder et al. 2012] or other (e.g. fire resistance [Carosio et al. 2012]) properties of the material used as substrate for the deposition. The main advantages of the LbL assembly technique is that it is possible to deposit almost all kind of chemical compounds and to very precisely control the quantity deposited and the thickness of the film created.

Outside of the protein-based materials field, LbL deposition of synthetic polymers (such as PEI) and nano-clays has already been used to create highly transparent and resistant materials that feature amazingly high mechanical and gas barrier properties [Jang et al. 2008; Priolo et al. 2010]. With 2 sides deposition of $[PEI/MMT]_{40}$ onto PET substrate, Priolo et al. [Priolo et al. 2010] in 2010 obtained a final material featuring a Young modulus of 12.5GPa and a P_{O2} as low as $2.89x10^{-19}$ mol.Pa^{-1}.m^{-1}.s^{-1}. Furthermore, pH was found to have a great influence on all properties, because it affects the capacity of PEI to unfold and so to form real nano layer. At neutral pH, the mechanical properties were favored (E of 33GPa at pH 7 against 12.5 at pH 10) whereas at basic pH the barrier properties were favored (P_{O2} of $2.89x10^{-19}$ mol.Pa^{-1}.m^{-1}.s^{-1} at pH 10 against 7.17 $x10^{-19}$ mol.Pa^{-1}.m^{-1}.s^{-1}). It is also interesting to note that even at high RH the barrier properties can remain very high (10 folds better than ones of common PET). But high RH has a negative effect nonetheless as it induce swelling of the clay layers [Wong et al. 2004; Jang et al. 2008; Holder et al. 2012].

These results pointed out the key role of nanoclays in mechanical and barrier properties enhancement obtained through LbL assembly. Such structure appears of great interest to modulate either mechanical or transfer properties of protein based materials. But to our knowledge there is still no study on that topic. However it is possible to produce protein / nanoclays composites with LbL deposition as demonstrated in previous studies applied to biological and medical fields. Szabo et al. in 2007 [Szabo et al. 2007] and Miao et al. un 2010

[Miao et al.] proved that it was possible to alternatively deposit Protamine, Lysozyme or Papain alternatively with nanoclays like saponite (up to 15 pairs of layers). The deposition was found to be more regular with lysozyme and papain than with protamine as a part of the protamine was lost during the dipping in the clay solution. It is also possible to alternatively deposit other protein with nanoclays [Lvov et al. 1995; Ariga et al. 2006]. However, as for glucose oxidase, it is often necessary to dilute the protein into a synthetic polymer such as PEI to ensure good adhesion between the monomolecular layers. But, even with such technique, the proteins immobilized in the LbL structure were able to maintain their activity [Ariga et al. 2006] which was very promising because if protein activity and properties are maintained through LbL deposition thanks to the mild conditions of the process (water, moderate temperature, low mechanical constraints), there is hope to maintain transfer properties (especially selectivity) of proteins such as WG or SP in similar LbL structure.

PERSPECTIVES

Through the various formulation, shaping, and structuration strategies discussed, it appears possible to overcome the mechanical weaknesses of protein-based materials and to structure them for packaging applications. The different strategies presented here to modulate mechanical properties of protein based materials might also be applied to modulate their gas and moisture transfer properties within a broad range; larger than the one of synthetic polymers. Then it would permit to cover the needs of most of fresh produce for modified atmosphere packaging. However, to improve and expand the use of such materials for fresh produce MAP, it seems mandatory to deepen the current knowledge on their properties at high relative humidity, and especially on carbon dioxide permeability and the impact of the modulation strategies on this property. Indeed, up to now, most of the studies are conducted at low to moderate RH and focused on water vapor permeability and oxygen permeability (to a lesser extent) only, neglecting carbon dioxide permeability despite its equal importance when considering the conception of materials for MAP applications. Also, deeper investigation on the structure/properties relationship in current modulation strategies such as coating and on new and innovative modulation strategies such as layer-by-layer deposition should be considered in the future as interesting packaging applications could be foreseen.

Table S.3. Gas transfer properties of "neat" protein-based materials and impact of various modulation strategies

Protein	RH	Gas Transfer Properties							
		P_{O_2} (10^{-18} mol.Pa^{-1}.m^{-1}.s^{-1})		P_{CO_2} (10^{-18} mol.Pa^{-1}.m^{-1}.s^{-1})		S		WVP (10^{-10} mol.Pa^{-1}.m^{-1}.s^{-1})	
Wheat Gluten									
Casted – Neat	Low	1.24[1]	-	35.76[1]	-	5.96	-	0.02[2]	-
Casted – Neat	Med	60[1] to 1200[3]	-	360[1] to 2500[3]	-	2.08[3] to 6[1]	-	0.14[3] to 4.16[4]	-
Gamma Irradiated	Med	-	-	-	-	-	-	-33% (4.16)[4]	⬇
Nanoclays (up to 10%)	Med	+4% (1250)[3]	➡	+0% (1250)[3]	➡	+0% (2.08)[3]	➡	-53% (0.15)[3]	⬇
Casted – Neat	High	1290[1]	-	36700[1]	-	28.45[1]	-	0.05[5] to 227[2]	-
Coated – On White Paper	High	14700[6]	-	176400[6]	-	12[6]	-	-	-
Coated – On Kraft Paper	High	21000[6]	-	10920[6]	-	5.20[6]	-	-	-
Coated – On Lab-made Untreated Paper	High	8328[7]	-	16927[7]	-	2.03[7]	-	0.13[7]	-
Moulded – Neat	High	1201[6]	-	21861[6]	-	18.2[6]	-	-	-
Compression-moulded on paper – Neat (900bar)	Low	5.18[8]	-	-	-	-	-	-	-
Low pressure (350bar)	Low	-42% (5.18)[8]	⬇	-	-	-	-	-	-
Compression-moulded on carboard – Neat (266bar)	Low	3.56[8]	-	-	-	-	-	-	-
Extruded – Neat	Med	23.4[9]	-	-	-	-	-	-	-
Wheat Gluten Fractions									
Gliadin Casted – Neat	Low	-	-	-	-	-	-	0.04[10] to 0.27[11]	-
Heat Treatment	Low	-	-	-	-	-	-	-75% (0.04)[10]	⬇
50% Chitosan	Med	-	-	-	-	-	-	+85% (0.27)[11]	⬆
Glutenin Casted – Neat	Low	-	-	--	-		-	0.02[10]	-
Heat Treatment	Low	-	-	-	-	-	-	-50% (0.02)[10]	⬇
Soy Protein									
Casted – Neat	Med	25[12] to 279[13]	-	-	-	-	-	0.14[14] to 1.58[15]	-
Crosslinked	Med	-	-	-	-	-	-	-36% (0.14)[14]	⬇
SDS	Med	-	-	-	-	-	-	-45% (1.58)[15]	⬇
Beeswax	Med	-	-	-	-	-	-	-43% (1.09)[16]	⬇
Cysteine	Med	-17% (279)[13]	➡	-	-	-	-	-7% (1.49)[13]	➡
Multilayered Composite with CZ	Med	+32% (25)[12]	⬆	-	-	-	-	-35% (0.52)[12]	⬇
Casted – Neat	High		-	-	-	-	-	0.46[17]	-
Extruded – Neat	Med	-	-	-	-	-	-	0.59[18]	-

MMT nanoclays (up to 15%)	Med	-	-	-	-	-	-	-41% (0.59)[18]	⬇
Moulded – Neat	High	-	-	-		-	-	0.94[19]	-
Soy Protein Fractions									
P1 or R1P3 or R3 Casted – Neat	High	-	-	-	-	-	-	0.46[17]	-
Corn Zein									
Casted – Neat	Low	66[20]	-	-	-	-	-	-	-
Casted – Neat	Med	23.1[20] to 35[21]	-	216[21]	-	6.17[21]	-	0.07[22] to 5.5[23]	-
MMT nanoclays (3%)	Med	-	-	-	-	-	-	-57% (0.07)[22]	⬇
Coated – On High Amylose Starch film	Med	-	-	-	-	-	-	9.72[23]	-
Casted - Neat	High	262[20]	-	-	-	-	-	-	-
Blown Extruded – Neat	Med	-	-	-	-	-	-	0.05[22]	-
MMT nanoclays (1%)	Med	-	-	-	-	-	-	-40% (0.05)[22]	⬇
Whey Protein									
Casted – Neat	Low	2.07[24] to 340[24]	-	-		-	-	0.014[25] to 1.87[26]	-
Casted – Neat	Med	36.6[24]	-	-		-	-	17.75[27]	-
Soya Oil	Med	-	-	-	-	-	-	+9% (17.75)[27]	➡
Sodium Caseinates									
Casted – Neat	Low	~ 4[28]	-	~4[28]	-	~1[28]	-	0.08[28]	-
Oleic Acid:Beeswax (1:1)	Low	+5000% (4)[28]	⬆	+32500% (4)[28]	⬆	+650% (1)[28]	⬆	-75% (0.08)[28]	⬇
Casted – Neat	Med	162.5[28] to 366.48[29]		910[28] to 2198.87[29]		5.6[28] to 6[29]		1.26[30] to 2.24[30]	
Oleic Acid:Beeswax (1:1)	Med	+92% (162.5)[28]	⬆	+184% (910)[28]	⬆	+48 (5.6)[28]	⬆	-60% (0.23)[28]	⬇
Casted – Neat	High	104.15[28]		625[28]		6[28]		1.8[28]	
Oleic Acid:Beeswax (1:1)	High	+365% (104.15)[28]	⬆	+600% (625)[28]	⬆	+50% (6)[28]	⬆	-86% (1.8)[28]	⬇
Gelatin									
Casted – Neat	Low	-		-		-		0.0003[31] to 0.096[32]	
Crosslinked (tannic acid)	Low	-		-		-		+0% (0.0003)[31]	➡
Casted – Neat	Med	0.12[33] to 0.34[33]		-		-		0.03[34] to 0.43[35]	
Cellulose NanoCristals (4%)	Med	-		-		-		-33% (0.03)[34]	⬇
Myofibrillar									
Casted – Neat	High	873[36]		11095.8[36]		12.71[36]		0.04[36] to 0.15[37]	

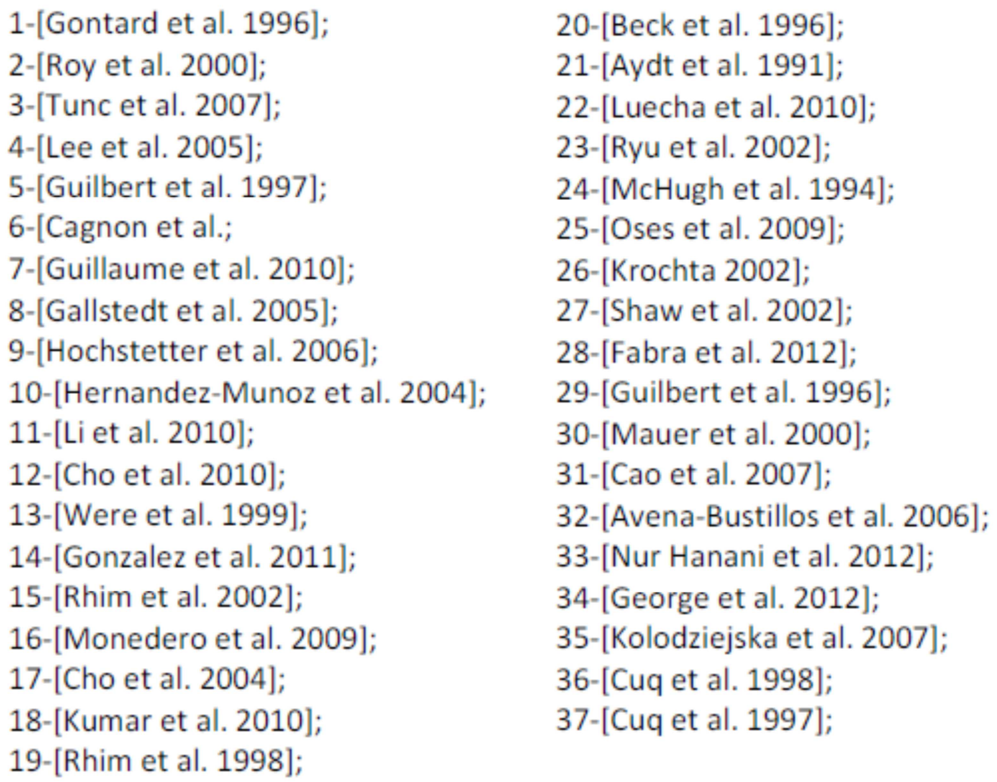
*Notes regarding Table S.3:

-“Neat” proteins materials: plasticizer content lower than 30%

-For the “modified” proteins materials, reduction (⬇), stability (➡) or increase (⬆) of the property compared to the “neat” material in the same study is indicated

-Low RH (<40%); Med RH (40<…<80); High RH (>80%)

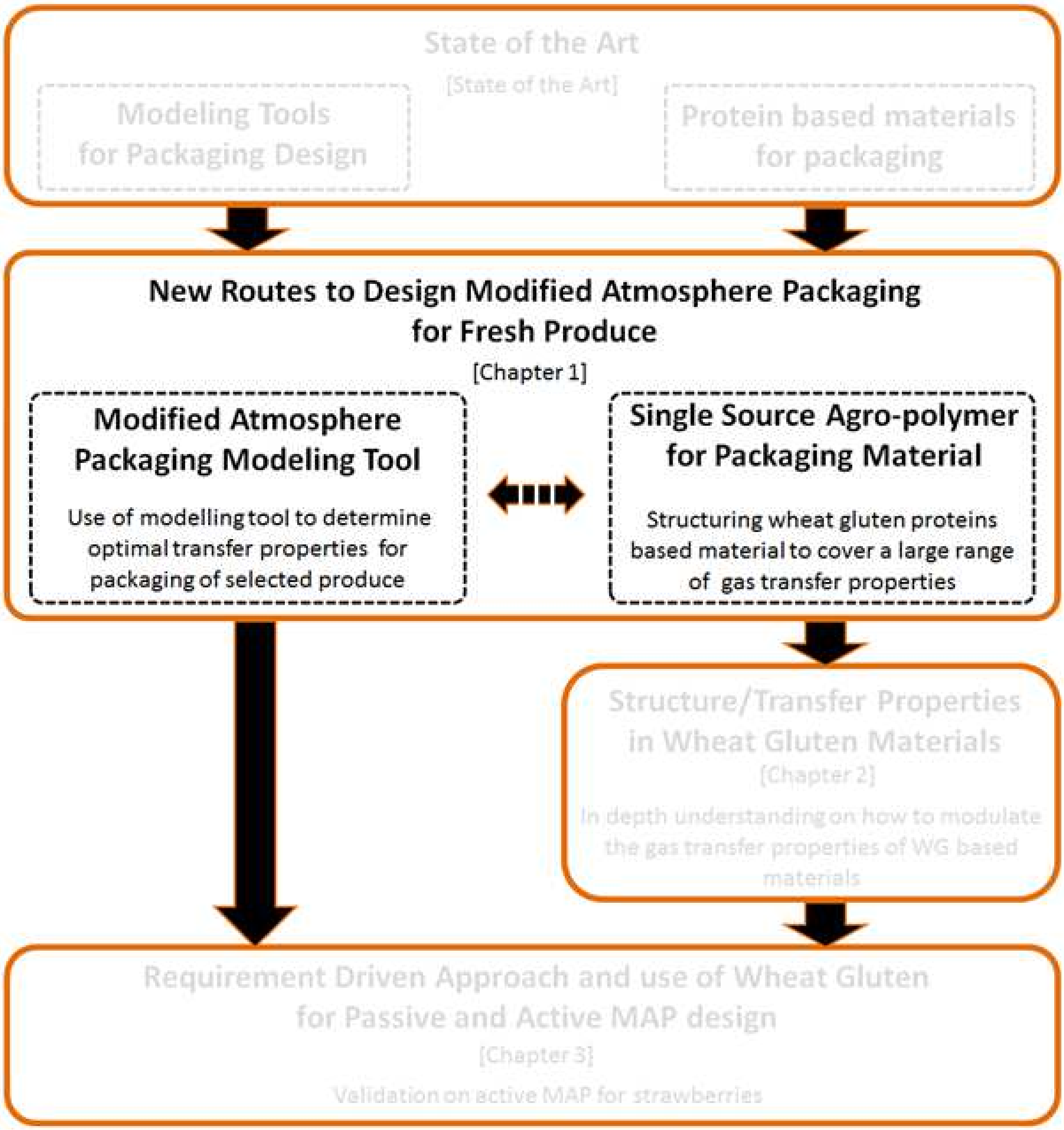
*References in Table S.3:



1-[Gontard et al. 1996];
2-[Roy et al. 2000];
3-[Tunc et al. 2007];
4-[Lee et al. 2005];
5-[Guilbert et al. 1997];
6-[Cagnon et al.;
7-[Guillaume et al. 2010];
8-[Gallstedt et al. 2005];
9-[Hochstetter et al. 2006];
10-[Hernandez-Munoz et al. 2004];
11-[Li et al. 2010];
12-[Cho et al. 2010];
13-[Were et al. 1999];
14-[Gonzalez et al. 2011];
15-[Rhim et al. 2002];
16-[Monedero et al. 2009];
17-[Cho et al. 2004];
18-[Kumar et al. 2010];
19-[Rhim et al. 1998];
20-[Beck et al. 1996];
21-[Aydt et al. 1991];
22-[Luecha et al. 2010];
23-[Ryu et al. 2002];
24-[McHugh et al. 1994];
25-[Oses et al. 2009];
26-[Krochta 2002];
27-[Shaw et al. 2002];
28-[Fabra et al. 2012];
29-[Guilbert et al. 1996];
30-[Mauer et al. 2000];
31-[Cao et al. 2007];
32-[Avena-Bustillos et al. 2006];
33-[Nur Hanani et al. 2012];
34-[George et al. 2012];
35-[Kolodziejska et al. 2007];
36-[Cuq et al. 1998];
37-[Cuq et al. 1997];

CHAPTER 1

NEW ROUTES TO DESIGN OPTIMAL MAP FOR FRESH PRODUCE



This chapter aims to illustrate the interest of the combination of the two new routes considered for MAP optimal design. First the interest of the requirement driven approach (focused on respiration and gas transfer) and the MAP modeling tools will be exposed and then the ability of materials from a single protein (wheat gluten (WG)) to cover the needs of various produce thanks to a large range of gas transfer properties; through different structuring scales (nano/micro), formulations and shaping processes will be discussed.

Publication 1

Nano and micro-structuring of materials from a single agro-polymer for sustainable MAP preservation of fresh food

Thibaut Cagnon, Carole Guillaume, Valérie Guillard, Nathalie Gontard

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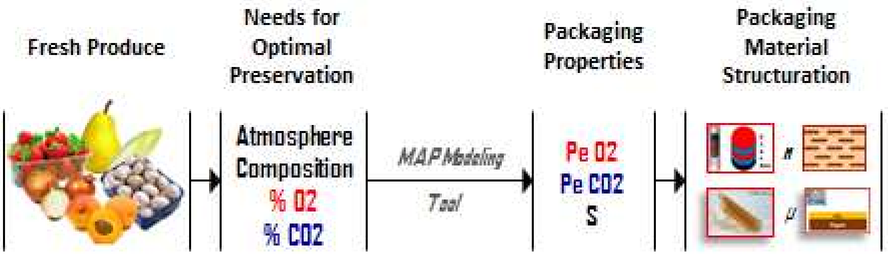
ABSTRACT: The main objective of the present work was to determine whether a single agro-polymer (wheat gluten) could fit the modified atmosphere packaging (MAP) requirements of a range of 6 different fresh produce in key terms of oxygen permeation (Pe_{O2}) and CO_2/O_2 permselectivity (S) values. The required properties for optimal packaging of fresh fruits and vegetables were first evaluated using the Tailorpack MAP modeling software with packaging dimensions, and respiratory and optimal atmosphere data as input parameters. Then, the modeled values obtained were compared to the properties of a range of wheat gluten (WG) composite films: mono-layer self-supported or multi-layer at micro or nano-scale, cast or thermo-plasticized, with different formulations (percentage of plasticizers or nano-fillers). The experimental gas transfer properties that could be covered by these materials ranged from 0.05×10^{-10} to 2.00×10^{-10} $mol.m^{-2}.s^{-1}.Pa^{-1}$ for Pe_{O2} and up to 18.0 for S. These ranges are much larger than for conventional plastics which exhibit Pe_{O2} from 0.10×10^{-10} to 0.20×10^{-10} $mol.m^{-2}.s^{-1}.Pa^{-1}$ and S up to 4.5. It was demonstrated from a food-requirements driven (Tailorpack modeling) and a multi-scale film structuring (WG based composites) approaches, that transfer properties of WG based films would fit the requirements of the six selected fruits and vegetables better than conventional plastics.



KEY WORDS: Modified atmosphere packaging; Fresh produce; Wheat gluten; Mass transfer properties; MAP modeling tool

INTRODUCTION

Important losses of fresh fruits and vegetables by spoilage between harvest and consumption have always been a concern for all stake-holders, from the producer to the consumer. The fragility and highly perishable character of these commodities lies in their living and respiring characteristic after harvest and through the entire distribution chain. The key factor for good preservation is to lower the respiration of the produce as it delays physiological and biochemical changes [Kader 1987; Kader et al. 1989; Solomos et al. 1989; Fonseca et al. 2002] and thus degradation rate. This can be achieved by decreasing the temperature or using modified atmosphere conditions.

Modified atmosphere compositions ranging from 2 to 15% O_2 and 0 to 15% CO_2 are commonly used for produce storage. According to numerous studies, each type of produce has its own requirements in terms of optimal storage conditions [Kader et al. 1989; Toivonen et al. 2009; Sandhya 2010; Guillaume et al. 2011].

Modified atmosphere packaging (MAP) aims to establish an optimal atmosphere for food preservation inside the packaging. It relies on modification of the headspace atmosphere through both gas transfer properties of the packaging materials and produce respiration (O_2 consumption and CO_2 production) [Kader et al. 1989; Sandhya 2010; Guillaume et al. 2011]. Therefore packaging, with suitable O_2 permeation (Pe_{O2}) and CO_2/O_2 permselectivity (S), should be properly chosen in order to allow the setting up of an optimal headspace atmosphere. Most of the MAP in the food industry is still based on a costly and time-consuming trial and error approach. But recent developments of MAP modeling tools such as the Tailorpack (www.tailorpack.com) or the PackinMap (www.packinmap.com) online applications, allow to design packaging materials specially adapted for MAP via reverse engineering [Gontard et al. 2011; Guillard et al. 2011; Guillaume et al. 2011]. Respiratory parameters of the produce to be packed, its optimal atmosphere for preservation and the packaging dimensions are the input data for the model.

Conventional plastic packaging materials are known for their very low O_2 permeation (Pe_{O2}) [Gontard et al. 2011] and limited CO_2/O_2 permselectivity (S) [Exama et al. 1993; Fishman et al. 1995; Al-Ati et al. 2003; Guillaume et al. 2011] that would often lead to excessive anoxia and induce detrimental metabolic deviations. Macro/micro perforations are used to limit this drawback but the permselectivity drops to a value of 1, unsuitable for generating the optimal modified atmosphere of most produce.

Other polymers, from renewable resources, such as protein (whey, soy proteins, and wheat gluten proteins) are more and more investigated for their unique mass transfer properties [Cuq et al. 1998; Gallstedt et al. 2005; Gastaldi et al. 2007; Guillaume et al. 2010a; Guillaume et al. 2010b; Gontard et al. 2011]. Among them, wheat gluten (WG) has been demonstrated to offer, at high relative humidity (RH), high gas permeation and permselectivity such as generally demanded for fresh fruit and vegetable packaging [Guillaume et al. 2010b]' [Gontard et al. 1996b]. Furthermore WG is processable with most industrial shaping technologies (casting, coating, thermomoulding, etc.) and specific plasticizers [Gontard et al. 1993] or fillers [Tunc et al. 2007] are susceptible to allow further modulation of its transfer properties.

Therefore, the present paper focuses on studying the ability of WG based materials to cover the requirements of a large range of fresh fruits and vegetables. In a first step, a reverse engineering approach has been conducted: starting from optimal MAP for 6 representative selected produce, required packaging material transfer properties (Pe_{O2} & S) have been determined using a MAP modeling tool. In a second step the gas transfer properties of WG based materials were analyzed as a function of the type of structuration (mono or multi-layers at microscopic and nanoscopic scales), the shaping process (casting or thermo-plasticizing) and the composition (percentage of plasticizers or nano-fillers). Finally adequacy of gas transfer properties (permeability and perm-selectivity) of WG based materials to fit requirements of the selected produce were analyzed and compared to conventional plastics.

MATERIALS AND METHODS

Materials

Wheat gluten (WG) powder, containing 7.2 wt. % of moisture and 76.5 wt. % of protein was provided by Amylum (France). Acetic acid and sodium sulphite, also used to prepare the coating solution were purchased from Aldrich. Glycerol was obtained from Sigma Co. (St Louis, MO, USA). HPS montmorillonites nanoclays (MMT) were purchased form Süd-Chemie AG (Choisy le Roi, France). Kraft paper (Teranna 36g.m^{-2}) was provided by Gascogne Paper (Mimizan, France) to serve as support paper for coatings along with white untreated paper (36g.m^{-2}) provided by Smurfit WR (Mortagne, France). Optigel SH synthetic nanoclays (short montmorillionites-like clays without heavy metals in the layer lattice) and polyethylene imine (PEI) for layer-by-layer assembly were kindly provided by the Institute Charles Sadron (Strasbourg, France). The commercial synthetic films used for comparison were a polyamide/polyethylene copolymer (PA/PE), a low density polyethylene (LDPE) and an oriented polypropylene (OPP) from Sudpack France S.A.S. (Marne La Vallée, France), Bolloré Division Films Plastiques (Quimper, France) and Lawson Mardon Morin (Sarrebourg, France) respectively.

Preparation of the WG based materials

Self-supported casted film. The film forming solution (500mL) was prepared using 75g of gluten, 0.050g of sodium sulfite, 25g of glycerol (30% w/dry w) and distilled water. The pH of the solution was brought to 4 using acetic acid. All the components were mixed vigorously using an Ultra Turrax mixer (Janke & Kernke, KG Illawerk, Germany) at ambient temperature (25°C). The film-forming solution was then kept 2 h at 25°C to remove all bubbles.

To form the "Mono-layer Self Supported – Casting – 90%RH" material, the film forming solution was spread onto a Plexiglas leveled surface using a film-making apparatus (Braive Instruments, Chécy, France) adjusted to 0.4mm height and dried at 30°C in a ventilated oven for 12 hours.

Self-supported thermo-molded film. Wheat gluten-plasticizer blends, so-called pre-film samples for thermomoulding, were obtained by mixing the gluten powder and plasticizer in a two blade counter-rotating batch mixer at a 3:2 differential speed (Plasti-corder W 50, Brabender, Duisburg, Germany) connected to a computer interface and controller unit (PL 2100, Brabender, Duisburg, Germany). The torque and product temperature were continuously recorded during the mixing process. Temperature of the mixing chamber was regulated using a regulation temperature unit (WTD4, Weinreich, Lüdenscheid, Germany) and water circulation in the double jacket of the mixer. Cold gluten (4°C) was first introduced in the mixer chamber (volume of 50cm^3); then the plasticizer (glycerol), equilibrated at 25°C was added 30 seconds after mixing began. A plasticizer content of 30% was used for all experiments. The mixer chamber was filled with 50 g total mass. The mixing speed was 30rpm. The mixing chamber was regulated at 25°C. Mixing was stopped 5 minutes after the time needed to reach the maximum torque.

Wheat gluten-plasticizer blend samples (5g) were placed between two flat polytetrafluoroethylene surfaces and pressed using a heated press (Sercom, Montpellier, France) (120°C, 200 bars) for 10 minutes to produce the “Mono-layer Self-Supported – Thermomoulded – 90%RH” films.

WG coated papers. The standard WG coating solution (21.23% dry w/v) for coated perm-selective materials was prepared at room temperature according to an adapted 3 step procedure [Guillaume et al. 2010a]. First, 30 g of WG powder was dispersed under shacking in 50 ml of a sodium sulphite solution (0.06g / 50mL) which worked as a reducing agent of the disulfide bonds. Then, after 30 minutes of settling, the pH of the solution was set to 4 by adding a 50% v/v solution of acetic acid. Finally the solution was adjusted to 130mL by adding deionized water and the whole mix was stirred and left to rest for a day.

The glycerol plasticized WG solution was prepared the same way except that 20% (w/dry w) of glycerol was added to the mix before the solution volume adjustment with deionized water.

For the WG MMT solution, 5% (w/dry w) of HPS montmorillonites were dispersed in water under magnetic stirring for 10 minutes. They were then added to the WG preparation just before the volume adjustment.

For coating on kraft papers: prior to coating, kraft support papers were stored in an RH controlled chamber set at 30% RH using $CaCl_2$. The coating was performed with the standard WG coating solution using an E409 blade coater from Erichsen (France). The coater was equipped with a blade n°8 featuring a spire width of 1mm. Coating speed was $10mm.s^{-1}$. To be spread all over the sheet surface, an excess (> 3mL) of the coating solution was deposited to produce coated papers. After coating the materials were left to dry at 20°C for 2 hours and the relative humidity was kept below 40%. The coated kraft paper will be referred as “Multi-layer Coated – Kraft – 100%RH” or “Multi-layer Coated – Kraft + Nanoclays – 100%RH” and “Multi-layer Coated – Kraft + Glycerol – 100%RH” when montmorillionites or glycerol were added to the coating solution respectively.

For coating on white papers: prior to coating, all papers were wet and then, flattened under vacuum on leveled aluminum plates to be stuck at the edges using masking tape, to avoid wrinkling during the coating process. Thereafter, vacuum was stopped and a layer (about 150 µm thick) was coated onto the surface of white base paper, using a thin-layer chromatography applicator (Braive instruments, Liege, Belgium), to produce WG coated white paper hereinafter referred as “Multi-layer Coated – White – 100%RH”. Drying was performed at 25°C and 50% RH until constant weight.

Layer-by-Layer assembly. The WG solution for the layer-by-layer (LbL) assembly consisted in a WG standard solution for coating (21.23% dry w/v without plasticizer) diluted to 0.2%.

Diluted WG solution (0.2%), Optigel SH solution (0.2%) and ultra-pure water were prepared and poured into separate spraying devices, AirBoy, purchased from Carl Roth (Karlsruhe, Germany). The layer-by-layer deposition was performed directly on kraft paper by alternate spraying (5 seconds) of the Optigel solution and the diluted WG solution. Between each layer deposition, washing (5 seconds) was performed with the ultra-pure water to remove excess deposit and weakly bound compounds. Deposition of 10 and 40 layers pairs were performed and referred as LbL $[WG/MMT]_{10}$ & LbL $[WG/MMT]_{40}$.

Material characterization

Thickness. The average thickness of each produced material was determined at room temperature and 30% RH with a hand-held digital micrometer (Mitutoyo instruments) from 10 measurements randomly taken over the paper surface.

Gas transfer properties. The O_2 and CO_2 permeation (Pe_{O2} & Pe_{CO2}) of WG coated papers were assessed in triplicate with an isostatic and dynamic method using gas phase chromatography. The materials were placed in a permeability cell. The inferior and superior chambers were each spread by a 30 mL.min^{-1} flux of permeant gas (O_2 or CO_2) and vector gas (He) respectively. The RH of the gas flux was set using a bubbling flask containing water and placed in a cryothermostat regulated at a properly chosen temperature. The differential temperature imposed between the cell and the cryothermostat permitted the establishment of the desired RH in the chamber. The self-supported materials were measured at lower RH due to their critical mechanical weakness at very high RH. The permeation cell was coupled to a gas chromatograph GC3800 from Varian (Les Ulis, France) equipped with an automatic valve to online analyze the evolution with time of permeant gas concentration in the superior chamber of the cell (analysis of 1ml of sample at prescribed times). The gas chromatograph was equipped with a filled column Porapak-Q from Chrompack (Les Ulis, France) of 2m length and 0.32m diameter for separating O_2 and CO_2, with a thermal conductibility detector (TCD). The gas (O_2 or CO_2) permeation of the material was determined as follows in the international system unit:

$$Pe_x = \frac{\Delta Q}{\Delta t \times A \times \Delta P} \qquad eq.1.1$$

where subscript x referred to O_2 or CO_2, ΔQ was the number of moles of gas that passed through the film, Δt was the time for which permeation occurred, A was the exposed film area and ΔP is the difference in pressure exerted by the gas on each side of the film. Gas permeation was expressed in mol.m^{-2}.s^{-1}.Pa^{-1} unit. For total O_2 and CO_2 desorption and RH stabilization, materials were placed prior to measurements in the permeation cell using helium to spread both chambers.

Material permselectivity (S) was calculated as the ratio of Pe_{CO2} on Pe_{O2}

MAP simulation

Tailorpack MAP modeling tool. The evolution with time of oxygen and carbon dioxide partial pressures in packaging headspace was simulated using the web-application Tailorpack (www.tailorpack.com) to evaluate viability of the selected packaging. This mathematical model is based on the mass balance between the oxygen and carbon dioxide flux through the packaging material on one hand and the O_2 and CO_2 consumption/ production of the produce respiration on the other hand as described previously. This model, as most of those previously established [Kok 1985; Wade et al. 1987; Kader et al. 1989; Edmond et al. 1991; Talasila et al. 1994; Fishman et al. 1995; Peppelenbos et al. 1996b], was based on Michaelis-Menten equations for produce respiration and Fick's laws for gas transfers. Briefly,

$$p_{O2}^{\cdot pkg} = \frac{Pe_{O2} \times S}{e} - \left(p_{O2}^{ext} - p_{O2}^{pkg}\right) \times RR_{O2} \times m = f_1 \qquad eq.1.2.1$$

$$p_{CO2}^{\cdot pkg} = \frac{Pe_{CO2} \times S}{e} - \left(p_{CO2}^{ext} - p_{CO2}^{pkg}\right) \times RR_{O2} \times m \times RQ = f_2 \qquad eq.1.2.2$$

with, considering no CO_2 inhibition

$$RR_{O2} = \frac{RR_{O2max} \times p_{O2}^{pkg}}{Km_{appO2} + p_{O2}^{pkg}} \qquad eq.1.3.1$$

or, considering non-competitive CO_2 inhibition

$$RR_{O2} = \frac{RR_{O2max} \times p_{O2}^{pkg}}{(Km_{appO2} + p_{O2}^{pkg}) \times \left(1 + \frac{p_{CO2}^{pkg}}{Ki_{CO2}^{n}}\right)} \qquad eq.1.3.2$$

where the first part of the right-hand side of f_1 and f_2 describes gas flux per time unit through the packaging material, while the second part describes gas consumption (and emission) by the vegetable or fruit modeled using a Michaelis-Menten-type equation.

In these 4 equations, Pe_{O2} and Pe_{CO2} are respectively the O_2 and CO_2 permeabilities (mol.m^{-1}.s^{-1}.Pa^{-1}), S is the packaging surface (m^2), e is the packaging thickness (m), p_i^j is the partial pressure of j in i (kPa), RR_{O2} is the O_2 respiration rate (mmol.kg^{-1}.h^{-1}), m is the mass of food (kg), RQ is the respiration quotient (-), RR_{O2max} is the maximal O_2 respiration rate (mmol.kg^{-1}.h^{-1}) and Km_{appO2} is the Michaelis-Menten constant (kPa) and Ki^n_{CO2} is the non-competitive CO_2 constant.

Numerical solving of the nonlinear ODE system constituted by these 3 equations was carried out using the ode45 solver from Matlab software (The Mathworks Inc., Natick, MA, USA). This routine adjusts the time step size for calculations according to the importance of partial pressure variations. All simulations were performed using the user-friendly interface (www.tailorpack.com).

This mathematical model can be run in two modes available on the Tailorpack website: optimization and simulation. In the case of optimization, the optimal atmosphere is previously required (%O_2 and %CO_2) and the optimal permeabilities of the packaging material could be then identified using an optimization procedure (e.g., Levenberg-Marquard algorithm) to fit predicted partial pressures to optimal ones. In the case of simulation, the packaging permeabilities (i.e. permeation value multiplied by thickness) are required and the evolution with time of O_2 and CO_2 headspace composition is predicted.

RESULTS AND DISCUSSION

Determination of required mass transfer properties for optimal MAP

The Tailorpack software was used in optimization mode to determine the required mass transfer properties for the packaging of the 6 selected produce. Three types of input data were necessary: optimal storage conditions, dimensions of the packaging and respiratory parameters of each produce. Two outputs describing the optimal gas transfer properties of the packaging were calculated: optimal O_2 permeation (Pe_{O2}) and permselectivity (S) values of the materials.

Optimal storage atmosphere. The optimal atmosphere composition for preservation of each of the six selected fresh produce are presented in Figure 1.1. For pears, onions, chicories & apricots the input data were taken from a single well known and recognized source [Kader et al. 1989] which was checked to be consistent with updated data from UC Davis [UCDavis 2012]. For strawberries and mushrooms, the values selected came from a compromise elaborated from the latter sources [Kader et al. 1989; UCDavis 2012] and from other studies [Woodward et al. 1972; El-Goorani et al. 1979; Burton et al. 1987; Lopez Briones et al. 1992; Shamaila et al. 1992; Lopez Briones et al. 1993; Roy et al. 1995]. Regarding mushrooms, Kader & al. (1989) recommended an atmosphere containing more than 1% O_2 and less than 15% CO_2 whereas other sources recommended slightly different values [Burton et al. 1987; Lopez Briones et al. 1992; Lopez Briones et al. 1993; Roy et al. 1995; UCDavis 2012]. As an example, it has been demonstrated that a CO_2 concentration above 5% could have a rapid and strong phytotoxic effect when mushrooms are back to normal air [Lopez Briones et al. 1992]. Consequently, the selected range was set at 2-10% O_2 and 2.5-5% CO_2. For the strawberries, recommended compositions by Kader & al. (1989) and UC Davis were 2-5% of O_2 and up to 15% of CO_2. Due to the risk of off flavor development at low O_2 content [El-Goorani et al. 1979; Shamaila et al. 1992], there is a consensus on the lowest O_2 value acceptable (2%), but the high CO_2 percentage is subject to controversy as it can induce off flavor development while it is well known to inhibit *B.Cinerea* growth [Woodward et al. 1972; Shamaila et al. 1992]. Therefore an O_2 content ranging from 2 to 5% and CO_2 from 0 to 10% were chosen for this study.

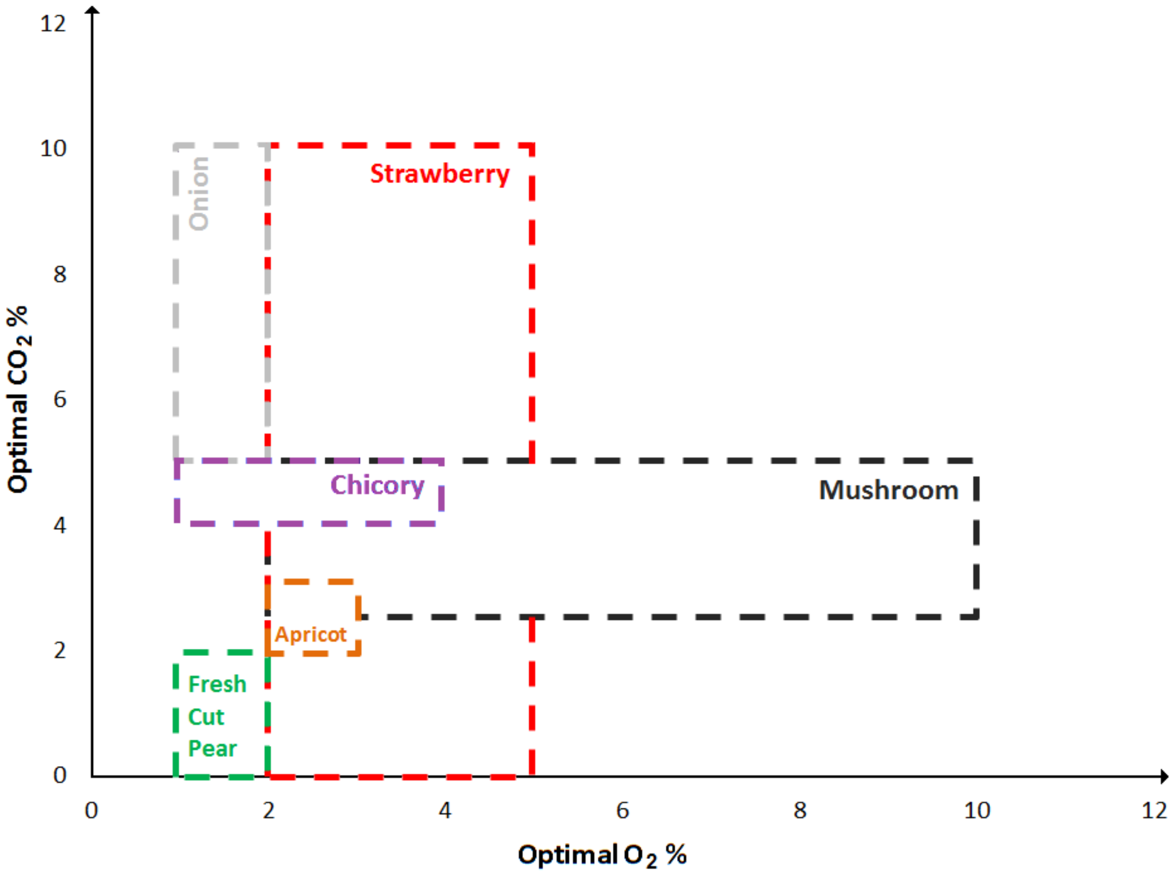


Figure 1.1. Optimal atmosphere compositions for preservation of 6 fresh produce [Varoquaux et al. 1999; Benkeblia et al. 2000; Charles et al. 2005; Gomes et al. 2010; Gouble et al. 2011a; Gouble et al. 2011b].

Packaging dimensions. Packaging dimensions (mass, volume, surface) were chosen to be as close as possible to commercial packaging (Table 1.1). The overall surface was available for transfer when “Sachet” or wrapping combined with “Light wood punnet” was selected. In case of “PET punnet + Lid” only the lid surface was available for transfer as the PET walls are assumed to be highly barrier to gases and therefore not significantly contributing to the modified atmosphere set up.

Respiratory parameters of the produce selected. Data for respiratory parameters of the 6 fresh fruits and vegetables selected were taken from the literature and unpublished database (Table 1.1). It should be noted that obtaining respiratory parameters is not an easy task due to the variability of biological produce, the absence of a standardized method and a huge dependency of these parameters on the produce variety.

In the case of fresh cut pears, apricots and onions the only thoroughly completed studies to our knowledge were considered [Benkeblia et al. 2000; Gomes et al. 2010; Gouble et al. 2011a; Gouble et al. 2011b]. For mushrooms and chicory, values presented in Table 1.1 for these two produce were chosen among the most deeply investigated and MAP oriented studies [Varoquaux et al. 1999; Charles et al. 2005]. For strawberries no thoroughly completed source was found and therefore a personal database was used.

Determination of required mass transfer properties. By running the Tailorpack software in optimization mode, a range of required/optimal Pe_{O2} and permselectivity values were obtained for each produce (Figure 1.2, cross representations). Values obtained covered a broad range of Pe_{O2} and S values: from 0.02×10^{-10}mol.m^{-2}.s^{-1}.Pa^{-1} to more than 6×10^{-10} mol.m^{-2}.s^{-1}.Pa^{-1} and from 2.5 to nearly 20, respectively. Within this interval a same range of Pe_{O2} or S values could fit the requirements of several produce (e.g. similar optimal values of Pe_{O2} for strawberries and fresh cut pears and similar S for strawberries and onions). However, a combined range of both Pe_{O2} and S never fitted the needs of more than one commodity highlighting that no unique material is able to fit the requirements of all produce.

Gas transfer properties of WG based materials and possible modulations

WG features unique permselectivity at high relative humidity (e.g. S value of 28.2 for a cast gluten film at 95%RH [Gontard et al. 1996b]) and good film forming properties that are adapted to all kinds of structuration (from mono to multi-layer at micro or nanoscopic scales). It is also compatible with most of the usual shaping processes (e.g. casting, thermo-plasticizing) [Gontard et al. 1992; Gallstedt et al. 2004; Guillaume et al. 2010a] and with various natural plasticizers [Gontard et al. 1993; Irissin-Mangata et al. 2001] or fillers [Tunc et al. 2007] opening a broad range of modulation possibilities that are discussed hereinafter.

Table 1.1. Respiratory parameters of 6 fresh produce and dimensions of their packaging

Produce		Produce characteristics and properties				Packaging Dimensions			
Type	**Variety**	**RR_{O2max} (mmol.kg^{-1}.h^{-1})**	**RQ**	**Km_{appO2} (kPa)**	**Ki_{CO2} (kPa)**	**Type**	**Surface (m^2)**	**Volume (m^3)**	**Mass (g)**
Apricot	*Bergeron*	1.000	0.78	4.50	/	Light Wood Punnet+Wrap	0.0756	0.00100	500
Chicory	/	1.400	0.74	9.26	1515	Sachet	0.2140	0.00200	500
Mushroom	*Agaricus bisporus*	5.270	0.78	0.10	/	Light Wood Punnet+Wrap	0.0960	0.00184	500
Onion	*Allium Cepa*	0.077	0.91	6.30	/	PET Punnet+Lid	0.0256	0.00100	500
Pear (fresh cut)	*Rocha*	0.900	1.00	2.59	/	PET Punnet+Lid	0.0245	0.00184	400
Strawberry[*]	*Charlotte*	0.800	0.91	8.10	/	PET Punnet+Lid	0.0256	0.00100	500

[*] Personal database

Processing of monolayer self-supported films. Oxygen permeation and permselectivity of cast wheat gluten film at 25° C and 90% RH were found to be 6.6×10^{-12}mol.m^{-1}.s^{-1}.Pa^{-1} and 18.2 (Figure 1.2 , orange square) respectively, in accordance with previous published results on cast materials in the same conditions [Mujica Paz et al. 1997; Irissin-Mangata et al. 2001]. O_2 permeation rates and permselectivities of cast WG film were about 1.5 fold higher than thermomoulded WG film (Figure 1.2, light brown square) and consequently CO_2 permeation rate was 2.25 times higher, in similar conditions of relative humidity, temperature and glycerol content. This indicated that the CO_2 permeation of wheat gluten films seemed more influenced by the film-forming technique than O_2 permeation. It can be related to a modification of the gluten network under high pressure and temperature conditions during the thermomoulding process affecting gas permeation. Previous studies [Micard et al. 2001; Hernandez-Munoz et al. 2004; Mangavel et al. 2004] suggested that casting of wheat gluten films seems to build a loose and open network while thermo-molding creates either a high polymeric network (due to the formation of additional covalent bonds like iso-peptide bonds, methylene bridge etc.) or a cross-linked network during heat treatment and especially at temperatures of above 120°C.

Despite such interesting gas transfer properties, self-supported WG films exhibit poor mechanical resistance and alternative solutions should be used to maintain the good transfer properties while improving the mechanical ones. One of these routes is WG coating onto paper substrate.

Processing and formulation of multilayer micro-structured WG coated papers.

All the coated papers studied in this work featured a high enough coating weight (around 15g.m^{-2}) and a homogeneous and thick enough apparent gluten layer on top of the paper in order to fill the paper pores and exhibit gas transfer properties as recommended in a previous study [Guillaume et al. 2010a].

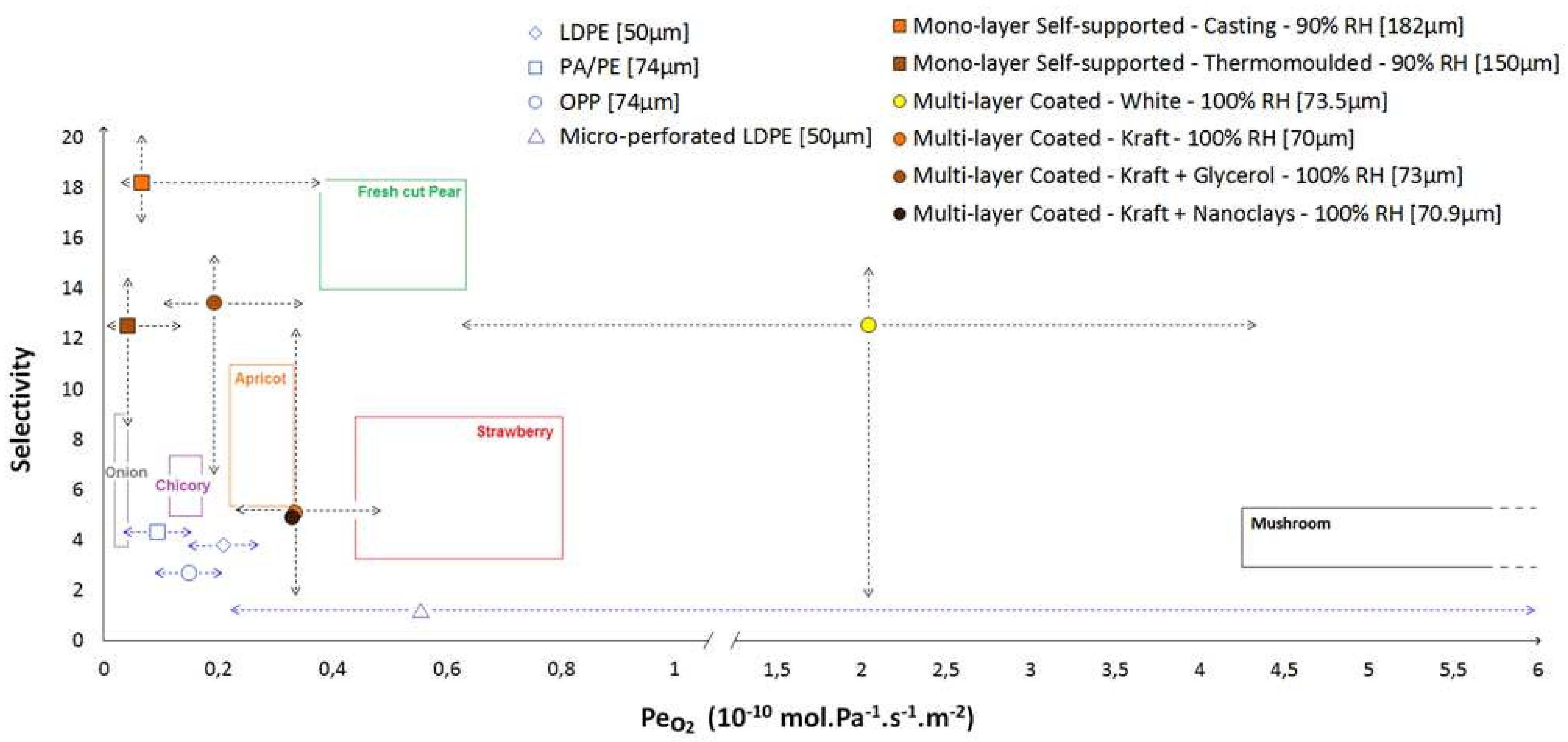


Figure 1.2. Optimal oxygen permeation and permselectivity to reach MAP requirements (optimal atmosphere of 6 fresh produce and gas transfer properties (Pe_{O2} & S) of various synthetic and WG based materials and their possible modulations

A white coated paper (bleached fibers, loose fiber network, coating weight 24g.m^{-2}) and a kraft coated paper (kraft fibers, tight fiber network, coating weight 15g.m^{-2}) were produced from a pure WG solution and compared. Both papers exhibited moderate O_2 barrier and interesting permselectivity values. The white WG coated paper (Figure 1.2, yellow circle) was 4.5 five more permeable to O_2 and 2 times more selective than the kraft (Figure 1.2, orange circle) WG coated paper. Such difference was mainly attributed to higher coating weight deposited in the white paper than in the kraft paper. An increased amount of protein deposited on the paper is likely to enhance the wheat gluten behavior (highly permselective at high RH) of the multi-layered material. However it should not increase the Pe_{O2} (2.04×10^{-10} mol.m^{-1}.s^{-1}.Pa^{-1} against 0.33×10^{-10}mol.m^{-1}.s^{-1}.Pa^{-1} for white coated paper against kraft coated paper). This unexpected higher Pe_{O2} may be due to the nature of the support papers used as gas transfer properties of WG coated paper are dependent on the surface properties of the support paper used (e.g. hydrophilicity and roughness) as evidenced in a previous study [Guillaume et al. 2010a].

The effect of the plasticizer content was then studied on kraft paper coated with a WG solution containing 0% or 20% of glycerol. The addition of glycerol induced a 2.5 times higher permselectivy but did not significantly affect Pe_{O2}. Increasing the content of the plasticiser into the solution is known to increase polymeric chain mobility, providing a better accessibility to active sites for CO_2 sorption onto the protein. As a consequence, CO_2 permeation increases as well as the selectivity [Gontard et al. 1993; McHugh et al. 1994; Pouplin et al. 1999; Irissin-Mangata et al. 2001; Shaw et al. 2002b]. It could be noted that similar effects have been evidenced by increasing WG materials water content (the most ubiquitous WG plasticizer) in high moisture environments [Gontard et al. 1993; Gontard et al. 1996a; Gontard et al. 1996b].

The effect of the addition of nano-fillers on gas transfer properties of coated paper was assessed by comparing a kraft coated paper with and without 5% (w/dryw) of MMT nanoclays. No significant difference was observed between the two papers. This is in agreement with previous results obtained on self-supported nano-composite WG materials [Tunc et al. 2007] demonstrating that an increase of the quantity of nanoclays up to 7.5% does not affect the O_2 and CO_2 transfer properties of the WG film. Spatial organization of the clays was demonstrated not to be exfoliated and oriented enough to form a complex pattern that would slow down gas transfers by a tortuosity effect.

Multilayer nano-structuration of WG films via LbL assembly. One new way to modulate the permeation values of film materials is to form a layer-by-layer assembly onto a substrate by depositing successive nano-layers of opposite electronic charge. This technique, which was first studied by Decher and coworkers [Decher et al. 1991; Decher et al. 1992; Decher 1997] and Lvov and coworkers [Lvov et al. 1995] in the 1990's, has been recently demonstrated to be able to create gas barrier materials when used on synthetic polymers [Jang et al. 2008; Priolo et al. 2010]. However, to our knowledge no attempt has been performed on structuring LbL material directly onto paper.

For this study, alternative LbL deposition of WG and clays nanolayers were performed 10 ($[WG/MMT]_{10}$) and 40 times ($[WG/MMT]_{40}$) on kraft paper. The material built with only 10 layers pairs was highly permeable to oxygen and non permselective. Increasing the number of layers deposited decreased the Pe_{O2} from around 500×10^{-10}mol.m^{-2}.s^{-1}.Pa^{-1} to around 200×10^{-10}mol.m^{-2}.s^{-1}.Pa^{-1} when depositing 40 layers pairs instead of 10. However, no increase of the permselectivity value was observed. Kraft paper exhibited a very irregular surface and lot of pores, so it was very likely that such thin depositions did not fill these pores sufficently. It can be concluded from these first results that mechanical and/or chemical pretreatment of the paper support should be performed prior to LbL deposition in order to create a slick appropriate surface for continuous LbL deposition.

CONCLUSION

It is possible to modulate gas transfer properties of WG based materials through structuration, shaping process and formulation. From the above-presented results, these factors have been classified according to their ability to provide increasing Pe_{O2} or S values of WG based films:

- Pe_{O2}: Thermomoulded self-supported material < Cast self-supported material < Coated kraft paper (with or without glycerol/MMT) < Coated white paper < LbL $[WG/MMT]_{40}$ < LbL $[WG/MMT]_{10}$
- S: LbL $[WG/MMT]_{10/40}$ < Coated kraft paper (with and without MMT) < Coated white paper < Coated kraft paper with glycerol and Thermomoulded self-supported material < Cast self-supported material

It should be noted that the layer-by-layer assembly could be a promising way to modulate gas transfer properties of WG based material if the support paper is properly previously treated or if another adequate support is chosen.

The above-mentioned modulations allowed the creation of WG based materials (Figure 1.2, yellow to brown symbol) which were able to cover a far broader range of Pe_{O2} and S values than conventional synthetic films (Figure 1.2, white and blue symbols). They ranged from around $0.05\times10^{-10}mol.m^{-2}.s^{-1}.Pa^{-1}$ to $2.00\times10^{-10}mol.m^{-2}.s^{-1}.Pa^{-1}$ for Pe_{O2} and up to 18.0 for S whereas the selected conventional synthetic materials were limited to values from 0.10×10^{-10} to $0.20\times10^{-10}mol.m^{-2}.s^{-1}.Pa^{-1}$ for Pe_{O2} and up to 4.5 for S. The Pe_{O2} of synthetic material could be increased by the use of micro-perforations as a function of hole density (Figure 1.2, blue dotted line) or thickness modulation. However in both cases, their permselectivity cannot be increased and remain very low (<4.5 for the considered plastics, 1 for micro-perforated materials) and not compatible with main produce requirements except onions. On the contrary, by regulating or combining structuration, shaping process and formulation it appeared possible to obtain WG based material with different Pe_{O2} and especially S values that could meet the requirements of all considered fruits and vegetable (Figure 1.2, black dotted arrows). For example, a monolayer self-supported thermomoulded WG film containing less glycerol and processed at a higher temperature is likely to exhibit suitable transfer properties for onion packaging. To obtain an optimal material for apricot packaging, a coated kraft paper containing 10% of glycerol would be appropriate. And finally, fresh cut pear could benefit from suitable MAP with a white WG coated paper with a slightly increased WG coating weight and glycerol content, while reducing WG coating weight could easily fit mushroom MAP requirements.

This demonstrates that a reverse engineering approach combined with micro and nano-structuring of agro-polymer based materials offers interesting perspectives for the design of eco-friendly packaging better adapted to fresh fruits and vegetables preservation.

ACKNOWLEDGMENTS

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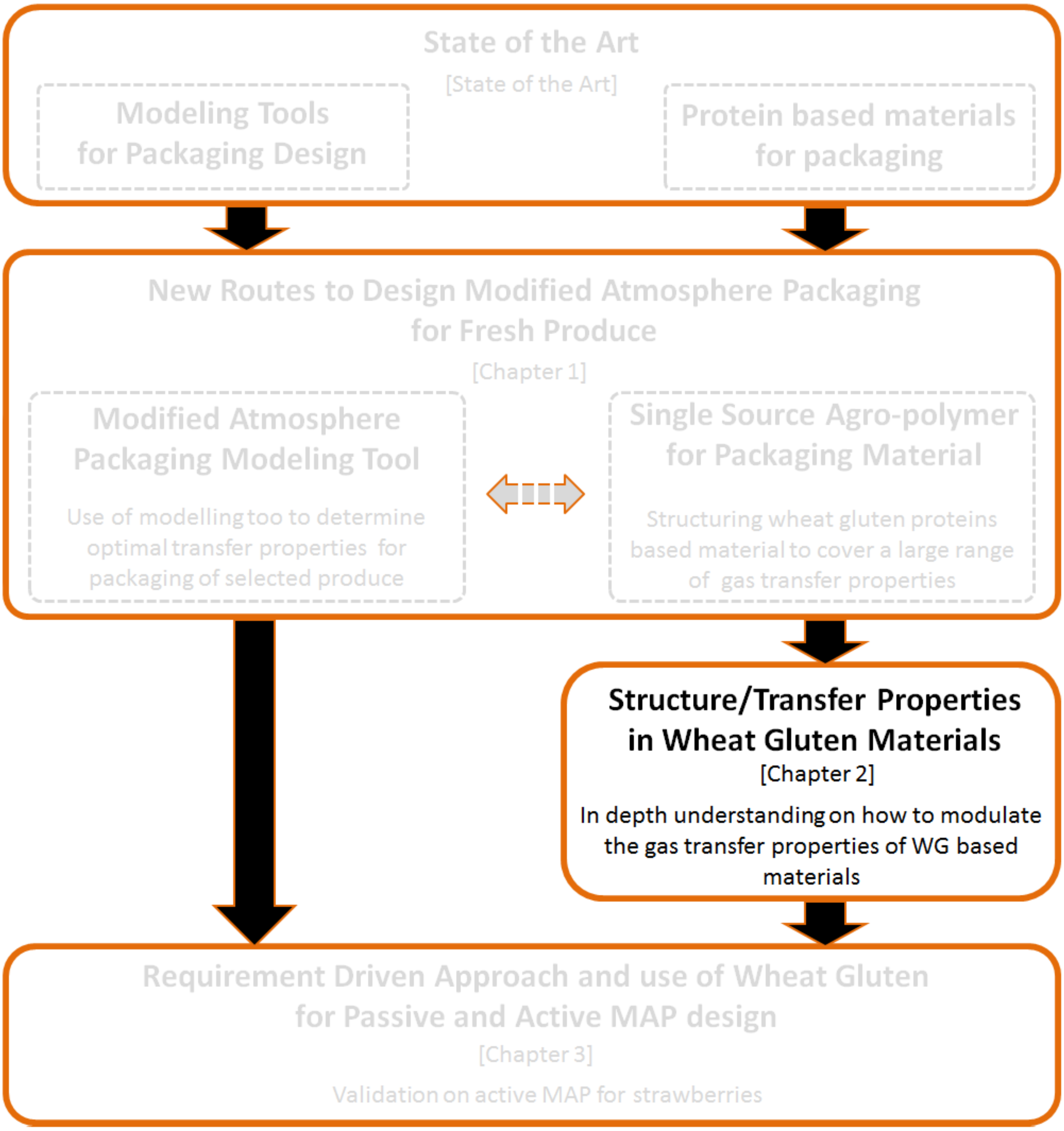
CHAPTER 2

IN DEPTH STUDY OF THE STRUCTURE/TRANSFER PROPERTIES RELATIONSHIP IN WHEAT GLUTEN BASED MATERIALS



In this chapter an in-depth study on the structure/transfer properties relationship will be performed to better understand how structuring process (including formulation) and architectures at different scales can modulate the gas transfer properties of wheat gluten based materials. First the influence of the support in the properties of a protein coated paper will be discussed. Then the possibility to perform layer-by-layer deposition involving protein onto paper based substrates will be assessed and the gas barrier performances of the built material evaluated. Finally, the impact of a CO_2 sorption treatment on the properties of wheat gluten coated paper will be measured.

Publication 2

Importance of the structure of paper support in gas transfer properties of protein-coated paper

Thibaut Cagnon, Carole Guillaume, Emmanuelle Gastaldi, Nathalie Gontard

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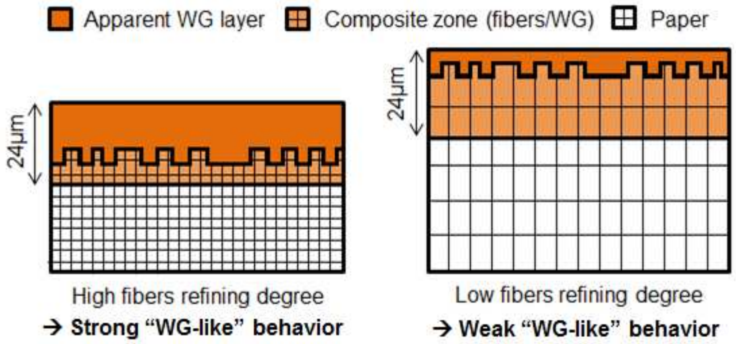
ABSTRACT: Composites made from fibrous support coated with agro-polymers have only been sparingly studied, especially in terms of transfer properties, despite their evidenced interest for applications such as food packaging. Furthermore most of past studies on the topic have been focused on the impact of surface properties of the support on the final coated material structure and its properties; leaving unexplored the potential impact of the in-bulk structure of the support. This study demonstrated the influence of in-bulk structure and especially fibers refining degree of 4 kraft papers (SP28, 36, 60 & 80) on the structure, and surface and gas transfer properties of their respective wheat gluten coated paper (WGP28, 36, 60 & 80). Paper presenting a high fibers refining degree (SP28) exhibited very tight and narrow in bulk fibers network which prevented most of the WG coated layer penetration, maintaining an important WG apparent layer on top of the paper and a small fibers/wheat gluten composite zone inside (WGP28). Such structure gave strong "WG-like" properties to the final coated material with moderate oxygen permeation and high permselectivity (1.50×10^{-11} mol.m^{-2}.s^{-1}.Pa^{-1} and 8.09 for WGP28) whereas the highly impregnated structure of coated papers built on lowly refined papers (SP80, wide and loose structure) gave coated materials presenting weak "WG-like" properties, supposedly due to a thick composite zone presenting interfacial defects, with higher oxygen permeation and very limited permselectivity (11.90×10^{-11} mol.m^{-2}.s^{-1}.Pa^{-1} and 1.06 for WGP80).



KEYWORDS: Fibrous support; Wheat gluten coating; Structure; Gas permeation; Surface properties

INTRODUCTION

During the last 20 years, protein based polymers have been the focus of many studies on food packaging materials due to their low price, and their biodegradable and renewable character [Tang et al. ; Kumar et al. 2009; Abdul-Khalil et al. 2012], and their unique functional properties as gases and vapor permeations [Gontard et al. 1996b; Cuq et al. 1998]. However, their poor mechanical properties, either too brittle at low relative humidity or not rigid enough at high relative humidity [Gontard et al. 1992; Cuq et al. 1998], needs to be improved for further industrial applications and the route of (nano-)composite materials has been already explored. Among the availability of a large range of processes and (nano-)fillers, protein based matrixes reinforced in bulk with micro [Kayserilioglu et al. 2003; Yu et al. 2006; Kunanopparat et al. 2008; Mastromatteo et al. 2008; Kumar et al. 2009] or nano-scale [Oksman et al. 2006; Yu et al. 2006; Hubbe 2008; Sanchez-Garcia et al. 2008; Zuo et al. 2009; Siqueira et al. 2010; Siro et al. 2010; Abdul-Khalil et al. 2012; Chang et al. 2012; George et al. 2012] fibrous fillers have been, by far, the most studied despite finding limited industrial applications up to now. In comparison, the yet already widely commercialized polymer coated fibrous supports (such as papers or cardboards) have been subjected to less scientific attention and the existing studies have been mostly focused on the surface properties of these composites [Trezza et al. 1994; Han et al. 1999; Han et al. 2001; Andersson 2008; Khwaldia et al. 2010] or their mechanical properties (to a lesser extent) [Aloui et al. ; Gallstedt et al. 2005; Khwaldia et al. 2010]. Only limited considerations have been given to their gas (O_2 and CO_2) or vapor transfer properties, which are of main interest in food packaging science considering the importance of oxygen, carbon dioxide and moisture on the preservation of food products [Gallstedt et al. 2005; Guillaume et al. 2010a; Guillaume et al. 2010b; Cagnon et al. 2012]. In addition, to better understand the final properties of protein coated on fibrous support, several studies have been conducted on coating and drying conditions [Ben Arfa et al. 2007b; Chalier et al. 2007a], the nature of proteins [Chalier et al. 2006; Gastaldi et al. 2007], the chemical composition of the support [Gallstedt et al. 2005; Guillaume et al. 2010a], the surface treatment of the support [Trezza et al. 1994; Gallstedt et al. 2005]. But surprisingly, no study has taken into consideration the impact of the structure of the support paper that is correlated to the refining degree of fibers.

This study proposes to investigate the impact of the in-bulk fibers network organization of a fibrous support on the resulting structure and gas (O_2 and CO_2) transfer and surface properties of protein coated material. Four industrial untreated kraft papers of same chemical composition but different basis weight and pulp refining degree have been chosen as fibrous supports for coating due to their widely available character, and common use for food packaging applications (e.g. sachet for bread or fresh fruits and vegetables). Wheat gluten (WG) have been considered for coating because of its very interesting gas transfer properties: relative humidity dependent gas transfer and surface properties and high permselectivity ratio (CO_2 permeation / O_2 permeation) at high relative humidity [Gontard et al. 1996b], especially for fruits and vegetable packaging [Cuq et al. 1998; Barron et al. 2002; Gontard et al. 2011]. Four WG coated papers were produced in identical conditions from the four different supports and their final properties (surface and gas transfer) were discussed in relation with their structure and the in-bulk structure of their respective coating support.

MATERIALS AND METHODS

Materials

Kraft papers (Terrana) of different basis weights 28, 36, 60 & 80g.m^{-2} (supplier data) were provided by Gascogne Paper (Mimizan, France) to serve as support papers hereinafter named Support-Papers (SP) 28, 36, 60, and 80, respectively. Samples of pastes used to process the kraft papers were also kindly provided by Gascogne Paper. Wheat gluten (WG) powder, containing 7.2 wt. % of moisture and 76.5 wt. % of protein was provided by Amylum (Mesnil St Nicaise, France). Acetic acid and sodium sulphite, also used to prepare the coating solution were purchased from Aldrich (St Quentin, France). Sodium dodecyl sulphate (SDS), 1,4-dithioerythitol (DTE) and iodoacetamide (IAM) for WG fractions quantification were purchased form Aldrich (St Quentin, France). For sample preparation to transmission electron microscopy, Technovit 7100 embedding kit was purchased from Labonord (Templemars, France).

WG solution preparation

A WG coating solution (21.23 wt. %) was prepared at room temperature according to a 3 steps procedure [Guillaume et al. 2010a], slightly adapted to match the desired quantities. First, 30 g of WG powder was poured into a box and dispersed under shaking in 50mL of a sodium sulphite / deionized water solution (0.06 g/50mL). This solution worked as a reducing agent of the disulfide bonds. Then, after 30min of settling, the pH of the solution was set to 4 by adding a 50/50 v/v. solution of acetic acid and deionized water. Finally the solution was adjusted to 130mL by adding deionized water and the whole mix was stirred and left to rest for a day.

WG coating process

Adequate procedure for coating process was determined empirically with the goal to reach a sufficient coating weight (>10g.m^{-2}) and to obtain a continuous and homogenous gluten layer.

Prior to coating, all Support-Papers were stored in a relative humidity (RH) controlled box set at 30% RH. The coating was performed on the raw side of the paper at room temperature using an Erichsen coater equipped with the blade n°8 featuring a spire width of 1mm and at a speed of 10 mm.s^{-1}. Three milliliters of the WG coating solution were necessary to perform the coating. These WG coated papers will be referred hereinafter as WG-Paper (WGP) 28, 36, 60 or 80 depending on the Support-Paper coated. After coating WG-Papers were dried slowly at room temperature and below 50% RH during 2 hours.

Following the same procedure, some Support-Papers of each basis weight were also swelled by coating with acidified (pH 4) deionized water for impregnation calculation purposes.

All materials were stored at 30%, 60%, 90% or 100% RH and 25°C, depending on their further characterization.

Thickness measurements

Average thicknesses of Support-Papers, swelled Support-Papers and WG-Papers were determined at room temperature and 30% RH with a hand-held digital micrometer (Mitutoyo instruments) from ten measurements randomly taken over the paper surface.

Basis weight and Coating weight

To evaluate the basis weight of each Support-Paper, 9 square pieces (5x5 cm) of paper were cut and weighed at room temperature and 30% RH with a precision balance (Adventurer pro, Ohaus). The results were then processed to get back to the meter square scale.

To assess the coating weight, 9 square pieces (5x5 cm) of both Support-Paper and WG-Paper were cut and left to dry during 24 h in a ventilated oven at 103°C. They were then placed to cool into a desiccator containing silica-gel. After one hour they were taken out and weighted with a precision balance. The coating weight (Cw) in grams per meter square was calculated as follows:

$$Cw = \frac{W_{WGP} - W_{SP}}{A} \qquad \textit{eq. 2.1}$$

where W_{WGP} (g) is the weight of a WG coated paper piece, W_{SP} (g) is the average weight of Support-Paper pieces and A (m²) is the area of a piece.

Impregnation calculation

To evaluate the part of the WG coating solution that penetrated into the paper, the percentage of impregnation of the coated layer was calculated as follows:

$$\%\, Impregnation = 100 \times \left(1 - \frac{e_{WGapp}}{e_s}\right) \qquad \textit{eq. 2.2}$$

where e_{WGapp} (µm) is the apparent thickness of the WG coated layer (remaining on top of the paper) and e_s (µm) is the thickness of self-supported wheat gluten film (here present inside and on top of the paper).

e_{WGapp} was calculated from:

$$e_{WGapp} = e_{WGP} - e_{SwP} \qquad \textit{eq. 2.2.1}$$

where e_{WGP} (µm) is the total thickness of WG-Paper and e_{SwP} (µm) the thickness of its respective swelled paper.

e_s was determined according to the following equation previously demonstrated [Gastaldi et al. 2007]:

$$e_s = 1.6 \times Cw \qquad \textit{eq. 2.2.2}$$

where cw ($g.m^{-2}$) is the coating weight of the WG-Paper.

Microscopy observation

Scanning electron microscopy. Scanning electron microscopy (SEM) observations of the paper pulps and wheat gluten coated papers cross sections were performed on small pieces (1x1 cm) directly mounted on stub with double sided carbon tape. After degassing under vacuum, samples were observed with a Scanning Electron Microscope S-4800 Hitachi (Japan). All micrographs were obtained using an accelerating voltage of 2.5 kV.

Transmission electron microscopy. Transmission electron microscopy (TEM) observations of the WG-Papers cross-sections were performed on thin slices after embedding of the samples in Technovit epoxy resin. A transmission electron microscope H7100 from Hitachi (Tokyo, Japan) was used for observation at a voltage acceleration of 75kV.

Wheat gluten fractions quantification by SE-HPLC

Extraction of proteins and SE-HPLC analysis were performed on WG powder grinded from the apparent WG layers of each WG-Paper and on lyophilized WG powder from a control "WG casted film" (WG coating solution casted onto a Plexiglas plate following the "WG coating process procedure). The procedure was carried out as previously described by Morel et al.in 2010 [Morel et al. 2000c], by performing two sequential extractions. The first with a 0.1 M sodium phosphate buffer (pH 6.9) containing 1% SDS, and the second with the same buffer and a sonication step to disperse SDS-insoluble glutenin polymers. Supernatants obtained from these extractions were injected onto a size exclusion column TSK-G 4000-SW (Merck, France) (7. mm × 30cm) equipped with a TSK 3000-SW (Merck, France) guard column (7.5mm, 7.5cm). SDS-insoluble glutenin polymers (*Fi*) content was obtained from the total area of the chromatogram of the second extract. The contents in the other protein fractions were obtained from the first extract profile: early-eluted fractions, *F1* and *F2*, included SDS-soluble glutenin polymers; following fractions, *F3* and *F4*, included mainly gliadin; the last eluting fraction, *F5*, included mainly albumin and globulin. The area under the peak corresponding to each fraction was expressed in % of total protein, estimated from the sum of the total areas under the chromatograms of the two extracts, once corrected for their different solid-to-solvent ratios [Morel et al. 2000a]. The percentage of total glutenin polymers can be calculated as the sum of *Fi*, *F1* and *F2*; *Fi* alone gives the percentage of unextractable glutenin polymers.

Contact angle measurements

Wettability. Wettability of the Support-Papers was evaluated by contact angle measurement using a contact angle meter Digidrop from GBX (Bourg de Peage, France), equipped with a diffuse light source, a CDD camera (25 frames per second). The measurements were taken at 30% RH and 25°C. Prior to the measurements, the papers were cut in 5x50mm bands, stuck on a glass slide using double sided adhesive and left during 2 days at the desired RH inside RH controlled chambers. The sensitivity of Support-Papers surface to wetting was investigated by measuring the behavior of a liquid drop (3 µL) of WG coating solution, ultra-pure water and paraffin oil, onto the paper surface. Both the initial wettability and the rate of change in wettability were investigated. Wettability (°.s^{-1}) was determined by measuring changes in averaged value of contact angles (measured on both sides of the drop) of the liquid drops with time. The evolution of volume and width basis of the drop as a function of time were also recorded to assess the contribution of absorption (mL.s^{-1}) and spreadability (mm.s^{-1}) which are two phenomenon involved in wettability. All measurements were performed in triplicate.

Surface Energy. The surface energy of both Support-Papers and WG-Papers was calculated using the Owen-Wendt equation and linear regression with value of wettability equilibrium angles for diiodomethane, ultra-pure water and ethylene-glycol as entries. These wettability angles were determined via the technique described above. The Owen-Wendt equation is written as follows:

$$\gamma_{\mathrm{L}} \times (1+\cos\theta) = 2\sqrt{\gamma_L^d \times \gamma_S^d} + 2\sqrt{\gamma_L^p \times \gamma_S^p} \qquad \textit{eq. 2.3}$$

where θ is the angle of the drop at equilibrium in degree, γ_L the energy of the liquid phase and $\gamma_L{}^d$ and $\gamma_L{}^p$ its disperse and polar component and γ_S the energy of the solid phase and $\gamma_S{}^d$ and $\gamma_S{}^p$ its disperse and polar component. All energies are expressed in mN.m^{-1}.

Wetting envelope. The wetting envelope of a material can be determined knowing its surface's polar ($\gamma_S{}^p$) and disperse ($\gamma_S{}^d$) surface energy components. The polar and disperse fractions of the liquid ($\gamma_L{}^p$ and $\gamma_L{}^d$) for which the contact angle is 0° ($\cos\theta=1$) were calculated with the same equations after Owen-Wendt. When this polar fraction was plotted against the disperse fraction, a closed contour for $\cos\theta=1$ was obtained. This contour was the wetting envelope, the limit between total and partial wetting of the material.

Bendtsen roughness measurements

The Bendtsen roughness (ISO 5636-3) of the Support-Papers was measured by Gascogne Paper (Mimizan, France) using a Bendtsen porosimeter model 6 with the entering air pressure set at 200kPa. Five measurements were taken on each Support-Paper and the result was expressed in ml.min^{-1}. Bendtsen roughness allows evaluation of the fibers refining degree of a paper: the higher the Bendtsen roughness, the lower the refining degree.

Gas transfer measurements

To assess the O_2 and CO_2 permeation of materials, a dynamic method using gas chromatography was used for WG-Papers and a static method using oxygen and carbon dioxide sensitive optical sensors for Support-Papers (due to high permeation of Support-Paper that saturated the gas chromatograph detector).

The O_2 and CO_2 permeation of Support-Papers were assessed in triplicate at 25°C with a static method using O_2 and CO_2 content monitoring spots and optic fibers from Presens (Regensburg, Germany) [Penicaud et al. 2011]. Prior to measurements, the material was placed at the desired RH for one day to equilibrate. It was then placed on top of a permeation cell containing both one O_2 spot and one CO_2 spot situated in a RH controlled chamber (Froidlabo, France). The cell was flushed with a gas mix consisting in 50% nitrogen and 50% carbon dioxide until the amount of O_2 inside went under 1hPa and the amount of CO_2 around 5hPa. The flush was then stopped and the cell hermetically closed. The amount of O_2 and CO_2 inside the cell was monitored via the spots and optic fibers and the permeation was calculated as follows:

$$Pe_x = \frac{J}{\Delta P \times A} \qquad \textit{eq. 2.4}$$

where Pe_x referred to O_2 or CO_2 permeation of the sample (mol.Pa^{-1}.m^{-2}.s^{-1}), J the flux of gas going through the material in mole per second, ΔP to the difference in pressure exerted by the gas on each side of the film in Pascal and A to the surface of the material in meter square.

The O_2 and CO_2 permeation of WG-Papers were assessed in triplicate with an isostatic and dynamic method using gas phase chromatography at 25°C. Materials were placed in a permeation cell. The inferior and superior chambers were each spread by a 30 mL/min flux of permeant gas (O_2 or CO_2) and vector gas (Helium) respectively. The RH of the gas flux was set thanks to a bubbling flask containing water and placed in a cryothermostat regulated at a properly chosen temperature. The differential temperature imposed between the cell and the cryothermostat permitted the establishment of the desired RH in the chamber (60 or 100%). The permeation cell was coupled to a gas chromatograph GC3800 from Varian (Les Ulis, France) equipped with an automatic valve to online analyze the evolution with time of permeant gas concentration in the superior chamber of the cell (analysis of 1 mL of sample at prescribed times). The gas chromatograph was equipped with a filled column Porapak-Q from Chrompack (Les Ulis, France) of 2m length and 0.32mm diameter for separating O_2 and CO_2, with thermal conductibility detector (TCD).The gas (O_2 or CO_2) permeation of the material was determined as follows in the international system unit:

$$P_{ex} = \frac{\Delta Q}{\Delta t \times A \times \Delta P} \qquad \textit{eq. 2.5}$$

where subscript Pe_x referred to O_2 or CO_2 permeation of the sample (mol.Pa^{-1}.m^{-2}.s^{-1}), ΔQ was the number of mol of gas that pass through the film, Δt was the time for which permeation occurs, A was the exposed film area and (ΔP) is the difference in pressure exerted by the gas on each side of the film. Gas permeation was then expressed in mol.m^{-2}.s^{-1}.Pa^{-1} unit. For total O_2 and CO_2 desorption and RH stabilization, materials were placed prior to measurements in the permeation cell using Helium to spread both chambers.

Calculation of WG composite layer theoretical oxygen permeability

To calculate the theoretical WG composite layer (Figure 2.7, apparent WG layer + interaction zone) permeability to oxygen ($P_{O2(WGcomp)}$), the permeability (i.e. permeation time thickness) of the Support-Papers and the WG coated paper were first calculated by dividing their permeation value by their respective thicknesses. Then the $P_{O2(WGcomp)}$ was calculated as follows (adapted from the conventional equation for global permeability of layered composite):

$$P_{O2(WGcomp)} = \frac{e_{WGcomp}}{\left(\frac{e_{WGP}}{P_{O2(WGP)}} - \frac{e_{SP}}{P_{O2(SP)}}\right)} \qquad \textit{eq. 2.6}$$

where e_{WGcomp}, e_{WGP} and e_{SP} are the thicknesses of the WG composite layer, the WG-Paper and the Support-Paper respectively, $P_{O2(WGP)}$ and $P_{O2(SP)}$ are the oxygen permeability of WG-Paper and Support-Paper respectively.

RESULTS AND DISCUSSION

Characterization of Support-Papers

In bulk structure. Four Terrana kraft Support-Papers of identical chemical composition but different basis weights were provided under the form of sheets: SP28, SP36, SP60 and SP80. Their basis weight varied from 29.67g.m^{-2} for SP28 to 79.84g.m^{-2} for SP80 together with their thickness from 40.55µm for SP28 to 92.04µm for SP80 and their Bendtsen roughness from 36.8 for SP28 to 231 for SP80 (Table 2.1). Bendtsen roughness value, which relies on the ability of air to penetrate in-bulk paper, is considered as a good indicator of paper porosity and so far is related to the refining degree of fibers (the lower the Bendtsen roughness value the higher the refining degree of the fibers) [Fardim et al. 2003]. When decreasing thickness of paper produced at the industrial scale, basis weight concomitantly decreases and pulp needs to be more refined to ensure sufficient mechanical resistance [Bhardwaj et al. 2007]. Higher refining implies a higher inter-fiber bonding capacity and so a better paper cohesion because of an enhanced surface fibrillation with a stronger cohesion between fibers due to the presence of many microfibrils [Chevalier-Billosta et al. 2007].

Table 2.1. Support Paper structure characterization (20°C and 30% RH)

Sample	**Support Paper Basis Weight ($g.m^{-2}$)**	**Thickness of Support Paper (µm)**	**Bendtsen Roughness ($ml.min^{-1}$)**	**Fiber's Refining Degree**
SP 28	29.67 ±0.13	40.55 ±1.25	36.8	↑
SP 36	35.38 ±0.37	49.72 ±1.47	102	
SP 60	62.40 ±0.38	75.68 ±2.21	246	
SP 80	79.84 ±1.03	92.04 ±2.22	231	

As evidenced by SEM observations of the different paper pulps (Figure 2.1), SP28 and SP36 exhibited more microfibrils than SP60 and SP80, and their Bendtsen roughness was lower, suggesting a tighter and less porous in bulk structure.

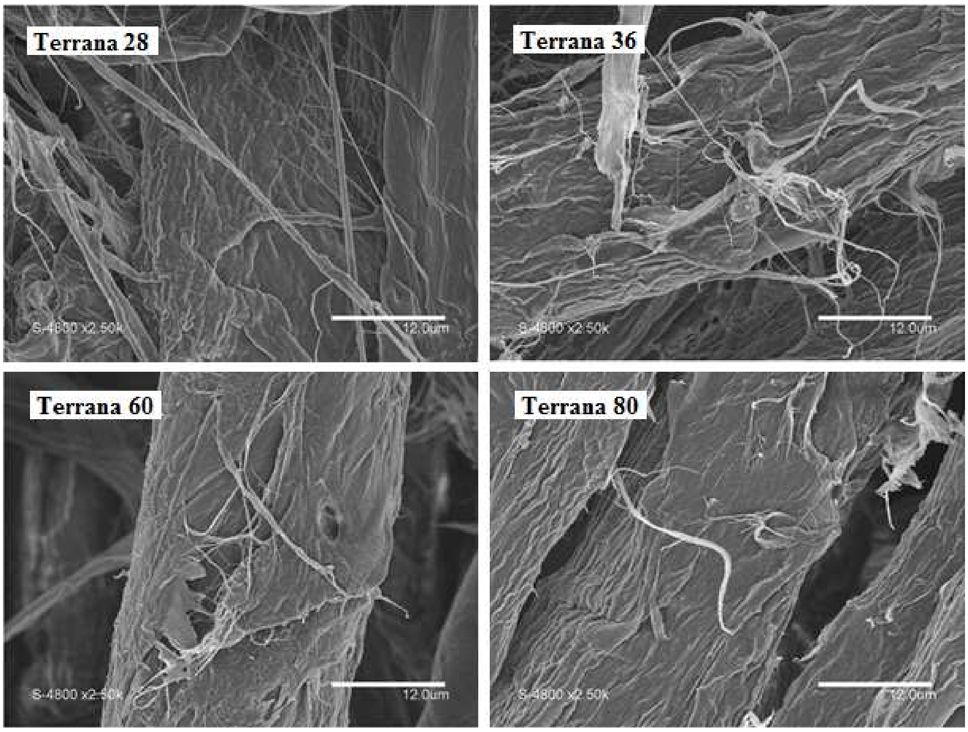


Figure 2.1. SEM views of Terrana Support-Papers – Impact of the refining degree on the presence of micro-fibrils. (x2500)

This was confirmed by oxygen and carbon dioxide permeation measurements at 25°C and 100% RH (Table 2.2): gases permeation slightly decreases with increase of the refining degree of the Support-Papers. For example SP80 was 4.4 times more permeable to O_2 and 1.7 times more permeable to CO_2 than SP28. That is in agreement with previous work on eucalyptus pulps where reduction of gases permeation was also attributed to a more intricate fibers network [Fardim et al. 2003]. It is also worth noting that all Support-Papers appeared to be somewhat less permeable to gases at moderate humidity (data not shown) than at very high humidity as previously demonstrated [Polat et al. 1993; Desobry et al. 1997]; e.g. SP28 presented a Pe_{CO2} of $0.35x10^{-8}$ $mol.m^{-2}.s^{-1}.Pa^{-1}$ at 60% RH against $9.17x10^{-8}$ $mol.m^{-2}.s^{-1}.Pa^{-1}$ at 100% RH. This is attributed to the swelling of cellulosic fibers in high RH conditions, that increases gases diffusivity [Desobry et al. 1997]. Whether differences in gases permeation were correlated to the different porosity of support papers, whatever the RH considered, all values remained characteristics of highly porous systems.

Table 2.2. Gas transfer properties (Pe_{O2}, Pe_{CO2}, S) of Support-Papers and Wheat Gluten coated Papers at 25°C & 100% RH

Support Papers	**Pe_{O2}** **10^{-11} (mol.m^{-2}.s^{-1}.Pa^{-1})**	**Pe_{CO2}** **10^{-11} (mol.m^{-2}.s^{-1}.Pa^{-1})**
SP 28	27900.00 ±660.00	9170.00 ±170.00
SP 36	40800.00 ±1780.00	5030.00 ±140.00
SP 60	43000.00 ±0.92.00	6820.00 ±160.00
SP 80	51900.00 ±0.65.00	6770.00 ±140.00

WG Coated Papers	**Pe_{O2}** **10^{-11} (mol.m^{-2}.s^{-1}.Pa^{-1})**	**Pe_{CO2}** **10^{-11} (mol.m^{-2}.s^{-1}.Pa^{-1})**	**S**
WGP 28	1.50 ±0.04	12.10 ±0.13	8.09 ±0.27
WGP 36	3.35 ±0.02	17.40 ±0.07	5.21 ±0.04
WGP 60	8.78 ±0.14	13.90 ±0.35	1.58 ±0.05
WGP 80	11.80 ±0.10	12.50 ±0.37	1.06 ±0.04

Surface properties. Surface properties of the four support-papers were assessed through different contact angle measurements. First, the wetting envelope of each Support-Paper was calculated at both 60 and over 90% RH (Figure 2.2). No significant difference in wetting envelope was demonstrated; surface energies were circa 40N.m^{-1} at 60% RH and 33N.m^{-1} at 90% RH, whatever the Support-Paper, and the dispersive component remained higher than the polar one whatever the RH. When RH increased, the dispersive component was slightly lowered without significantly affecting the polar one and then the hydrophobic character of papers was slightly reduced. Considering that liquid placed on the same chart is supposed to wet the material partially if outside the wetting envelope or totally if inside, Support-Papers were supposed to all be only partially wet by hydrophilic compounds such as water and partially to fully wet by hydrophobic ones such as paraffin oil. Then, droplets of the WG-coating solution, water and paraffin oil were deposited at the surface of support papers and static and dynamic contact angle measurements were performed at 30%RH and 25°C to simulate coating conditions (data not shown). Wettability (combination of absorption and spreading phenomena) of all support papers to WG coating solution and water was negligible with initial contact angle measured close to 90° and 110°, respectively. This indicated a low affinity between the Support-Papers and aqueous solution such as the WG coating solution. Initial contact angle and wettability of paraffin oil were around 30° and 10°.s^{-1}, respectively, whatever the support paper tested. As expected, both results confirmed that all Support-Papers were of hydrophobic nature at low RH.

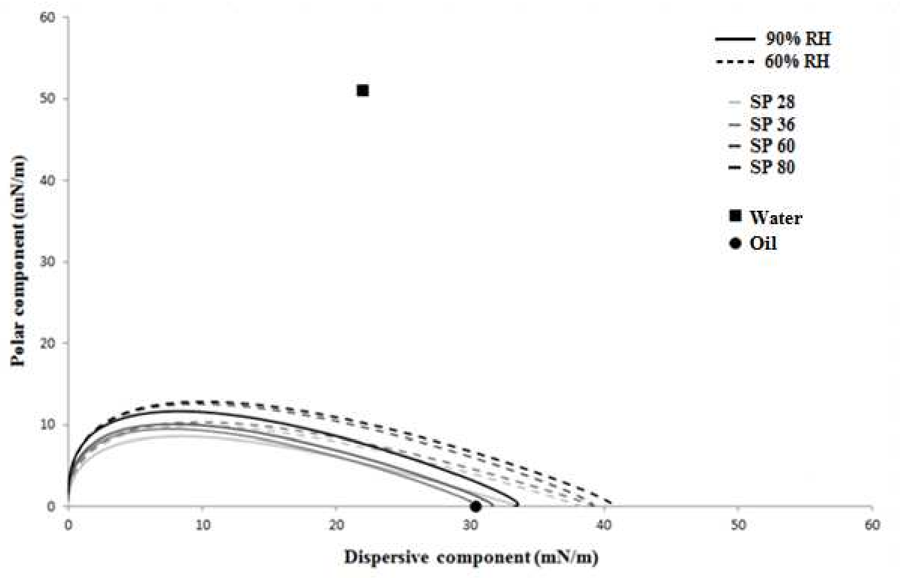


Figure 2.2. Wetting envelopes of the Support-Papers at 25°C and 60%RH and 90%RH

With differences in fibers refining and then porosity of support papers, difference in their wettability to various liquids was also expected. Indeed the hydrophobic nature of paper has been demonstrated to be emphasized by surface roughness [Quere 2002] which is generally correlated to the refining degree of the fibers. Thus the lowest refined papers (SP60 and SP80) were supposed to be more hydrophobic than the two others (SP36 and SP28). But, all papers used in this study were subjected to a mechanical treatment inherent to the Terrana paper production. This treatment implies friction on felt inducing folding of both fibers and micro-fibrils onto the paper surface that likely blotted out the difference in surface properties.

Structure of WG-Papers

As described in the material and method section, Support-Papers were coated with a WG solution and dried slowly at room temperature (2h and 20 °C). Depending on the basis weight of the Support-Papers, these coated materials were referred to as WG-Paper 28, 36, 60 or 80 (WGP28, 36, 60, 80).

The thicknesses of WG-Papers ranged from 62.78 to 108.17µm, for WGP28 and WGP80 respectively. Their coating weights (Cw) were almost identical with values from 14.48 to 15.60g.m^{-2} and would led to a WG coated layer with a theoretical thickness around 24µm (e_s), assuming no penetration of the layer inside the papers (Table 2.3). TEM (Figure 2.3) observations of the WG-Papers cross sections evidenced the presence of a continuous WG layer in all samples. However, a significant decrease of the apparent WG layer thickness was observed with decrease of fibers refining. It should be noted that measurement of the accurate thickness of each apparent WG layer could not be performed on TEM micrographs because of the swelling induced by the preparation of samples for TEM observations (for instance, apparent thickness of WGP28 was 40 µm, much more than the maximal and theoretical value of 24 µm). SEM observations of the cross sections of the WGP were also performed and confirmed the presence of a continuous WG layer on top of every paper. As there was no swelling during preparation, measurement of the apparent thickness of this layer was possible on WGP36 and WGP60 (Figure 2.4). However, sample preparation was difficult and important spreading of gluten from the apparent WG layer onto the fibers was observed when cutting WGP28 and WGP80, thus no estimations were done.

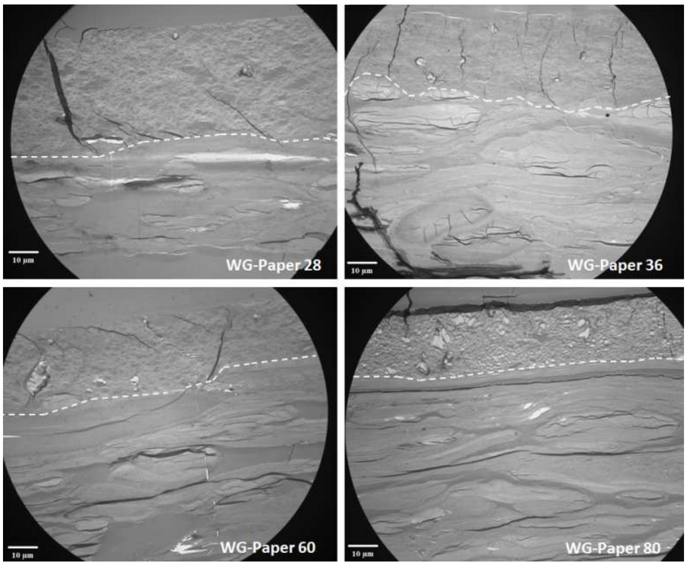


Figure 2.3. TEM cross-section views of the WG-Papers (x1000) – Illustration of the impact of the Support-Paper on the thickness and regularity of the WG layer. White dotted line indicates the Paper/WG separation.

Note: Vertical black marks on the micrograph came from self-folding of the thin layers of material before embedding and are not representative of any potential defect on the WG-layer

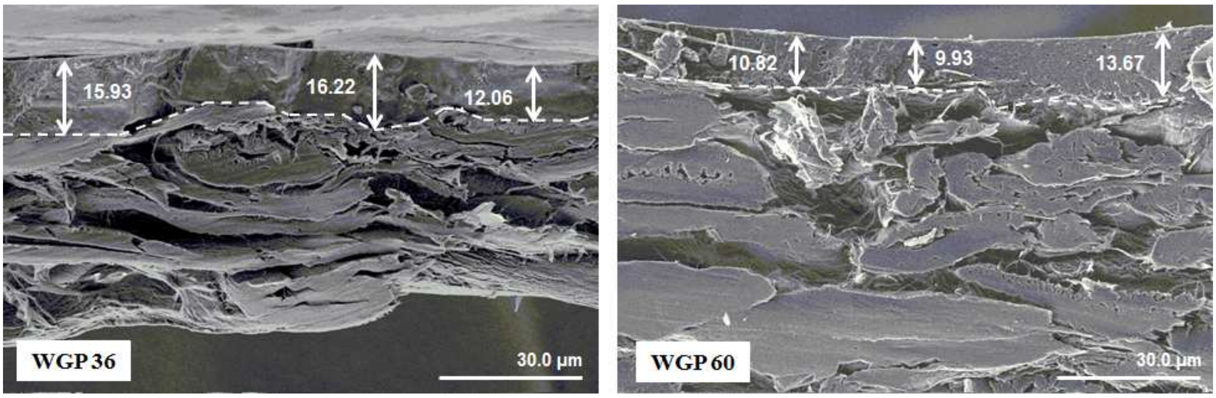


Figure 2.4. SEM cross-section views of the WG-Papers (x1000) – Evaluation of the thickness of the apparent WG layer. White dotted line indicates the Paper/Gluten separation.

To more accurately assess the apparent thickness of WG layers in each of the WG-Papers, this parameter was determined from calculation (*eq. 2.2.1*). Results (Table 2.3) were coherent with SEM measurements for WGP36 and WGP60 even if they slightly differed probably because of minor WG spreading onto fibers and the local character of SEM observations. As noticed on TEM micrographs, the apparent thickness of the WG layer decreased with decrease of fibers refining and, most of all, WG layers were all thinner than the theoretical thickness (24µm), whatever the WG paper considered. Albeit there was no affinity between support papers and the WG coating solution during contact angle measurement, forced spreading (due to mechanical action of the coating blade) led to penetration of the coating solution into paper. This was quantified with the percentage of impregnation (*eq. 2.2*) and it appeared that the higher the refining degree of the fibers, the lower the percentage of the WG coated layer impregnated: from 15% impregnation for the WG layer of WGP28 against circa 70% for WGP60 and WGP80 (Table 2.3). WG-papers were then all considered as tri-layered composite materials with an apparent layer of WG proteins, a composite zone with a combination of WG proteins and fibers.

Differences in impregnation of WG papers were related to the in bulk structure of their relative support papers. They were considered as porous systems exhibiting different size of pores related to their refining degree, from a tight and narrow network in highly refined paper (as SP28) to a loose and coarse network in lowly refined paper (as SP80). After deposition at the surface of each support, WG proteins – consisting in molecules of various molecular weights (MW): glutenin polymers between 150000g.mol^{-1} and more than 5 millions g.mol^{-1}, monomeric gliadins between 17000 and 70000g.mol^{-1}, and then small proteins (albumin and globulin) with less than 17000 g.mol^{-1} [Bietz et al. 1972; Lasztity 1986; Morel et al. 2000b; Lagrain et al. 2010] – elute and split through the fibers network depending on their size (molecular weight) and/or the size of voids (pores) within the support papers. Then protein composition of the apparent layer of WG papers may be affected by this elution profile. But, no differences were observed in the protein composition of the apparent layers of the WGP. Indeed, with SE-HPLC, it was possible to determine that they all presented a composition of 1.5% high MW glutenins, 17.0% low MW glutenins, 11.5% ω-gliadins, 52.0% α-β-γ-gliadins, 14.5% albumins-globulins and 3.5% insoluble fractions which was very similar to the protein composition of a control “casted wheat gluten film”: 1.4% high MW glutenins, 13.8% low MW glutenins, 10.0% ω-gliadins, 55.7% α-β-γ-gliadins, 16.2% albumins-globulins and 2.9% insoluble fractions. However it remains possible that a separation took place within the apparent layer with the high MW proteins remaining at the very surface of the material and the low MW proteins in the depth of the WG layer (towards the composite zone). In any case, given the differences in impregnation observed, the penetration speed of the WG coating solution was different depending on the refining degree. The lower the refining degree of the fibers (and the bigger the pore size, e.g. SP80), the faster and deeper the penetration of the coating solution (e.g. WGP80).

Table 2.3. WG-Papers structure characterization (20°C and 30%RH)

Sample	**Coating Weight [Cw]**	**Theoretical thickness of apparent WG-Layer [e_s]**	**Thickness of WG-Paper [e_{WGP}]**	**Thickness of respective Swelled Support-Paper [e_{SWP}]**	**Thickness of apparent WG-Layer (calculated) [e_{WGapp}]**	**Impregnation**
	($g.m^{-2}$)	**(µm)**	**(µm)**	**(µm)**	**(µm)**	**(%)**
WGP 28	14.64 ±0.46	23.43 ±1.03	62.78 ±0.47	43.10 ±1.13	19.68 ±1.41	15.00 ±3.21
WGP 36	15.01 ±0.69	24.02 ±1.56	69.61 ±0.08	53.71 ±1.31	15.90 ±1.52	33.51 ±8.87
WGP 60	15.60 ±0.78	24.96 ±1.77	93.39 ±1.03	86.30 ±2.27	7.09 ±2.87	71.45 ±13.61
WGP 80	14.48 ±0.14	23.165 ±0.32	108.17 ±0.87	100.01 ±2.59	8.10 ±3.20	65.02 ±9.97

Surface and gas permeation properties of WG-Papers related to their structure

Surface Properties of WG-Papers. The surface energy of each WG-Paper was determined at 60 and over 90% RH with different reference liquids to obtain their wetting envelopes (Figure 2.5). For each considered RH, no significant difference between WG-Papers was observed which was consistent with the identical apparent WG layer protein compositions observed earlier.

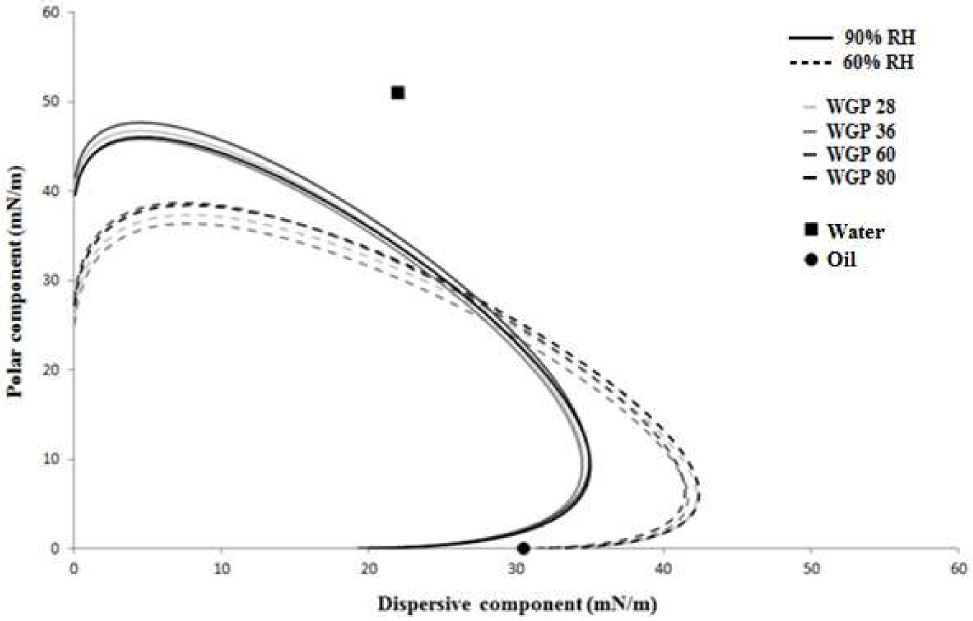


Figure 2.5. Wetting envelopes of the WG-Papers at 25°C and 60%RH and 90%RH

Whatever the humidity, surfaces of the wetting envelopes of the WG-Papers (Figure 2.5) were higher than those of their respective Support-Papers (Figure 2.2): surface energy reached 55 or 56N.m^{-1} for WG papers against 40 or 33N.m^{-1} for support papers, respectively at 60% or 90% RH. Considering the dispersive and polar components of wetting envelopes of the WG-Papers and their respective support papers, it appeared that the WG coating increased the resistance to wetting by dispersive liquids (such as oil) but decreased the resistance to polar ones (like water). This was predictable since WG films exhibit a strong hydrophilic character at the considered relative humidities [Gontard et al. 1993]. It can be noted that contrary to Support-Papers, the surface properties of WG-Papers were significantly affected by the relative humidity. Despite a very similar surface energy at both 60 and 90%RH, they differed in their behavior towards polar or dispersive liquids. The WG-Papers were more sensitive to polar liquids such as water and less sensitive to dispersive ones than at 60% RH. For instance, WG-papers were totally wet by oil at 60% RH but only partially wet at 90% RH.

The higher sensitivity to polar liquids was expected since WG films are known to exhibit a RH-dependent water sorption behavior characterized by an exponential shape between 60 and 100% RH, as previously evidenced [Gontard et al. 1993]' [Guillard et al. 2003]. As a consequence, the affinity between WG-Papers and hydrophobic liquid is reduced at high RH.

Gas transfer properties of WG-Papers. Gases (oxygen and carbon dioxide) permeation of each WG-Paper was assessed at 25 °C and 100% RH (Table 2.2). Values, ranging from 1 to $17x10^{-11}mol.m^{-2}.s^{-1}.Pa^{-1}$ were almost in the same range than previous published data on WG coated papers [Guillaume et al. 2010a] (coating weight higher than 20 $g.m^{-2}$) at 80% RH, between 11 $x10^{-11}$ and $38x10^{-11}mol.m^{-2}.s^{-1}.Pa^{-1}$. All WG-Papers exhibited far lower (10^3 to 10^4 times) O_2 and CO_2 permeation values than their respective Support-Paper, proving that (i) the apparent WG layer was continuous and (ii) coating weight was above the $10g.m^{-2}$ limit in all WG papers. Those two conditions have been previously demonstrated to be compulsory to bring enhanced barrier properties to the WG coated materials in comparison to their supports [Gallstedt et al. 2005; Guillaume et al. 2010a].

While Pe_{CO2} of WG-Papers remained quite the same whatever the WG-Paper tested at 100% RH, Pe_{O2} of WG papers increased when increasing the impregnation and decreasing the thickness of the apparent WG layer: WGP60 and WGP80 presented an O_2 permeation 3 to 12 times higher than WGP36 and WGP28 (Table 2.2). As a consequence, the permselectivity ratio was concomitantly decreased from 8 for WGP28 to 1 for WGP80. And so, only WGP28 (and to a lesser extent WGP36) presented the high permselectivity characteristic of WG materials at high RH [Gontard et al. 1996b; Mujica Paz et al. 1997; Pochat-Bohatier et al. 2006]. Furthermore, only WGP28 displayed the RH-dependent gas permeation behavior of WG materials which is marked by an increase in O_2 and CO_2 permeation together with an increase in permselectivity ratio (Pe_{CO2}/Pe_{O2}) when increasing RH from 60 to 100% RH [Gontard et al. 1996b; Mujica Paz et al. 1997; Pochat-Bohatier et al. 2006]. Indeed, it presented a Pe_{O2} of $1.23x10^{-11}mol.m^{-2}.s^{-1}.Pa^{-1}$, Pe_{CO2} of 1.10 $x10^{-11}mol.m^{-2}.s^{-1}.Pa^{-1}$, and a permselectivity ratio of 0.9 at 60% RH (data not shown) against $1.50x10^{-11}mol.m^{-2}.s^{-1}.Pa^{-1}$, 12.10 $x10^{-11}mol.m^{-2}.s^{-1}.Pa^{-1}$, 8.09, respectively, at 100% RH.

To go deeper in the understanding of the gas transfer behavior of the WG-Papers, the oxygen and carbon dioxide permeability (P_{O2} & P_{CO2}, permeation multiplied by thickness) of the apparent WG layer of the WGPs, were calculated using equation 6 for bilayer composite and assuming the composite layer presented a very permeable character, as paper (Table 2.4).

Table 2.4. Gas transfer properties of the apparent WG layer of each WG-Paper and of some self-supporter WG films from the litterature

Sample	**RH**	**Thickness of apparent WG-Layer**	**P_{O2} of apparent WG-Layer**	**P_{CO2} of apparent WG-Layer**	**S of apparent WG-Layer**
	%	**(µm)**	**10^{-18} (mol.m^{-1}.s^{-1}.Pa^{-1})**	**10^{-18} (mol.m^{-1}.s^{-1}.Pa^{-1})**	
Studied Wheat Gluten coated papers					
WGP 28	100	19.68	300	2430	8.1
WGP 28	60	19.68	231	218	0.9
WGP 36	100	15.90	536	2790	5.2
WGP 60	100	7.09	615	975	1.6
WGP 80	100	8.10	945	1010	1.1
WG self-supported films from literature					
Casted WG-Film (20% glycerol) [Gontard et al. 1996]	95	100	1290	36700	28.5
Casted WG-Film (20% glycerol) [Cagnon et al.]	90	182	1201	21861	18.2
Casted WG-Film (20% glycerol) [Gontard et al. 1996]	60	100	100	750	7.5
Casted WG-Film (20% glycerol) [Gontard et al. 1996]	0	100	1	7	7.0

At 100% RH, it appeared that the P_{O2}, P_{CO2}, and S of the apparent WG layers were different depending on the WGP. Such results were not expected given the similar protein composition observed for every apparent WG layer. These unexpected differences could be attributed to a bias in the estimation of the real apparent and continuous WG layer thickness. Indeed, the interface between the composite zone and the apparent WG layer is likely not flat since some fibers may have penetrated into the apparent WG layer illustrated on TEM micrograph of WGP60 (Figure 2.6).

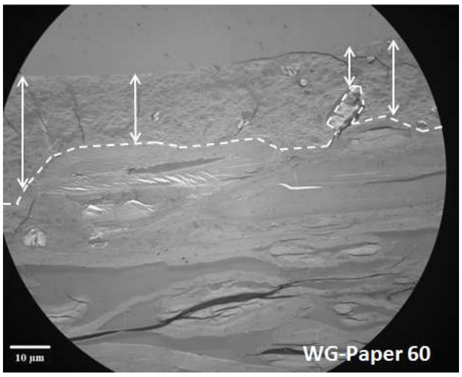


Figure 2.6. TEM cross-section views of the WGP60 (x1000) – Illustration of the non-flat character of the composite zone / apparent WG layer interface. White dotted line indicates the Paper/Gluten separation

In case of coating on highly refined papers (e.g. WGP28), which presents a thick apparent WG layer, the impact of this bias would remain very limited whereas in case of coating on lowly refined papers (e.g. WGP60 and WGP80), which present a very thin apparent WG layer, it would be very important, as illustrated in (Figure 2;7).

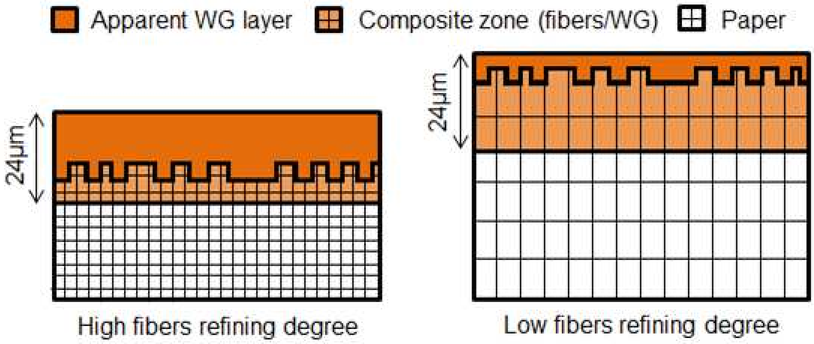


Figure 2.7. Schematic structure of WG coated paper

Such considerations may explain why the P_{O2} of the apparent WG layer increased from 300 $x10^{-18}mol.m^{-1}.s^{-1}.Pa^{-1}$ to 945 $x10^{-18}mol.m^{-1}.s^{-1}.Pa^{-1}$ for WGP28 and WGP80 respectively. Consequently, for lowly refined papers, the P_{O2} of the apparent WG layer may have been overestimated due to the presence of an important fibers/WG composite part in the thickness considered; a composite part presenting a high permeability towards oxygen due to the presence of preferential diffusion path at the fibers/WG interface. Knowing that the driving force in CO_2 permeability of WG materials is the sorption capacity [Pochat-Bohatier et al. 2006], the presence of fibers in the apparent WG layer may reduce the CO_2 accessibility to target amino-acid of WG [Pochat-Bohatier et al. 2006]. As a consequence, the CO_2 permeation of WGP 60 and WGP 80 is underestimated compared to a pure WG layer. Concomitantly this bias affect the permselectivity of lowly refined coated papers and explained their weak WG-like behavior compared to the strong WG-like behavior of highly refined coated papers.

Finally the transfer properties of the apparent WG layer of the material presenting the strongest WG-like behavior, WGP28, was compared to the ones of self-supported casted WG films from the literature. It appeared that the RH dependent gas transfer properties were well correlated between the apparent WG layer of WGP28 and the self-supported casted WG films with a strong increase of P_{O2} and S with RH increase from 60% to over 95% in each case. However, at 100% RH, the P_{O2} and P_{CO2} calculated for the apparent WG layer of WGP28 were significantly lower than the one of the self-supported casted WG film (4.3 times and 15 times lower for P_{O2} and P_{CO2} respectively). But these differences could easily be explained by the mandatory presence (for mechanical purposes) of glycerol inside the self-supported casted WG films that greatly enhance their permeabilities by acting as a plasticizer [Gontard et al. 1993; Irissin-Mangata et al. 2001].

CONCLUSION

While the enhancement of gas barrier properties of paper when coated with wheat gluten (WG) proteins has been previously reported, this work brings a new understanding on the underlying mechanisms affecting structuring and consequently gas transfer properties of WG-coated paper. It appeared possible to modulate the gas transfer properties of WG coated papers (WGP) by selecting fibrous support presenting different fibers refining degrees. Increasing fibers refining led to a tighter and narrower network that would limit the penetration of the coating solution within the paper during the coating / drying process and thus reduce the impregnation of the coating solution in the support. Marginal impregnation ensured a limited thickness for the fibers/WG composite zone and the presence of a thick apparent WG layer that would bring WG-like transfer properties to the coated material. On the contrary, low fibers refining created a loose and coarse in bulk network, that led to a thick composite layer and a thin apparent WG layer and thus to a material presenting weak WG-like behavior. This principle was not considered specific to gluten coating on kraft paper and can certainly be applied to any protein coating (such as soy proteins, whey proteins, etc…) on any fibrous support (bleached pulp for instance).

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Publication 3

Control of O_2 barrier properties of papers coated with wheat gluten / nanoclays multilayers

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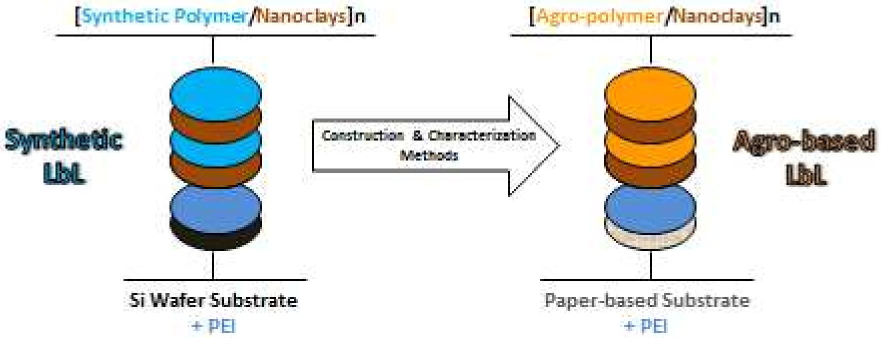
Journal of Composite Science and Technology – In preparation for submission

ABSTRACT: Layer-by-layer (LbL) assembly is a powerful technique allowing construction of nanostructured multicomponent materials with tailorable properties depending on composition and structure of the deposited films. This study aims to fabricate LbL films adapted to fresh produce packaging (moderate oxygen permeability (P_{O2}) to allow produce respiration) and based on wheat gluten agro-polymer (WG) and montmorillonites nanoclays (MMT) deposited by spraying on paper substrates. WG is an uncommon molecule in LbL assembly and to our knowledge, paper has never been used as substrate for LbL deposition due to its rough and non-homogenous character. Therefore, a fluorescence based method was developed to verify successful LbL deposition on paper prior the fabrication of LbL films [WG/MMT]n on paper and paper coated with heat reticulated wheat gluten (WGr paper). Whereas LbL films built on paper appeared to be still porous and thus too permeable for the targeted application, the ones built on WGr paper featured P_{O2} values from 4.70×10^{-15} to 1.27×10^{-15} mol.Pa^{-1}.m^{-1}.s^{-1} that perfectly fitted the requirements for most of fresh produce.



KEYWORDS: Layer-by-Layer Assembly, Paper, Gluten, Nano-clays, O_2 permeability

INTRODUCTION

Layer-by-layer (LbL) fabrication of molecularly organized films is studied since the 1930's [Blodgett et al. 1937]. From complicated procedures restricted to small classes of molecules, it is now extended to a large range of molecules (either organic from biological or fossil origin or mineral) [Lvov et al. 1995; Decher et al. 1998; Izquierdo et al. 2005; Ariga et al. 2006; Jang et al. 2008; Priolo et al. 2010] and mainly applied in medical, pharmaceutical or electronic fields [Ariga et al. 2006]. This came possible thanks to methods of sequential adsorption of cationic and anionic molecules elaborated in the 1990's by Decher and co-workers [Decher et al. 1991; Decher et al. 1992]. It consists in the alternative deposition of polyanions and polycations on a substrate by contact via simple methods such as dipping or spraying. Dipping was the first method deposition process used but is time consuming. In contrary, spraying is a very fast technique [Schlenoff et al. 2000] and it takes less than an hour to obtain 20 layers pairs films of comparable quality with dipping ones (several hours for 20 layers pairs in this case) [Izquierdo et al. 2005]. Up to now, atomically smooth, reflecting, or transparent surfaces are used as model substrates to be able to characterize the film formation by conventional surface characterization techniques (atomic force microscopy, x-ray reflectometry, neutron reflectometry, elipsometry, UV/Vis spectroscopy). Among the few techniques that could work on more exotic surfaces, there are optical microscopy, fluorescence microscopy, x-ray photoelectron spectroscopy (XPS), or reflection UV/Vis spectroscopy.

In the field of food packaging, nano-structuring is more and more studied to improve mechanical properties [Sinha Ray et al. 2003] and functional properties of materials [Choudalakis et al. 2009]. But most studies are focused on bulk nano-composite materials prepared by incorporation of nano-particles inside a polymeric matrix either from fossil or biological resources [Sinha Ray et al. 2003; Rhim et al. 2007; Choudalakis et al. 2009; De Azeredo 2009; Abdul-Khalil et al. 2012]. Among nano-particles, the ones presenting a platelet form such as nano-clays are particularly studied [Chivrac et al. 2009; Choudalakis et al. 2009; Rhim 2011]. Recently, LbL assembly of nano-clays has been used to reduce oxygen permeability of a synthetic substrate material [Jang et al. 2008; Priolo et al. 2010]. It evidenced the great potential of LbL deposition involving nano-clay layers onto synthetic polymer to drastically reduce the O_2 permeability of materials by increasing the number of layer pairs deposited. This opens new routes for modulating gas transfer properties of packaging material, and could be appropriate to develop material with tailored gas transfer properties. From highly barrier to oxygen, for inert food, to medium or low barrier to oxygen, for respiring food, a large range of oxygen permeation rate is expected. To design packaging material for respiring produce, as fruits and vegetables, through LbL deposition, the substrate must be sufficiently permeable to allow tailored reduction of its oxygen permeability (P_{O2}) with alternate deposition of adequate polymers or molecules. Due to their high barrier properties (P_{O2} usually lower than $1x10^{-15}$ $mol.Pa^{-1}.m^{-1}.s^{-1}$), conventional synthetic polymers used in food packaging are obviously not adapted as substrate and porous support need to be considered. Paper, a widely available solid material, which presents high gas permeability, could be a good candidate even if it has never been considered as substrate for LbL deposition. Only cellulosic fibers were considered for LbL coating of their surfaces [Zheng et al. 2006; Peng et al. 2008; Johansson et al. 2012].

The aim of this study was to control oxygen permeability of paper substrates modified by LbL films made of agro-polymer (wheat gluten)/nanoclays (montmorillonites) for fresh food packaging applications. A Method to characterize quantitatively LbL assembly on rough, inhomogeneous, and non-transparent substrates was developed by transposing LbL assembly from a synthetic model substrate (silicon wafer) to paper. First, the possibility to use wheat gluten (WG) and montmorillonites (MMT) in LbL assembly was assessed by depositing each of them alternately with model polymers (poly(sodium-4-styrene sulfonate) (PSS) and poly(diallyldimethylammonium chloride) (PDDA) respectively), and then spray deposition of gluten/nanoclays layer pairs was tested onto a Si wafer substrate. Consecutively, the technique was transposed to paper or paper coated with reticulated WG, along with the development of a characterization method for LbL assembly on such substrates based on fluorescence staining. Finally the oxygen permeabilities of the built LbL materials were measured.

MATERIALS AND METHODS

Materials

Wheat gluten (WG) powder, containing 7.2 wt. % of moisture and 76.5 wt. % of proteins was provided by Amylum (Mesnil St Nicaise, France). Acetic acid and sodium sulphite, also used to prepare the WG solution were purchased from Aldrich (St Quentin, France). Bleached paper sheets (T75) was provided by Arjowiggins (Boulogne Billancourt, France) to serve as support for LbL assembly along with silicon (Si) wafers purchased from Wafernet, Inc. (San Jose, CA, USA). Optigel SH synthetic nanoclays (short montmorillonites-like clays without heavy metals in the layer lattice), poly(ethylene imine) (PEI, Lupasol WF, MW=25 000 g.mol^{-1} PEI, BASF), poly(diallyldimethylammonium) chloride (PDDA, MW=100000-200000g.mol^{-1}, 20 wt. %) and poly(sodium-4-styrene sulfonate) (PSS, M= 206g.mol^{-1}, Sigma-Aldrich) have been used for layer-by-layer assembly without further purification. Optigel montmorillonite has been sonicated for 1h hour and left to rest for one week prior to use. The spray deposition was carried out by using air pump spray cans purchased from Carl Roth (Karlsruhe, Germany).

Preparation of the support for LbL Assembly

Paper coated with reticulated wheat gluten. A WG solution (21.23% dry w/v) was prepared at room temperature according to an adapted 3 steps procedure [Guillaume et al. 2010a]. First, 30g of WG powder was dispersed under shacking in 50ml of a sodium sulphite solution (0.06g / 50mL) which worked as a reducing agent of the disulfide bonds. Then, after 30 minutes of settling, the pH of the solution was set to 4.7 by adding a 50% v/v solution of acetic acid. Finally the solution was adjusted to 130mL by adding deionized water and the whole mix was stirred and left to rest for one day.

Prior to coating, T75 support papers were stored in a relative humidity (RH) controlled chamber set at 30% RH using $CaCl_2$. The coating was performed with the standard WG coating solution using an E409 blade coater from Erichsen (Reuil-Malmaison, France). The coater was equipped with a blade n°8 featuring a spire width of 1mm. Coating speed was set at 10mm.s^{-1}. To be spread all over the sheet surface, an excess (> 3mL) of the coating solution was deposited to produce coated papers. After coating the materials were left to dry at 20° C for 2 hours and the relative humidity was kept below 40%. The average coating weight (determined using the method detailed in Guillaume et al. 2010 [Guillaume et al. 2010a]) of the built WG coated papers was 21g.m^{-2}. Finally the gluten layer deposited was thermally cross-linked by exposing the coated papers to a temperature of 110°C during 1 hour. These substrates were referenced as WGr papers.

LbL assembly by spraying

Diluted WG solution (0.2w%) at pH 4.7 (to stabilize the solution and to make WG positively charged), Optigel SH solution (0.2w%), PEI solution (2.5 mg/mL) at pH 10 (for better LbL assembly and barrier properties [Priolo et al. 2010]), PDDA solution (2.0mg/mL in 0.5M of NaCl), PSS solution (0.6mg/mL in 0.5M of NaCl) and ultra-pure water were prepared. All materials have been used without further purification. All solutions have been poured into separate spray cans and were kept refrigerated before use. Spraying was carried out perpendicularly to the receiving surface (paper / WGr Coated Paper / Si wafer) which was fixed in a vertical orientation. The spray rate of the spraying bottles was approximately 0.6 $mL.s^{-1}$ during the entire deposition process. A preliminary layer of PEI was deposited during 15s then rinsed by ultra-pure water during the same amount of time. Alternative depositions of $[PSS/WG]_n$, $[MMT/PDDA]_n$, $[MMT/WG]_n$ or $[MMT/PEI]_n$ (n: number of layer pairs) were then performed by spraying each of the solution during 5s until the desired number of pairs of layers was reached. Between each layer deposition, a rinsing step with ultra-pure water was carried out during 5s. After LbL assembly, the samples were placed on a Plexiglas plate and left to dry in a ventilated climatic chamber at 20°C and 50% relative humidity (RH).

LbL characterization

Global thickness of the materials. For permeability value calculation, average global thicknesses of the materials were determined at room temperature and 100% RH with a hand-held digital micrometer (Mitutoyo instruments) from ten measurements randomly taken over the material surface.

Elipsometry. Measurement of the produced LbL film thickness on Si wafers (not taking the SiO_2 substrate thickness into account) was carried out with a PLASMOS SD 2100 instrument operating at the single wavelength of 632.8nm and a constant angle of 45°. The refractive index of the polyelectrolytes was considered constant at the value of n=1.465. Such assumption will lead to slightly incorrect values in measurement of the absolute film thicknesses but it will allows quick and precise enough determination of relative film thicknesses. The values obtained with this assumption are of better precision than required for the comparison of film growth data as in this report. For each substrate studied several points were measured to obtain the average value for the film thickness and to determine the film homogeneity.

Staining. Staining was done with two different dyes: one conventional dye, amido black (AB), and one fluorescent dye, fluorescein isothiocyanate (FITC).

The staining with AB was performed in 2 different ways, either by direct staining of WG and performing LbL deposition with the stained material, or by doing LbL deposition of native WG and staining of the whole material after deposition. For the first way 1g of amido black in 50mL ethanol/acetic acid (9:1 v/v) was added to 200mL of a solution of centrifuged

2% WG solution at pH 4. The solution has been stirred overnight and the excess of AB was removed by dialysis against distilled water brought to pH 4 by adding acidic acid during 10 days. For the second way, the final material was dipped into a solution of 1w% of AB in ethanol:acetic acid (9:1 v/v) for 2h. After staining the excess of AB has been removed by rinsing 3 times with ethanol:acetic acid solution for 30min each time.

Fluorecein isothiocyanate staining was performed by adding 4 mg of FITC in 20mL DMSO to 200mL 2% WG solution at pH 4. The purification was done in the same way as described previously for the AB stained WG.

Fluorescence Microscopy and Spectroscopy. Fluorescence images through reflected light were taken with a Zeiss Axiotech^{vario} microscope from Carl Zeiss SAS (Le Pecq, France) using a conventional filter set (n°9 from Zeiss) for FITC and a Nikon COOLPIX 4500 camera with a MDC2 relay lens. The intensity of the fluorescence was also evaluated by fluorescence spectroscopy using a FluoroMax-4 spectrometer (Horiba Jobin-Yvon, Chilly Mazarin, France). Wavelength were scanned from 200 to 950nm (maximum excitation pic of FITC at 495nm), the slit opening was 1 mm, the signal/noise ratio was 3000.1 and the minimum resolution 7ps/channel.

SEM observations

Scanning electron microscopy (SEM) observations of the materials surfaces were performed on small pieces (1x1 cm) directly mounted on stub with double sided carbon tape. After degassing under vacuum, samples were observed with a Scanning Electron Microscope S-4800 Hitachi (Tokyo, Japan). All micrographs were obtained using an accelerating voltage of 2.5kV.

Oxygen permeability measurements

The O_2 permeability of materials was assessed in triplicate, thanks to a static method using O_2 monitoring spots and optic fibers from PreSens (Regensburg, Germany). Prior to measurements, the material was placed at the desired RH (100% RH) for one day to equilibrate. It was then placed on top of a permeability cell containing one O_2 spot situated in a RH controlled chamber Meditest 600/1300 from Firlabo (Meyzieu, France). The cell was flushed with nitrogen until the amount of O_2 inside went under 1hPa. The flush was then stopped and the cell hermetically closed. The amount of O_2 inside the cell was monitored via the spots and optic fibers. The permeability was calculated as follows:

$$P_x = \frac{J \times e}{\Delta P \times A} \qquad \textit{eq1.}$$

where *x* refers to the analyzed gas, *J* to the flux of gas going through the material in mole per second, ΔP to the difference in pressure exerted by the gas on each side of the film in Pascal and A to the surface of the material in meter square.

RESULTS AND DISCUSSION

(WG/MMT) LbL assembly method: From Si Wafer to Paper substrate

Characterization of $(\mathbf{MMT/WG})_n$ films on silicon wafer. LbL deposition of WG (considered as a polycation) and MMT (considered as a polyanion) was studied on smooth and well-defined silicon wafer substrates (model surface). For a better film adhesion on the surface, PEI was deposited as a preliminary layer and then, the LbL deposition was performed to obtain the desired multilayer system. First, WG and MMT were deposited alternatively with model polycation (PDDA) and polyanion (PSS) respectively to obtain $\mathrm{PEI(PSS/WG)_n}$ and $\mathrm{PEI(MMT/PDDA)_n}$ assemblies. Then $\mathrm{PEI(MMT/WG)_n}$ assembly was prepared. Resulting film thicknesses were measured by elipsometry after each deposition step in order to characterize the film growth as shown in Figure 2.8.

In all cases, a linear increase of film thickness with sprayed layer number was obtained. Only slight deviations were observed within the first 3-5 layers due to the influence of the Si surface and/or the PEI layer. Therefore the increment per layer pair was constant, even for a very high number of layers (18 layers max i.e. 9 pairs of layers). The increment was 4.0 nm/layer pair for the (PSS/WG) system, 2.8 nm/layer pair for (MMT/PDDA) and 8.5 nm/layer pair in the case of (MMT/WG). It can be noted that the MMT layers seemed to be thinner than WG and PDDA ones as evidenced by the “stairs like” appearance of the thickness curve. The error-bars, especially in the case of (MMT/PDDA) and (MMT/WG), were quite small indicating an homogeneous film thickness distribution. Also the photographs in Figure 2.8 showed that the films are optically homogeneous with no visible defects. Nano clays and other sheet-like materials are known to act as leveling agents [Kotov et al. 1997], which means that they were able to flatten surfaces when used for multilayer formation.

These results showed that LbL composites with various compositions could be formed on flat surfaces in a good quality. As LbL deposition is known to work on almost any wettable surface [Decher et al. 2012] results were very promising for the LbL deposition of (WG/MMT) layers on a more exotic material such as paper.

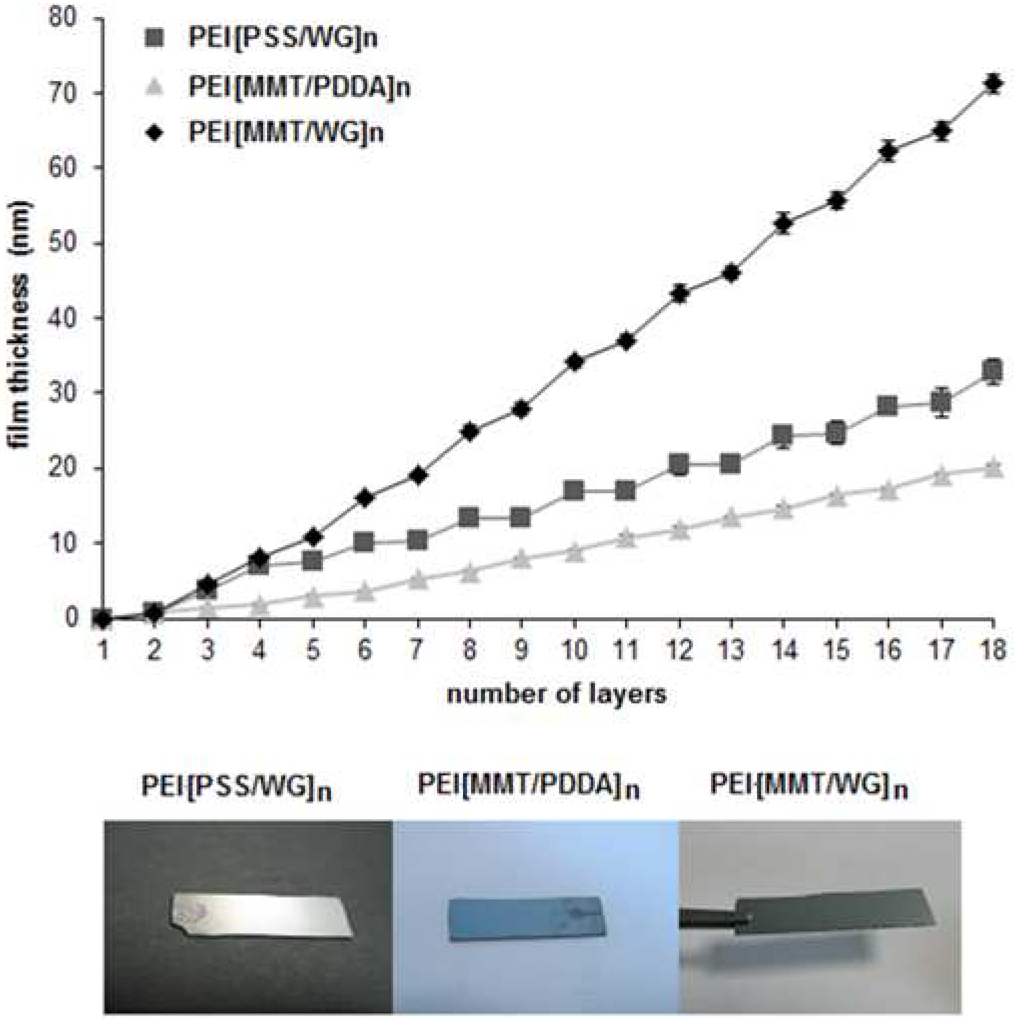


Figure 2.8. Film construction curves and photograph of PEI[PSS/WG]$_n$, PEI[MMT/PDDA]$_n$ and PEI[MMT/WG]$_n$

Characterization of (MMT/WG) films on paper substrate. LbL architectures (PEI[PSS/WG]$_n$, PEI[MMT/PDDA]$_n$ and PEI[MMT/WG]$_n$) established on Si wafer were carried out onto paper substrate. Standard characterization techniques like UV/vis spectroscopy, AFM or elipsometry cannot be used to characterize LbL films onto paper due to the heterogeneity and roughness of this support. Therefore, two methods were developed to estimate or to semi-quantify the amount of amino-containing material in the films, i.e. WG. Both approaches were based on the staining of WG proteins with dyes that are able to couple with amino moieties.

The first dye was amido-black (AB) which is usually used to stain amino acids and proteins. WG was stained either prior or after the LbL deposition and the intensity of coloration was used to estimate the amount of gluten in the film as a function of the number of gluten layer deposited. As can be seen in Figure 2.9, the coloration intensity increases with the number of gluten layers deposited whatever the chosen WG staining process (before or after LbL deposition). Thus, the successful LbL deposition of PEI(PSS/WG)$_n$ multilayers films on paper could be proven by this AB staining method. In case of staining WG before LbL deposition, the coloration intensity was rather small whereas a much higher contrast and a better dye distribution were obtained when staining was performed on deposited film. It can be noted that in the case of staining after LbL deposition, the control sample that did not contained any WG (i.e. the bare paper) showed a light blue coloration coming most probably from the AB dye embedded within the wood fibers tissue. However, as the blue coloration is a value that is quite difficult to quantify, the AB staining was only useful to validate the LbL assembly on paper and could not be used for a quantitative analysis of the film construction.

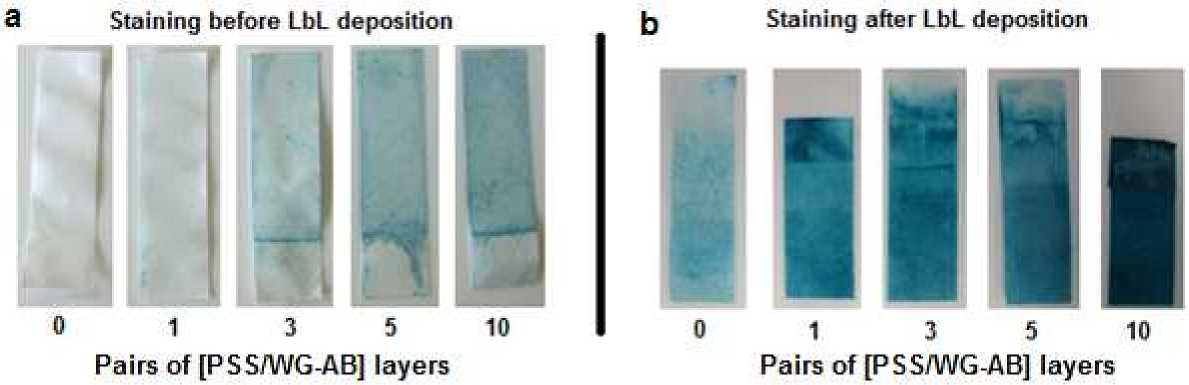
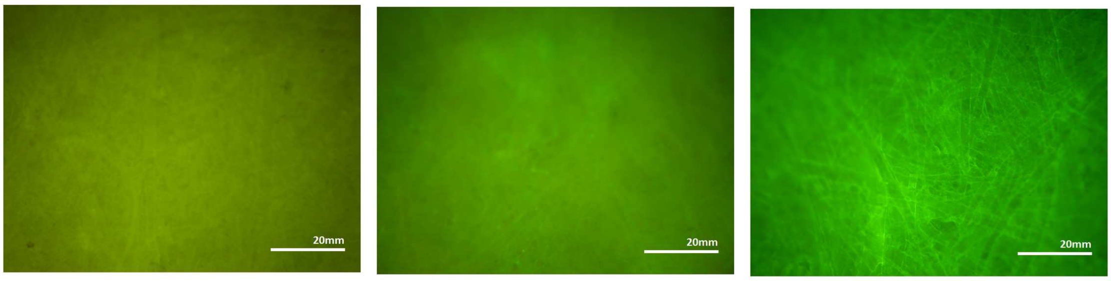


Figure 2.9. Paper coated with multilayers of PEI[PSS/WG-AB] by spraying. (a) staining before LbL deposition, (b) staining after LbL deposition; from left to right: bare paper, 1, 3, 5 and 10 layer pairs of PEI[PSS/WG-AB].

To overcome the classical absorption dye limitations and to be able to better quantify the film growth, an approach based on a fluorescent dye, fluorescein isothiocyanite (FITC), was developed. Fluorescent dyes such as FITC present the advantage to be more easily and precisely quantifiable than standard ones using fluorescence spectroscopy. Figure 2.10 shows fluorescence micrographs of a blank paper and papers modified with PEI(PSS/WG-FITC)$_1$ and PEI(PSS/WG-FITC)$_{10}$ films. A clear increase of fluorescence intensity with the number of WG-FITC layers deposited is observed. Furthermore, at higher magnification, it becomes visible that the fluorescent material is concentrated on the borders of the paper fibers (Figure 2.11).

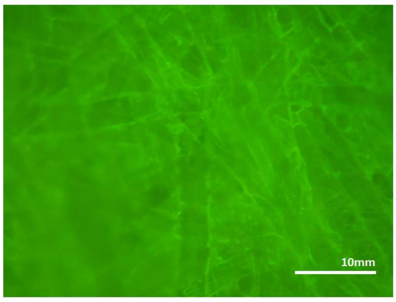


Paper

Paper PEI[PSS/WG-FITC]$_1$

Paper PEI[PSS/WG-FITC]$_{10}$

Figure 2.10. Fluorescence micrographs of PEI[PSS/WG-FITC]$_n$ (x200)



Paper

PEI[PSS/WG-FITC]$_{10}$

Figure 2.11. Fluorescence micrograph of PEI[PSS/WG]$_{10}$, magnification and contrast increase (x400)

The fluorescence spectra of 1, 3, 5 and 10 layer pairs deposited on paper also showed an increase in peak intensity with the number of WG-FITC layers (Figure 2.12). When the maximum of intensity recorded for each system was plotted against the number of layers deposited, a linear relationship was observed indicating that a regular growth of the layer thickness was obtained for LbL deposition of PEI[PSS/WG-FITC]$_n$ on paper (n from 1 to 10). Based on fluorescence spectroscopy method, it was possible to establish a relationship between the number of layer deposited on paper and the fluorescence intensity during the buildup of PEI[PSS/WG-FITC]$_n$ film.

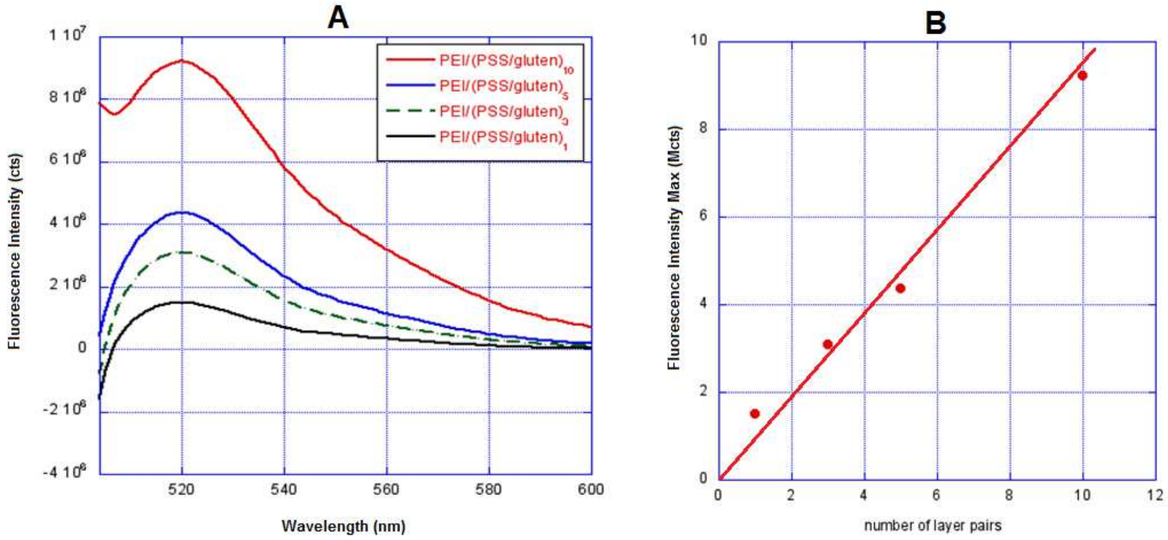


Figure 2.12. Fluorescence spectroscopy spectrum (A) and maximum fluorescence intensity / number of layers pairs deposited relationship (B) for a Paper-PEI[PSS/WG-FITC]$_n$ film

The same experiments were carried out for a PEI[MMT/WG-FITC]$_n$ film deposited onto paper (Figure 2.13). Contrary to what was observed for PEI[PSS/WG-FITC]$_n$ deposited on the same support, there was no linear correlation between the number of layer pairs deposited and the maximum fluorescence intensity for the PEI[MMT/WG-FITC]$_n$ film. Instead, a saturation of the maximum intensity recorded was observed for 2 or more layer pairs deposited and the intensity recorded for 1 pair of [MMT/WG] was 7.5 times higher than for 1 pair of (PSS/WG). This saturation behavior for the montmorillonite/gluten system is still not fully understood, but previous works on other systems involving nanoclays have evidenced that a migration of the fluorescent dye towards the nano-clay layer could lead to the formation of highly fluorescent FITC aggregates at the interface between the 2 layers of the system causing saturation of the fluorescence spectrometer [Mobius 1995; Chibisov et al. 1999; Pevenage et al. 1999; Chibisov et al. 2001; Tsukanova et al. 2002].

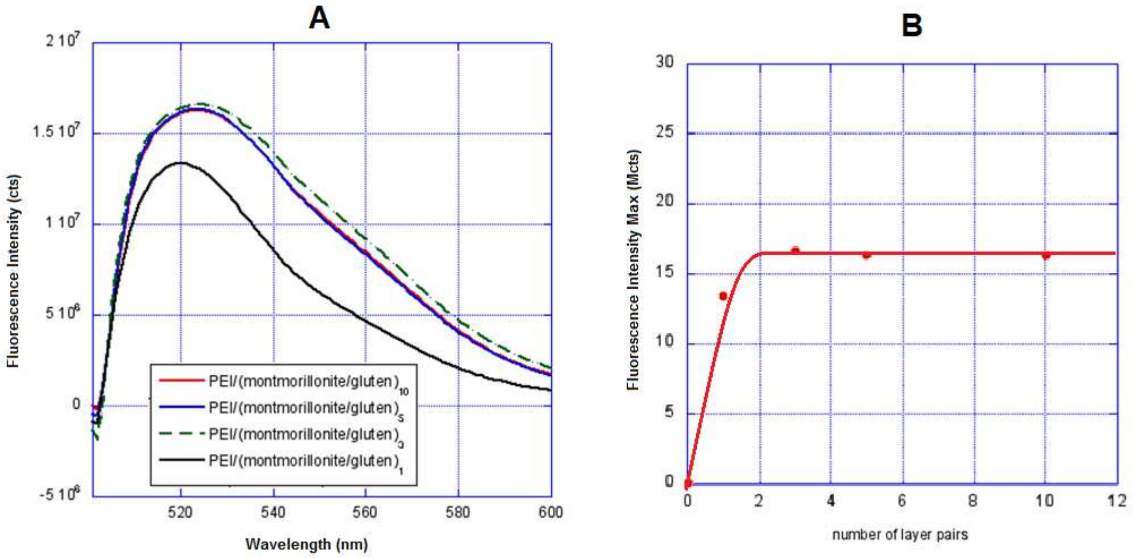


Figure 2.13. Fluorescence spectroscopy spectrum (A) and maximum fluorescence intensity / number of layers pairs deposited relationship (B) for a Paper-PEI[MMT/WG-FITC]$_n$ film

Oxygen permeability of LbL assembly (PEI+[MMT/WG]n) on paper. Given the possibility of performing LbL assembly on paper, construction of such nanostructured composites (PEI+(MMT/WG)$_n$) with tailorable O_2 permeability was attempted. Due to the foreseen application O_2 permeation measurements were performed only at high relative humidity (>90%) to mimic the one encountered when packing respiring produce and results are presented in Table 2.5.

Depositing a preliminary layer of PEI on the paper did not significantly affect its oxygen permeability (2.97 x10^{-11} against 2.19x10^{-11}mol.Pa^{-1}.m^{-1}.s^{-1} for Paper and Paper-PEI, respectively). On the contrary, depositing 10 or 40 pairs of [MMT/WG] in addition of the preliminary layer reduced the P_{O2} by 6 and 15 times respectively. However such values remained high and it was likely due to the highly porous character of the paper. The ultrathin layers deposited were not able to fill and block pores of paper, as evidenced in Figure 2.14. Thus, it created a still porous final material whatever the type or number of layers deposited and P_{O2} was not reduced enough to meet requirements of fresh fruits and vegetables [Cagnon et al. 2012].

Table 2.5. Oxygen permeability of paper based LbL materials

	Support Materials		**« Agro » LbL Materials**	
	Paper	**Paper** **PEI**	**Paper** **PEI[MMT/WG]$_{10}$**	**Paper** **PEI[MMT/WG]$_{40}$**
P_{O2} **(10^{-15} mol.Pa^{-1}.m^{-1}.s^{-1})**	29710 ± 3050	21930 ± 960	4900 ± 510	1960 ± 220

Table 2.6. Oxygen permeability of WGr coated paper based LbL materials

	Support Materials		**« Agro » LbL Materials**		**« Synthetic» LbL Materials**	
	WGr Coated Paper	**WGr Coated Paper** **PEI**	**WGr Coated Paper** **PEI[MMT/WG]$_{10}$**	**WGr Coated Paper** **PEI[MMT/WG]$_{40}$**	**WGr Coated Paper** **PEI[MMT/PEI]$_{10}$**	**WGr Coated Paper** **PEI[MMT/PEI]$_{40}$**
P_{O2} **(10^{-15} mol.Pa^{-1}.m^{-1}.s^{-1})**	1.16 ± 0.19	10.05 ± 1.58	4.70± 0.13	1.27 ± 0.09	2.41 ± 0.14	0.19 ± 0.02

Oxygen permeability of LbL assembly PEI[MMT/WG]$_n$ on paper coated with reticulated WG. To overcome the porosity of paper substrate and fill pores, a thick layer (thickness around 20µm coating weight around 21g.m^{-2}) of WG was coated onto paper and then heat-reticulated. This new substrate for LbL was named WGr paper hereinafter. While fibers and surface pores remained visible when the LbL assembly was performed on paper, they were fully covered when the deposition was performed on WGr paper (Figure 2.14) and consequently reducing the O_2 permeability of the substrate.

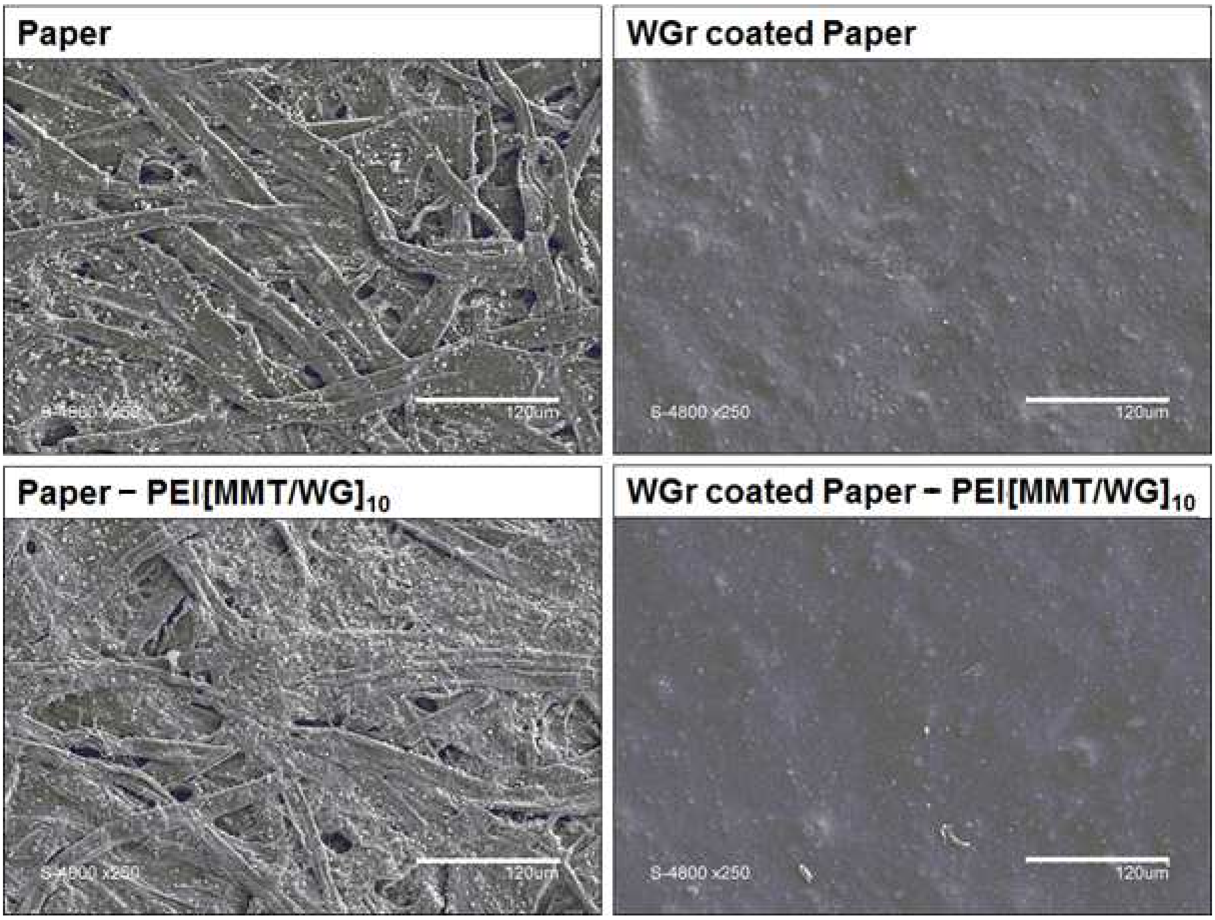


Figure 2.14. SEM observation of Paper, WGr Coated Paper, Paper-PEI[MMT/WG]$_{10}$, WGr Coated Paper-PEI[MMT/WG]$_{10}$

Materials built on WGr paper substrate were far less permeable than the ones built on paper and the impact of the number of layers deposited was more significant (Table 2.6). Indeed, WGr paper-PEI[MMT/WG]$_{10}$ exhibited P_{O2} of circa 5x10^{-15}mol.Pa^{-1}.m^{-1}.s^{-1} against a value around 5x10^{-12} for Paper-PEI[MMT/WG]$_{10}$. Depositing 40 pairs of layers instead of 10 reduced the P_{O2} of the material by 3.7 when depositions were performed on WGr paper against 2.5 when performed on paper. It is likely that the increased barrier properties were due to a tortuosity effect brought by the MMT layers (which are considered as impermeable platelets [DeRocher et al. 2005]) as the WG is known to feature only moderate oxygen barrier properties at high relative humidity. While it has been proven that incorporating MMT in the bulk of a WG matrix gave poor result in term of barrier properties enhancing because of incomplete exfoliation and non iso-directional orientation [Tunc et al. 2007]; use of LbL deposition allowed to overcome such problems and to create a tortuosity that reduce the oxygen transmission [Nielsen 1967; Cussler et al. 1988; Bharadwaj et al. 2002; Ranade et al. 2003; Jang et al. 2008; Priolo et al. 2010]. P_{O2} values of around 10^{-15}mol.Pa^{-1}.m^{-1}.s^{-1} obtained with LbL assembly on WGr paper were interesting for fresh food packaging application. A range of materials presenting numbers of layers pairs from 1 to 40 would indeed cover the optimal range of oxygen transfer previously defined for packaging material of various fresh produce [Cagnon et al. 2012]. It should be noted that O_2 values of LbL assembly on WGr paper (10 to 40 pairs) were in the same order of magnitude than the ones obtained with WG based materials structured at micro-scale by Cagnon et al in 2012. But, the LbL technique offer higher potential in terms of precise modulation within this range of permeabilities than standard micro structuration techniques.

Oxygen permeability of LbL assembly PEI [MMT/PEI]$_n$ on paper coated with reticulated WG. The agro-based PEI[MMT/WG]$_n$ materials were then compared to synthetic-based PEI[MMT/PEI]$_n$ materials built on the same WGr paper substrate (Table 2.6). As expected, since synthetic polymer are known to be more barrier than agro-ones at high relative humidity, PEI[MMT/PEI]$_n$ based materials were more barrier to O_2 than PEI[MMT/WG]$_n$ based materials: 2 and 10 fold more for 10 and 40 layers pairs respectively. As for the agro based LbL reduction of the permeability can mostly be attributed to the tortuosity effect brought by the nano-clay layers. The clear decrease of the oxygen transfer properties with synthetic LbL assembly was also consistent with previous works where deposition was performed by dipping [Jang et al. 2008; Priolo et al. 2010]. However, higher reduction of the P_{O2} were observed in these studies as Priolo et al. in 2010 recorded a 16 time reduction for a [MMT/PEI] assembly when increasing number of layer deposited form 10 to 40 and Jang et al. in 2008 observed a nearly 30 times reduction for a [MMT/Polyacrylamide] assembly when increasing number of layer deposited form 10 to 20. In the present work, the P_{O2} of a PEI [MMT/PEI]$_n$ assembly was reduced 13 folds when increasing number of layer deposited form 10 to 40. This difference was attributed to both the nature of polycation (Poly(acrylamide) instead of PEI at pH 10 in Jang et al.) and polyanion (Cloisite instead of

Optigel in both studies) used for the deposition and also to the different RH used in P_{O2} measurement (0% RH for Jang et al. and Priolo et al. against 100% RH in the present study) that could induce swelling of the nanoclays.

Finally, it appeared that all the LbL films created on WGr paper (agro and synthetic both), except the PEI[PEI/MMT]$_{40}$, remained more permeable to O_2 than the WGr paper itself. The increase in P_{O2} value between WGr paper and WGr paper-PEI material (10 times higher) it seemed safe to assume that this was due to a lixiviation of the WGr coating by the preliminary PEI layer deposited. As can be seen in Figure 2.15, the surface of the WGr paper-PEI was very different of the one of the WGr paper. Compared to the smooth and homogenous WGr paper surface, a wave like pattern was observed, indicating degradation due to the preliminary PEI layer deposition. So when aiming for very high O_2 barrier behavior, the paper must be first coated with a substance resistant to PEI or the PEI layer must be suppressed or replaced by a substance harmless for WGr.

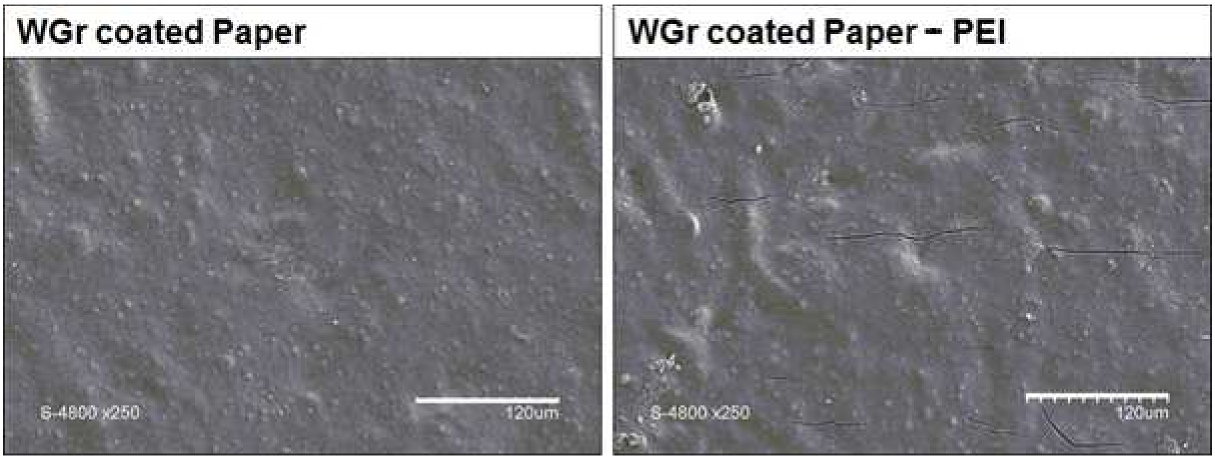


Figure 2.15. MEB observations of WGr Coated Paper, WGr Coated Paper-PEI

CONCLUSION

This study, through establishment of a new characterization method based on fluorescence spectroscopy, proved the possibility to create LbL structures with agro-based materials (wheat gluten agro-polymer and MMT nanoclays) and to perform depositions of such molecules by spraying onto relatively rough and non-homogenous substrates (like Paper or WGr Coated Paper) to tailor their gas transfer properties. The substrate for deposition remained of major importance when targeting such properties as paper finally proved to be too porous to allow creation of barrier LbL materials whereas WGr paper brought far better results. Indeed, the relatively rough but non-porous WGr paper substrate allowed creation of moderately barrier LbL materials (based on PEI[MMT/WG]$_n$ or PEI[MMT/PEI]$_n$) that would be of major interest in the field of fresh food packaging due to the said transfer properties and their highly tailorable character. Furthermore, given proper adaptation of the substrate or the preliminary layer, agro-based LbL could also be of interest to pack foods that require low to very low oxygen permeability packaging.

Publication 4 (short note)

Impact of CO_2 sorption on the gas transfer properties of wheat gluten coated papers

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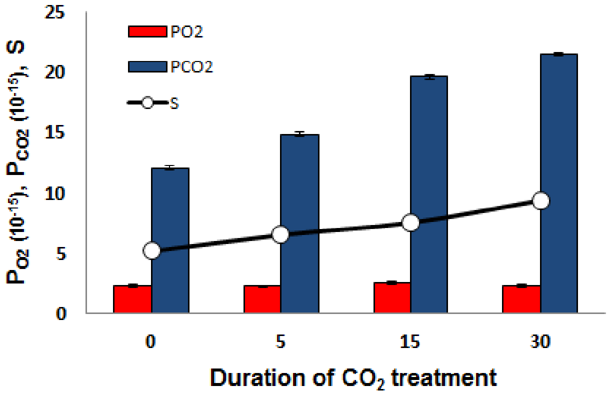
Journal to be determined – In preparation for submission

ABSTRACT: A new kind of CO_2 treatment under standard condition of temperature and pressure was experimented on wheat gluten coated papers (WG-Papers) with the aim of modifying their gas transfer properties. Increasing CO_2 treatment duration led to higher CO_2 content bound in the materials (from 40.57×10^{-3} mol.cm^{-3} for untreated material to 60.71×10^{-3} mol.cm^{-3} for 30h CO_2 treated material) and increased CO_2 permeability and O_2/CO_2 permselectivity (up to 2 time higher for a 30h treatment) for the WG-Papers. Modification of the WG coated layer structure and/or chemistry of the WG-Papers and its CO_2 sorption capacity was assumed to be the source of modified gas transfer properties. However further experiments are still required to validate this hypothesis and go deeper in the understanding of the underlying mechanisms of the gas transfer modulation observed.



KEYWORDS: Wheat Gluten; Carbon dioxide treatment; Gas transfer properties

INTRODUCTION

Carbon dioxide is already used under supercritical conditions as a plasticizer to facilitate processing of many polymeric materials [Sauceau et al. 2011], including bio-polymers [Knez et al. 2011] and nano-composites [Hwang et al. 2008; Akbarinezhad et al. 2011]. As other plasticizing mediums, CO_2 under supercritical conditions had a clear impact on mechanical [Asai et al. 2007] and mass transfer properties [von Schnitzler et al. 1999] of the final materials. But to our knowledge, CO_2 under normal conditions of temperature and pressure is neither studied nor used for similar purposes. This can be explained by the limited carbon dioxide sorption capacity of conventional synthetic polymers. However, oppositely to the conventional plastics, protein based materials and especially wheat gluten (WG) based one present interestingly high CO_2 sorption capacity [Gontard et al. 1996b; Pochat-Bohatier et al. 2006] and are known to be able to react and bond with CO_2 molecules in many ways [Gontard 1998]. Thus, use of CO_2 under standard conditions of temperature and pressure could be envisaged to modify the structure and then properties of WG-based materials.

WG based materials have been long studied for their peculiar gas transfer properties at high relative humidity (very high permselectivity, ratio of CO_2 permeation rate and O_2 permeation rate, and moderate oxygen permeability) that made them of interest for applications such as fresh produce packaging. Despite such interesting properties, their weak mechanical resistance led to combine them with fillers or mechanical supports as paper. But, when coating WG onto paper, the WG like behavior is reduced compared to self-supported films, notably the high permselective character [Cagnon et al. 2012]. Then new ways of modulating the properties of WG coated paper (and hopefully increasing its permselectivity) are of major interest.

In this study, the impact of a CO_2 treatment under standard conditions of temperature and pressure on the gas transfer properties of WG coated papers was evaluated and understanding of the underlying mechanisms of the modifications observed was attempted.

MATERIALS AND METHODS

Materials

Kraft papers (Terrana 36g.m^{-2}) were provided by Gascogne Paper (Mimizan, France) to serve as support papers. Wheat gluten powder, containing 7.2 wt. % of moisture and 76.5 wt. % of protein was provided by Amylum (Mesnil St Nicaise, France). Acetic acid and sodium sulphite, also used to prepare the coating solution were purchased from Aldrich (St Quentin, France). Carbon dioxide and helium were purchased from Air Liquide (Montpellier, France) for sorption treatments. Barium hydroxide, hydrochloric acid, perchloric acid, anhydrous ethanol, and phenolphthalein were purchased from Sigma Aldrich (St Quentin, France) for absorbed CO_2 quantification. Barium chloride for high relative humidity (RH) conditioning of the materials was purchased from Aldrich (St Quentin, France).

WG solution preparation

A WG coating solution (21.23 wt. %) was prepared at room temperature according to a 3 steps procedure [Guillaume et al. 2010a], slightly adapted to match the desired quantities. First, 30g of WG powder was poured into a box and dispersed under shaking in 50mL of a sodium sulphite / deionized water solution (0.06g/50mL). This solution worked as a reducing agent of the disulfide bonds. Then, after 30min of settling, the pH of the solution was set to 4 by adding a 50/50 v/v. solution of acetic acid and deionized water. Finally the solution was adjusted to 130ml by adding deionized water and the whole mix was stirred and left to rest for a day.

WG casting process (WG-films)

The WG coating solution was casted onto a Plexiglas plaque with a casting blade featuring a 2mm height and left to dry under slow and soft conditions at room temperature and below 50% RH during 180 minutes. Wheat gluten self-supported casted materials will be referred hereinafter as WG-films. All WG-Films were 175µm thick and exhibited a density of 1.6 cm^3.g^{-1}.

WG coating process (WG-Papers)

Prior to coating, all Kraft papers were stored in a RH controlled box set at 30% RH. The coating was performed on the untreated side of the paper at room temperature using an

Erichsen coater equipped with the blade n°8 featuring a spire width of 1mm and at a speed of 10mm.s^{-1}. Three milliliters of the WG coating solution were necessary to perform the coating. After coating WG-Papers were dried under slow and soft conditions at room temperature and below 50% RH during 90 minutes. Once processed, all materials were sealed into LDPE pouches waiting for characterization. Wheat gluten coated papers will be referred hereinafter as WG-Papers. All WG-Papers featured a thickness of 70µm and a coating weight of 14.5 g.m^{-2}.

Thickness and coating weight measurements

Average thicknesses of WG-films and WG-Papers were determined at room temperature and 30% RH with a hand-held digital micrometer (Mitutoyo instruments) from ten measurements randomly taken over the paper or WG-film surface.

To assess the coating weight, 9 square pieces (5x5cm) of both Kraft paper and WG-Paper were cut and left to dry during 24h in a ventilated oven at 103°C. They were then placed to cool into a desiccator containing silica-gel. After one hour they were taken out and weighted with a precision balance. The coating weight (Cw) in grams per meter square was calculated as follows:

$$Cw = \frac{W_{WGP}-W_{SP}}{A} \qquad eq.2.7$$

where W_{WGP} (g) is the weight of a WG coated paper piece, W_{SP} (g) is the average weight of Kraft paper pieces and A (m²) is the area of a piece.

CO_2 sorption treatment

Both WG-films and WG-Papers were subjected to CO_2 sorption treatments in a permeation cell at 100%RH. Materials were placed in an aluminum permeation cell and the inferior and superior chambers were both spread by a 30ml.min^{-1} flux of CO_2. The RH of the CO_2 flux was set thanks to a bubbling flask containing water and placed in a cryothermostat regulated at a properly chosen temperature. The differential temperature imposed between the cell and the cryothermostat permitted the establishment of the desired RH in the chamber (100%). The materials underwent the treatment during 0, 5, 15 or 30 hours. Such treated materials were referred as WG-film or WG-paper 5Ø, 15Ø, or 30Ø. Then all materials were subjected to a helium (He) desorption treatment to remove non-bonded CO_2 molecules. Materials were referred as WG-film 0, WG-film 5, WG-film 15, WG-film 30 or WG-Paper 0, WG-Paper 5, WG-paper 15, WG-Paper 30 according the length of the CO_2 treatment applied.

Quantification of CO_2

The quantity (in mol) of carbon dioxide inside the WG-films and WG-Papers was evaluated thanks to a titration method adapted from Gill in 1988 [Gill 1988] and Jakobsen et al. in 2004 [Jakobsen et al. 2004].

Prior to measurements, the samples were weighted (all between 0.05 and 0.15 g). They were then immersed in a mixture of perchloric acid and ethanol (50/50 v/v) which forced the CO_2 adsorbed in WG based materials to be released into the headspace of a first bottle ("extraction bottle") and then transferred into the second bottle ("titration bottle") where it reacted with a $Ba(OH)_2$ (0.0016N) aqueous solution (9mL in excess) to form solid $BaCO_3$. CO_2 was instantly consumed by the reaction with $Ba(OH)_2$, so the overall equilibrium of the reaction promoted the release of CO_2 from the material until none remained. Finally, after 48hours, the residual quantity of $Ba(OH)_2$ in the "titration bottle" was titrated directly into the flask against a standard HCl (0.0032N) solution using phenolphtalein as color indicator.

To take in consideration possible impact of atmospheric CO_2 contained in the bottle at the beginning of the experiment or leaking in during the experiment, two control bottle containing only $Ba(OH)_2$ (9mL in excess) were prepared. The first one was titrated right away and the second one at the end of the experiment.

So given the chemical reactions implied:

[CO_2 extraction] : $CO_3^{2-} + 2H^+ \rightarrow HCO_3^- + H^+ \rightarrow H_2CO_3 \rightarrow CO_2(gas) + H_2O$

[CO_2 consumption] : $Ba(OH)_2 + CO_2 \rightarrow BaCO_3 + H_2O$

[Residual $Ba(OH)_2$ titration] : $Ba(OH)_2 + 2HCl \rightarrow BaCl_2 + 2H_2O$

the amount of CO_2 per volume unit of material exposed (in $mol.cm^{-3}$) was calculated as follows:

$$(CO_2) = \frac{\frac{[HCl]}{2} \times \left((V_{tf} - V_s) - (V_{t0} - V_{tf})\right)}{vm} \qquad \textit{eq2.}$$

where [HCl] (in $mol.L^{-1}$) is the concentration of the HCl solution used for titration, V_{tf} (in L) and V_{t0} the volume of HCl necessary to titrate the control solution at the end and at the beginning of the experiment respectively, V_s (in L) the volume of HCl necessary to titrate the sample solution from the titration bottle, and vm (cm^3) the volume of the sample placed in the extraction bottle.

The absorbed amount of carbon dioxide was assessed in triplicate for each treatment conditions.

Permeability measurements

The O_2 and CO_2 permeability of WG-Papers were assessed in triplicate with an isostatic and dynamic method using gas phase chromatography. Materials were placed in a permeability cell. The inferior and superior chambers were each spread by a 30mL.min^{-1} flux of permeant gas (O_2 or CO_2) and vector gas (Helium) respectively. The RH of the gas flux was set thanks to a bubbling flask containing water and placed in a cryothermostat regulated at a properly chosen temperature. The differential temperature imposed between the cell and the cryothermostat permitted the establishment of the desired RH in the chamber (100%). The permeability cell was coupled to a gas chromatograph GC3800 from Varian (Les Ulis, France) equipped with an automatic valve to online analyze the evolution with time of permeant gas concentration in the superior chamber of the cell (analysis of 1ml of sample at prescribed times). The gas chromatograph was equipped with a filled column Porapak-Q from Chrompack (Les Ulis, France) of 2m length and 0.32mm diameter for separating O_2 and CO_2, with thermal conductibility detector (TCD). The gas (O_2 or CO_2) permeability of the material was determined as follows in the international system unit:

$$P_x = \frac{\Delta Q \times e}{\Delta t \times A \times \Delta P} \qquad eq3.$$

where subscript P*x* referred to O_2 or CO_2 permeability of the sample (mol.Pa^{-1}.m^{-1}.s^{-1}), ΔQ was the number of mol of gas that pass through the film, e is the thickness of the material, Δt was the time for which permeation occurs, A was the exposed film area and (ΔP) is the difference in pressure exerted by the gas on each side of the film. Gas permeability was then expressed in mol.m^{-1}.s^{-1}.Pa^{-1} unit. For total O_2 and CO_2 desorption and RH stabilization, materials were placed prior to measurements in the permeation cell with helium flux in both chambers.

Glass transition temperature measurements

Measurement of the glass transition temperature (Tg) and differential of heat capacity (ΔCp) of WG-films and WG-Papers were carried out, in triplicate, using a DSC2000 in modulated scanning mode and T-zero hermetic pans and lids, all from T.A Instruments (Geneva, Switzerland). The temperature was set to go from -40°C to 80°C with a slope of 3°C.min^{-1} and a modulation amplitude and modulation frequency were 0.796°C and 100s^{-1} respectively.

Prior to measurements "pan-size" samples of the materials were cut and placed in hermetic boxes containing $BaCl_2$ solution for conditioning at high RH (92%) during 3 days (Note: the DSC Q2000 is limited to analysis of sample conditioned at 95%RH maximum). Just before measurement, the weight of the sample to be tested was measured on a precision balance.

FTIR measurements

FTIR measurements were carried out on a Nicolet 6700 from Thermo (Illkirch, France) equipped with a triple reflection diamond crystal and a MCT detector. The wavelength range was 800-4000nm, the resolution was set at 4cm^{-1} and 32 scans were performed per run. The recorded spectra were analyzed for discrimination with the Omnic software. 6 measurements were carried out on each sample. Prior to measurements, the samples were conditioned at 100%RH and 25°C during 3 days.

RESULTS AND DISCUSSION

Impact of CO_2 on the gas transfer properties of WG-Papers

The O_2 and CO_2 permeation of all WG-Papers were performed at high relative humidity (>90%RH) where WG based materials exhibit their more interesting properties [Gontard et al. 1996b; Mujica Paz et al. 1997].

Impact on CO_2 transmission. When comparing the evolution of the percentage of CO_2 transmitted through the material during the permeation measurement of untreated WG-Paper (WG-Paper U) and WG papers previously treated with CO_2 (whatever the duration of the CO_2 treatment), it appeared that the CO_2 sorption treatment increased the CO_2 permeation of WG paper, as illustrated in Figure 2.16 for material CO_2 treated during 15hours (WG-Paper 15Ø). Indeed, the steady value of CO_2 transmitted was almost 2 times higher for WG-Paper 15Ø than for WG-Paper U. And more interestingly, it also appear that forced desorption under He flux did not permit to restore the CO_2 transmission profile found for the untreated WG-Paper. As an example, the steady value recorded for WG-Paper 15 (after He desorption) was equivalent to the one of WG-Paper 15Ø. Such results proved that the CO_2 treatment induced irreversible changes within the material.

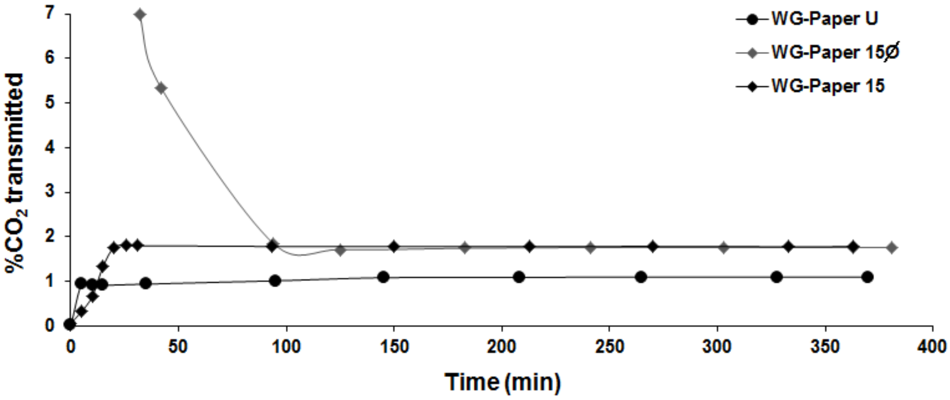


Figure 2.16. Illustration of the irreversible character of the CO_2 treatment. Percentage of CO_2 transmitted against time during the P_{CO2} measurement by gas chromatography

Therefore, for practical purposes, all CO_2 treated WG papers were subjected to He desorption prior to further measurements or analysis.

Impact on CO_2 and O_2 permeations. Permeability of the WG-Papers 0, 5, 15 and 30 towards O_2 and CO_2 were measured. As can be seen on Figure 2.17, all WG-Papers exhibited quite the same oxygen permeability (between $2.2x10^{-15}$ and $2.6x10^{-15}mol.Pa^{-1}.m^{-1}.s^{-1}$). However, the permeability towards CO_2 of these materials increased with increasing content of CO_2. WG-Paper 30 presented a P_{CO2} of $21.48x10^{-15}mol.Pa^{-1}.m^{-1}.s^{-1}$ and a permselectivity of 9.40 whereas WG-Paper 0 presented a P_{CO2} and permselectivity of only $12.09x10^{-15}mol.Pa^{-1}.m^{-1}.s^{-1}$ and 5.18 respectively. Consequently, permselectivity also increased from 5.18 for WG paper 0 to 9.40 for WG-Paper 30 and was in the same range than data from previous work: from 2 when WG was coated onto kraft paper (and same coating weight) [Guillaume et al. 2010a] to 12 when coated onto white bleached pulp paper (with highest coating weight) [Cagnon et al. 2012]. It is worth to notice that comparing to other modulation strategies as increasing coating weight or changing paper support, CO_2 treatment did not seem to be the most efficient. But, considering the constant increase in P_{CO2} and S, it was assumed that these properties could be further increased by longer treatments.

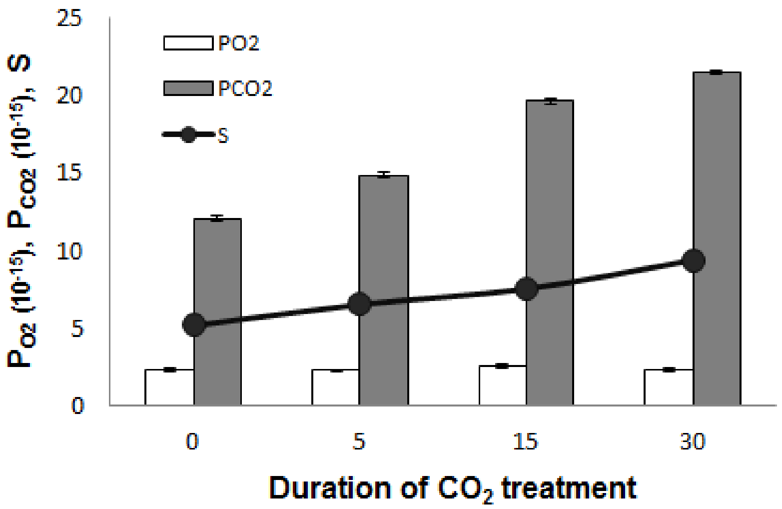


Figure 2.17. Evolution of the gas transfer properties of WG-Papers with the duration of the CO_2 treatment (P_{O2}: O_2 permeability, P_{CO2}: CO_2 permeability, S: ratio of permselectivity)

Relation between the structure of the CO_2 treated WG-Papers and their transfer properties.

In order to better understand how the CO_2 treatment affected the gas transfer properties of WG-Papers and what were the underlying mechanisms of its action, structural characterizations were undertaken in comparison with WG-films. Albeit support paper may react with CO_2 as previously demonstrated (CO_2 sorption coefficient of $107x10^{-6}$ mol.Pa^{-1}.m^{-3} in laminated white paper [Desobry et al. 1997]), only WG was supposed to bring transfer properties to the final material and it is known to interact with CO_2 through different interactions with amino-acids (CO_2 sorption coefficient of $18757x10^{-6}$ mol.Pa^{-1}.m^{-3} in WG self-supported film with glycerol as plasticizing agent [Pochat-Bohatier et al. 2006]).

WG-Papers CO_2 content. First, the quantity of CO_2 per volume unit present inside the WG-Papers materials was measured thanks to an acid/base titration method (Table 2.7). It is important to note that the amount of CO_2 measured here did not contain any residual "free" CO_2 molecules as the last step of material treatment implied desorption under He flux. Therefore all the CO_2 molecule that reacted with $Ba(OH)_2$ during the extraction phase of the measurement were bound with the gluten matrix or the paper. It appeared that, the longer the CO_2 treatment, the higher the content of CO_2 trapped in the WG-Papers (and likely bonded as all non-bonded CO_2 was removed through He desorption at the end of the CO_2 sorption treatment). While the CO_2 content in WG-Paper 0 was 40.6mmol.cm^{-3}, WG-Paper 30 contained 60.7 mmol.cm^{-3} of CO_2. These results showed that WG-paper naturally contained CO_2 molecules. In addition, no steady value was obtained after 30 hours of treatment, which led to assume that the maximal CO_2 retention capacity of the WG papers was not reached even after 30h treatment.

Table 2.7. Quantity of CO_2 adsorbed in WG-Film and WG-Paper materials after CO_2 sorption treatment

CO_2 treatment duration (h)	**0**	**5**	**15**	**30**
Quantity of CO_2 present in WG-Film *In 10^{-3} mol.cm^{-3}*	19.37	22.46	26.23	31.83
Quantity of CO_2 present in WG-Paper *In 10^{-3} mol.cm^{-3}*	40.57	47.57	53.29	60.71

For comparison, WG-films were subjected to same CO_2 treatments (and He desorption). Ideally, WG-films would have to exhibit the same coating weight (and thickness) than the WG layer coated onto paper (theoretically, 24 µm for 14g.m^{-2}, [Gastaldi et al. 2007]); but due to the brittleness of such films, thicker materials were produced (175µm). Such difference in thickness considerably acted on the quantity of bonded CO_2 during treatments since in none of the tested conditions (from 0 to 30 hours) the equilibrium was reached and then the CO_2 gradient effect should be more pronounced in thick materials than in thinner ones. Then WG films exhibited nearly 2 fold less CO_2 than WG papers whatever the duration of the treatment. To roughly estimate what the amount of CO_2 would have been in a 2 µm WG film, values obtained were multiplied by 7.3 (ratio between the actual thickness of film, 175µm, and its expected one, 24µm): from 141.4mmol.cm^{-3} in WG-film 0 to 232.3mmol.cm^{-3} in WG-film 30. This approximation showed that, even if paper may contribute to CO_2 fixation, it also seemed to restrict CO_2 bonding in WG proteins. Whatever, the amount of bonded CO_2 to WG papers was correlated to the increase in CO_2 permeability and permselectivity.

Structural modifications. Even if paper contributed to the CO_2 retention of WG-Paper, its impact on the transfer properties of the composite (and so the impact of the modulation it may underwent) was likely very limited due to its porous character. Therefore, explanation to P_{CO2} and S changes with CO_2 treatment should be found in changes in the WG coated layer.

Increased P_{CO2} after CO_2 sorption treatment can be related to an increase of CO_2 sorption and/or CO_2 diffusion coefficients of the WG layer (if the Fick's law is obeyed: permeability (P) equals diffusion (D) multiplied sorption (S), P=DxS). However it has been proven that the diffusion coefficient of oxygen and carbon dioxide often witness changes of the same order when submitted to the same treatments (e.g. D_{CO2} and D_{O2} were increased 1.5 and 1.9 time, respectively, for a plasticization by 80% to 93% RH increase) [Pochat-Bohatier et al. 2006] and no evolution on P_{O2} (and so on D_{O2}) of WG-Paper was observed with the CO_2 treatment. So the focused was placed on possible sorption carbon dioxide capacity modification through the CO_2 treatment.

- ***WG coated layer plasticization***

First the possible plasticization of the material by the treatment was assessed as a WG network presenting higher molecular mobility may present more available sorption sites for the CO_2 molecules. To do so, the glass transition (Tg) temperature of the different WG-Papers at 92% RH was measured by modulated diffusion scanning calorimetry (MDSC, Figure 2.18). No clear trend appeared as the Tg varied around a mean value of 20°C with relatively high standard deviation.

Same observation was made on WG-films but their Tg was significantly lower (around -10°C). This value was significantly lower than the ones recorded previously by Gontard et al. in 1996 [Gontard et al. 1996a] in similar conditions of RH. However the WG self-supported were not prepared the same way as the WG solution used by the aforementioned authors contained, glycerol and ethanol and was heated at 40°C prior to casting. Chiou et al. in 2009 also studied the Tg of WG self-supported films and found a much higher value (circa 50°C) [Chiou et al. 2009], but this could be attributed both to the lower RH of the measurement (50% instead of >90%) and to the fact that the film preparation implied prolonged stay in an oven at a temperature up to 70°C which could have induced cross-linking of the gluten network.

The fact that no significant difference was observed neither on WG paper nor WG films submitted to various CO_2 treatments could indicate that either, no plasticization or physical structure modification of the gluten network of WG-Papers was induced by the CO_2

sorption treatment, or that the plasticization was too fine to be detected by this method. It is possible that a slight Tg evolution was masked by the high standard deviations observed. These deviations could be attributed to undesired RH changes of the WG-Paper samples due to the fact that it was difficult to maintain their RH at very high level during their preparation for MDSC measurement. Indeed, properties of WG based material (including Tg) [Gontard et al. 1996a] are known to be very RH sensitive and especially as high RH such as the one considered.

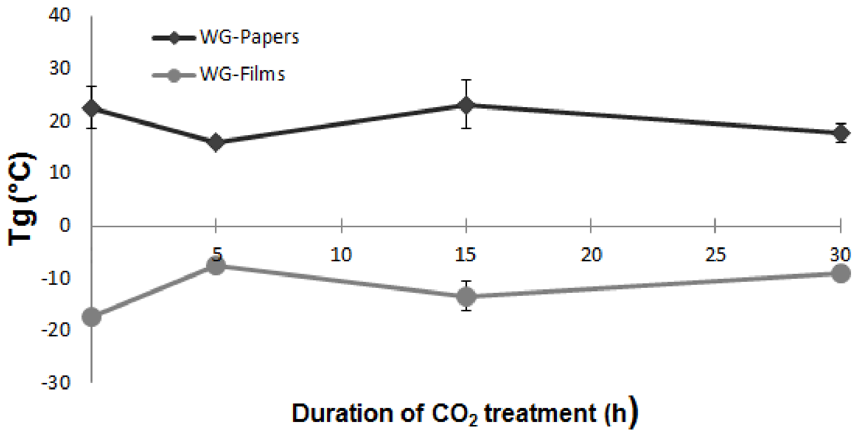


Figure 2.18. Evolution of the Tg of WG-Papers and WG-films with the duration of the CO_2 treatment

- ***WG proteins structure modifications***

Carbon dioxide is known to be able to react and bound with many chemical groups present in the gluten network: covalent binding through carbamination with lysine, electrostatic bindings with ionized lateral groups, hydrogen binding with glutamine residue, hydrophobic lateral groups or peptidic chain [Gontard 1998]. Creation of such new bindings or the alteration of native ones by the CO_2 treatment could modify the secondary structure of the WG proteins and so their CO_2 sorption capacity. To assess these possible structural changes of the protein chain, WG-Paper 0 and WG-Paper 30 as well as WG-film 0 and WG-film 30 were analyzed by FTIR spectroscopy.

After discriminating analysis, no significant differences were observed between the respective spectra of materials submitted to 0 or 30 hours treatment, either for WG-Films (Illustrated in Figure 2.19) or WG-Paper, even when focusing at the amide band (1650nm^{-1} to 1600nm^{-1}) where change in secondary structure might be detected or amine band (3400nm^{-1} to 3000 nm^{-1}) where main interactions could take place. This indicated that either the CO_2

treatment induced no changes in such structures or that these changes were too subtle to be measured through standard FTIR spectroscopy.

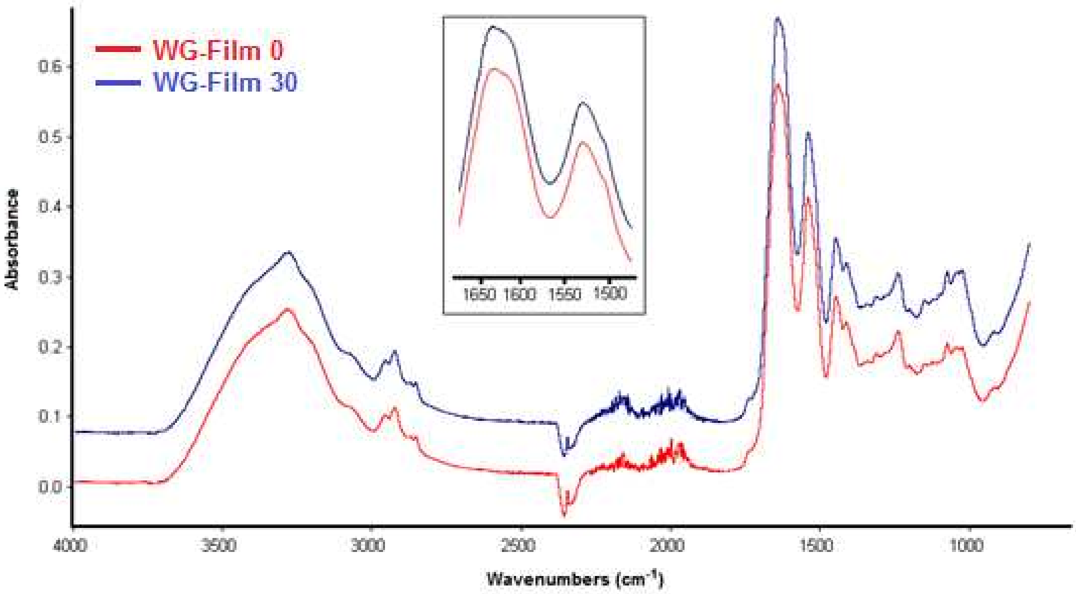


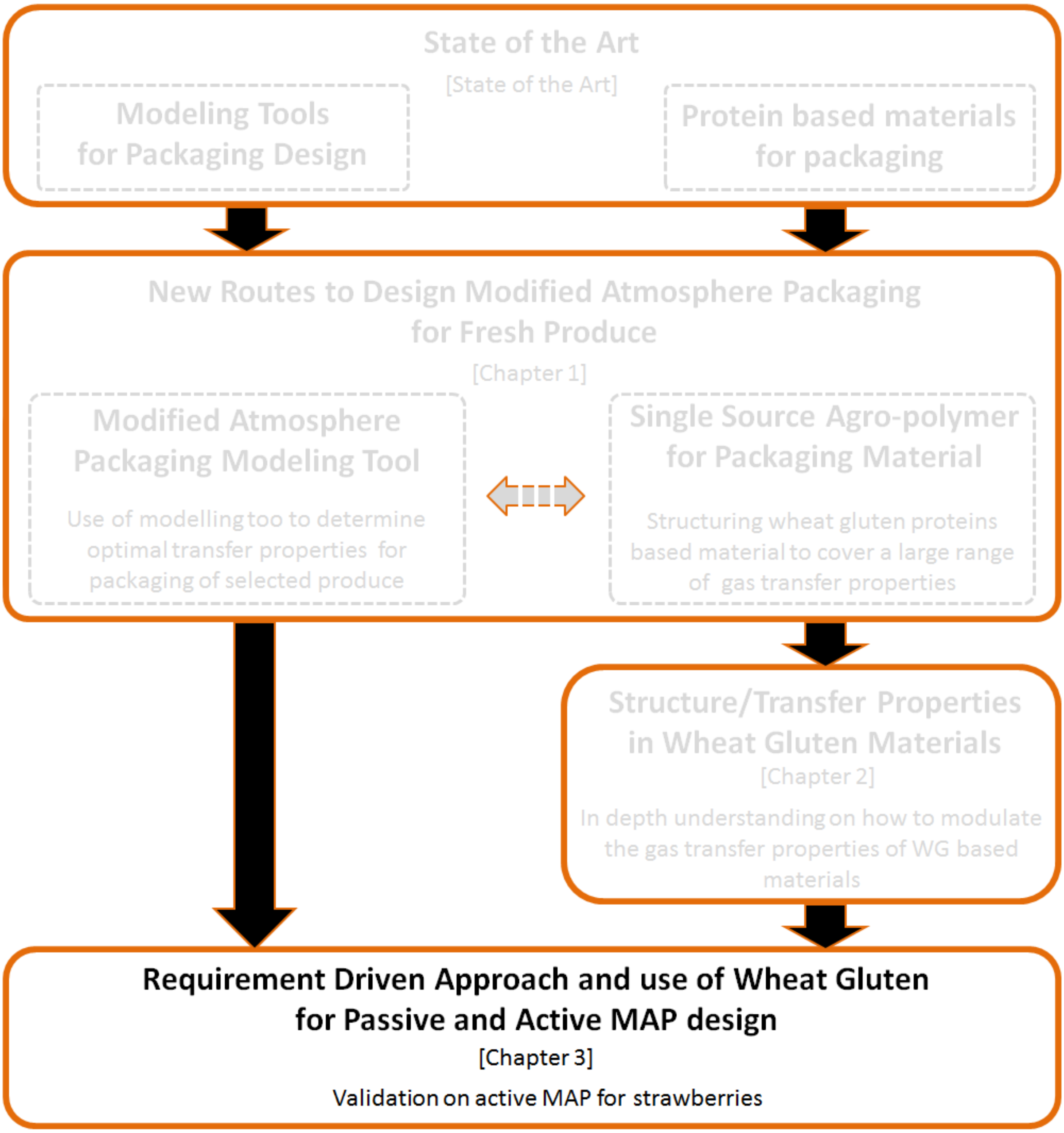
Figure 2.19. FTIR spectra of WG-Film 0 and WG-Film 30

CONCLUSION

Through this study it appeared that CO_2 treatments at normal conditions of temperature and pressure can modify the gas transfer properties of protein (WG) based material and interestingly enhance their CO_2/O_2 permselectivity. However further studies are still required to i) evaluate the full potential of this kind of treatment with determination of the maximum CO_2 retention capacity of the materials and concomitantly the maximal permselectivity value reachable and ii) understand the underlying mechanisms that induced these gas transfer properties modifications. By applying longer treatment (until reaching plateau values of CO_2 retention and then permselectivity), characterization techniques used would more discriminative. The real respective contribution of paper and WG should be evaluated by also submitting uncoated paper to the same CO_2 treatments than the WG-Papers. Then, more precise and powerful techniques to characterize the organization and the chemistry of the WG network submitted to the CO_2 treatment can be considered. For instance, quantification of the gluten fractions before and after CO_2 treatment by SE-HPLC could be envisaged to assess creation or destruction of new bonds within the WG network.

CHAPTER 3

REQUIREMENT DRIVEN APPROACH AND USE OF WHEAT GLUTEN PROTEINS FOR PASSIVE AND ACTIVE OPTIMAL MAP DESIGN



For this last chapter, the efficiency of the combined use of the requirement driven approach (applied to all the produce requirements) and wheat gluten based materials for passive but also active MAP design through a case study on strawberry will be demonstrated. This work will lead to the conception of a prototype active tailor-made packaging for strawberry to enhance produce preservation. Then this prototype will be scaled-up from laboratory-scale to industrial-scale and the efficiency of the industrial-scale packaging will be assessed on the berries.

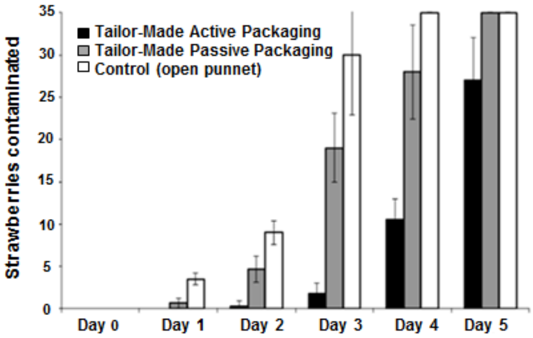
Publication 5

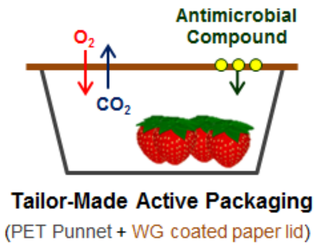
Fresh food packaging:
A requirement driven approach
(case study on strawberries packed with agropolymers)

Thibaut Cagnon, Aurore Méry, Pascale Chalier, Carole Guillaume and Nathalie Gontard

Innovative Food Science and Emerging Technologies - Submitted

ABSTRACT: To overcome the limitations of current trial and error approaches used to design food packaging, a requirement driven approach was adapted from the software and service industries to the field of fresh fruits and vegetable packaging. Based on 5 steps, it relies on the knowledge of produce needs and the use of modeling tools to turn produce requirements into packaging properties before designing the packaging. It was then successfully applied to dimension and design an active packaging, tailor made for optimal preservation of strawberries at 20°C. The solution consisted in a PET punnet providing protection against physical damage and a lid ensuring both gas transfer for optimal atmosphere and release of an active agent acting against mold growth. This active lid was made of wheat gluten proteins containing 2-nonanone that was quickly released only in high RH conditions in an amount equal to the minimal inhibition dose for Botrytis cinerea.





KEYWORDS: Requirement driven approach; Active packaging; Strawberry; Modify atmosphere packaging; Antimicrobial aroma compound

INTRODUCTION

Up to now, most of food products are packed into material selected for economic or marketing reasons, or for interesting transfer properties (e.g. water vapor and gas barrier) and technical features (e.g. UV barrier). To choose an appropriate material, the costly and time consuming "trial and error" method is still commonly used taking into account the knowledge and experience of food industries. But, in some cases and mainly for respiring foods (e.g. fruits and vegetable), it may lead to detrimental changes in quality, inducing a depression of the commercial value and affecting the brand reputation. This appears when focusing on one property of the material (e.g. oxygen barrier property) while neglecting the others (e.g. carbon dioxide barrier property), and because materials are commonly produced to cover a broad range of food products that exhibit different requirements and face different conditions through their supply chains. In the current context of competitiveness, market globalization and sustainability, optimal packaging solutions designed for a given product – so-called tailor-made packaging – should be urged and could be based on a requirement driven approach (RDA) taking into account product needs since the very beginning of its conception.

RDA has been used for almost 20 years in fields like information technology, software design [Castro et al. 2002] or product design and innovation in the industry [Kusiak 2009] to reduce the risk of inadequacies between the functionalities of a product, software or service and the specific needs of potential users. The RDA is often based on a 4 steps method as described by Castro et al. [Castro et al. 2002] which was first centered on products requirement and was then optimized to take into consideration both expectations from consumers [Stein 1994; Castro et al. 2002] and innovations from research and development departments [Kusiak 2009]. Focusing on fresh produce quality, the RDA could be transposed to the design of tailor-made packaging, and enhanced by implementation of reverse engineering on specific points such as optimal atmosphere for Modified Atmosphere Packaging (MAP) preservation.

Among fresh fruits and vegetable, strawberries are especially interesting because of their fragility and mold sensitivity that cause high depreciation in sales at retailing point. This makes the development of an optimal packaging solution to maintain the quality of these produce during storage of economic interest. Various researches have been conducted to improve the storage of these berries such as modified atmosphere packaging [Nielsen et al. 2008] or edible coatings [Tanada-Palmu et al. 2005; Hernandez-Munoz et al. 2008] sometimes combined to essential oils [Bhaskara Reddy et al. 1998] or aroma compounds

[Almenar et al. 2009] to produce active materials with antimicrobial property. Albeit some solutions appeared to extend the shelf-life of strawberries at temperature lower than 10°C, none of them gave satisfactory results or have been tested at 20°C, which is the most common temperature at European retailing points. Then there is still a need to design packaging fully adapted to the storage of strawberries at ambient temperature.

This study aims to adapt the RDA to fresh produce packaging and apply it to the development of an optimal active packaging for strawberries with agro-polymer based materials. The RDA relies on one hand on bibliographical data collection on the needs of strawberries and current knowledge on its preservation, and on the other hand on experimental data acquisition for material development and validation of the designed packaging in conditions of use.

MATERIALS AND METHODS

Materials.

Commercial kraft paper (Teranna 36g/m²) was provided by Gascogne Paper (Mimizan, France) in sheet form to serve as support for coating. Syral (Marckolsheim, France) supplied vital wheat gluten (WG) powder (AG110), containing 7.2 wt. % of moisture and 76.5 wt. % of proteins. All chemical products as acetic acid and sodium sulphite used to prepare the WG coating solution and sodium sulphate, dichloromethane and tween 80 were purchased from Aldrich (St Quentin, France), and Potato dextrose agar (PDA) medium from Biokar Diagnostics (Allone, France). Trans-2-hexenal (purity: 98%; density: 0.846; saturated vapor pressure: 879.8 Pa; LogP: 1.7; all at 25°C) and 2-nonanone (purity 99%; density: 0.830; saturated vapor pressure: 75 Pa; LogP: 2.9; all at 25°C) used as antimicrobial compounds and 2-nonanol (purity 97%) used as internal standard were purchased from Aldrich (St Quentin, France). *Botrytis cinerea* (MUCL30158) was kindly provided by the Catholic University of Leuven (Leuven, Belgium) and *Rhyzopus Stolonifer* (harvesting from contaminated strawberries) was provided by the Centre Technique Interprofessionnel des Fruits et Légumes (CTIFL, Lanxade, France). Strawberries (*var. Charlotte*) were harvested by CTIFL (Prigonrieux, France) for testing the impact of antimicrobial compounds on the fruits or purchased from a local farmer for testing packaging efficiency in conditions of use. PET punnets were purchased from a local retailer (Metro, France).

Minimal inhibition concentration (MIC) determination.

To prepare inoculums of *B. cinerea and R. stolonifer*, spores of 7 day-old-cultures grown of PDA were harvested in saline distilled water with 0.1% (v/v) tween 80. Concentration of spores was determined using a haemocytometer (Malassez cell) with an optical microscope at 400x magnification and adjusted to 10^4 spores/mL.

Petri dishes containing 10mL of PDA medium were then inoculated by 100µL of 10^4 spores solution (total of 10^3 spores) and placed in sterilized 1L jars containing 5mL of water to adjust Relative Humidity (RH) to 100% RH. Filter papers impregnated with a known amount of active volatile compound (from 0, for the control, to 40µL) were stuck on the lids of jars. Jars were closed and incubated at 22°C for 10 days. Three jars were daily opened to measure the mycelia diameter as well as 3 control jars to check the viability of molds. The MIC was then determined as the minimal amount of active volatile compound for which no mold growth was observed during 10 days at 22°C and 100%RH and expressed in µL.L^{-1} of air.

Impact of MICs on the quality of strawberries.

The effect of MICs on the quality of strawberries was assessed. Twenty strawberries were put inside a 1L punnet, whose lid was perforated (to avoid anoxia) and fitted with a filter paper impregnated with the tested active compound at its MICs, and stored at 20°C during 5 days. Visual aspect of the berries was assessed and presence or absence of the characteristic odor of the tested active volatile compound was noted.

MAP simulation.

The evolution with time of oxygen and carbon dioxide partial pressures in packaging headspace was simulated using the web MAP modeling tool Tailorpack (www.tailorpack.com, UMR IATE, France) to evaluate viability of the selected packaging. This modeling tool is built on a mathematical model based on the mass balance between the oxygen and carbon dioxide flux through the packaging material on one hand and the O_2 and CO_2 consumption/ production of the produce respiration on the other hand. This model, as most of those previously established [Kok 1985; Wade et al. 1987; Kader et al. 1989; Edmond et al. 1991; Talasila et al. 1994; Fishman et al. 1995; Peppelenbos et al. 1996b], was based on Michaelis-Menten equations for produce respiration and Fick's laws for gas transfers as detailed in Cagnon et al. 2012 [Cagnon et al. 2012].

It can be run in two modes available on the Tailorpack website: optimization and simulation. In both cases, produce characteristics such as maximum respiratory rate (RR_{O2max}), respiratory quotient (RQ), apparent Michaelis constant (Km_{O2app}) and inhibition constant (Ki_{CO2}) as well as packaging dimensions such as surface, volume, thickness and the mass of produce to be packed are required. In the case of optimization, the optimal atmosphere is previously required (%O_2 and %CO_2) and the optimal permeabilities (i.e. permeation value multiplied by thickness) of the packaging material could be then identified using an optimization procedure (e.g., Levenberg-Marquard algorithm) to fit predicted partial pressures to optimal ones. In the case of simulation, O_2 and CO_2 packaging permeabilities are required and the evolution with time of %O_2 and %CO_2 headspace composition is predicted.

Preparation of the coating solution.

WG coating solution (21.23% dry w/v) was prepared at room temperature according to a 3 steps procedure [Guillaume et al. 2010a]. First, 30g of WG powder were dispersed under shacking in 50mL of a sodium sulphite solution (0.06g/50mL), which worked as a reducing agent of the disulfide bonds. Then, after 30min of settling, the pH of the solution was set to 4 by adding a 50% v/v solution of acetic acid. Finally the solution was adjusted to 130mL by adding deionized water and the whole mix was stirred and left to rest for a day.

The desired active compound was then added (15, 30 and 45% w/dry-w) to prepare the active WG coating solution used to create the antimicrobial materials. The whole mix was stirred again for 5min at 8000rpm with a L4RT high speed mixer from Silverson (Chesham, England). This active solution was used immediately for coating or analyses (microscopy observations or viscosity measurements).

Optical microscopy on WG solutions.

Each solution was deposited onto a glass microscope slide and observed at 20x magnification, under a DM2000 optical microscope from Leica Microsystems (Nanterre, France) equipped with a JVC color digital camera (Tokyo, Japan) for recording.

Viscosity and consistency of WG solutions.

The viscosity measurements were carried out on a Physica Rheolab MC1 viscometer from Anton Paar (Stuttgart, Germany) equipped with a CC27st measurement module and a TEZ peltier. Samples were equilibrated at 25°C before analysis, and then measured. The shear rate of the module (γ in s^{-1}) increased from 1 to $1250s^{-1}$ and the shear stress of the sample (τ in Pa) was measured for every shear rate value. The consistency (k in Pa.s) and the flow behavior index (n) were calculated thanks to the following equation:

$$\tau = k \times \gamma^n \qquad \textit{eq. 3.1}$$

Preparation of passive or active materials.

Prior to coating, all support papers were stored in a RH controlled chamber set at 30% RH using $CaCl_2$. The coating was performed on the raw side of the sheets immediately after the preparation of the coating solution using an E409 blade coater from Erichsen (France) equipped with the blade n°8 featuring a spire width of 1mm. Coating speed was set at $10mm.s^{-1}$. To be spread all over the sheet surface, an excess (>3mL) of coating solution was deposited to produce coated papers. After coating, materials were left to dry at 20°C for 2 hours and the relative humidity was kept below 40%. Material made from the WG coating solution were referred as Passive WG-Papers (identical to Kraft WG-Paper [Cagnon et al. 2012]) and materials made from the active WG coating solution were referred as Active WG-Papers.

Uncoated papers used for coating weight assessment were subjected to the same treatment except the coating solutions were devoid of proteins.

Coating weight measurement.

Coating weight was calculated from 9 replicates by subtracting the dry basis weight of a defined area (25cm²) of uncoated paper from the dry basis weight of the same area of coated paper. Dry basis weights were measured on an Ohaus precision balance after 24 h of drying at 103°C.

Oxygen and carbon dioxide permeation measurements.

The O_2 and CO_2 permeation (Pe_{O2} and Pe_{CO2}) of coated materials were assessed in triplicate thanks to an isostatic and dynamic method using gas phase chromatography detailed in Cagnon et al. 2012 [Cagnon et al. 2012]. The permselectivity (S) of the materials was also calculated as the ratio of Pe_{CO2} on Pe_{O2}.

Determination of the residual amount of aroma compound in active materials and losses during processing.

Extraction of aroma compounds was performed by immersing square pieces of materials (3x3cm) in a mixture consisting in 5mL of deionized water and 5mL of dichloromethane. 100µL of a 3% 2-nonanol solution were added to the mixture as internal standard. After 16h under magnetic stirring (500rpm), the organic phase containing the aroma compound and the internal standard was removed and dried over anhydrous sodium sulphate to ensure complete elimination of aqueous phase. The analysis of dried organic phase was carried out on a Varian 3800 GC-FID equipped with a DB5 column (30mx0.25mm, film thickness of 25µm, T8W Scientific) and a flame ionization detector (FID, hydrogen: 30mL.min^{-1}, air: 300 mL.min^{-1}, nitrogen: 30mL.min^{-1}). Temperature of the detector was set at 300°C and the injector at 250°C. Hydrogen was used as the carrier gas with a flow rate of 2mL/min. The column temperature was programmed to first rise from 50 to 90°C at a rate of 4°C/min and then from 90 to 250°C at a rate of 15°C.min^{-1} to be finally maintained at 250°C for 10min. The injections were done in split mode with a ratio of 1:20. The residual amount of aroma compound in the material was calculated by correlation between the aroma compound peak and the internal standard peak, extrapolated to a surface unit and then expressed in g/m². Loss of aroma compound (in percentage) during material processing (i.e. solution preparation, coating and drying) was calculated by taking into account the residual (w_r) and theoretical (w_t) amount of aroma compound as follows:

$$\%Loss = \frac{(w_t - w_r)}{w_t} \qquad eq.\ 3.2$$

$$with\ w_t = Cw \times (\%\ Aroma\ compound\ added) \times (\frac{1}{E_{ef}})$$

where Cw (g/m²) is the coating weight of the coated papers previously evaluated and E_{ef} the extraction efficiency for each aroma compound. (E_{ef}) was determined at about 0.917 and 0.937 for trans-2-hexenal and 2-nonanone respectively.

Kinetics of active compound release from the coated papers.

Square pieces of materials (3x3cm) were put in a chamber set at 20°C and desired RH (<50% and 100%). The RH was adjusted by spreading humidified air through the chamber (air flux: 25ml/min). Air flux was humidified by bubbling in a gas washing bottle containing water and placed in a cryothermostat regulated at a properly chosen temperature. The differential temperature imposed between the chamber and the cryothermostat allowed the establishment of the desired RH in the chamber. The RH was verified by a probe placed inside the chamber. Materials were taken out from the controlled chamber at prescribed time intervals (between 0 and 16 days) and aroma compound residual content was immediately determined as described above and expressed for a surface equal to the lid (256cm²).

Packaging structures.

Three different packaging were used. The Control consisted in an open 1L PET punnet. The Passive Tailor-Made Packaging was made of the same punnet closed with a passive material based lid (256cm²) and the Active Tailor-Made Packaging of the same punnet closed with an active material based lid (256cm²).

Tests of the packaging in operating conditions.

Strawberries were picked up and stored for one night at 10°C prior to experiment. 500g of strawberries (±5%) were placed in each of the 3 packaging structures described above. Packed produce were stored during 5 days at 20°C and 100% RH in a controlled chamber (Meditest 600/1300, Firlabo, France). Mold spot formation, changes in the overall aspect of the berries, and traces of exudates were daily assessed in triplicate. The amount of O_2 inside the packaging was monitored daily via an online and non-destructive method. An oxygen sensitive optical sensor (Fibox/Oxy-view, Presens, Germany) was placed inside each punnet and the evolution of the amount of oxygen was recorded in triplicate during the storage. The amount of CO_2 inside the packaging was also measured on the last day of storage using a µGC analyzer (R3000, SRA Instruments, Agilent, France) equipped with a filled column M5A and a TCD (Chrompack, France). Helium was used as carrier gas and the temperature was set at 80°C for 3min.

RESULTS AND DISCUSSION

This section is presented according to the different steps of the requirement driven approach (RDA) we proposed to design an optimal packaging for fresh produce. This approach was adapted from Castro's methodology [Castro et al. 2002] and is illustrated in Figure 3.1 for optimal packaging of strawberries:

-Step 1. "Determination of produce needs" (*"Early requirements"* in the Castro method): evaluation of parameters (physical, chemical, physiological, microbiological, and environmental) that could affect quality attributes and shelf life of the produce in conditions of use, and could have an impact on its commercial value (closely related to consumer's expectations);

-Step 2. "Foreseen solutions for packaging" (*"Late requirements"* in the Castro method): transcription of the early requirements into possible packaging solutions, taking into account experience and knowledge of all the stakeholders that contribute to the innovation;

-Step 3a. "Dimensioning of Tailor made packaging" (*"Architectural design"* in the Castro method): determination of the format of the packaging depending on the produce weight, turning packaging solutions into packaging/material properties, and selection of the packaging/material constituents to obtain these properties;

-Step 3b. "Detailed design of material" (*"Detailed design"* in the Castro method): if materials do not already exist, conception of the material(s) to meet the desired properties

-Step 4. "Validation of tailor-made packaging in condition of use" (additional step to the Castro method): validation of efficiency of the tailor made packaging in the conditions of use.

The two first steps rely on acquisition of data and knowledge from a state of the art combined to critical considerations from producers, suppliers, retailers, and consumers. The third one is based on reverse engineering and needs modeling tool(s) to determine format and optimal properties of the packaging. The two last steps consist in experimental procedures for conception of material(s), when necessary, and packaging validation.

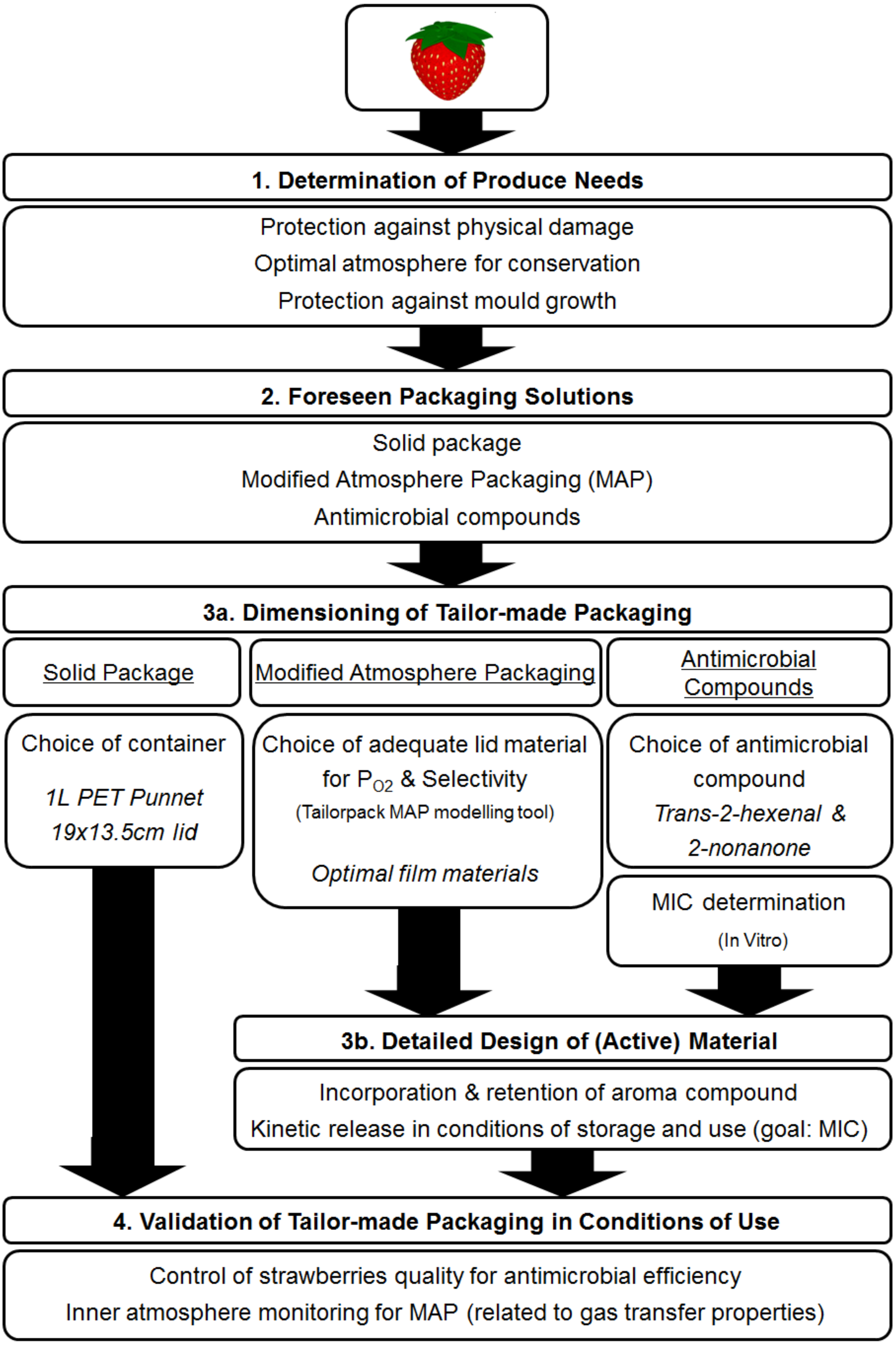


Figure 3.1. Requirement Driven Approach adapted to the development of optimal MAP for strawberries

Determination of produce needs (Step 1 of the RDA).

As highly perishable commodities, strawberries exhibit a short shelf life during post-harvest. They are characterized by delicate tissues easily damaged by mechanical stress [Moras 2005], fungal spoilage mainly caused by the growth *Botrytis cinerea* (the most common) and *Rhyzopus stolonifer* (the most resistant) [Maas 1981; Bhaskara Reddy et al. 1998], and high respiration rates [Kader et al. 1989] inducing their senescence and consequently changes in quality attributes such as texture, color, odor, flavor, acidity, and sweetness [Shamaila et al. 1992]. To extend their shelf life, main requirements have been identified [UCDavis 2012] and are presented in order of decreasing importance: protection against physical damage, inhibition/delay of fungal growth, and reduction of the respiration rate (while avoiding anoxia) to delay senescence. It should be noted that in the specific case of strawberries, senescence is generally not observed due to the fast degradation induced by mold proliferation.

Foreseen solutions for packaging (Step 2 of the RDA).

To conceptualize an efficient packaging solution for strawberries, it was necessary to prospect the solutions already considered.

Protection against physical stress. Physical damages might occur during handling and transportation all along the supply chain as well as at selling points since customers might touch the product (which is also a source of microbial contamination). For this reason, strawberries require a physical protection that can only be brought by a solid packaging structure. The solution already widely applied is to use punnets either made of PET, cardboard or light wood [Moras 2005] (sometimes with a bubble wrap layer on the bottom of the punnet).

Protection against molds. Due to their sensitive and irregular surface, external washing is poorly efficient toward microbial decontamination. Coating strawberries with films based on starch, chitosan or wheat gluten have also been experienced [Vu et al. ; Tanada-Palmu et al. 2005; Vargas et al. 2006] but was not fully satisfactory and some coatings even had detrimental effects. High level of CO_2 (10% or higher) and/or use of volatile antifungal agents as essential oil (red thyme, oregano, clove, cinnamon, tea tree) or aroma compounds (as 2-nonanone) have been demonstrated efficient to delay or inhibit mold growth from strawberries [Vu et al. ; El-Goorani et al. 1979; El-Kazzaz 1983; Bhaskara Reddy et al. 1998; Fernandez-Trujillo et al. 1999; Hertog et al. 1999; Almenar et al. 2007; Rodriguez et al. 2007; Almenar et al. 2009]. However, high amount of CO_2 (higher than 15%) is questionable as some author demonstrated that such treatment could induce off-odor [El-Kazzaz 1983; Ke et al. 1991; Shamaila et al. 1992]. When using essential oils or aroma compounds, part of them can be absorbed by fresh tissues and therefore induce organoleptic changes as the modification of strawberries aromatic profile or produce browning. Moreover, even if they are chosen among generally recognized as safe (GRAS) compounds, they can exhibit toxicological effect on consumers if present at high level in the produce. Then it is necessary to verify if their minimal inhibition concentration (MIC) towards the molds considered is below the oral toxicity value of LD_{50}. Whether essential oils appeared interesting for economical reason and large spectrum efficiency [Pirbalouti et al.], volatile aroma compounds were retained in the present work for inhibiting/delaying fungal decay in strawberries because their side-effects can be more easily controlled and understood.

Delaying senescence. Even if lowering of the fruit respiration rate is not of major importance for strawberries preservation, the inner atmosphere composition need to be considered in order to avoid anoxia (or fermentative catabolism). So a minimum oxygen level should be maintained inside the packaging, but the limit is not precisely set. Some authors witnessed fermentation below 2.3% of O_2 [El-Goorani et al. 1979] but other studies have demonstrated that short storage at 1% O_2 and 5°C was harmless for the fruit and that treated strawberries were not differentiable from untreated ones (stored at air composition) by an untrained panels [Ke et al. 1989; Ke et al. 1991]. Too high amounts of CO_2 (>15%) and its subsequent acidification of the fruit also needed to be avoided to meet consumers’ expectations as discussed previously. So it was decided that atmosphere composition featuring O_2>1% and CO_2<15% should be targeted.

As a consequence of this bibliographical work, the packaging solution proposed was based on a two parts packaging (Figure 3.1): a solid punnet (part 1) for mechanical protection; and a lid (part 2) for antimicrobial volatile compounds release for inhibition of mold growth and gas transfer regulation for establishment of an optimal atmosphere for preservation (O_2>1% and CO_2<15%).

Dimensioning of tailor-made packaging (Step 3a of the RDA).

The format of the packaging was first selected in order to remain close to commercial strawberry packaging encountered for 500g of produce: volume was fixed to 1 L and the opening surface to 256cm². A PET punnet has been chosen as a solid punnet against physical damage. Then the lid material constituents were selected to ensure gas transfer regulation and antifungal activity. A MAP modeling tool was used to determine the optimal range of O2 permeability (Pe_{O2}) and permselectivity (S), equal to CO_2/O_2 permeability ratio. This allowed the identification of materials exhibiting these gas transfer duties as potential lid materials of the packaging. At the same time, choice of the antimicrobial compounds and evaluation of the minimal inhibitory concentrations (MIC) towards B. cinerea and R. stolonifer (targeted amount of active agent required in the further packaging headspace) were performed.

Choice of Adequate Lid Material. The lid material must exhibit adequate Pe_{O2} and S to reach an optimal atmosphere range for strawberries (at least 1% O_2 and below 15% $CO_{2,}$ as previously defined). The Tailorpack MAP modeling tool was used in optimization mode to determine targeted Pe_{O2} and S values, with input data from Table 3.1 and neglecting gas exchanges through PET (Pe_{O2}: $0.02x10^{-11}mol.Pa^{-1}.m^{-2}.s^{-1}$) all along the short time span of strawberry. These values were then compared to the ones of several films including wheat gluten based materials, selected due to the interesting intrinsic transfer properties of WG based materials [Gontard et al. 1996b; Cagnon et al. 2012] (Table 3.2).

Low density polyethylene (LDPE), oriented polypropylene (OPP), Untreated Coated Paper and Kraft WG-Paper were found to fit the optimal Pe_{O2} and S requirements ($Pe_{O2}>1.43x10^{-11}$ $mol.Pa^{-1}.m^{-2}.s^{-1}$ and S>1.60). Oppositely, the micro-perforated plastic could exhibit the required Pe_{O2} but would never be selective enough. Only OPP (conventional plastic) and the Kraft WG-Paper (agro-material) were retained as lid material since they exhibited lower Pe_{O2} than their counterparts (LDPE and Untreated Coated Paper respectively) and so would likely fare better in the matter of reducing strawberry respiration.

Table 3.1. Input data to run the Tailorpack web application (MAP modeling tool) in optimization mode

Produce		Produce characteristics and properties*				Packaging Dimensions				Optimal Atmosphere	
Type	Variety	RR_{O2max} (mmol.kg^{-1}.h^{-1})	RQ	Km_{appO2} (kPa)	Ki_{CO2} (kPa)	Type	Lid Surface (m²)	Volume (m³)	Mass (kg)	O_2 (%)	CO_2 (%)
Strawberry	*Charlotte*	0.800±0.1	0.91	8.10±1.5	/	PET Punnet +Lid	0.0256	0.00100	500±5	>1	<15

*strawberry respiratory data from personal database

Table 3.2. Gas transfer properties of the targeted lid material (from simulation with input data mean values) and of a selection of existing materials

Lid Material	RH (%)	S	Pe_{O2} (mol.Pa^{-1}.m^{-2}.s^{-1})	Thickness (µm)
Target (O_2>%1 & CO_2<15%)	NA	> 1.6	> 1.43×10^{-11}	50*
UTP (untreated WG coated paper)[19]	80%	2.03	11.3×10^{-11}	73.5
Kraft WG-Paper[18]	100%	5.1	3.33×10^{-11}	69.5
LDPE[58]	50%	3.8	2.10×10^{-11}	50
OPP[18]	50%	2.7	1.49×10^{-11}	74
Micro-perforated plastic[59]	50%	1	2.74×10^{-12} to 5.48×10^{-7}	35

*Theoretical thickness set for simulation

Choice of antimicrobial compounds. To limit the growth of both *B. cinerea* and *R. stolonifer* two volatile natural antimicrobial agents, 2-nonanone and trans-2-hexenal have been identified. The former because it is an aroma compound of strawberry that has been already tested in active packaging for strawberries [Almenar et al. 2007; Almenar et al. 2009] and the latter because it is produced by strawberries in response to wounding as a protection against fungal growth [Myung et al. 2006] and have also been found efficient in delaying mold growth on other fruits such as apples [Corbo et al. 2000].

MIC determination. Various amounts of trans-2-hexenal and 2-nonanone were tested to evaluate the MIC of both compounds towards each mold. The mold charge was set at 10^3 spores per Petri dish, a slightly over evaluated value of the mold charge present in commercial 500g strawberries punnets (unpublished data from CTIFL, France, years 2008-2009). Figures 3.2a & 3.2b showed examples of the growth of molds (*B. cinerea*) on Petri dishes via the diameter of the contaminated area on the agar gel, thus allowing the determination of a mold growth delay or the MIC (no growth during 10 days). It is important to mention that MIC is very useful to compare compounds in their antimicrobial efficiency but is determined in conditions that may not match realistic storage, especially in terms of temperature, mold charge and time.

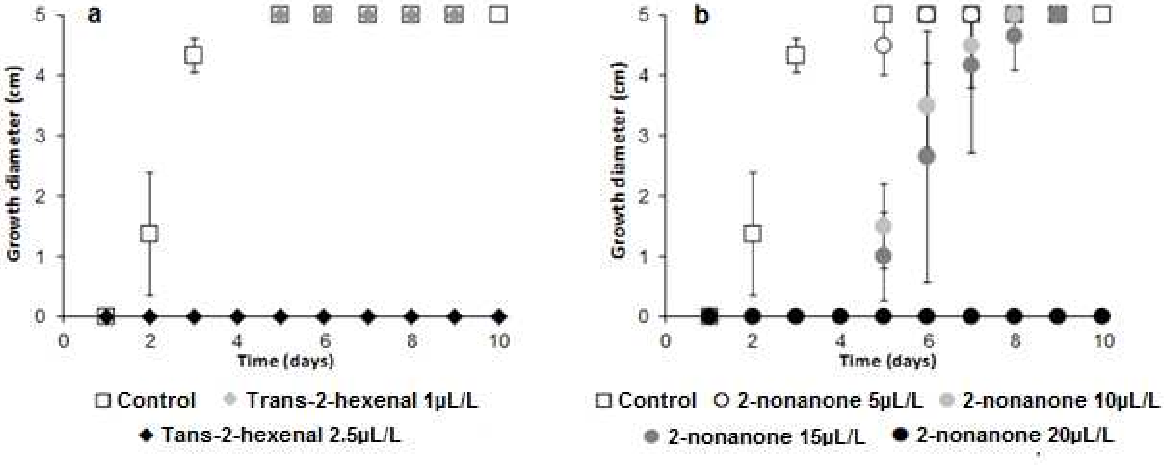


Figure 3.2. Growth of B. cinerea at 100% RH and 22°C expressed in diameter for different concentrations of (a) trans-2-hexenal and (b) 2-nonanone

The MIC for trans-2-hexenal towards both molds was interestingly low: only 2.5µL.L^{-1} of air (i.e. 2.11mg.L^{-1}) were required for *B. cinerea* inhibition and 5µL.L^{-1}of air (i.e. 4.23 mg.L^{-1}) for *R. stolonifer* inhibition (data not shown)). This compound was more efficient than carvacrol, which exhibit a MIC of 5µL.L^{-1} of air towards *B. cinerea* [Ben Arfa et al. 2007b]. Such efficiency was not previously suspected. Vaughn et al. [Vaughn et al. 1993] proved a total inhibition of *B. cinerea* by 20µL.L^{-1} of air of trans-2-hexenal but did not test any lower concentration. In regard to 2-nonanone, its MIC was significantly higher as 20µL.L^{-1} of air (i.e. 16.4mg.L^{-1}) were required to inhibit *B. cinerea*. Vaughn et al. [Vaughn et al. 1993] determined a MIC value of 40µL.L^{-1} for 2-nonanone toward the same mold but the exact conditions of the experiment (number of spores inoculated) were not given. For *R. stolonifer* more than 40µL.L^{-1} of 2-nonanone prevented its growth (data not shown). Whatever the aroma compound, MIC calculated here were far lower than LD$_{50}$ (3200mg/kg for 2-nonanone and 850 mg.kg^{-1} for trans-2-hexenal) thus guaranteeing no risk of health hazard even if other input occur.

It has been proven that compounds with strong hydrophobic characteristics (2<LogP<4) solubilize easily in cell walls and so tend to reach the toxic concentration leading to cell destruction [Sikkema et al. 1995; Weber et al. 1996; Ben Arfa et al. 2006]. So, looking at their respective log P (1.7 against 2.9 for trans-2-hexenal and 2-nonanone respectively), 2-nonanone should have been slightly more efficient than trans-2-hexenal. But the chemical nature and the ability of the compound to act as a proton exchanger have also to be taken into account [Ultee et al. 2002; Ben Arfa et al. 2006]. Being an aldehyde, trans-2-hexenal with a double bond in second position is more likely to exchange a proton with the cell structure than the 2-nonanone or be able to link DNA and protein through its aldehyde function. This would make it more efficient in making the cell wall collapse and thus damaging the mold.

Since reasons of their efficiency are also reasons of their possible phytotoxicity, strawberries were subjected to the determined MICs (as well as 40µL.L^{-1} of air of 2-nonanone) and their quality was daily assessed. It appeared that the highest doses of both aroma compound (5 and 40µL.L^{-1} of air for trans-2-hexenal and 2-nonanone, respectively) caused external injuries (darkening of berries or akenes) whereas their lower doses (corresponding to MICs towards *B. cinerea*) maintained the fruits quality compared to the control (Table 3.3). In addition, aroma smelling was detected for the highest concentrations only, whatever the compound studied. In such condition, only the lower doses (2.5 and 20µL.L^{-1} of air for trans-2-hexenal and 2-nonanone, respectively) were retained for the study even if they are not sufficient to inhibit the growth of *R. stolonifer*. They could at least delay its growth for few days.

Table 3.3. Effect of the determined MICs on fruit qualities (visual aspect and presence of aroma odor at punnet opening)

		Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Control	Aspect	++	+	- (decay)	- (decay)	- (decay)
(no aroma compound)	Aroma Odor	/	/	/	/	/
trans-2-hexenal 2.5µL	Aspect	++	++	+	+	+
(MIC towards *B. cinerea*)	Aroma Odor	No	No	No	No	No
trans-2-hexenal 5µL	Aspect	++	+	- (burn)	- (burn)	- (burn)
(MIC towards *R. stolonifer*)	Aroma Odor	Slight	Strong	Strong	Strong	Strong
2-nonanone 20µL	Aspect	++	++	+	+	+
(MIC towards *B. cinerea*)	Aroma Odor	No	No	No	No	No
2-nonanone 40µL	Aspect	++	+	- (burn)	- (burn)	- (burn)
(Delay of *R. stolonifer* growth)	Aroma Odor	Slight	Strong	Strong	Strong	Strong

Detailed design of (active) material (Step 3b of the RDA).

The aim of this step was to design the lid material with the ability to retain aroma compound at its above-mentioned MIC towards *B. cinerea* when stored and to release it quickly in condition of use (when put in presence of the fruits). The triggering effect identified here is the increase in RH that quickly reaches around 95-100% inside the package.

Among the selected candidate, synthetic polyolefin as OPP have been found to well retain and absorb volatile aroma compound but to present limited release capacity [Pezo et al. 2008]; moreover no possibilities of RH controlled released have ever been reported. It can be noted that it is possible to enhance the retention/release capacities of materials by in bulk incorporation [Kollengode et al. 1997; Almenar et al. 2007; Tonelli 2008; Astray et al. 2009] or surface application [Del Valle 2004] of cyclodextrins that get this ability. However this complex and costly process was not envisaged in this study since multiple studies already proved the interesting RH dependent retention/release of protein based materials [Chalier et al. 2006; Chalier et al. 2007b; Mascheroni et al. 2010] such as wheat gluten. Consequently incorporation of 2-nonanone and trans-2-hexenal was only tested on Kraft WG-Paper. Kraft WG-Paper containing aroma compound will be referred hereinafter as Active WG-Paper whereas the original Kraft WG-Paper (devoid of aroma compound) will be referred as Passive WG-Paper and used as a standard.

Incorporation & retention of aroma compounds (on lid size material). Solutions of WG containing 3 different concentrations of aroma compound (15%w/dry-w, 30%w/dry-w and 45%w/dry-w) were tested. Residual amount of aroma compound in the Active WG-Paper after coating and drying was assessed, along with the retention percentage compared to the initial quantity of the compound (Table 3.4). It has been previously demonstrated that increasing the concentration of volatile aroma compound in a protein based casting solution can improve its retention during the fabrication process [Chalier et al. 2007a].

Extremely poor retention of trans-2-hexenal by the WG material during the processing of the material was observed, as indicated by the low values of residual amount whatever the initial concentration. Moreover, increasing the incorporated dose brought no significant improvement of the quantity retained and as a consequence, the percentage retained decreased significantly. Optical microscopy observations on solutions (not showed) ruled out exclusion of trans-2-hexenal from the matrix as the mix were homogeneous whatever its concentration. This apparent low retention could be linked to the high volatility of trans-2-hexenal or a crosslinking of the matrix by the trans-2-hexenal, which would consume a large part of the aldehyde. The first point, expected because of the high saturation vapor pressure of the compound (879.8Pa) could explain the overall low retention but not the non-augmentation of retention along with the introduced quantity. The second one seemed in accordance with previous results on cinnamaldehyde incorporated into a soy protein matrix [Ben Arfa et al. 2007b] and the fact that aldehydes are known for their strong ability to cross-link proteins [Kupec et al. 2003; Balaguer et al. 2011]. In the end, whatever the concentration introduced, the MIC for trans-2-hexenal previously determined was never reached.

On the contrary, for the 2-nonanone, the MIC (16.4mg/L) was reached in Active WG-Paper made from WG solutions containing 30 and 45% w/dry-w of aroma compound (retention percentage of 27.6% and 30.1% respectively). However, the retention percentage remained lower than 50% whatever the initial concentration tested whereas past studies reported up to 76.4% of retention for carvacrol within a WG layer [Mascheroni et al. 2010]. It could be explained by a better compatibility of the WG matrix with carvacrol than with 2-nonanone. Indeed, the difference in compatibility with a matrix between two compounds can be assessed by looking at the evolution of the consistency between the matrix solution alone and the matrix solution with addition of compound [Mascheroni et al. 2010] . In the case of a 15% w/dry-w addition of aroma compounds, carvacrol increased the consistency index by almost $7Pa.s^n$ [Mascheroni et al. 2010] whereas 2-nonanone only brought an increase of $0.0275Pa.s^n$, which showed a far better compatibility between carvacrol and WG than between 2-nonanone and WG. The evolution of retention ratios with the increase of 2-nonanone introduced were in accordance with previous results obtained on carvacrol and soy protein coated papers by Ben Arfa et al. in 2007 [Ben Arfa et al. 2007b], even if the overall retention values were far lower with the Active WG-Paper containing 2-nonanone (27.8% against 82.4% retained for 30% w/dry-w introduced). It can also be noted that 2-nonanone is more volatile than carvacrol which can also explain the higher losses during processing (saturated vapor pressure: 75Pa for 2nonanone against 6.4Pa for carvacrol). But the goal of this part was not to achieve the highest retention inside the matrix but to reach the expected dose (MIC) with a minimal amount of aroma introduced in solution.

In conclusion, the best coating solution in terms of quantity of product introduced and amount of agent trapped was the one containing 30% w/dry-w of 2-nonaone and, only the materials coated with this solution were to be tested for the controlled release.

Table 3.4. Aroma compound retention capacity of Active WG-Papers as function of the type and amount of aroma compound added (and information about their coating weights and thicknesses)

Aroma Compound	Initial Amount in Solution (%w/dry weight)	Coating Weight ($g.m^{-2}$)	Thickness (µm)	Residual Amount on lid material (mg)	Retention (%)
None	0	13.44±1.03	69.55±1.33	NA	NA
Trans-2-hexenal	15	13.44±1.20	69.43±0.79	0.282±0.08	0.53±0.17
	30	14.01±0.89	70.02±1.06	0.31±0.01	0.4±0.01
	45	14.25±1.17	70.89±0.96	0.30±0.03	0.2±0.02
2-nonanone	15	14.02±0.93	69.50±1.47	14.2±1.04	20.2±2.02
	30	14.78±0.27	69.62±1.12	21.5±4.65	27.8±3.33
	45	14.75±0.42	68.98±0.93	46.2±6.65	30.1±4.32

Kinetic release of aroma compound in conditions of storage and use. It is necessary to ensure that the release of the trapped 2-nonanone happens only in conditions of use and not in storage conditions. In addition, quick and complete MIC release is expected as slow or incomplete release might allow mold growth. For these reasons, storage conditions and conditions of use were simulated as 50% RH and 20°C during 14 days and 100% RH and 20°C for 48 hours, respectively, and results appeared in Figure 3.3.

The release of 2-nonanone was inferior to 1mg when the RH was kept below 50% even after 14 days of storage. But when the RH was raised to 100%, the release was triggered and all the 2-nonanone was quickly released from the material (less than 48 hours); MIC was reached in less than 24 hours. This triggering effect of RH was attributed to wheat gluten proteins physicochemical properties change at high RH [Mujica Paz et al. 1997]. Indeed, at high RH wheat gluten films undergo plasticization and the chain mobility inside the matrix increases, which should increase the diffusivity of molecules such as the aroma compounds through the matrix. In previous studies, a multiplication by 2 or 20 of the diffusivity of 2-nonanone in gliadin [Balaguer et al. 2012] and carvacrol in WG [Mascheroni et al. 2011], respectively, were reported for an augmentation of relative humidity. These results confirmed that the Active WG-Paper should be able to endure storage without active compound losses (even if it still needs to be confirmed on a larger time span and more drastic conditions) and will quickly release the entire amount of active volatile compound available (and so the MIC) in conditions of use.

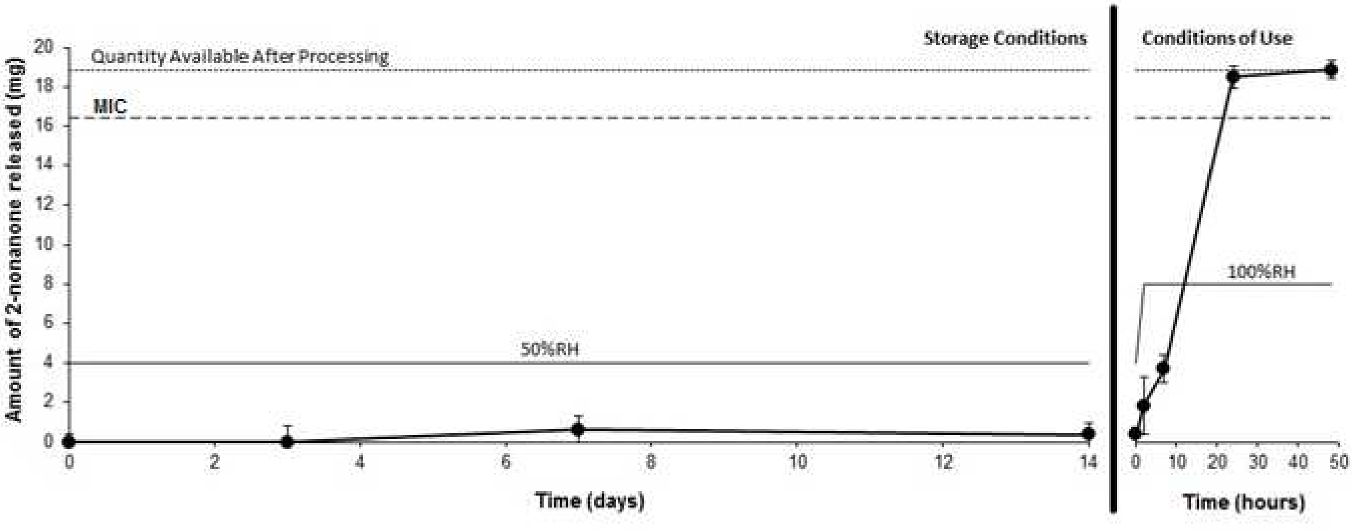


Figure 3.3. Kinetic release of 2-nonanone from Active WG-Paper (lid size: 256cm²) in conditions of storage (50% RH) and usage (100% RH) at 20°C

Validation of tailor-made packaging in condition of use (Step 4 of the RDA).

In order to complete the packaging development, the Tailor-Made Active Packaging was tested and compared to a Tailor-Made Passive Packaging and the Control in terms of quality of the fruits (35 berries, i.e. around 500g) and inner oxygen content. RH was set at 100% for maximum efficiency of the packaging (antimicrobial agent release and gas transfer properties) and the temperature was set at 20°C to simulate storage conditions at retailing points.

Inner atmosphere composition. The inner oxygen content was monitored, as it was a critical point to avoid anoxia. Passive (not showed) and Active Tailor-Made Packaging (Figure 3.4), both presented the same behavior. The oxygen content remained low (below 2.5%) but well above the 1% minimal limit, thus likely reducing the senescence of the fruit while avoiding risk of any detrimental effects due to low oxygen content. The similar evolution in both packaging was expected since O_2 permeation and permselectivity values of the Active WG-Paper (Pe_{O2}: $3.42x10^{-11}mol^{-1}.Pa.m^{-2}.s^{-1}$ and S: 4.9) were not significantly different from the ones of the Passive WG-Paper material (Pe_{O2}: $3.33x10^{-11}mol^{-1}.Pa.m^{-2}.s^{-1}$ and S: 5.1) and remained in the optimal ranges previously determined. However, experimental values did not exactly fit the one predicted thanks to the Tailorpack web application, which may be due to the variability of fresh produce and the fact that transfer properties of PET punnet were neglected. Guillard & al. [Guillard et al. 2011] recently found a way to deal with such variability problems (from the produce or the packaging materials) by implementing parameters uncertainties and error propagation into MAP modeling tools. Finally, in both Passive and Active Tailor-Made Packaging, the recorded CO_2 contents were well below the 15% limit at the end of the experiment.

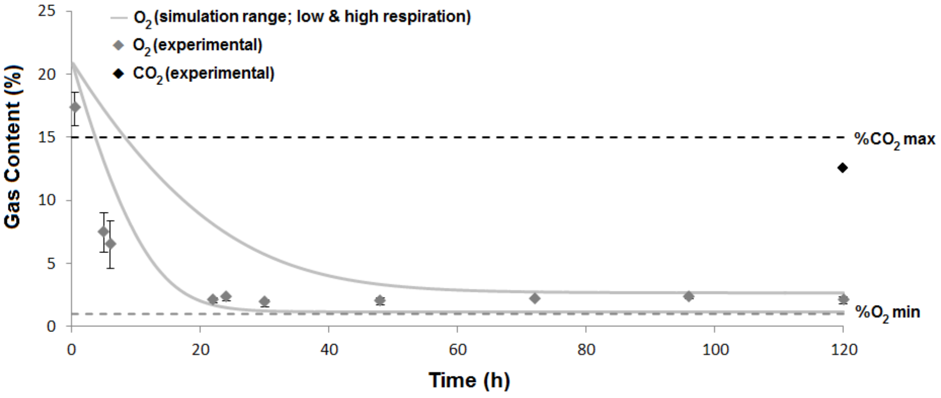
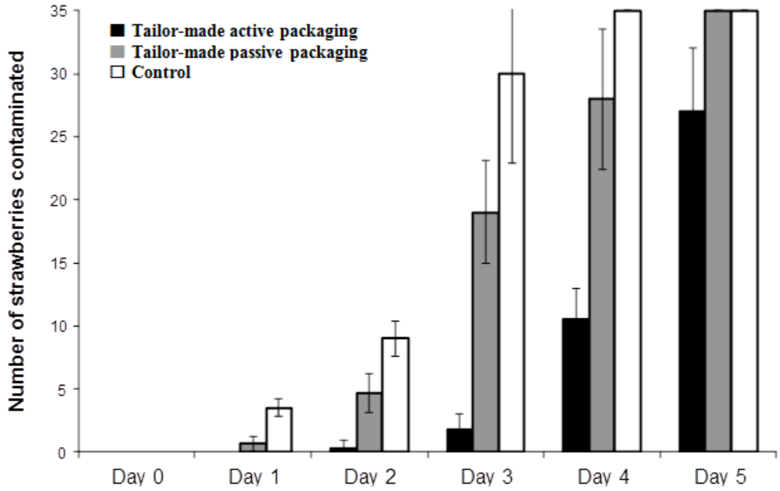


Figure 3.4. Evolution of the atmosphere composition inside the Active Tailor-Made Packaging for strawberries stored at 100%HR and 20°C

Quality of the strawberries. The number of contaminated strawberries visible from outside the packaging was assessed each day along with the aspect of the berries and the presence of exudate.

The Active Tailor-Made Packaging delayed contamination start (considered as more than 5 contaminated strawberries per package) for 3 days, and the Tailor-Made Passive packaging delayed it for 1 day compared to the control for which contamination was detected at day one (Figure 3.5). CO_2 was suspected to act as an antimicrobial agent in both Passive and Active Tailor-Made Packaging (present at around 12% inside both packaging). Increased shelf- with the active packaging life (2 more days compared to the passive packaging) was thus attributed to the release of the antimicrobial agent (2-nonanone). The delay in mold growth observed in the Active Tailor-Made Packaging instead of the expected total inhibition (due to the MIC) could be explained by both the bi-directional release of the volatile agent (towards the berries and towards the outside) and adsorption into the PET punnet. Apparition of exudate inside the Tailor-Made Packaging was delayed by 2 days compared to the control and passive packaging and the visual quality of the berries was better in this packaging at every step of the experiment (Figure 3.5). This confirmed both the antimicrobial efficiency and the positive effect on berries quality of 2-nonanone already observed by Almenar et al. in 2009 [Almenar et al. 2009].



		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Control	Exudate	o	💧	💧💧	💧💧💧	💧💧💧💧	💧💧💧💧
	Aspect	++++	+++	++	+	-	-
TM Passive	Exudate	o	💧	💧	💧💧	💧💧💧	💧💧💧💧
	Aspect	++++	++++	++	+	-	-
TM Ative	Exudate	o	o	o	💧	💧	💧💧💧
	Aspect	++++	++++	++++	++++	++	+

Figure 3.5. Test of packaging antimicrobial efficiency (100%RH and 20°C): Strawberries contamination and quality (overall aspect and exudate)

CONCLUSION

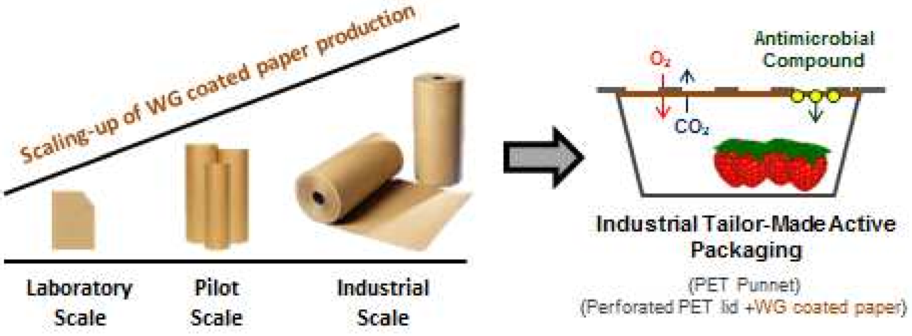
The requirement driven approach was successfully adapted to the field of fresh food packaging. A new active modified atmosphere packaging tailored to the needs of strawberry (protection against physical stress and microbial growth, and reduction of the respiration rate) was designed using this approach. It was based on a PET punnet (for mechanical protection) closed with a WG coated kraft paper lid which was in charge of both the establishment of the optimal atmosphere composition for preservation and the RH triggered release of a sufficient dose of an antimicrobial agent (2-nonanone). This Active Tailor-Made Packaging was successfully tested in high RH conditions (100% RH), which could be easily obtained with water vaporization systems for example, and allowed to extend the shelf life of the strawberry by 3 days.

Additional Results

Up-scaling of Active WG-Paper production

Partners: UMR IATE, Centre Technique du Papier (Grenoble, France), Gascogne Paper (Mimizan, France), Adivec (Germany)

ABSTRACT: The production of active and passive wheat gluten coated papers (Active WGP and Passive WGP) was up-scaled from laboratory scale to industrial scale (with an intermediate pilot scale production). The materials produced were characterized in terms of coating weight, gas transfer properties and antimicrobial compound (2-nonanone) retention. The industrial materials were found to exhibit lower coating weight but higher gas transfer properties than their laboratory counterparts which was due to the different kraft paper used as support for coating and the industrial coating technique. But materials produced at industrial scale were found to retain slightly higher contents of 2-nonanone thanks to a very quick drying (less than 30 seconds instead of 2h at laboratory scale). This result indicated successful up-scaling in term of potential antimicrobial efficiency of the material.



KEYWORDS: Up-scaling, Industrial production, Wheat gluten, Paper, Active MAP, Strawberries

OBJECTIVES

Given the positive impact of the Active Tailor-made Packaging (with WG coated paper lid loaded with 2-nonanone) built at laboratory scale on strawberry, it was interesting to assess the feasibility of an industrial scale production of Active WGP.

Consequently production of WG coated paper was first tried at pilot scaled. Then, the Industrial Active and Passive WGP were produced and their gas transfer and aroma compound retention properties evaluated.

MATERIALS AND METHODS

Materials.

Commercial kraft papers (Teranna 36g/m² for laboratory and pilot production and Kalysack 70g/m² for industrial production) were provided by Gascogne Paper (Mimizan, France) in roll or sheet form to serve as support for coating. Jackering (Hamm, Germany) supplied vital wheat gluten (WG) powder, containing approximately 7.2wt. % of moisture and 76.5wt. % of proteins. All chemical products as acetic acid and sodium sulphite used to prepare the WG coating solution were purchased from Aldrich (St Quentin, France). 2-nonanone (purity 99%; density: 0.830; saturated vapor pressure: 75Pa; LogP: 2.9; all at 25°C) used as antimicrobial compounds and 2-nonanol (purity 97%) used as internal standard were purchased from Aldrich (St Quentin, France) as well as sodium sulphate for the extraction procedure. Strawberries (*var. Charlotte*) and PET punnets were provided by a local farmer for testing packaging efficiency in conditions of use.

Material production at laboratory scale.

Preparation of the Passive WG coating solution. The Passive WG coating solution (21.23% dry w/v) was prepared at room temperature according to a 3 steps procedure [Guillaume et al. 2010a]. First, 30g of WG powder were dispersed under shacking in 50mL of a sodium sulphite solution (0.06 g/50mL), which worked as a reducing agent of the disulfide bonds. Then, after 30min of settling, the pH of the solution was set to 4 by adding a 50% v/v solution of acetic acid. Finally the solution was adjusted to 130mL by adding deionized water and the whole mix was stirred and left to rest for a day.

Preparation of the Active WG coating solution. The desired active compound was added (15, 30 and 45% w/dry-w) to the Passive WG coating solution to prepare the Active WG coating solution used to create the antimicrobial materials. The whole mix was stirred again for 5min at 8000rpm with a L4RT high speed mixer from Silverson (Chesham, England). This active solution was used immediately for coating or analyses (microscopy observations or viscosity measurements).

Laboratory scale paper coating. The coating was performed on the raw side of Teranna 36g.m^{-2} sheets (A4 format) immediately after the preparation of the coating solution using an E409 blade coater from Erichsen (France) equipped with the blade n°8 featuring a spire width of 1mm (Figure 3.6). Coating speed was set at 10mm.s^{-1}. To be spread all over the sheet surface, an excess (>3 mL) of coating solution was deposited to produce coated papers. After coating, materials were dried under 2 different conditions:

- Room: 20°C for 2 hours with relative humidity kept below 40% and
- Tunnel: 110°C under pulsated air for less than 30 seconds (in a tunnel drier)

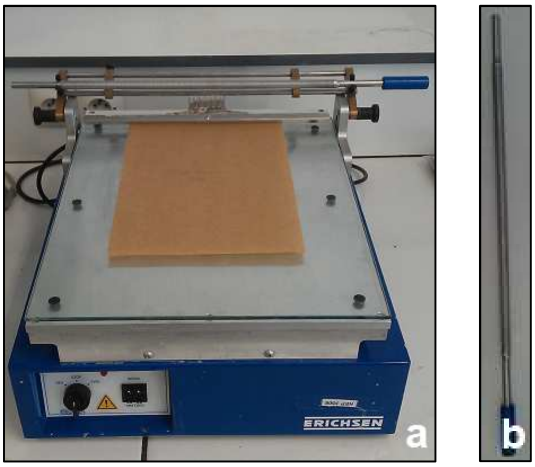


Figure 3.6. Laboratory coating machine Erichsen E409 (a) and view of the coating tool (b)

Material production at pilot scale.

Preparation of the Passive WG coating solution. The Passive WG coating solution (21.23 wt.%) for pilot production was prepared at room temperature according to a 3 steps procedure [Guillaume et al. 2010a], adapted to match the desired quantities. First, 2.3kg of WG powder was poured into the bowl of a Hobart planetary mixer, 3.85L of a sodium sulphite / deionized water solution (4.60g / 3.85L) were added and the mix was stirred until formation of homogenous dough. The sodium sulphite solution worked as a reducing agent of the disulfide bonds. Then, after 30min of settling, the pH of the solution was set to 4 by adding a 50/50 vol. solution of acetic acid and deionized water. Finally the solution was adjusted to 10L by adding deionized water and the whole mix was stirred and left to rest for a day. The procedure was repeated ten times to produce a 100L batch and the solution exhibited similar viscosity than its laboratory equivalent.

Pilot scale paper coating. A roll of Teranna 36g.m^{-2} kraft support paper was placed in the "Pilot-coating" machine of the Centre Technique du Papier (CTP, Grenoble, France). The coating tank was filled with the Passive WG solution (Figure 3.7). Given preliminary results, a "soft blade" was used as coating tool, the speed was set at 70m.min^{-1} and the drying was chosen as 108kW.h^{-1} (infra-red lamps) and 200°C (hot air) combined.

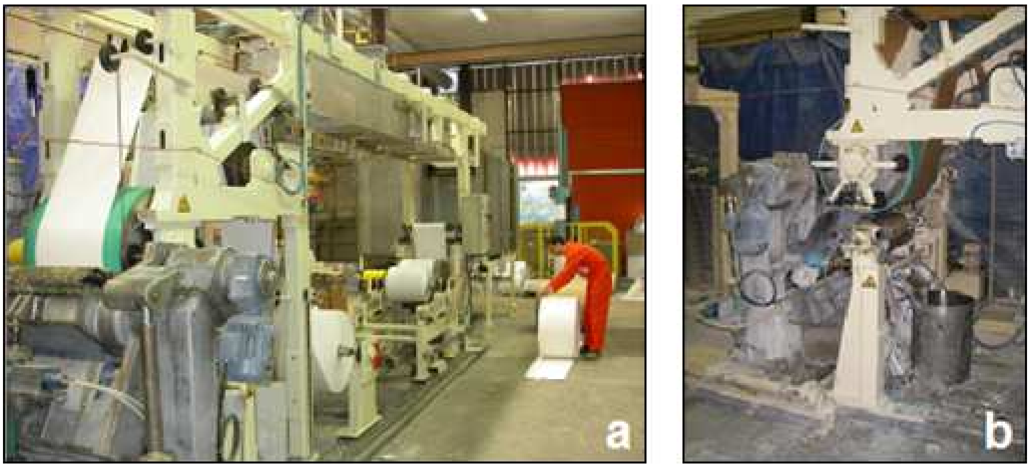


Figure 3.7. CTP "Pilot-Coating" machine (a) and view of the coating tank and tool (b)

Material production at industrial scale.

Preparation of the Passive WG coating solution. The Passive WG coating solution (21.23 wt.%) for industrial production was prepared by Adivec (Hamm, Germany) at room temperature according to the same 3 steps procedure adapted to match the desired quantities. A 1000L batch was prepared in a Z-mixer. The solution was used 4 days after preparation and exhibited similar viscosity than its laboratory equivalent.

Preparation of the Active WG coating solution. The Active WG coating solution (21.23 wt. %) for industrial production was initially prepared the same way by Adivec (Hamm, Germany). The only difference was the addition of 2-nonanone (30% w/dryw) at the end of the preparation. A 1000 L batch was prepared in a Z-mixer. The solution was used 4 days after preparation and exhibited similar viscosity than its laboratory equivalent.

Industrial scale paper coating. A roll of Kalysack 70g.m^{-2} kraft support paper was placed in the industrial paper coating machine of Gascogne Paper (Mimizan, France). The coating tank was filled with the Passive WG solution (Figure 3.8). Given preliminary results, an engraved cylinder was used as coating tool (pressure 200 mbar), the speed was set at 200m.min^{-1}and the drying temperature at 125°C (pulsated hot air).

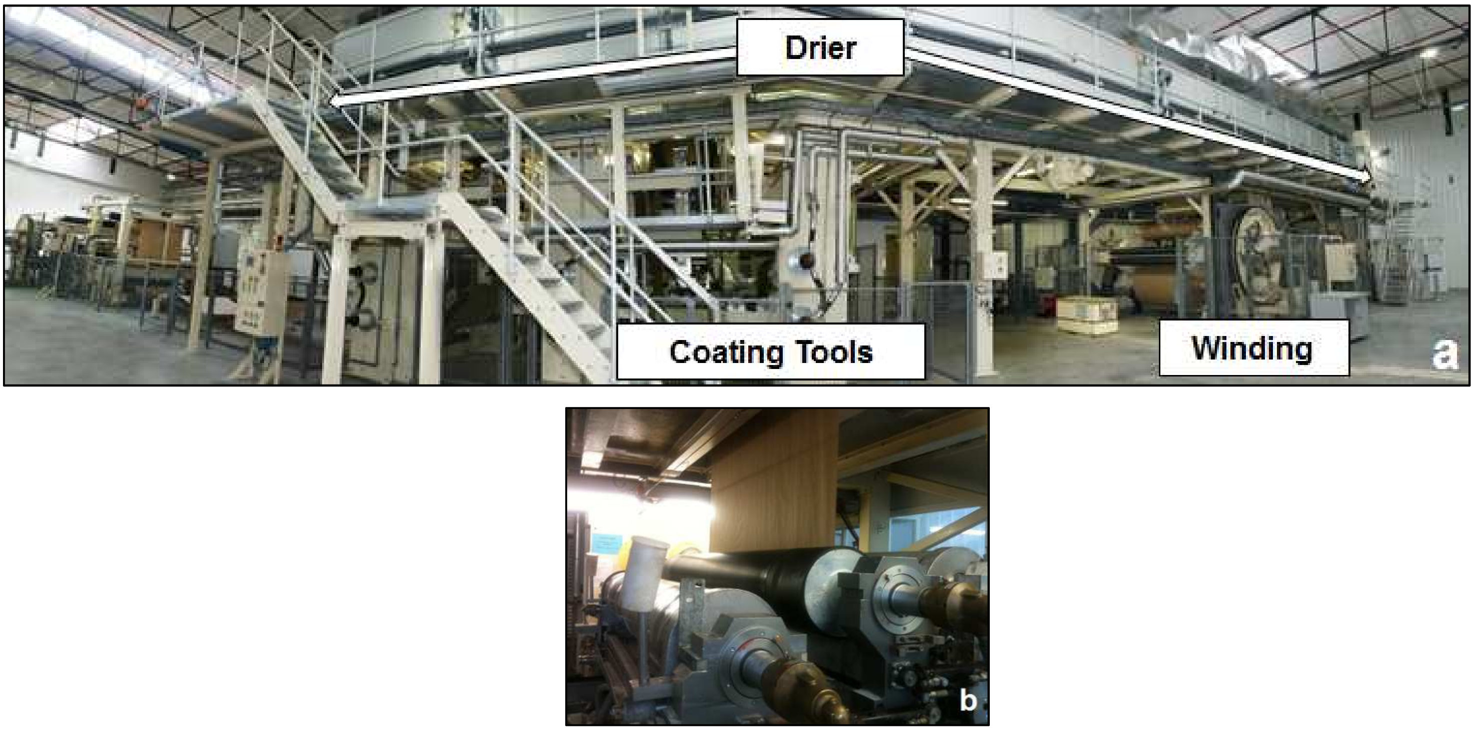


Figure 3.8. Gascogne Paper industrial paper coating machine (a) and close up on the coating tool (b)

Laboratory and up-scaled WG coated papers characterization.

Thickness. The average thickness of each produced material was determined at room temperature and 30% RH with a hand-held digital micrometer (Mitutoyo instruments) from 10 measurements randomly taken over the paper surface.

Coating weight. To assess the coating weight, 9 square pieces (5x5cm) of both Support-Paper (uncoated) and WG-Paper were cut and left to dry during 24h in a ventilated oven at 103°C. They were then placed to cool into a desiccator containing silica-gel. After one hour they were taken out and weighted with a precision balance. The coating weight (Cw) in grams per meter square was calculated as follows:

$$Cw = \frac{W_{WGP} - W_{SP}}{A} \qquad eq.\ 3.3$$

where W_{WGP} (g) is the weight of a WG coated paper piece, W_{SP} (g) is the average weight of Support-Paper pieces and A (m^2) is the area of a piece.

Gas transfer properties. The O_2 and CO_2 permeation (Pe_{O2} & Pe_{CO2}) of WG coated papers were assessed in triplicate with an isostatic and dynamic method using gas phase chromatography. The materials were placed in a permeability cell. The inferior and superior chambers were each spread by a $30mL.min^{-1}$ flux of permeant gas (O_2 or CO_2) and vector gas (He) respectively. The RH of the gas flux was set using a bubbling flask containing water and placed in a cryothermostat regulated at a properly chosen temperature. The differential temperature imposed between the cell and the cryothermostat permitted the establishment of the desired RH in the chamber. The self-supported materials were measured at lower RH due to their critical mechanical weakness at very high RH. The permeation cell was coupled to a gas chromatograph GC3800 from Varian (Les Ulis, France) equipped with an automatic valve to online analyze the evolution with time of permeant gas concentration in the superior chamber of the cell (analysis of 1mL of sample at prescribed times). The gas chromatograph was equipped with a filled column Porapak-Q from Chrompack (Les Ulis, France) of 2m length and 0.32mm diameter for separating O_2 and CO_2, with a thermal conductibility detector (TCD). The gas (O_2 or CO_2) permeation of the material was determined as follows in the international system unit:

$$Pe_x = \frac{\Delta Q}{\Delta t \times A \times \Delta P} \qquad eq.\ 3.4$$

where subscript x referred to O_2 or CO_2, ΔQ was the number of moles of gas that passed through the film, Δt was the time for which permeation occurred, A was the exposed film area and ΔP is the difference in pressure exerted by the gas on each side of the film. Gas permeation was expressed in $mol.m^{-2}.s^{-1}.Pa^{-1}$ unit. For total O_2 and CO_2 desorption and RH stabilization, materials were placed prior to measurements in the permeation cell using helium to spread both chambers.

Material permselectivity (S) was calculated as the ratio of Pe_{CO2} on Pe_{O2}

Determination of the residual amount of aroma compound in active materials and losses during processing. Extraction of aroma compounds from Industrial Active WGP was performed by immersing square pieces of materials (3x3cm) in a mixture consisting in 5mL of deionized water and 5 mL of dichloromethane. 100µL of a 3% 2-nonanol solution were added to the mixture as internal standard. After 16h under magnetic stirring (500rpm), the organic phase containing the aroma compound and the internal standard was removed and dried over anhydrous sodium sulphate to ensure complete elimination of aqueous phase. The analysis of dried organic phase was carried out on a Varian 3800 GC-FID equipped with a DB5 column (30mx0.25mm, film thickness of 25µm, T8W Scientific) and a flame ionization detector (FID, hydrogen: 30mL.min^{-1}, air: 300mL.min^{-1}, nitrogen: 30mL.min^{-1}). Temperature of the detector was set at 300°C and the injector at 250°C. Hydrogen was used as the carrier gas with a flow rate of 2mL.min^{-1}. The column temperature was programmed to first rise from 50 to 90°C at a rate of 4°C.min^{-1} and then from 90 to 250°C at a rate of 15°C.min^{-1} to be finally maintained at 250°C for 10min. The injections were done in split mode with a ratio of 1:20. The residual amount of aroma compound in the material was calculated by correlation between the aroma compound peak and the internal standard peak, extrapolated to a surface unit and then expressed in g.m^{-2}. Loss of aroma compound (in percentage) during material processing (i.e. solution preparation, coating and drying) was calculated by taking into account the residual (w_r) and theoretical (w_t) amount of aroma compound as follows:

$$\%Loss = \frac{(w_t - w_r)}{w_t} \qquad \textit{eq. 3.5}$$

$$with\ w_t = Cw \times (\%\ Aroma\ compound\ added) \times (\frac{1}{E_{ef}})$$

where Cw (g/m²) is the coating weight of the coated papers previously evaluated and E_{ef} the extraction efficiency for each aroma compound. (E_{ef}) was determined at about 0.917 and 0.937 for trans-2-hexenal and 2-nonanone respectively.

RESULTS AND DISCUSSION

Production of WG coated paper at laboratory scale.

The details regarding the coated paper produced at laboratory scale dried under Room conditions and their gas transfer properties can be found in the publication 5 in the Chapter 3. Their equivalents dried under Tunnel conditions featured the same surface aspect (under naked eye or SEM) and the same gas transfer properties. This indicated the possibility to dry WG coated paper quickly and at high temperature like during a pilot or industrial coating process without damaging the final material or deteriorating its transfer properties.

Production of WG coated paper at pilot scale.

With the settings selected, it was possible to produce WG coated papers at pilot scale without any tacking between the layers of the roll. The WGP produced at pilot scale exhibited a sufficient coating weight (>10g/m²) (Table 3.5) and a good and glossy surface aspect (no visible defects) similar to the one of WGP produced at laboratory scale.

Compared to the Passive WGP produced at laboratory scale, the one produced at pilot scale featured higher oxygen permeation (Pe_{O2}) and lower permselectivity (S) (Table 3.5). This loss of gas barrier properties and permselectivity could be explained by the combination of drastic drying conditions at the pilot scale and the lower coating weight deposited. In fact, infra-red lamp drying is known to damage the WG coated layer, even if the material is shortly exposed.

However, as the lowering of O_2 content in the packaging to reduce fruits respiration was only the third requirement for strawberries (the two first being protection against physical stress and protection against mold growth) and as the drying condition on the industrial coating machine would be less drastic (no infra-red involved) these results at pilot scale seemed promising enough to pursue the scaling-up process.

Table 3.5. Comparison of WG coated paper (passive and active) produce at laboratory, pilot and industrial scale

	Laboratory Scale			Pilot Scale	Industrial Scale	
	Passive WGP (Room dried)	**Passive WGP (Tunnel dried)**	**Active WGP (Room dried)**	**Passive WGP (IR – Hot air dried)**	**Passive WGP (Hot air dried)**	**Active WGP (Hot air dried)**
Support-Paper	Teranna 36	Teranna 36	Teranna 36	Teranna 36	Kalysack 70	Kalysack 70
Thickness(µm)	69.55 ±1.33	70.01 ±1.17	69.62 ±1.12	63.68 ±1.06	129,12 ±3.01	128,07 ±2.68
Coating Weight (g/m²)	13.44 ±1.03	14.12 ±0.96	14.78 ±0.27	11.30 ±0.92	12.26 ±2.06	11.25 ±1.92
Pe$_{O2}$ (10^{-11} mol/Pa.m².s)	3.33 ±0.07	3.18 ±0.13	3.42 ±0.09	25.68 ±1.34	217.05 ±6.56	224.13 ±8.02
Permselectivity	5.18 ±0.11	4.97 ±0.16	4.90 ±0.14	1.41 ±0.06	1.48 ±0.34	1.56 ±0.28
2-nonanone retained (g/m²)	NA	NA	0.84 ±0.18	NA	NA	0.86 ±0.08

Production and properties of WG coated paper at industrial scale.

As for the production at pilot scale, the settings selected allowed clean production of rolls of Industrial Passive and Active WGP without any tacking between the roll layers. Both kind of paper produced at industrial scale exhibited a sufficient coating weight (Table 3.5) but their surface aspect was very different from the one of the paper produced at smaller scales. They did not exhibit the usual glossy aspect of the WG coated paper and the Support-Paper fibers were apparent. Such observation indicated a non-homogenous and non-continuous WG layer.

Consequently, the gas barrier properties and permselectivity of the materials built at industrial scale were particularly poor for WG coated papers. The Passive and Active Industrial material exhibited Pe_{O2} of 217.05 $x10^{-11}$ and $224.13x10^{-11}mol.Pa^{-1}.m^{-2}.s^{-1}$) and S of 1.48 and 1.56 respectively. So the Industrial Passive WGP was not of interest for strawberries (or any other fresh food) and highlighted the need of further adjustments of the coating process at industrial scale to obtain interesting passive materials for MAP applications. However, the Industrial Active WGP could still be interesting due its antimicrobial activity (main requirement for strawberry packaging).

Regarding the active compound (2-nonanone) retention properties of the Industrial Active WGP, the material was able to retain slightly higher quantities than the Laboratory WGP despite a lower coating weight and a coating solution used 4 days after its preparation (instead of right away in laboratory conditions). This interestingly good result is probably due to the fast drying performed in the industrial coating machine (<30s) compared to the lengthy one performed at laboratory scale (2h). Finally, these good retention results proved the interest of the Industrial Active WGP for strawberry packaging and raised high hopes to obtain a good antimicrobial effect with the active materials built at industrial scale.

CONCLUSION

It was possible to coat kraft paper with WG-based solution at both pilot and industrial scale despite huge process differences. The papers produced after production up-scaling exhibited inadequately high gas transfer properties for passive MAP applications but presented an interestingly high retention capacity for volatile antimicrobial compounds such as 2-nonanone (slightly higher than the paper produced at laboratory scale). Hence, given the fact that 2-nonanone release was found to be the major factor in strawberry's preservation enhancement through active MAP, production at industrial scale of Active Tailor-Made Packaging appears possible and should be considered.

DISCUSSION GENERALE ET PERSPECTIVES

DISCUSSION GENERALE

Malgré l'ampleur que prend la technologie sous atmosphère modifiée pour le conditionnement des fruits et légumes frais, l'adéquation des matériaux d'emballage avec les besoins des produits est insuffisante. D'une part parce que la conception de ces emballages repose encore sur des approches empiriques de type essai-erreur, ne laissant pas de place à des outils d'optimisation ; et d'autre part parce que la plupart des matériaux disponibles sur le marché présentent des propriétés de transfert aux gaz trop restreintes compte tenu de la gamme de propriétés nécessaire pour couvrir les besoins de l'ensemble de ces aliments. Les travaux que j'ai réalisé ces trois dernières années ont été centrés sur la mise en place et la validation d'une approche basée sur l'ingénierie reverse visant à identifier les propriétés de transfert requises ou cibles pour un végétal donné. Pour concevoir des matériaux à façon, c'est à dire répondant à ces propriétés, j'ai également exploré différents moyens de modulation de ces propriétés à travers des procédés de structuration à différentes échelles de papiers enduits de protéines de blé, issus de ressources renouvelables et mieux adaptés aux végétaux que les matériaux synthétiques car plus perméables aux gaz (et aux vapeurs).

Approche basée sur l'ingénierie reverse pour la conception d'EAM et outils de simulation.

Les précédents travaux portant sur la modélisation des échanges de gaz dans les systèmes emballage/aliment a donné lieu à l'élaboration d'outils de simulation et de prédiction pour l'emballage sous atmosphère modifiée des fruits et légumes, tels que les outils en ligne Tailorpack (application gratuite) ou PackinMap (application payante). Même si dans leur état actuel, ils ne peuvent pas être considérés comme des outils d'aide à la décision complets, ils permettent de traduire des besoins d'un végétal en propriétés de transfert de l'emballage, en prenant en compte la physiologie du végétal considéré et son atmosphère optimale de conservation et les dimensions de l'emballage. L'intérêt de tels outils de simulation dans une démarche d'ingénierie reverse a été démontré à travers l'exemple de Tailorpack, en déterminant les propriétés de transfert d'une gamme représentative de fruits et légumes. En se basant sur les approches déjà mises en place dans le domaine des services et de l'industrie de pointe, cette démarche d'ingénierie reverse pour la conception d'EAM a été élaborée et comporte 2 étapes cruciales que sont l'identification des besoins des produits et leur transcription en propriété de transfert. Elle repose sur 5 étapes et a été validée pour l’élaboration d’EAM actif adapté à la conservation des fraises : définition des besoins du produit, solutions d’emballage envisageables, dimensionnement de l’emballage, conception détaillée (ex : fonction antimicrobienne) de l’emballage, et enfin validation de l’emballage. Les besoin de la fraise ont d’abord été définis et hiérarchisés avant d’identifier et sélectionner les solutions d’emballage. Les propriétés attendues de l’emballage ont été déterminées à l’aide de l’outil de modélisation, pour les propriétés de transfert aux gaz, et à l’aide de tests in vitro pour qualifier et quantifier la dose d’agent actif nécessaire à l’inhibition de croissance de contaminants fongiques. Le matériau actif présentant ces propriétés a été conçu : papier enduit d’une couche de gluten de blé contenant de la 2nonanone. Combiné (dans un rôle de couvercle) à une barquette en PET (pour la protection contre le stress physique), il a permis de rallonger la durée de conservation des fraises en bon état de 3 jours par rapport à une barquette ouverte classique, prouvant ainsi l’efficacité de l’approche par ingénierie reverse.

Il est important de noter que cette approche n’est bien entendu pas limitée au type d’emballage ou aux produits considérés et peut être étendue à tout produit dont les caractéristiques physiologiques et les besoins sont connus. La généralisation de son utilisation devrait permettre de mieux dimensionner les emballages et faciliter leur conception.

Modulation des propriétés de transfert de papiers enduits de gluten de blé.

Il est apparu que malgré des propriétés de transferts intéressantes, à forte humidité, pour l’EAM des fruits et légumes, les matériaux protéiques (gluten de blé, protéines de soja, caséines, gélatine, etc…) étaient surtout étudiés à des humidités moyennes voire basses et principalement pour leurs propriétés de transfert à la vapeur d’eau ou barrière aux graisses. Il résulte donc un manque de données et de connaissances sur les liens entre la structure des matériaux et leurs propriétés de transfert aux gaz a haute humidité et de ce fait, sur les mécanismes de structuration permettant de moduler ces propriétés.

Mes travaux ont permis de mettre en évidence qu’à partir d’une même matrice protéique, le gluten de blé, il était possible de couvrir des gammes de permsélectivités allant de 5 à 18 et de perméance à l’oxygène allant de $0,02x10^{-10}$ à $2x10^{-10}$ $mol.Pa^{-1}.m^{-2}.s^{-2}$, ce qui répondait parfaitement aux besoins d’une sélection représentative de produits frais respirant. Parmi ces matériaux, les papiers enduits de gluten ont particulièrement été étudiés pour comprendre quels étaient les mécanismes sous-jacents permettant de moduler leurs propriétés de transferts aux gaz dans différents cas de structuration.

Tout d'abord, il a été mis en évidence que la structure interne des papiers supports impactait considérablement les propriétés de transfert des papiers enduits de protéines de blé. Les papiers hautement raffinés présentent une structure fibreuse dense qui limite la pénétration des protéines dans le support. Dans ce cas, la structure des papiers enduits résulte ainsi en une couche protéique plus importante (dite zone apparente) et une couche composite (zone de pénétration) plus fine que lorsque les papiers sont faiblement raffinés (et donc avec un réseau fibreux plus lâche). Le rapport entre ces 2 couches affecte considérablement les propriétés de transfert aux gaz des papiers enduits : plus la zone apparente est importante et la zone de pénétration étroite, plus le papier enduit présente un caractère gluten important avec une haute sélectivité et des perméabilités aux gaz modérées. Par conséquent, en plus du poids de couche, de la composition de la pulpe, des traitements de surface des papiers, le degré de raffinage des fibres est un facteur à prendre en compte dans la modulation des propriétés de transfert des papiers enduits.

Les papiers enduits ainsi obtenus ont ensuite été traités avec du CO_2 à pression atmosphérique à l'aide d'un balayage gazeux plus ou moins long. Alors que la perméation à l'oxygène n'est pas affectée par ce traitement, la perméation au dioxyde de carbone est considérablement augmentée et par conséquent la permsélectivité des matériaux. De plus, il a été mis en évidence que cet effet était irréversible. En effet ces propriétés sont conservées même après une étape de désorption sous flux d'Hélium. Il semble que des interactions entre le CO_2 et les acides aminés des protéines de gluten se créent durant le traitement au CO_2, compte tenu que la quantité résiduelle de CO_2 dans les papiers enduits sorbés au CO_2 puis désorbés à l'He augmente en fonction du temps de traitement appliqué, tout comme dans les films de gluten, sans pour autant atteindre un maximum de sorption. Cependant le type d'interaction n'a pas pu être mis en évidence en analyse FTIR. De même, les analyses de température de transition vitreuse n'ont pas permis de démontrer un effet plastifiant de ces interactions. La sensibilité des outils analytiques utilisés semble insuffisante pour mettre en évidence ces interactions. Il serait judicieux d'augmenter les temps de traitement au CO_2 pour avoir des différences plus marquées et d'utiliser des méthodes plus sensibles comme par exemple la chromatographie d'exclusion qui pourrait permettre de mettre en évidence des réarrangements protéiques.

La structuration de papiers enduits a également été réalisée grâce à la méthode de construction couche par couche en combinant des couches de protéines de gluten de blé avec des couches de nanoparticules comme les feuillets d'argile. L'association de polymère synthétique avec ce type de nanoparticule a montré qu'il était possible de moduler les propriétés de transfert aux gaz (oxygène) en fonction du nombre de paires de couches. La principale difficulté ici était d'adapter cette technique à un support irrégulier (soit sur le support papier soit sur le support papier préalablement enduit de gluten) et de pouvoir évaluer l'épaisseur des couches. De ce fait, la construction des matériaux a été réalisée pas à pas au moyen de support et de molécules modèles dont l'épaisseur a été évaluée, entre autre, par microscopie par fluorescence (avec FITC comme marqueur fluorescent des protéines). L'étude des propriétés des matériaux papiers-protéines-montmorillonites ainsi obtenus a permis de montrer que la dépose de couches permettait de diminuer la perméabilité à l'oxygène du support papier grâce à un effet barrière apporté par les montmorillonites en fonction du nombre de couches déposées. Cependant, les couches (jusqu'à 40 paires de couches) ne permettaient pas de couvrir de façon continue la surface des papiers (due à leur hétérogénéité de surface) et seule la construction couche par couche sur les papiers enduits permet d'atteindre des perméabilités à l'O_2 compatible avec les applications visées. Dans tous les cas cette technique permet de moduler de façon précise les propriétés de transfert des matériaux, même sur des supports papiers.

PERSPECTIVES

Outil de simulation EAM.

Bien qu'il ait été prouvé que les outils de simulation/prédiction EAM actuellement disponibles permettaient de conduire avec succès une procédure de développement par ingénierie reverse d'emballages, il apparait clairement que ces outils peuvent encore grandement être améliorés sur le plan scientifique. Ainsi, il serait utile de leur permettre de prendre en compte des phénomènes aujourd'hui négligés tels que la transpiration du produit, la production d'éthylène, l'impact de l'humidité relative ou le transfert de molécules plus complexes que l'oxygène et le dioxyde de carbone (ex: composés antimicrobiens volatiles), ce afin d'améliorer la précision globale. Sur un plan plus général, il apparait que pour avoir un impact significatif sur les pertes en fruits et légumes frais constatées tout au long de la chaine de distribution, l'emploi de la méthode d'approche par ingénierie reverse doit être généralisée le plus rapidement possible. Or une telle généralisation nécessite des outils de simulation et des bases de données non seulement puissant et complets mais également accessibles à tous types d'utilisateurs (le plus souvent novice sur le sujet). Ainsi, l'utilisation massive des outils de simulation en ligne disponibles est aujourd'hui limitée par l'absence ou le caractère incomplet des bases de données associées. Il est en effet inenvisageable de demander à un cultivateur ou à un grossiste en fruits et légumes d'effectuer lui-même des recherches bibliographiques sur les besoins du produit à emballer ou sur notamment sa physiologie. De même, si l'approche scientifique ne se soucie que du produit emballé, de sa qualité et de sa conservation, il apparait évident que des critères annexes, notamment de coûts vont entrer en ligne de compte pour les membres de la filière fruits et légumes et de tels critères ne sont pour l'instant pas intégré aux outils de simulations actuel. Ainsi, il serait souhaitable d'envisager la construction d'outils d'aide à la décision complets, intégrant non seulement l'aspect prédiction MAP des outils existants mais aussi des critères de coûts ou de cycle de vie des matériaux d'emballage. De tels outils devant évidemment comporter des bases de données produits et matériaux complètes et tenues à jour.

Matériaux protéiques étudiés.

Pour compléter les 3 cas étudiés lors de ce travail, l'étude des matériaux protéiques pourrait être étendue à d'autres types de structuration (ex : nano-composites dans la masse) ou d'autres protéines que le gluten (ex : protéines de soja, zéine, etc…). Sachant que la construction couche par couche est possible sur des supports hétérogènes, il serait intéressant de poursuivre l'étude des propriétés de transfert (CO_2, vapeur d'eau) et notamment développer des modèles prédictifs des transferts en fonction du nombre de couche. Il serait également intéressant de rechercher des substances issues des ressources renouvelables pouvant jouer le rôle de couche préliminaire pour la dépose couche par couche (actuellement PEI) et compatible avec les protéines et/ou les feuillets d'argile. Concernant les matériaux traités au CO_2, des essais plus discriminants pourraient être conduits pour mieux comprendre les modifications engendrées et leur impact sur les propriétés de transfert aux gaz. Enfin, des tests de vieillissement pourraient être conduits sur tous les matériaux fabriqués, à la fois dans des conditions de stockage (basse humidité) et d'utilisation (haute humidité), afin de s'assurer de leur viabilité sur le moyen ou long terme que ce soit en termes de performances (tenue mécanique et transferts de masse) ou de sécurité alimenta ire (contamination par croissance de micro-organismes). De tels tests ont déjà été conduits sur divers types de matériaux protéiques (produits par casting, thermomoulage ou déposition ultrafine) [Orliac et al. 2003; Pompa et al. 2005; Oses et al. 2009b] et il devrait être possible de s'en inspirer pour test les matériaux produits au cours de la thèse. Enfin, il serait intéressant de se pencher plus précisément sur la scellabilité des matériaux conçus (sur la base de précédentes études conduites sur la scellabilité des matériaux à bases de protéines et polysaccharides [Cho et al. 2010; Abdorreza et al. 2011; Nur Hanani et al. 2013]) afin de pouvoir mieux envisager la production industrielle de l'emballage sous atmosphère modifiée actif conçu pour les fraises dans le chapitre 3.

Il faut également noter que sur un plan industriel, les agro-matériaux et en particulier les matériaux protéiques présentent actuellement certaines limitations. En effet les matériaux protéiques, répondant aux besoins des fruits et légumes, construits et utilisés dans cette étude n'ont pour le moment pas atteint le stade de production industriel (excepté un test concluant pour le papier enduit d'une solution de gluten « antimicrobienne » destiné à l'emballage des fraises) et ne sont donc pas encore prêts à remplacer les plastiques conventionnels sur le marché de l'emballage. De plus, il est probable que le changement d'échelle implique des changements de propriétés pour ces matériaux, les process de fabrications étant très différents, comme en témoigne la perte de propriétés barrière du matériau actif conçu pour l'emballage des fraises alors que ses propriétés antimicrobiennes ont-elles pu être conservées. Des études complètes sur les matériaux produits à cette échelle seront donc nécessaires avant une possible mise sur le marché.

Avenir des matériaux protéiques pour l'emballage.

Au-delà des considérations portant nos matériaux d'étude, il existe de plus amples problématiques liées à l'utilisation à grande échelle des matériaux protéiques dans l'emballage des fruits et légumes frais.

La première réside dans la possible compétition entre l'utilisation de ressources agronomiques pour la nutrition humaine et leur utilisation pour la fabrication de matériaux à une époque où plus de 850.000 de personnes souffrent de mal nutrition dans le monde (chiffres OMS, 2007). Si cette question demeure d'importance il faut savoir que la part de l'agriculture dédiée aux usages non alimentaires est très limitée en comparaison par exemple de l'alimentation animale et que la majorité des protéines utilisées pour la fabrication de matériaux sont aujourd'hui des coproduits ou des déchets de l'industrie agro-alimentaires et donc ne concurrence pas directement l'alimentation humaine. Il est également intéressant de noter que les protéines présentent un coup équivalent aux matières plastiques conventionnels

(PE, PP, PS,…) et peuvent être façonnées avec les procédés industriels (ex : injection moulage, coating) sans engendrer de sur-coûts de production. Il faut toutefois rester attentif à la fluctuation des prix des céréales et autres matières premières qui sont actuellement en constante augmentation.

La seconde problématique est liée à la sécurité alimentaire des matériaux protéiques au contact. Il n'existe actuellement pas de tests de migration spécifiquement adaptés au cas des protéines et la réglementation elle-même ne mentionne qu'une limite globale (et non des limites spécifiques) de migration. Ces aspects devront donc être complétés dans l'avenir. Cependant, compte tenu des temps de contact emballage/aliments très courts (généralement de l'ordre de la semaine) et de la taille des molécules protéiques, la migration devrait être limitée. Et il pourrait être possible de s'affranchir totalement du problème en plaçant le matériau protéique au cœur d'un composite multicouches en choisissant des couches additionnelles très perméables aux gaz (ex : support fibreux - protéine – support fibreux). De plus comparé aux plastiques synthétiques et à leurs additifs, les matériaux protéiques présentent uniquement un risque du point de vue allergies/intolérance tout comme la plupart des aliments. Un étiquetage spécifique et clair devrait permettre de limiter les risques, comme déjà existant pour les aliments allergènes.

Le comportement des matériaux protéiques vis-à-vis de différents facteurs environnementaux et industriels doit également être pris en considération et leurs domaines d'utilisations précisément définis en conséquence. Ainsi, l'humidité relative, la température et les conditions de pH (induites par l'aliment) doivent être anticipées tout comme la réaction du matériau à des environnements de stockage plus ou moins contaminés (un filmage des matériaux pourrait cependant solutionner ce problème précis). Et selon que le matériau protéique sera utilisé au contact direct de l'aliment ou comme membrane servant à réguler les échanges gazeux (sur la base d'un emballage conventionnel de type barquette par exemple, *cf. emballage sous atmosphère modifiée actif pour les fraises, chapitre 3*), les contraintes en termes de propriétés mécaniques seront de toute évidence différentes.

Enfin, une fois toutes ces problématiques prises en compte, il ne faut pas oublier que c'est l'acceptation de ces nouveaux emballages par le consommateur qui déterminera leur succès. Cela implique, en plus des traditionnels critères de coût et d'impact sur les qualités organoleptiques des produits, de nombreux facteurs supplémentaires tels que l'aspect visuel de l'emballage, son côté pratique ou encore son impact écologique (plutôt positif dans le cas présent grâce à la biodégradabilité des protéines).

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FIGURES AND TABLES

LISTS

FIGURES LIST

TABLES LIST

PUBLICATIONS ET COMMUNICATIONS

PUBLICATIONS SCIENTIFIQUES RELATIVES AUX TRAVAUX DE THESE

[1] **Review: Multi-scale structuration of protein based materials for MAP of fresh produce**
T. Cagnon, C. Guillaume, V. Guillard, N. Gontard.
Trends in Food Science and Technology – Submitted

[2] **Nano and micro-structuring of materials from a single agro-polymer for sustainable MAP preservation of fresh food.**
T. Cagnon, C. Guillaume, V. Guillard, N. Gontard.
Journal of Packaging Technology and Science, IAPRI2012– (DOI: 10.1002/pts.1973)

[3] **Of the importance of the structure of support paper in gas transfer properties of protein-coated paper.**
T. Cagnon, C. Guillaume, E. Gastaldi, N. Gontard.
Journal of Applied Polymer Science – Submitted

[4] **Control of O_2 barrier properties of papers coated with wheat gluten / nanoclays multilayers.**
T. Cagnon, R. Szamocki, C. Guillaume, O. Felix, G. Decher.
Journal of Composite Science and Technology – In preparation for submission

[5] **Short Note: Impact of CO_2 sorption on the gas transfer properties of wheat gluten coated papers.**
T. Cagnon, C. Guillaume, N. Gontard.
Journal to be determined – In preparation for submission

[6] **Fresh food packaging: A requirement driven approach (case study on strawberries and agro-based materials).**
T.Cagnon, A. Méry, C. Guillaume, P. Chalier, N. Gontard.
Innovative Food Science and Emerging Technologies – Submitted

AUTRES COMMUNICATIONS

CHAPITRE D’OUVRAGE

[1] **Modeling tools to design optimal packaging for fruits**
C. Guillaume, N. Gontard, B. Gouble, T. Cagnon
Fruit Preservation: Novel and Conventional Technologies (ed. A. Rosenthal)

CONFERENCES

[1] **Nano and micro-structuring of materials from a single agro-polymer for sustainable MAP preservation of fresh food.**
T. Cagnon, C. Guillaume, N. Gontard.
Iapri World Conference 2012 – San Luis Obispo, CA, USA – 17-21 Juin 2012

[2] **Emballages Actifs : Principes, applications, développements, aspects réglementaires**
T. Cagnon, N. Gontard.
Journées de l’emballage – Troyes – 3-4 Octobre 2012

[3] **Conception de matériaux d’emballage pour la conservation sous atmosphère modifiée de fruits et légumes frais : Utilisation combinée de l’ingénierie reverse et de matériaux agro-polymères**
T. Cagnon, C. Guillaume, N. Gontard.
Séminaire Annuel UMR IATE – Montpellier – 2 Juillet 2012

[4] **Emballages Actifs : Focus sur l’emballage des produits laitiers**
T. Cagnon, C. Guillaume, V. Guillard, N. Gontard
Conférence privée Lactalis – Laval – 8 Février 2013

POSTERS

[1] **Conception d’emballages sur mesure pour fruits et légumes frais selon une approche intégrée**
T. Cagnon, C. Guillaume, N. Gontard
Journée de l’école doctorale SP-SA – Montpellier, France – 10 Juin 2011

[2] **Tailorpack: Active tailor made and eco-friendly packaging for fresh fruits and vegetables preservation**
B. Gouble, T. Cagnon, P. Reling, C. Guillaume.
Fruit and vegetable processing, First Euro-Mediterranean symposium – Avignon – 2011

MANAGEMENT DE PROJET

CO-REDACTION DE DELIVRABLES

[1] **Composés Volatiles Antimicrobiens**
T. Cagnon, C. Guillaume, P. Chalier, R. Szamocki, N. Gontard
D X.X – Projet Tailorpack – 2011

REUNIONS

En présence de N. Gontard, C. Guillaume, E. Gastaldi, V. Guillard, H. Angelier-Coussy, P. Chalier, B. Gouble, P. Reling, S. Lurol, A. Faure, G. Decher, R. Sczamocki, O. Felix, F. Rambert and O. Bongrand

[1] **Réunion Semestrielle Projet Tailorpack (Propriétés de transfert de matériaux à base de gluten de blé)**
Montpellier, France – Décembre 2010

[2] **Réunion Semestrielle Projet Tailorpack (Matériaux actifs antimicrobiens à base de gluten de blé)**
Montpellier, France – Juin 2011

[3] **Réunion Semestrielle Projet Tailorpack (Bilan des travaux sur les matériaux à base de gluten de blé)**
Montpellier, France – Décembre 2011

ANNEXES

Annexe 1

Thesis Defense

Thibaut Cagnon

November 13th – Montpellier Supagro, Montpellier, France

CONTENT: This section contains the slides used as support for the defense of this thesis that was publicly held at Montpellier Sup'Agro on December 13th in the presence of the members of the jury.

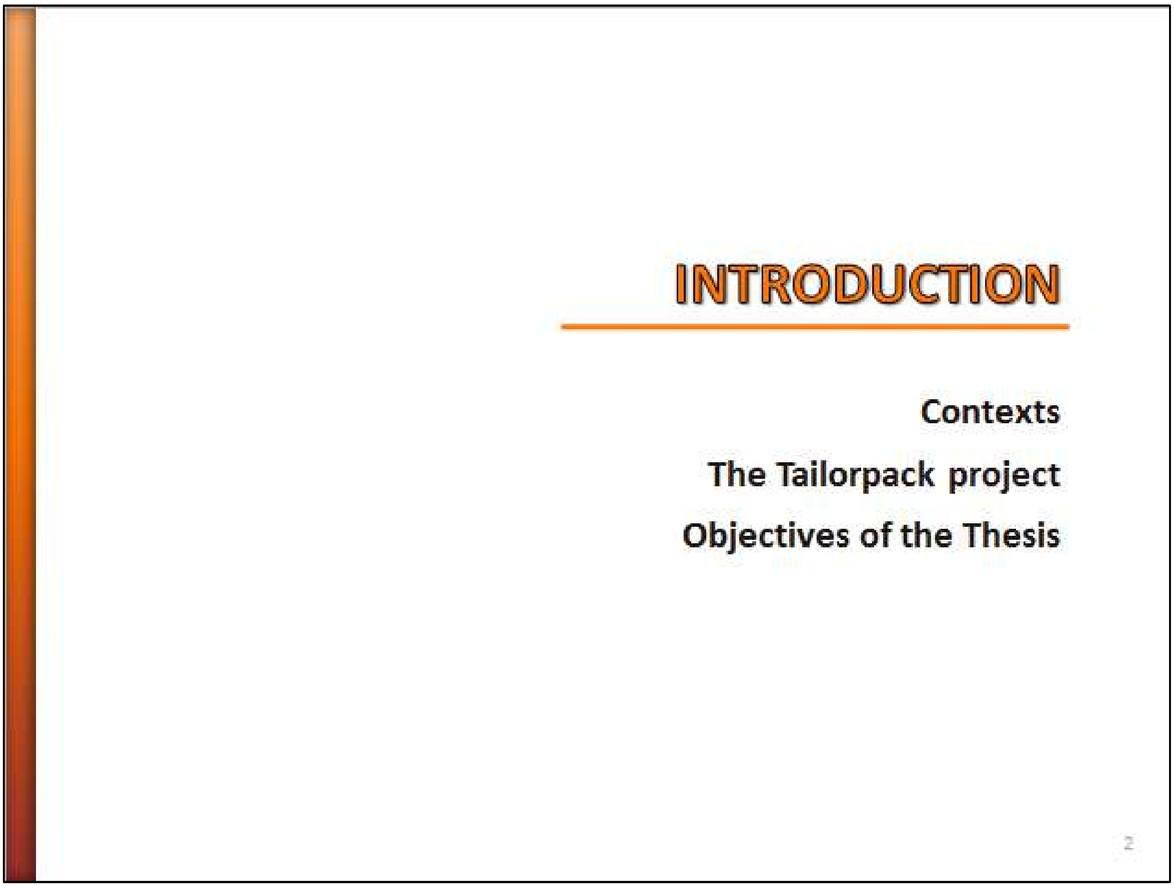
MASS TRANSFERS IN FOOD/PACKAGING SYSTEMS:

STRUCTURING TAILOR-MADE MULTILAYER MATERIALS FOR MODIFIED ATMOSPHERE PACKAGING OF RESPIRING PRODUCE

Thibaut Cagnon
UMR IATE – Agropolymer Engineering and Emerging Technologies

***Thesis Director:** Nathalie Gontard*
***Thesis Co-Director:** Carole Guillaume*

November 13th
Montpellier Sup'Agro

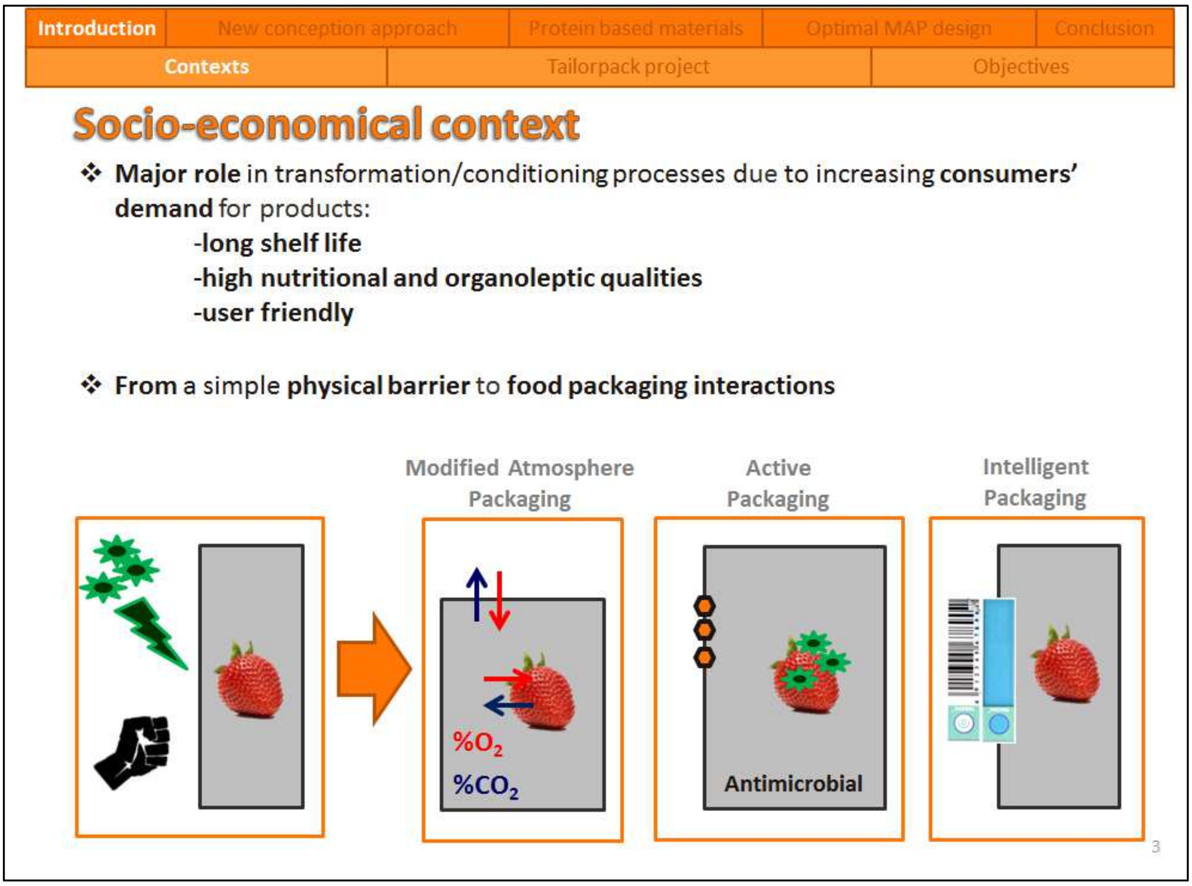


INTRODUCTION

Contexts

The Tailorpack project

Objectives of the Thesis



Introduction | New conception approach | Protein based materials | Optimal MAP design | Conclusion

Contexts | Tailorpack project | Objectives

Socio-economical context

- **Major role** in transformation/conditioning processes due to increasing **consumers' demand** for products:
  - **-long shelf life**
  - **-high nutritional and organoleptic qualities**
  - **-user friendly**
- **From** a simple **physical barrier** to **food packaging interactions**

Modified Atmosphere Packaging

Active Packaging

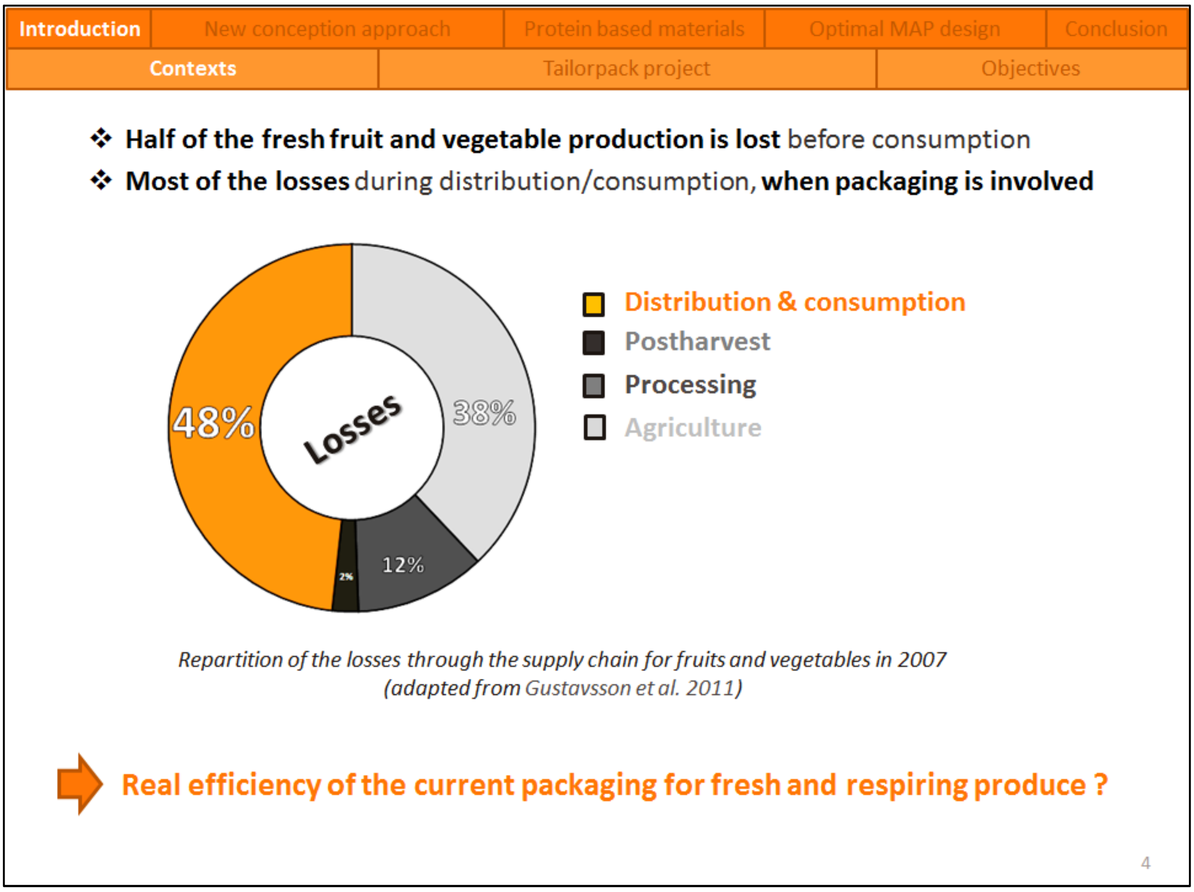
Intelligent Packaging

$\%O_2$

$\%CO_2$

Antimicrobial

3



Introduction | New conception approach | Protein based materials | Optimal MAP design | Conclusion

Contexts | Tailorpack project | Objectives

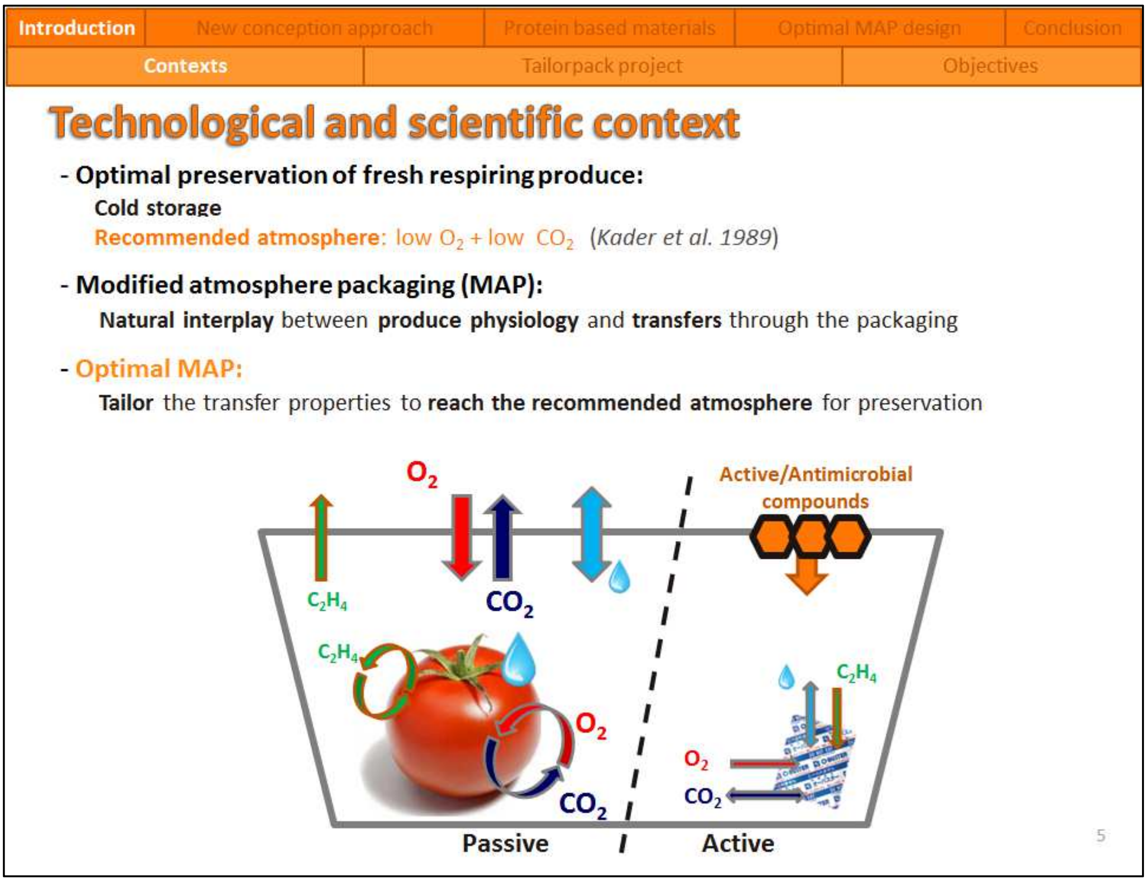
- **Half of the fresh fruit and vegetable production is lost** before consumption
- **Most of the losses** during distribution/consumption, **when packaging is involved**

- 🟧 Distribution & consumption
- ■ Postharvest
- ▨ Processing
- ☐ Agriculture

Repartition of the losses through the supply chain for fruits and vegetables in 2007 (adapted from Gustavsson et al. 2011)

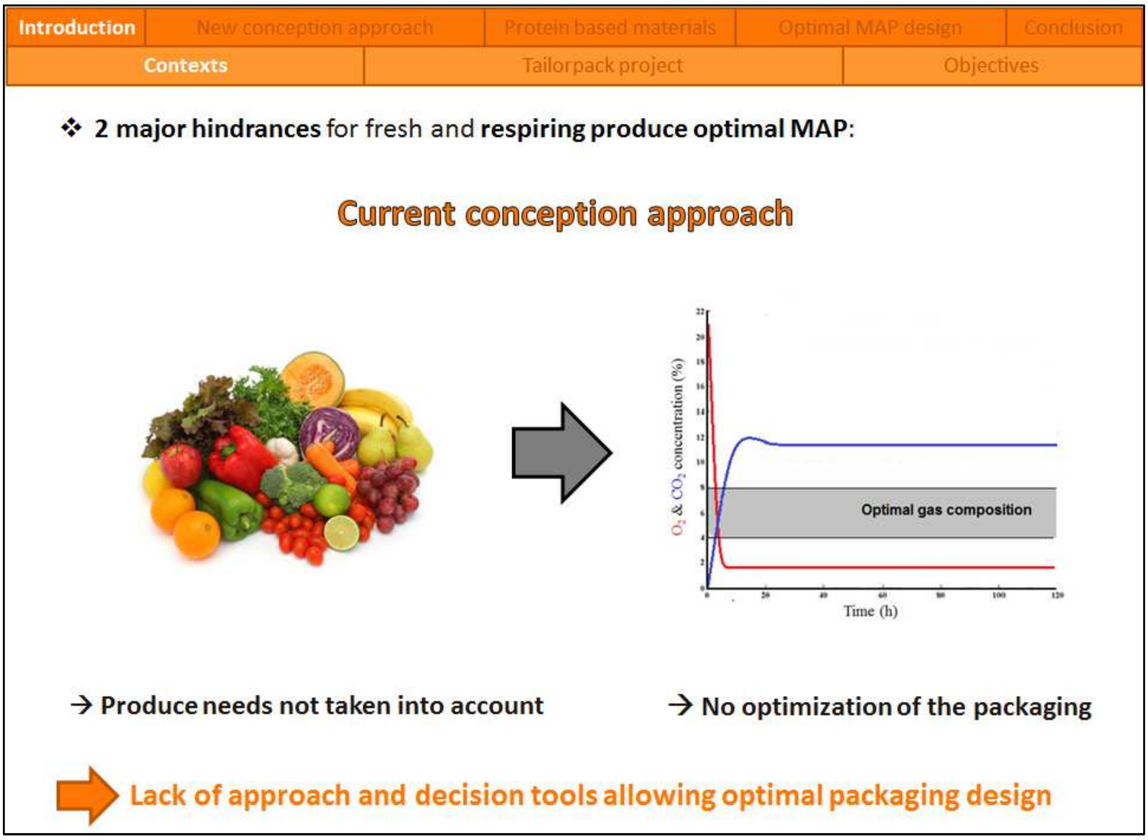
Real efficiency of the current packaging for fresh and respiring produce ?

4



Technological and scientific context

- **Optimal preservation of fresh respiring produce:**
  - Cold storage
  - Recommended atmosphere: low O_2 + low CO_2 (*Kader et al. 1989*)
- **Modified atmosphere packaging (MAP):**
  - **Natural interplay** between **produce physiology** and **transfers** through the packaging
- **Optimal MAP:**
  - **Tailor** the transfer properties to **reach the recommended atmosphere** for preservation



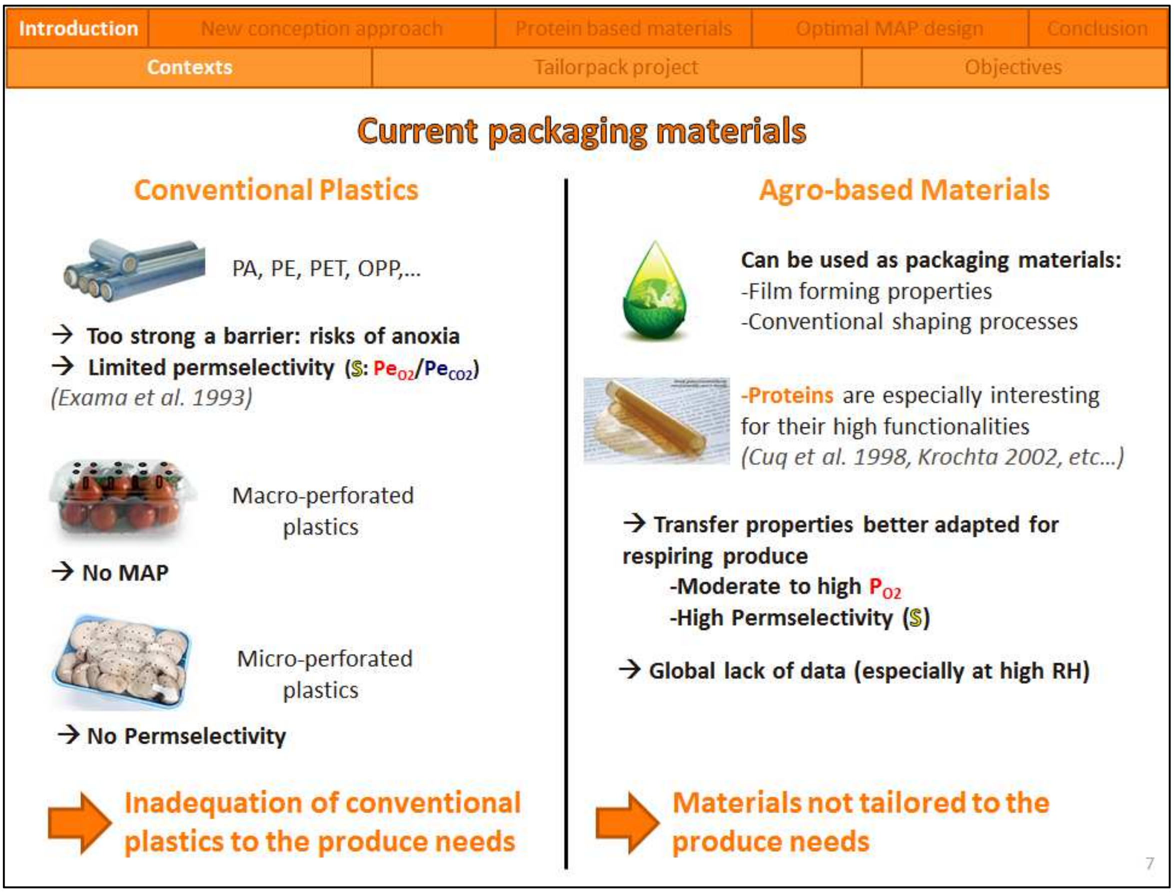
❖ **2 major hindrances** for fresh and **respiring produce optimal MAP**:

Current conception approach

→ **Produce needs not taken into account**

→ **No optimization of the packaging**

Lack of approach and decision tools allowing optimal packaging design



Current packaging materials

Conventional Plastics

PA, PE, PET, OPP,...

→ **Too strong a barrier: risks of anoxia**
→ **Limited permselectivity (S: Pe_{O2}/Pe_{CO2})**
(Exama et al. 1993)

Macro-perforated plastics

→ **No MAP**

Micro-perforated plastics

→ **No Permselectivity**

Inadequation of conventional plastics to the produce needs

Agro-based Materials

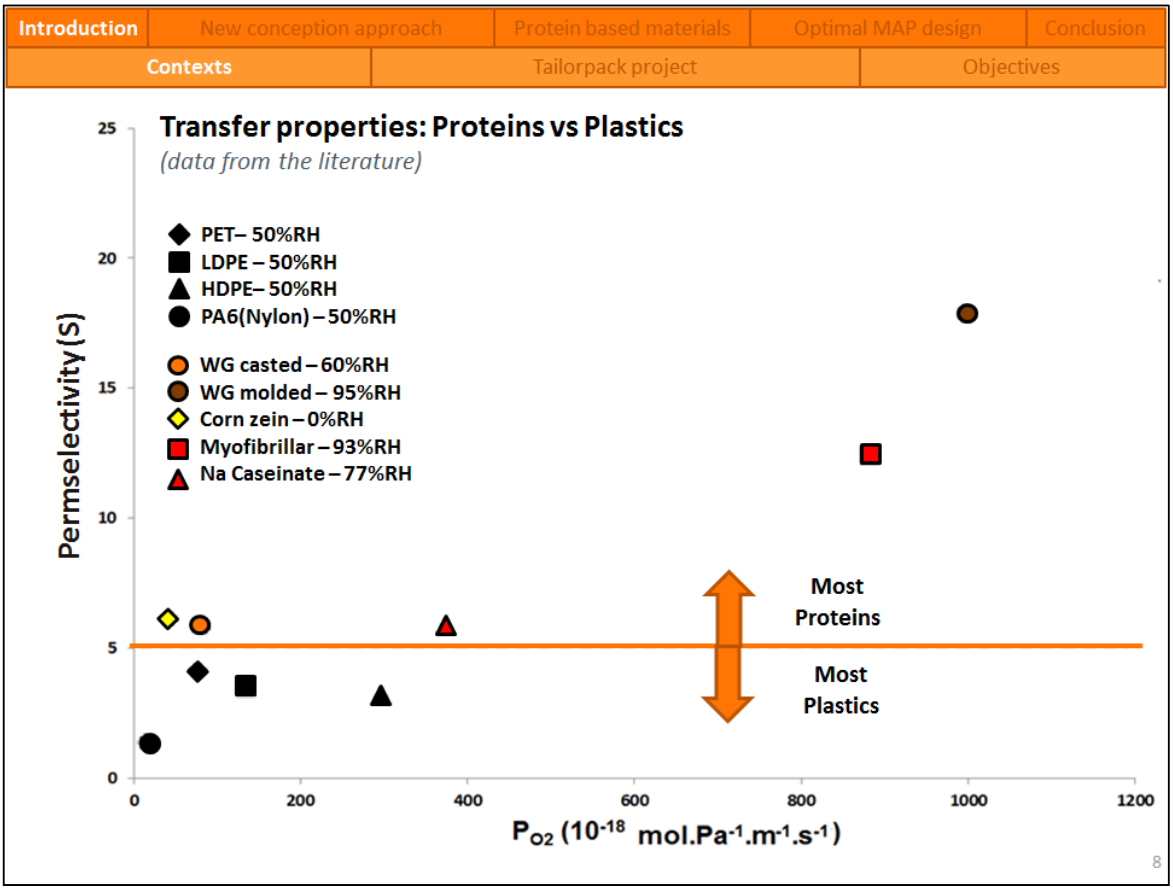
Can be used as packaging materials:
-Film forming properties
-Conventional shaping processes

-Proteins are especially interesting for their high functionalities *(Cuq et al. 1998, Krochta 2002, etc…)*

→ **Transfer properties better adapted for respiring produce**
-Moderate to high P_{O2}
-High Permselectivity (S)

→ **Global lack of data (especially at high RH)**

Materials not tailored to the produce needs



Transfer properties: Proteins vs Plastics

(data from the literature)

Introduction	New conception approach	Protein based materials	Optimal MAP design	Conclusion

Contexts	Tailorpack project	Objectives

Tailorpack project (2008-2011)

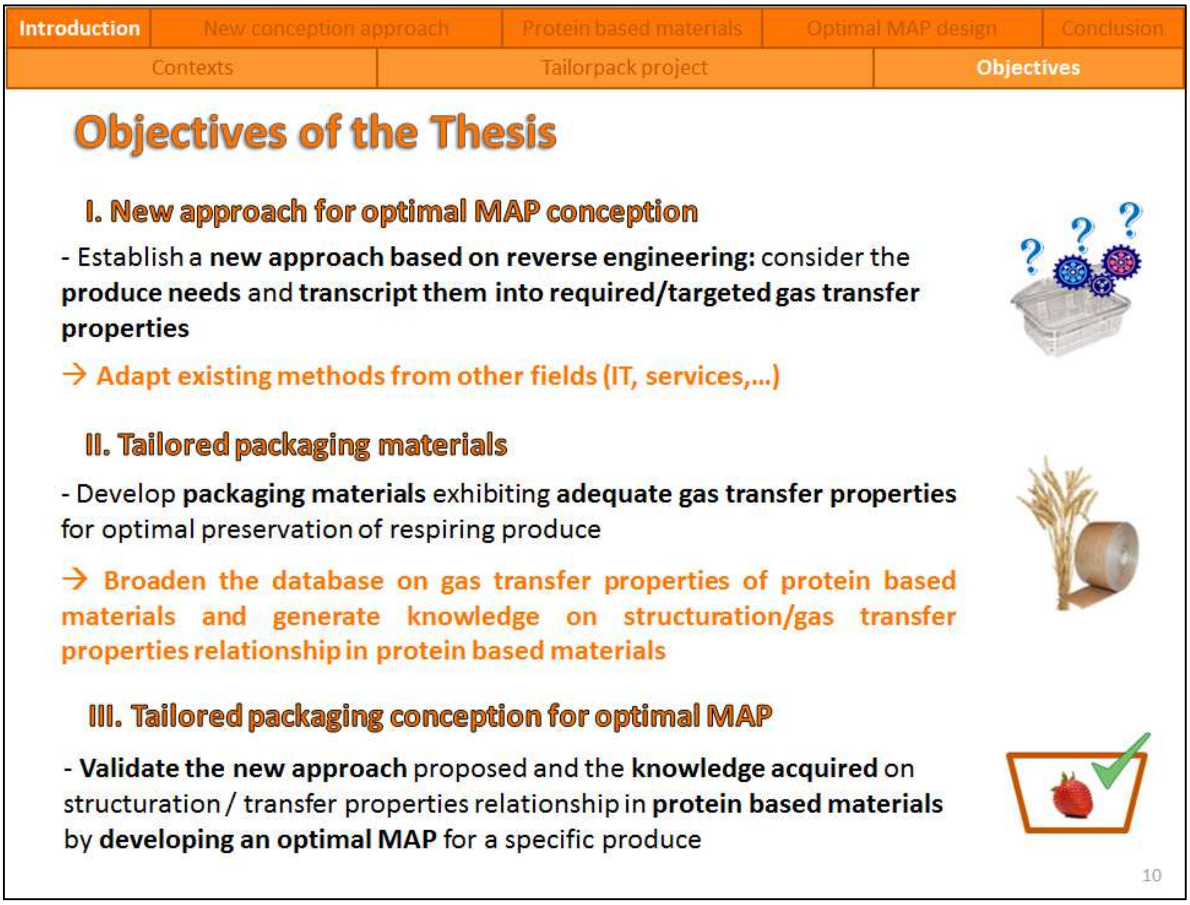
Conception of active and biodegradable packaging adapted to the preservation of fresh and respiring produce

- Develop a **user friendly decision tool** to help **design of tailored packaging materials** by predicting **targeted transfer properties** taking into account **the needs of the produce.**

- Design **agro-based packaging materials** (protein/fibrous material composites) able to **overcome the limitations of plastics**

Partners

UMR iAte Ingénierie des Agropolymères & technologies émergentes
UMR SQPOV
Institut Charles SADRON
Ctifl
Gascogne Paper
PACKERING
ADIVEC



Introduction	New conception approach	Protein based materials	Optimal MAP design	Conclusion

Contexts	Tailorpack project	Objectives

Objectives of the Thesis

I. New approach for optimal MAP conception

- Establish a **new approach based on reverse engineering:** consider the **produce needs** and **transcript them into required/targeted gas transfer properties**

→ Adapt existing methods from other fields (IT, services,…)

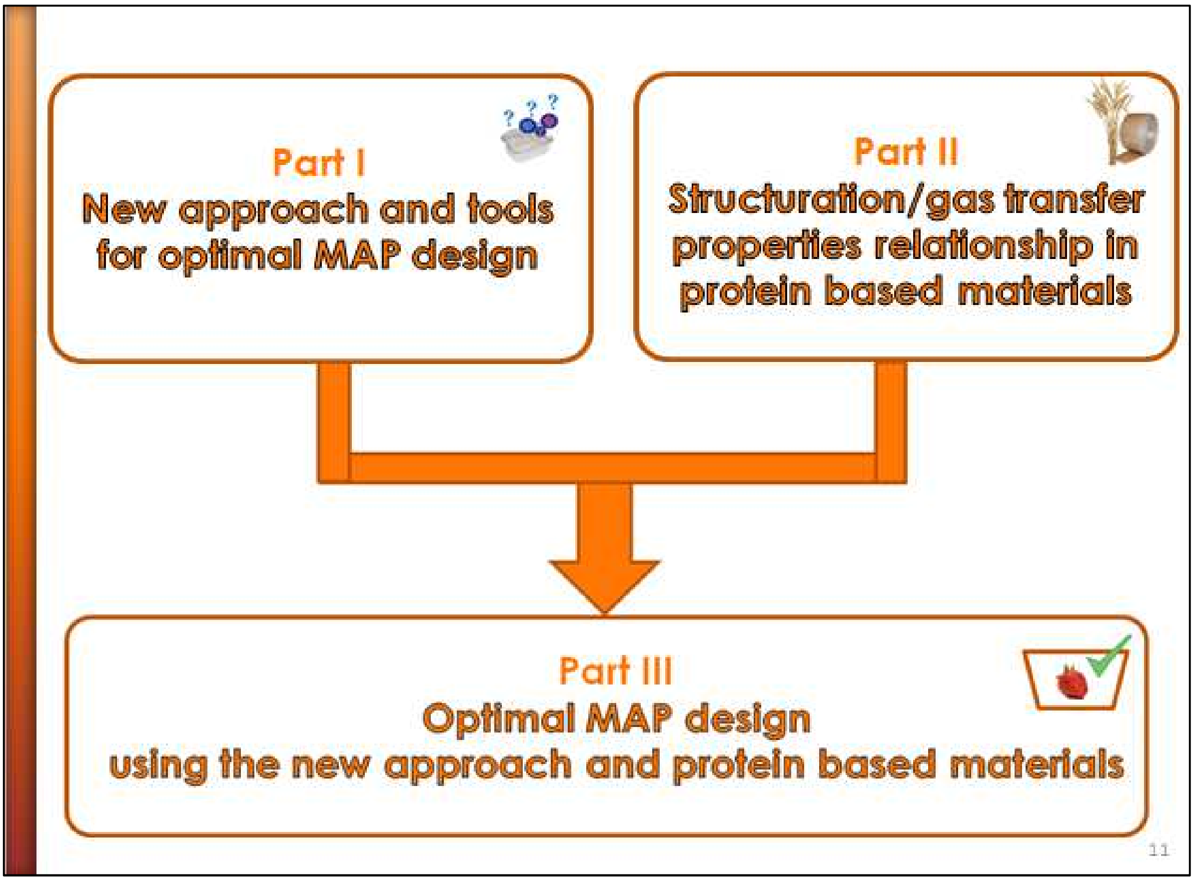
II. Tailored packaging materials

- Develop **packaging materials** exhibiting **adequate gas transfer properties** for optimal preservation of respiring produce

→ Broaden the database on gas transfer properties of protein based materials and generate knowledge on structuration/gas transfer properties relationship in protein based materials

III. Tailored packaging conception for optimal MAP

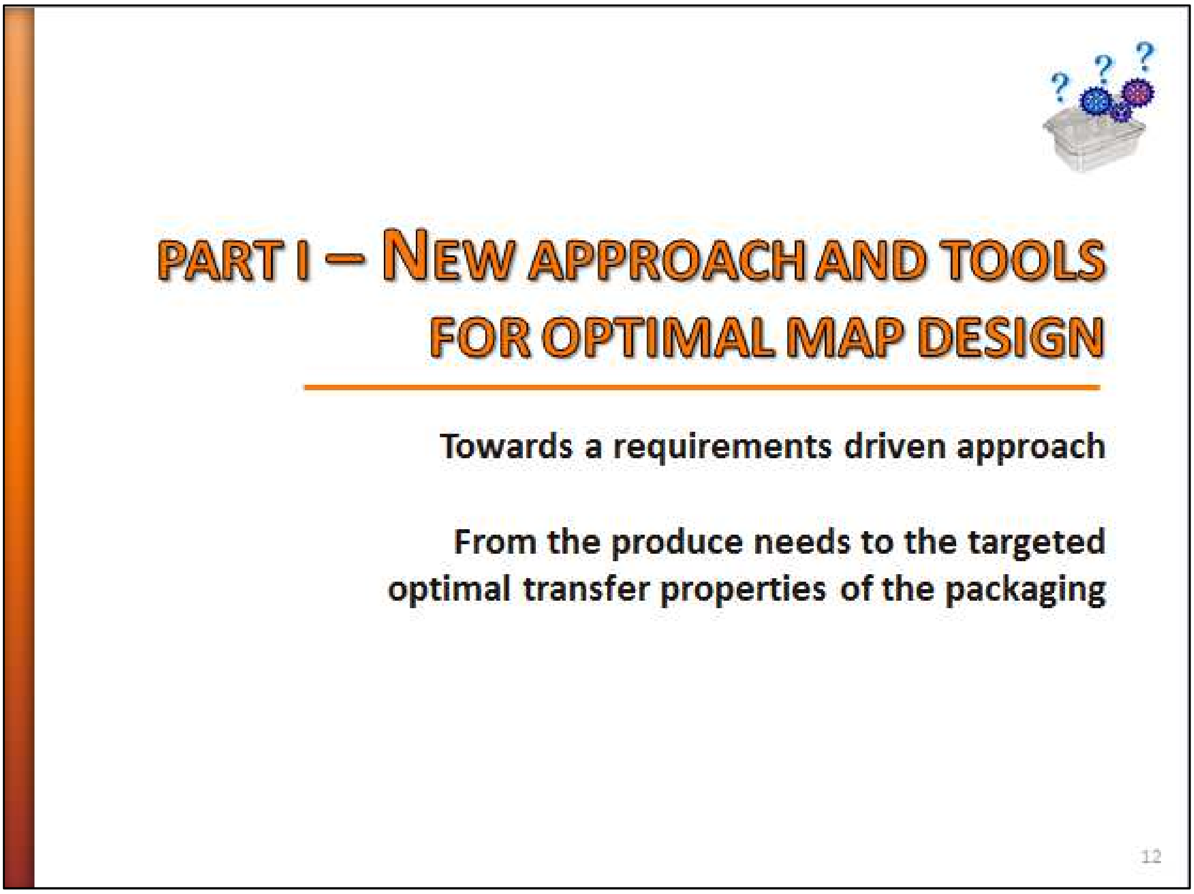
- **Validate the new approach** proposed and the **knowledge acquired** on structuration / transfer properties relationship in **protein based materials** by **developing an optimal MAP** for a specific produce



Part I
New approach and tools for optimal MAP design

Part II
Structuration/gas transfer properties relationship in protein based materials

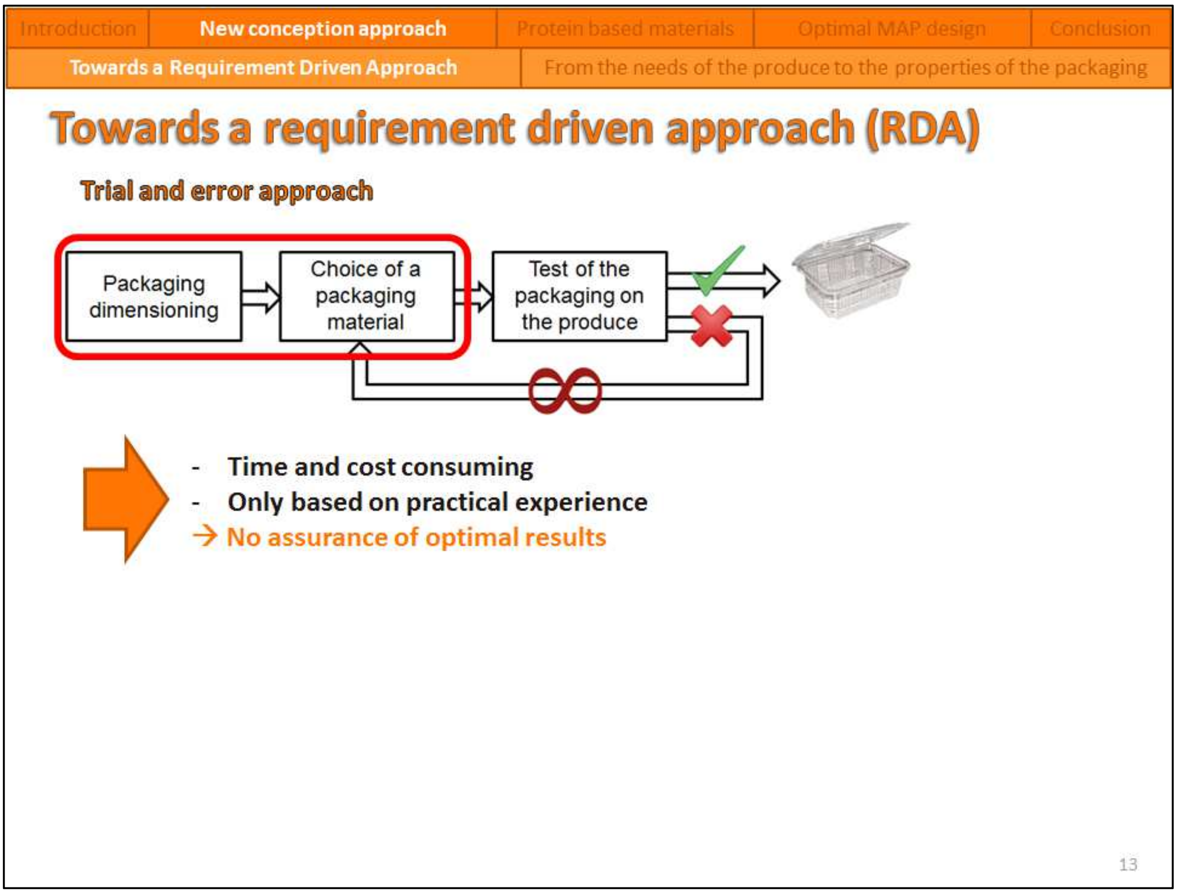
Part III
Optimal MAP design
using the new approach and protein based materials



PART I – NEW APPROACH AND TOOLS FOR OPTIMAL MAP DESIGN

Towards a requirements driven approach

From the produce needs to the targeted optimal transfer properties of the packaging

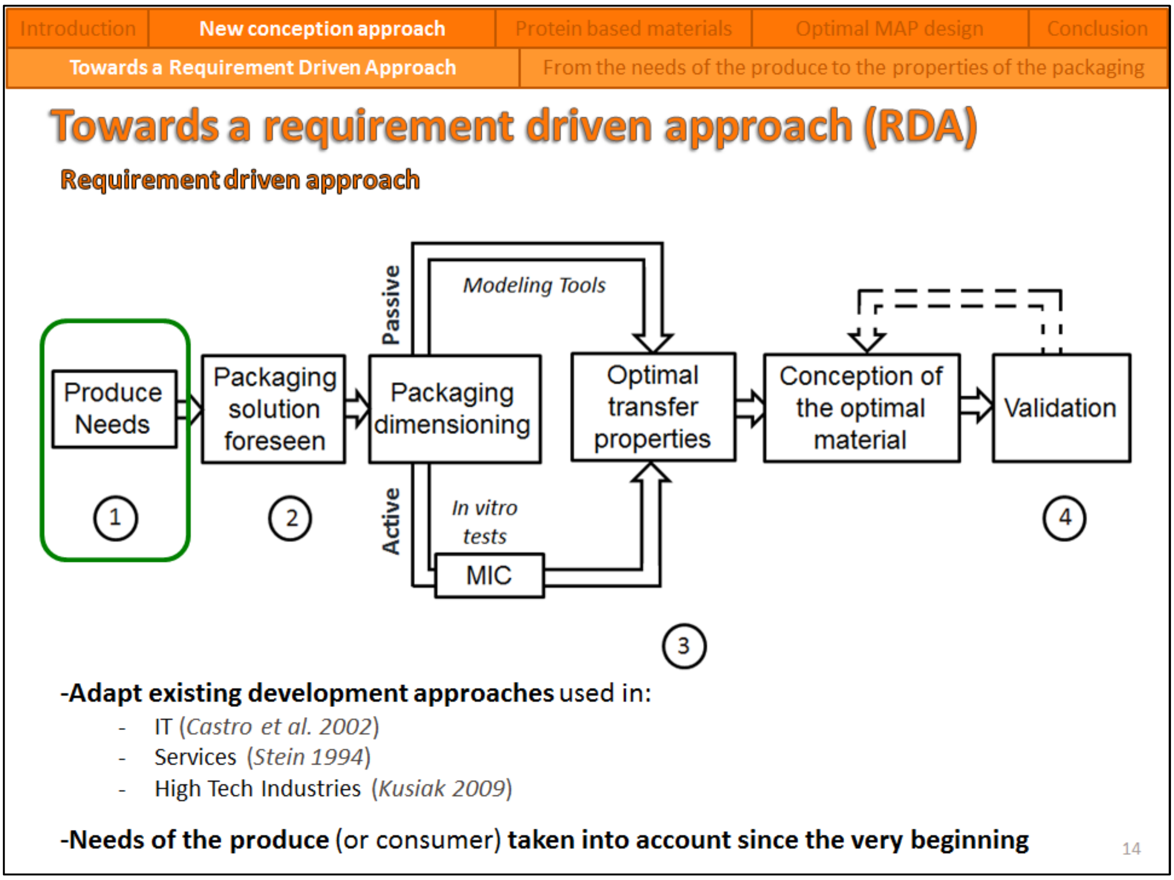


Towards a requirement driven approach (RDA)

Trial and error approach

- Time and cost consuming
- Only based on practical experience

→ No assurance of optimal results



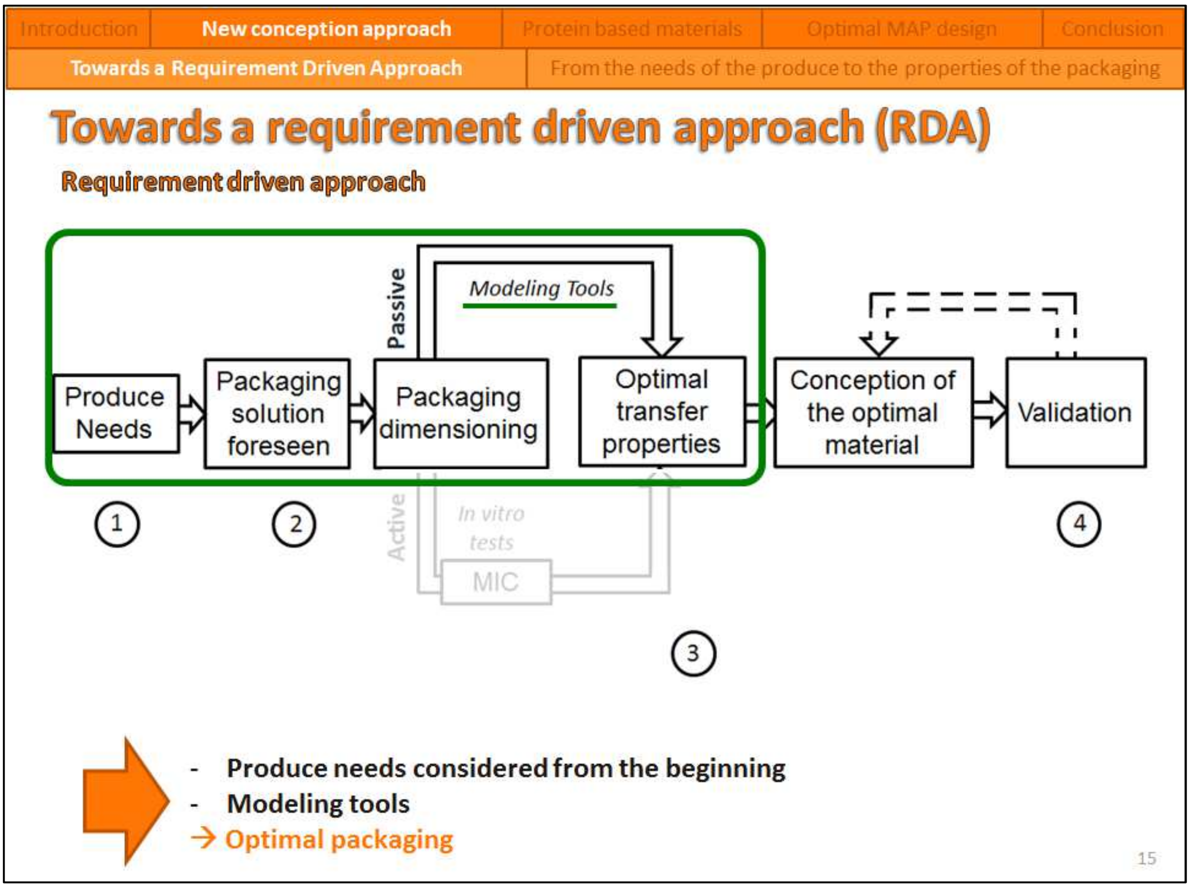
Towards a requirement driven approach (RDA)

Requirement driven approach

-Adapt existing development approaches used in:

- IT (*Castro et al. 2002*)
- Services (*Stein 1994*)
- High Tech Industries (*Kusiak 2009*)

-Needs of the produce (or consumer) **taken into account since the very beginning**



Towards a requirement driven approach (RDA)

Requirement driven approach

Passive

Modeling Tools

Produce Needs

Packaging solution foreseen

Packaging dimensioning

Optimal transfer properties

Conception of the optimal material

Validation

Active

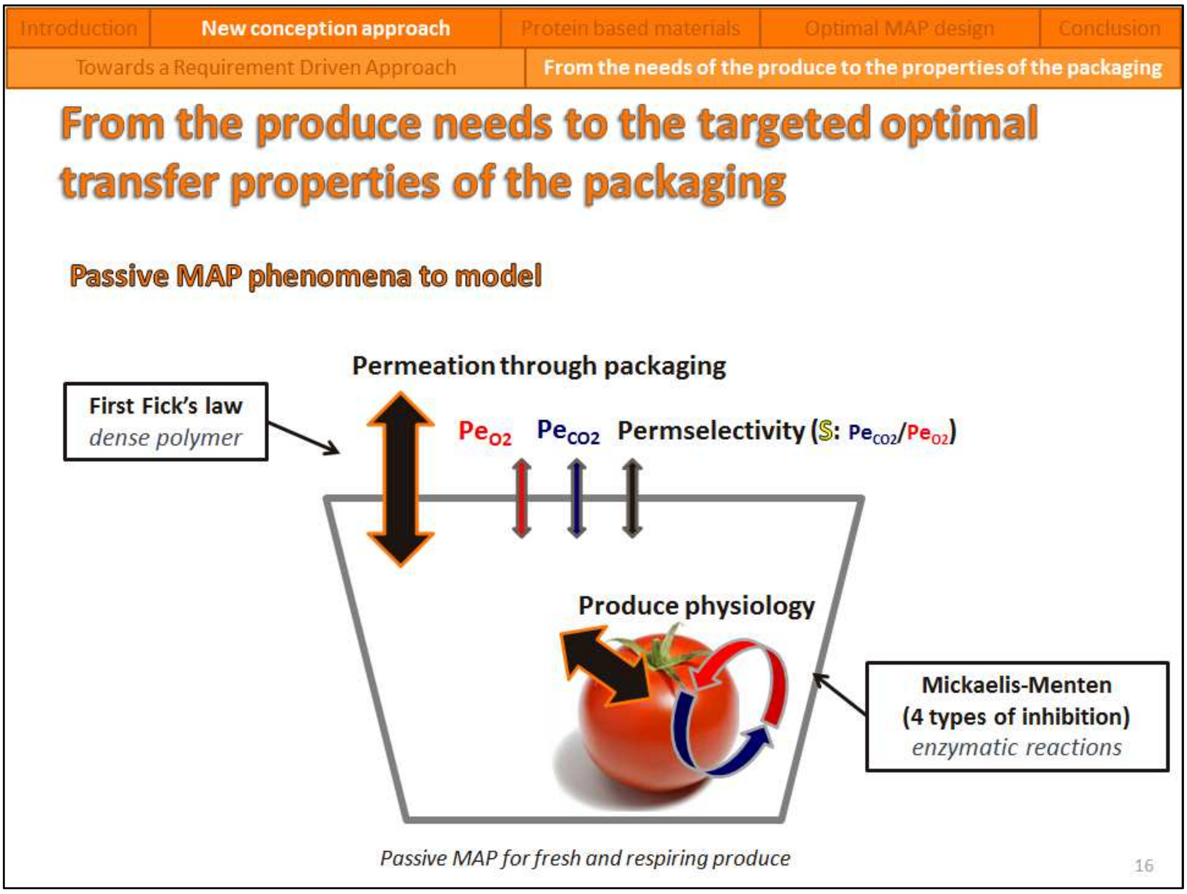
In vitro tests

MIC

① ② ③ ④

- **Produce needs considered from the beginning**
- **Modeling tools**

→ **Optimal packaging**



From the produce needs to the targeted optimal transfer properties of the packaging

Passive MAP phenomena to model

Permeation through packaging

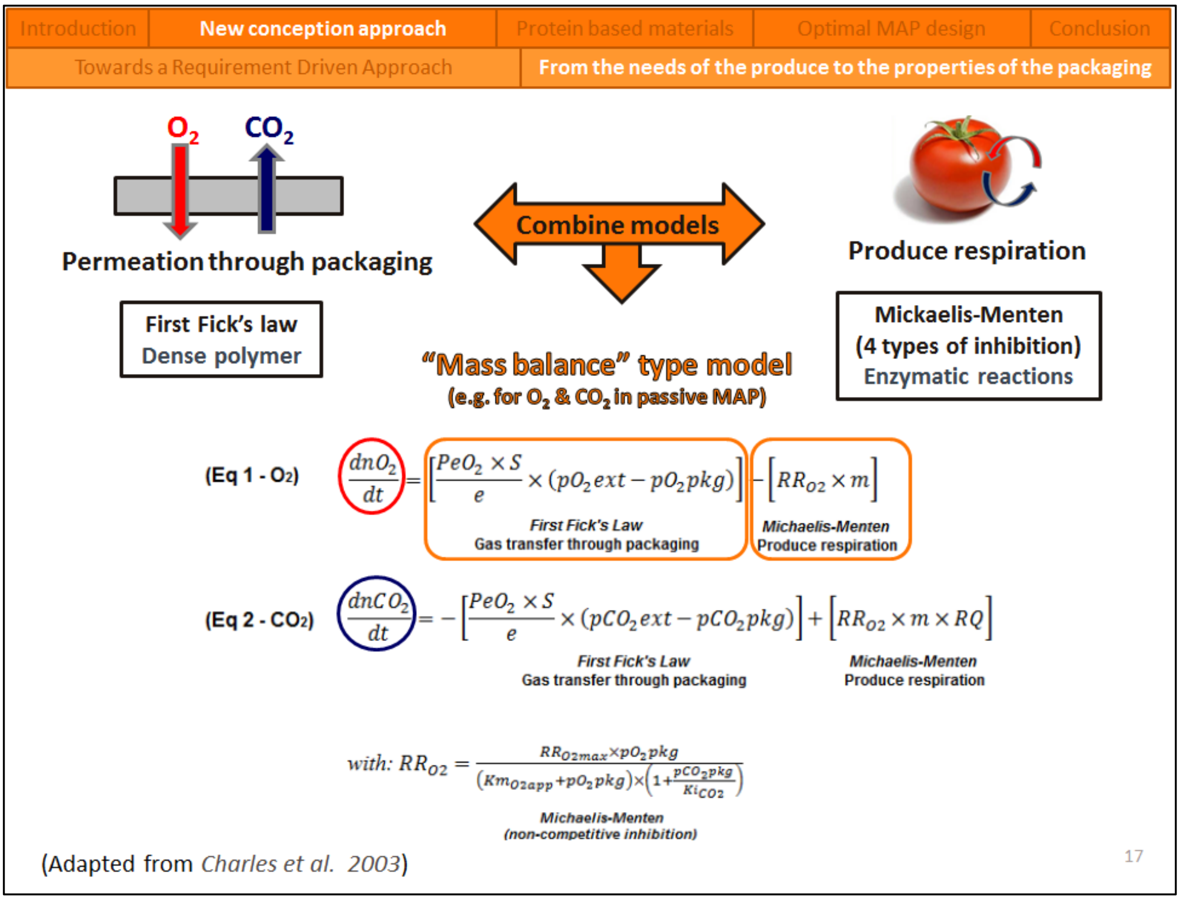
First Fick's law
dense polymer

Pe_{O2} Pe_{CO2} **Permselectivity** (S: Pe_{CO2}/Pe_{O2})

Produce physiology

Mickaelis-Menten
(4 types of inhibition)
enzymatic reactions

Passive MAP for fresh and respiring produce



Introduction | **New conception approach** | Protein based materials | Optimal MAP design | Conclusion

Towards a Requirement Driven Approach | **From the needs of the produce to the properties of the packaging**

Permeation through packaging

First Fick's law
Dense polymer

Combine models

Produce respiration

Mickaelis-Menten
(4 types of inhibition)
Enzymatic reactions

"Mass balance" type model
(e.g. for O_2 & CO_2 in passive MAP)

(Eq 1 - O2)

$$\frac{dnO_2}{dt} = \underbrace{\left[\frac{PeO_2 \times S}{e} \times (pO_2ext - pO_2pkg)\right]}_{\text{First Fick's Law, Gas transfer through packaging}} - \underbrace{\left[RR_{O2} \times m\right]}_{\text{Michaelis-Menten, Produce respiration}}$$

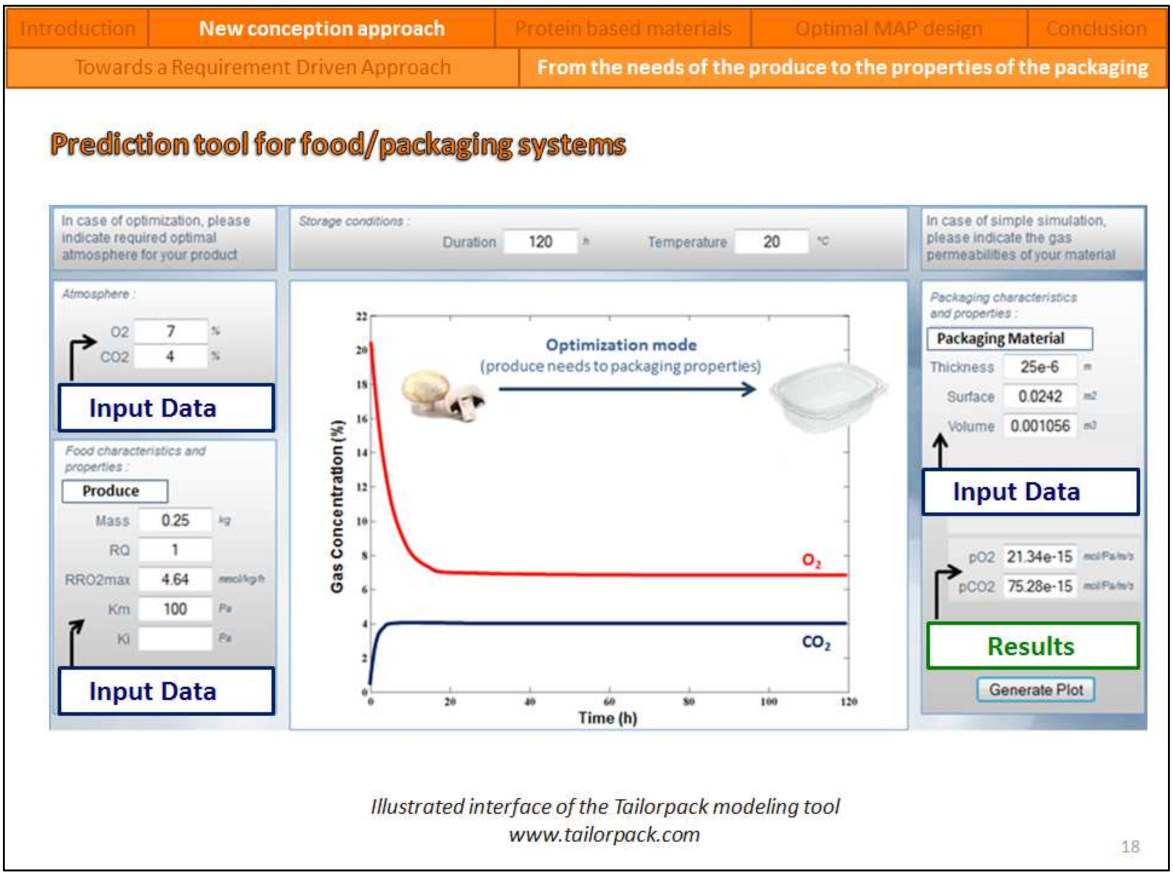
(Eq 2 - CO2)

$$\frac{dnCO_2}{dt} = -\underbrace{\left[\frac{PeO_2 \times S}{e} \times (pCO_2ext - pCO_2pkg)\right]}_{\text{First Fick's Law, Gas transfer through packaging}} + \underbrace{\left[RR_{O2} \times m \times RQ\right]}_{\text{Michaelis-Menten, Produce respiration}}$$

$$with:\ RR_{O2} = \frac{RR_{O2max} \times pO_2pkg}{(Km_{O2app} + pO_2pkg) \times \left(1 + \frac{pCO_2pkg}{Ki_{CO2}}\right)}$$

Michaelis-Menten
(non-competitive inhibition)

(Adapted from *Charles et al. 2003*)



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Towards a Requirement Driven Approach | **From the needs of the produce to the properties of the packaging**

Prediction tool for food/packaging systems

In case of optimization, please indicate required optimal atmosphere for your product

Atmosphere :
O2 7 %
CO2 4 %

Input Data

Food characteristics and properties :
Produce
Mass 0.25 kg
RQ 1
RRO2max 4.64 mmol/kg/h
Km 100 Pa
Ki Pa

Input Data

Storage conditions :
Duration 120 h
Temperature 20 °C

Optimization mode
(produce needs to packaging properties)

In case of simple simulation, please indicate the gas permeabilities of your material

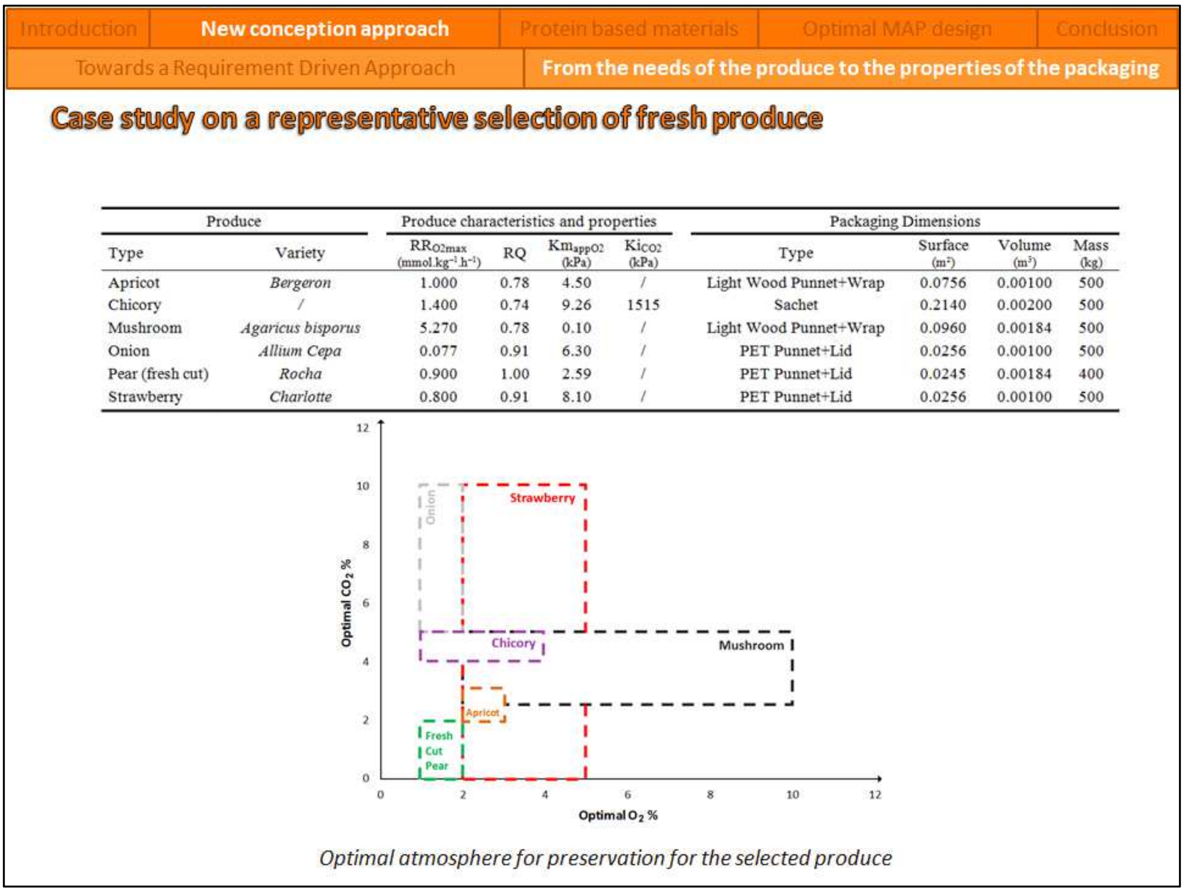
Packaging characteristics and properties :
Packaging Material
Thickness 25e-6 m
Surface 0.0242 m2
Volume 0.001056 m3

Input Data

pO2 21.34e-15 mol/Pa/m/s
pCO2 75.28e-15 mol/Pa/m/s

Results

Illustrated interface of the Tailorpack modeling tool
www.tailorpack.com



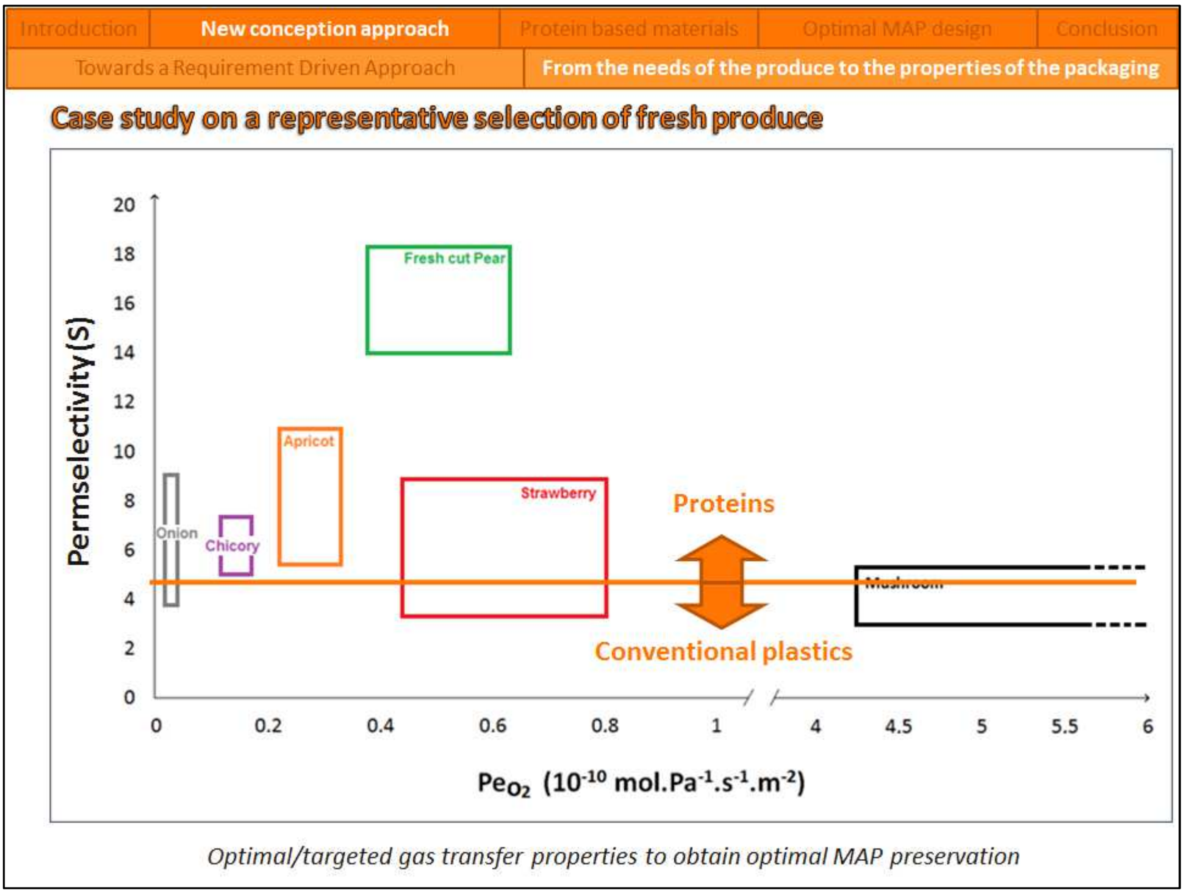
Introduction | **New conception approach** | Protein based materials | Optimal MAP design | Conclusion

Towards a Requirement Driven Approach | **From the needs of the produce to the properties of the packaging**

Case study on a representative selection of fresh produce

Produce		Produce characteristics and properties				Packaging Dimensions			
Type	Variety	RR_{O2max} ($mmol.kg^{-1}.h^{-1}$)	RQ	Km_{appO2} (kPa)	Ki_{CO2} (kPa)	Type	Surface (m^2)	Volume (m^3)	Mass (kg)
Apricot	*Bergeron*	1.000	0.78	4.50	/	Light Wood Punnet+Wrap	0.0756	0.00100	500
Chicory	/	1.400	0.74	9.26	1515	Sachet	0.2140	0.00200	500
Mushroom	*Agaricus bisporus*	5.270	0.78	0.10	/	Light Wood Punnet+Wrap	0.0960	0.00184	500
Onion	*Allium Cepa*	0.077	0.91	6.30	/	PET Punnet+Lid	0.0256	0.00100	500
Pear (fresh cut)	*Rocha*	0.900	1.00	2.59	/	PET Punnet+Lid	0.0245	0.00184	400
Strawberry	*Charlotte*	0.800	0.91	8.10	/	PET Punnet+Lid	0.0256	0.00100	500

Optimal atmosphere for preservation for the selected produce



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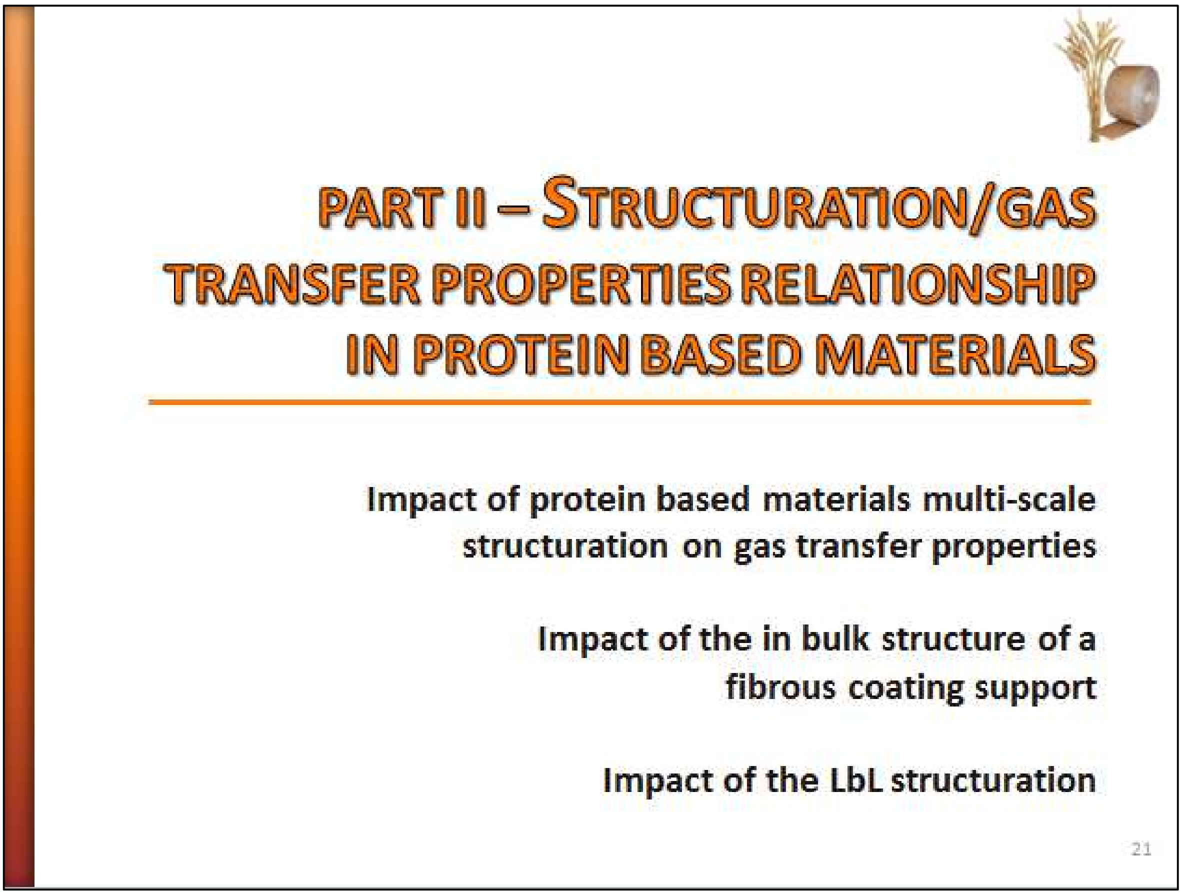
Towards a Requirement Driven Approach | **From the needs of the produce to the properties of the packaging**

Case study on a representative selection of fresh produce

Optimal/targeted gas transfer properties to obtain optimal MAP preservation



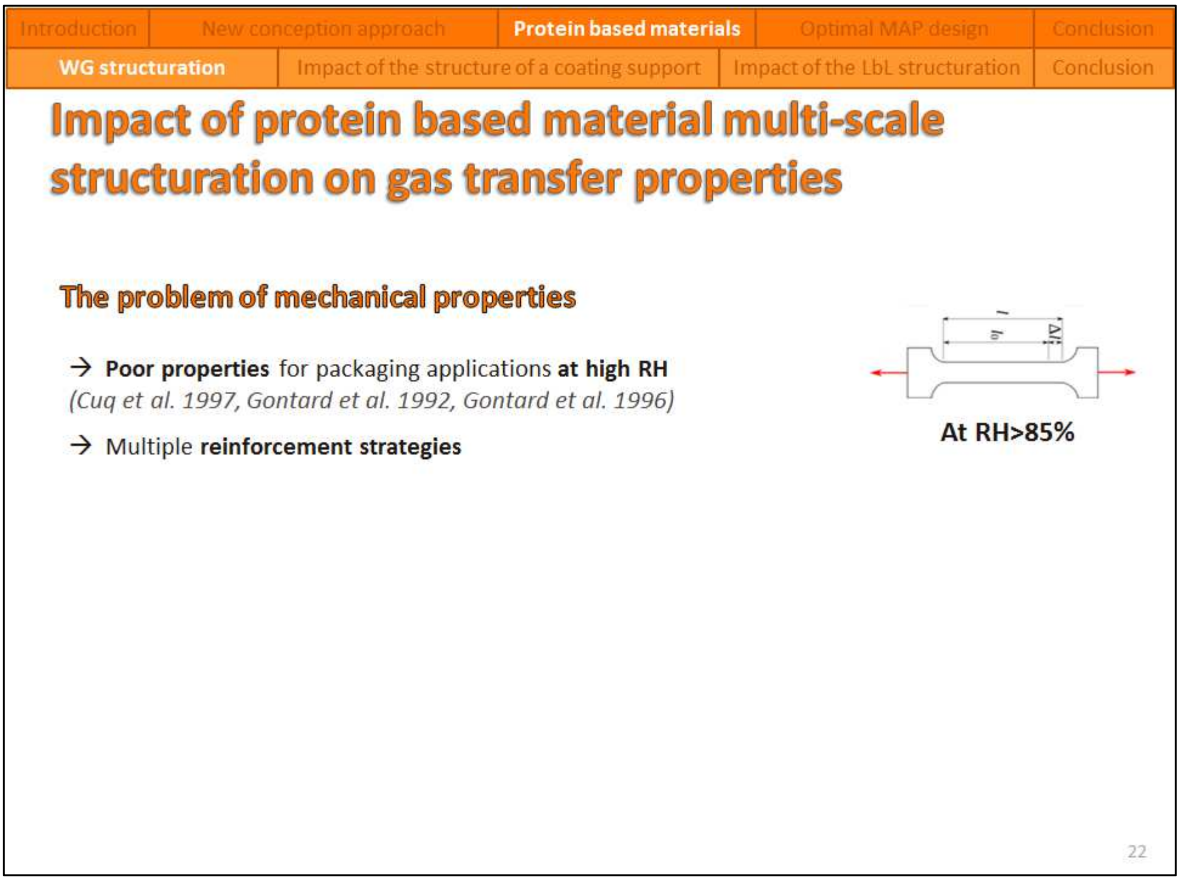
PART II – STRUCTURATION/GAS TRANSFER PROPERTIES RELATIONSHIP IN PROTEIN BASED MATERIALS

Impact of protein based materials multi-scale structuration on gas transfer properties

Impact of the in bulk structure of a fibrous coating support

Impact of the LbL structuration

21



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WG structuration | Impact of the structure of a coating support | Impact of the LbL structuration | Conclusion

Impact of protein based material multi-scale structuration on gas transfer properties

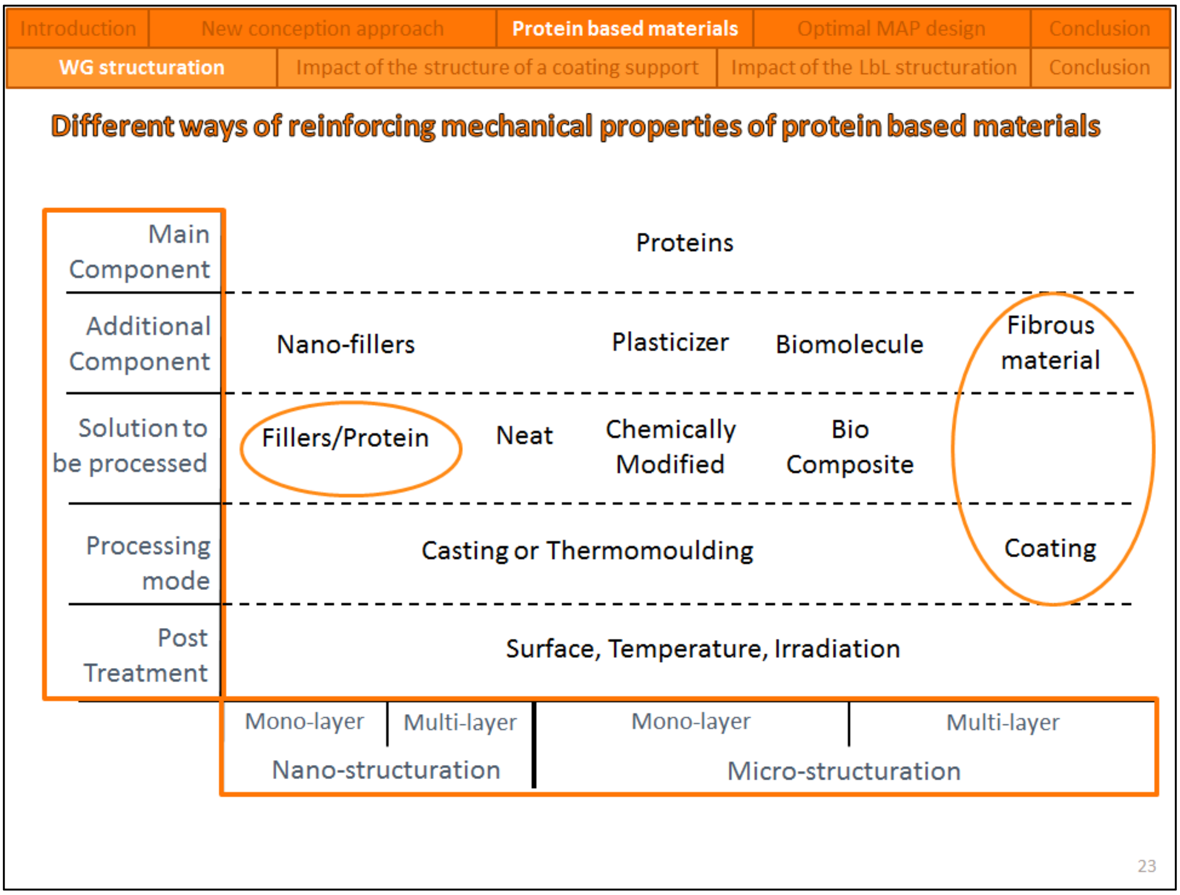
The problem of mechanical properties

→ **Poor properties** for packaging applications **at high RH** *(Cuq et al. 1997, Gontard et al. 1992, Gontard et al. 1996)*

→ Multiple **reinforcement strategies**

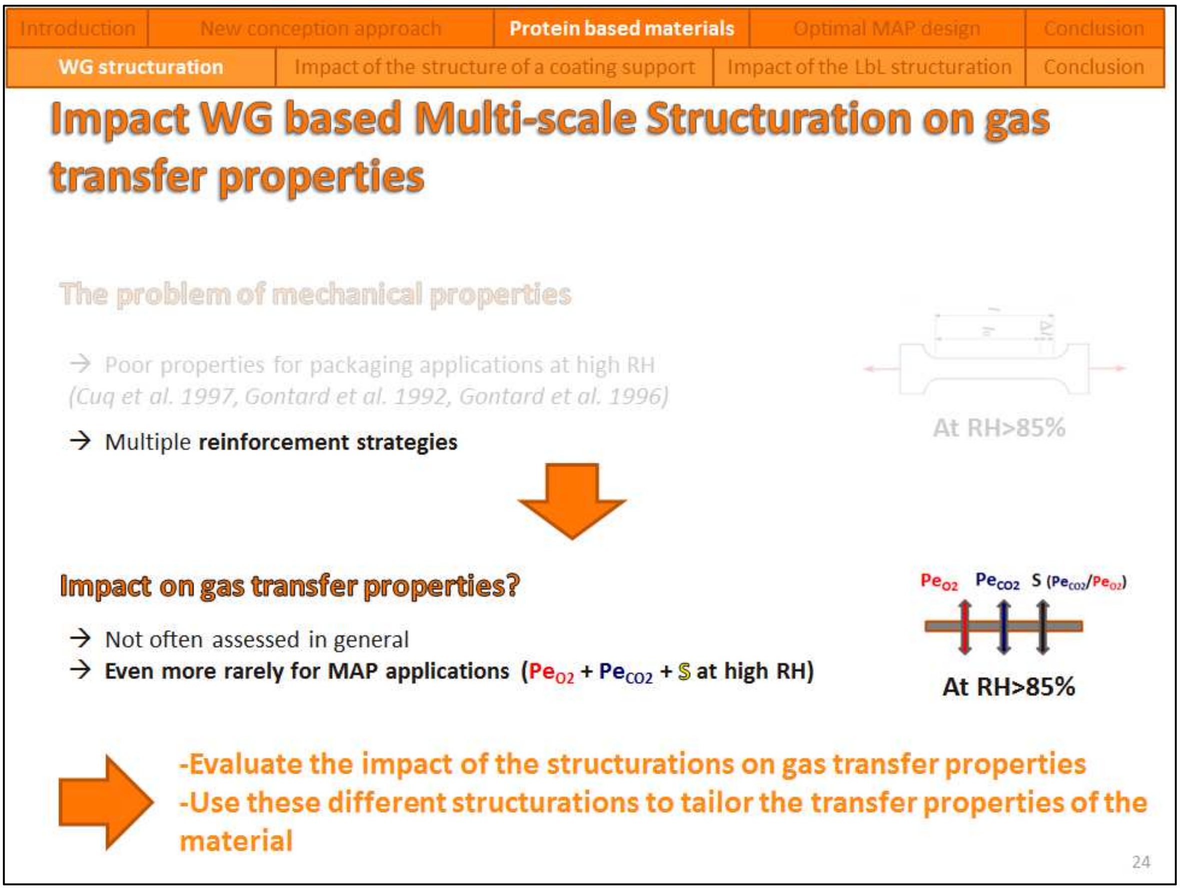
At RH>85%

22



Different ways of reinforcing mechanical properties of protein based materials

Main Component	Proteins				
Additional Component	Nano-fillers		Plasticizer	Biomolecule	Fibrous material
Solution to be processed	Fillers/Protein	Neat	Chemically Modified	Bio Composite	
Processing mode	Casting or Thermomoulding				Coating
Post Treatment	Surface, Temperature, Irradiation				
	Mono-layer	Multi-layer	Mono-layer		Multi-layer
	Nano-structuration		Micro-structuration		



Impact WG based Multi-scale Structuration on gas transfer properties

The problem of mechanical properties

→ Poor properties for packaging applications at high RH
(Cuq et al. 1997, Gontard et al. 1992, Gontard et al. 1996)

→ Multiple **reinforcement strategies**

At RH>85%

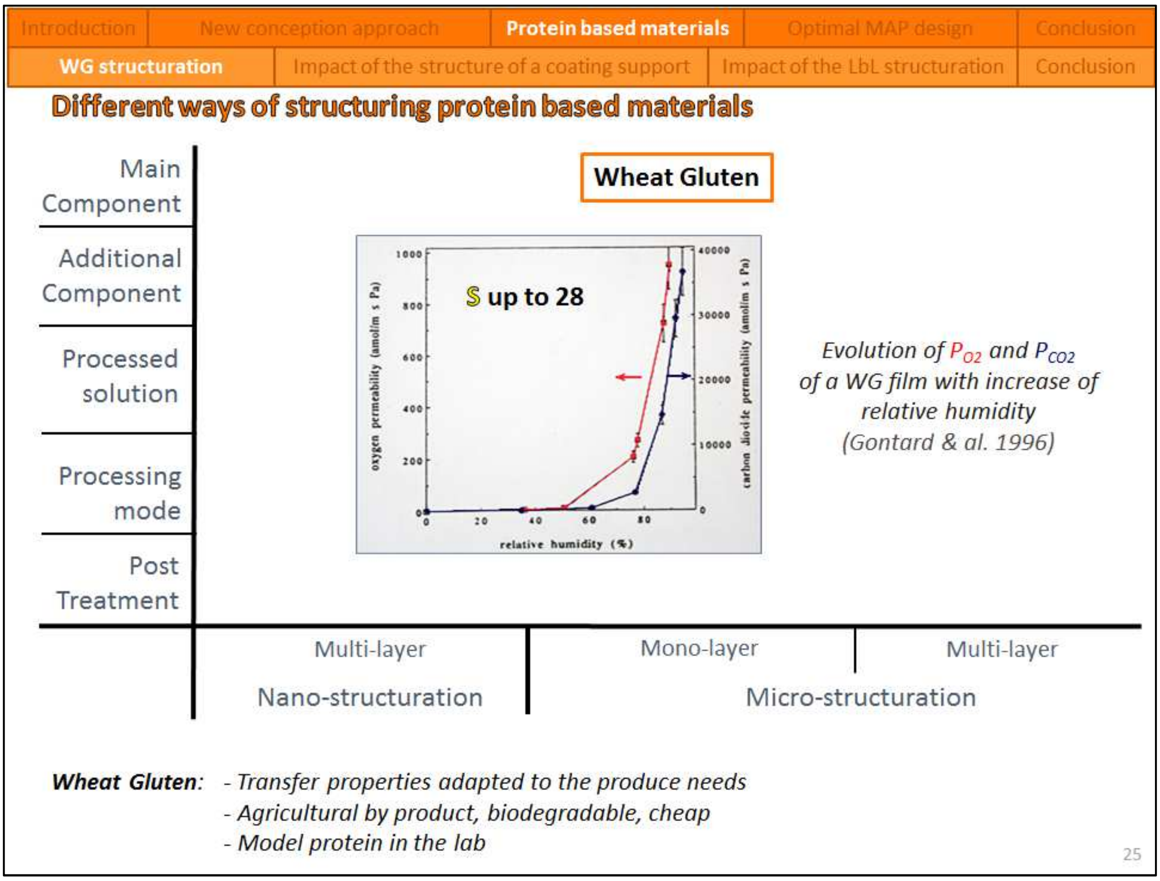
Impact on gas transfer properties?

→ Not often assessed in general

→ **Even more rarely for MAP applications (Pe_{O2} + Pe_{CO2} + S at high RH)**

At RH>85%

-Evaluate the impact of the structurations on gas transfer properties
-Use these different structurations to tailor the transfer properties of the material



Introduction | New conception approach | **Protein based materials** | Optimal MAP design | Conclusion

WG structuration | Impact of the structure of a coating support | Impact of the LbL structuration | Conclusion

Different ways of structuring protein based materials

Main Component

Additional Component

Processed solution

Processing mode

Post Treatment

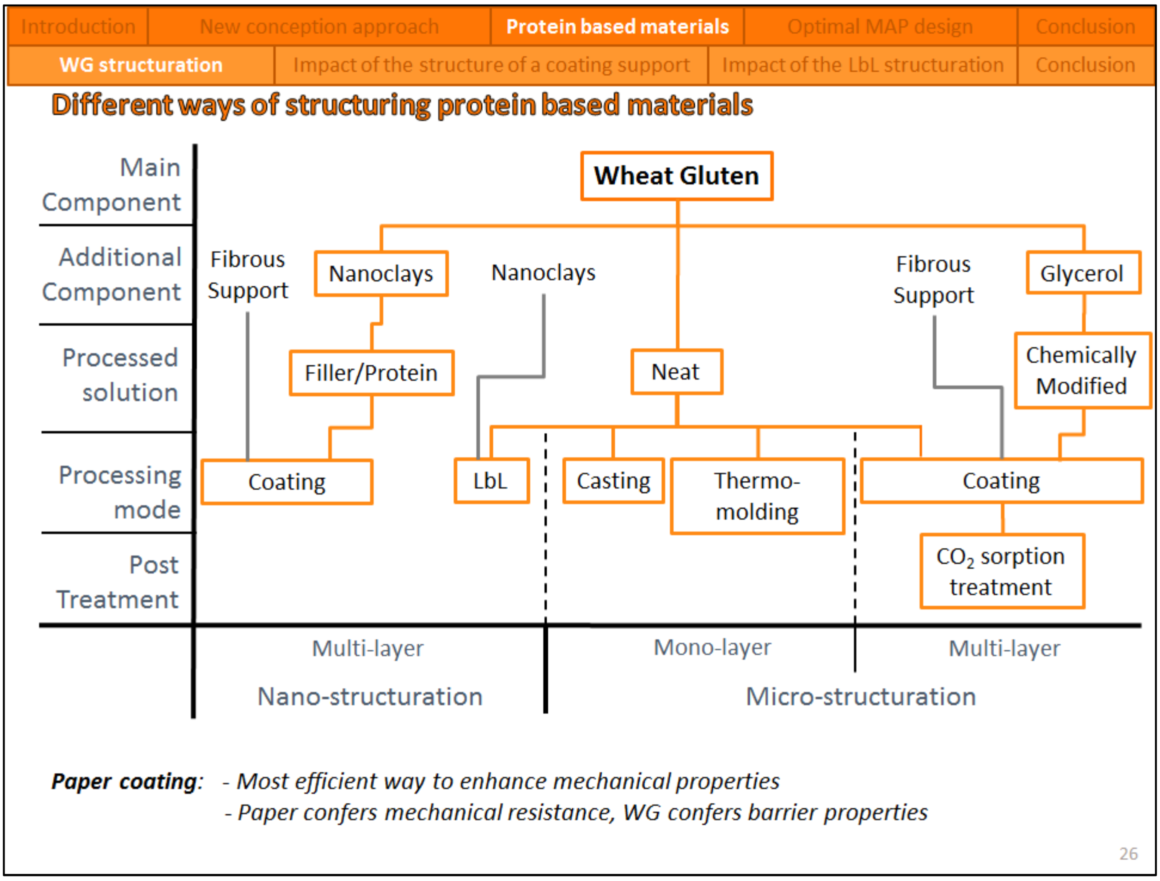
Wheat Gluten

Evolution of P_{O2} and P_{CO2} of a WG film with increase of relative humidity (Gontard & al. 1996)

Multi-layer | Mono-layer | Multi-layer

Nano-structuration | Micro-structuration

Wheat Gluten: - *Transfer properties adapted to the produce needs*
- *Agricultural by product, biodegradable, cheap*
- *Model protein in the lab*



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Different ways of structuring protein based materials

Main Component

Additional Component

Processed solution

Processing mode

Post Treatment

Wheat Gluten

Fibrous Support

Nanoclays

Nanoclays

Fibrous Support

Glycerol

Filler/Protein

Neat

Chemically Modified

Coating

LbL

Casting

Thermo-molding

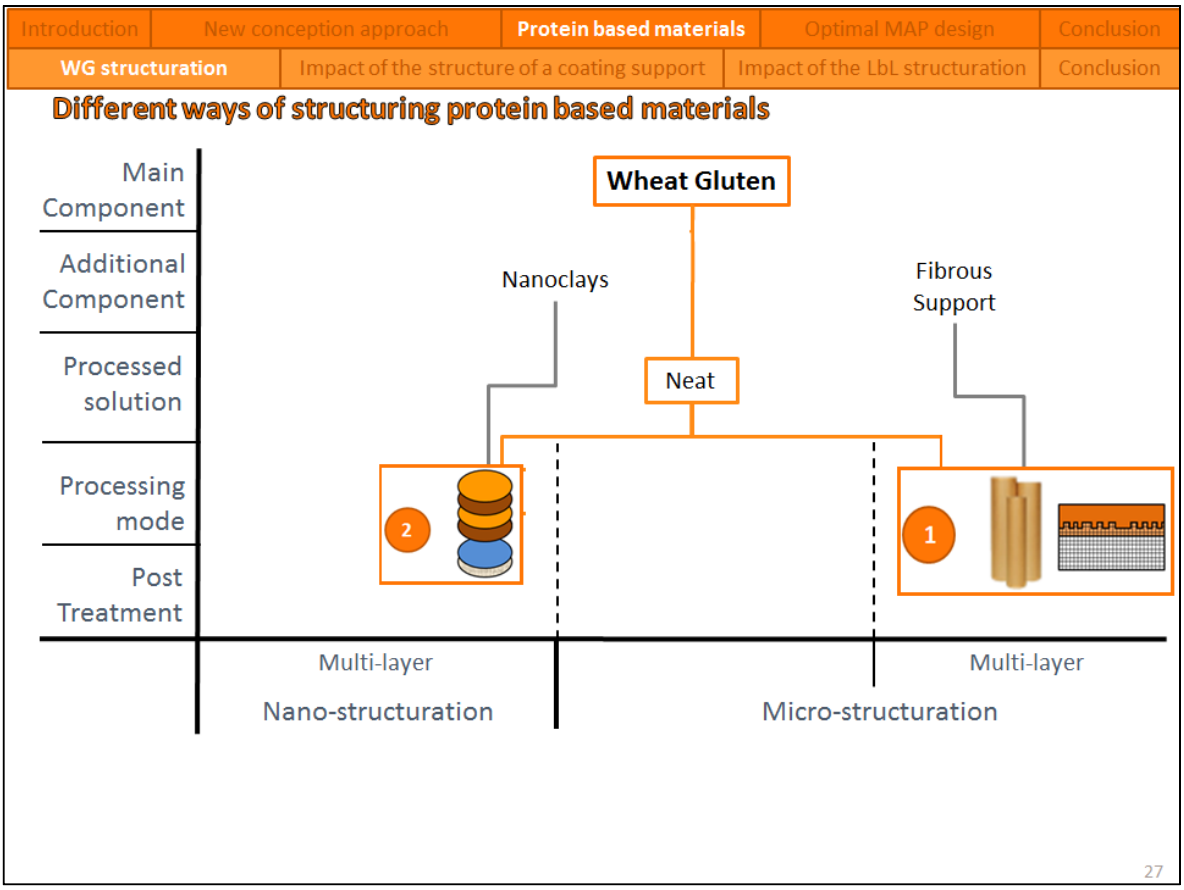
Coating

CO_2 sorption treatment

Multi-layer | Mono-layer | Multi-layer

Nano-structuration | Micro-structuration

Paper coating: - *Most efficient way to enhance mechanical properties*
- *Paper confers mechanical resistance, WG confers barrier properties*



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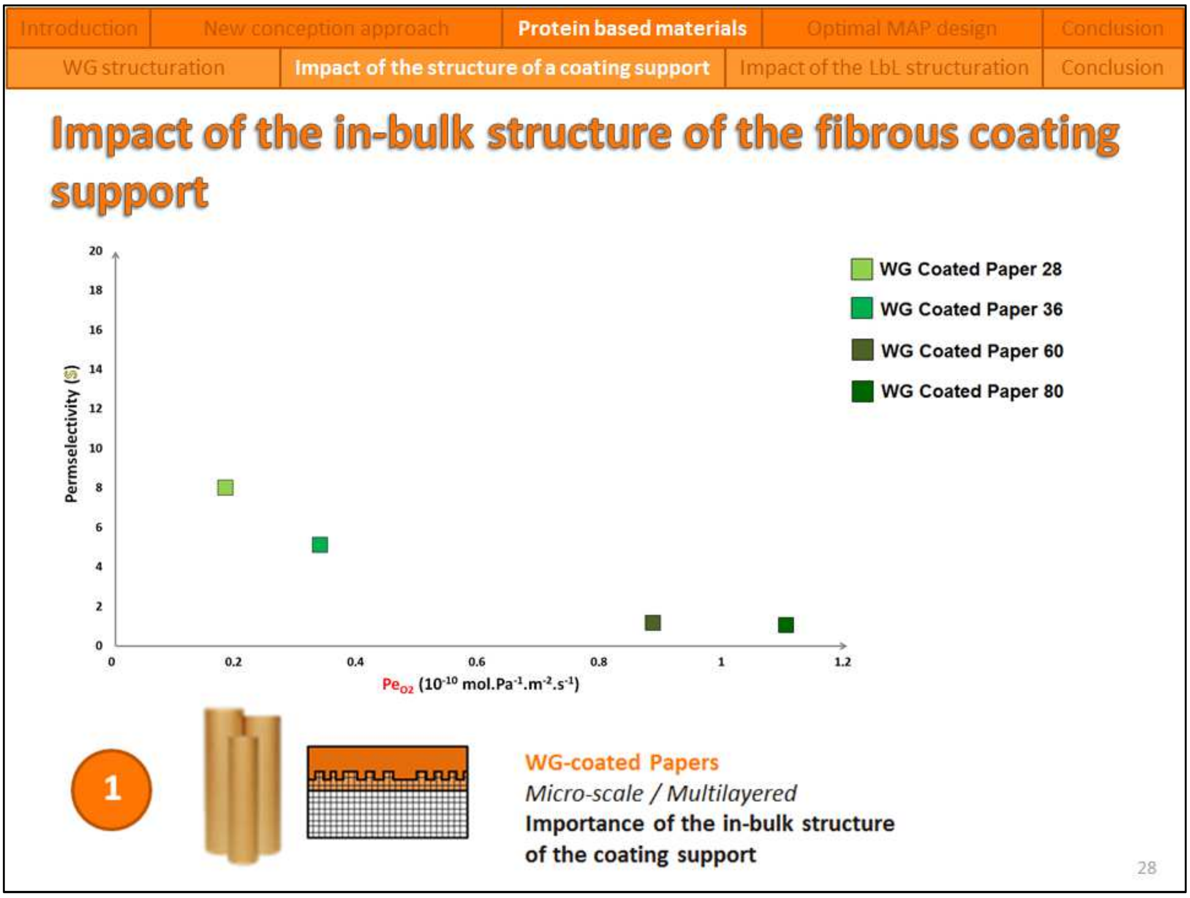

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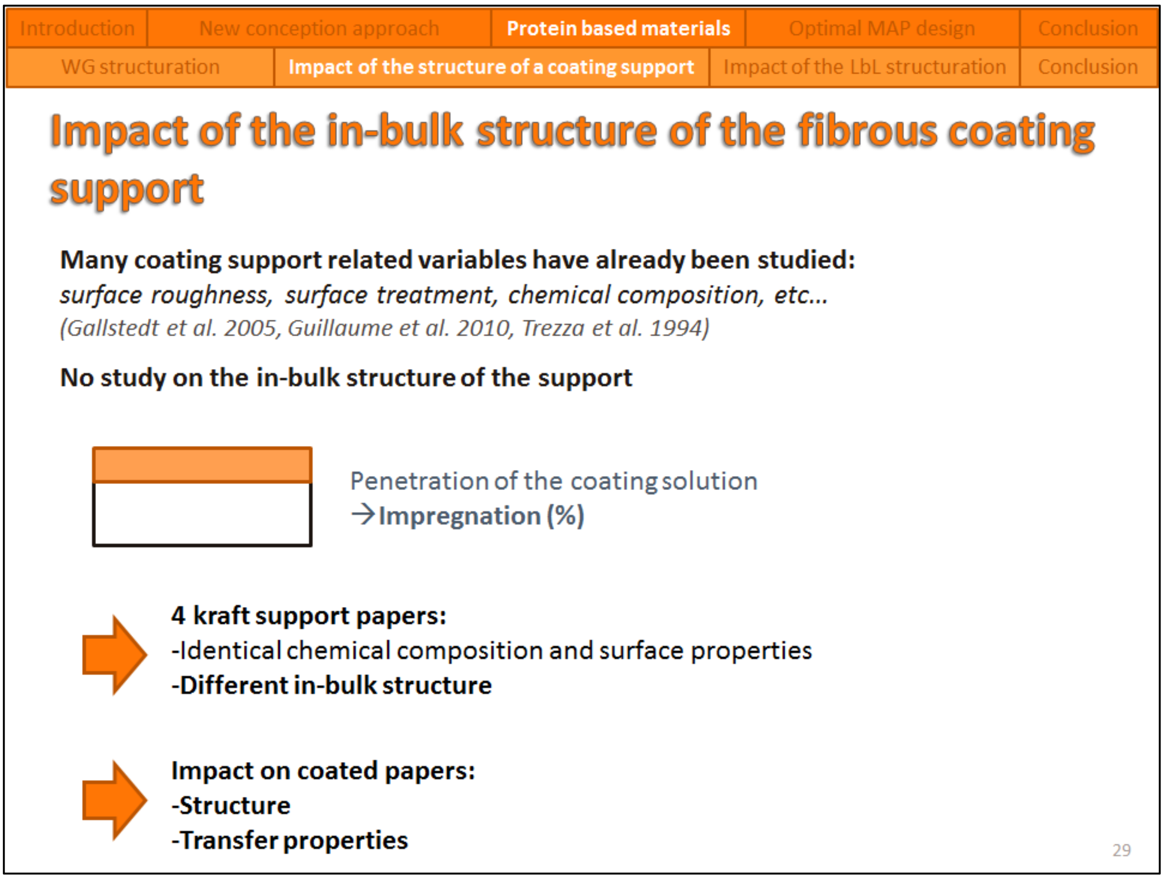
WG structuration | **Impact of the structure of a coating support** | Impact of the LbL structuration | Conclusion

Impact of the in-bulk structure of the fibrous coating support

WG-coated Papers

Micro-scale / Multilayered

Importance of the in-bulk structure of the coating support



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WG structuration | **Impact of the structure of a coating support** | Impact of the LbL structuration | Conclusion

Impact of the in-bulk structure of the fibrous coating support

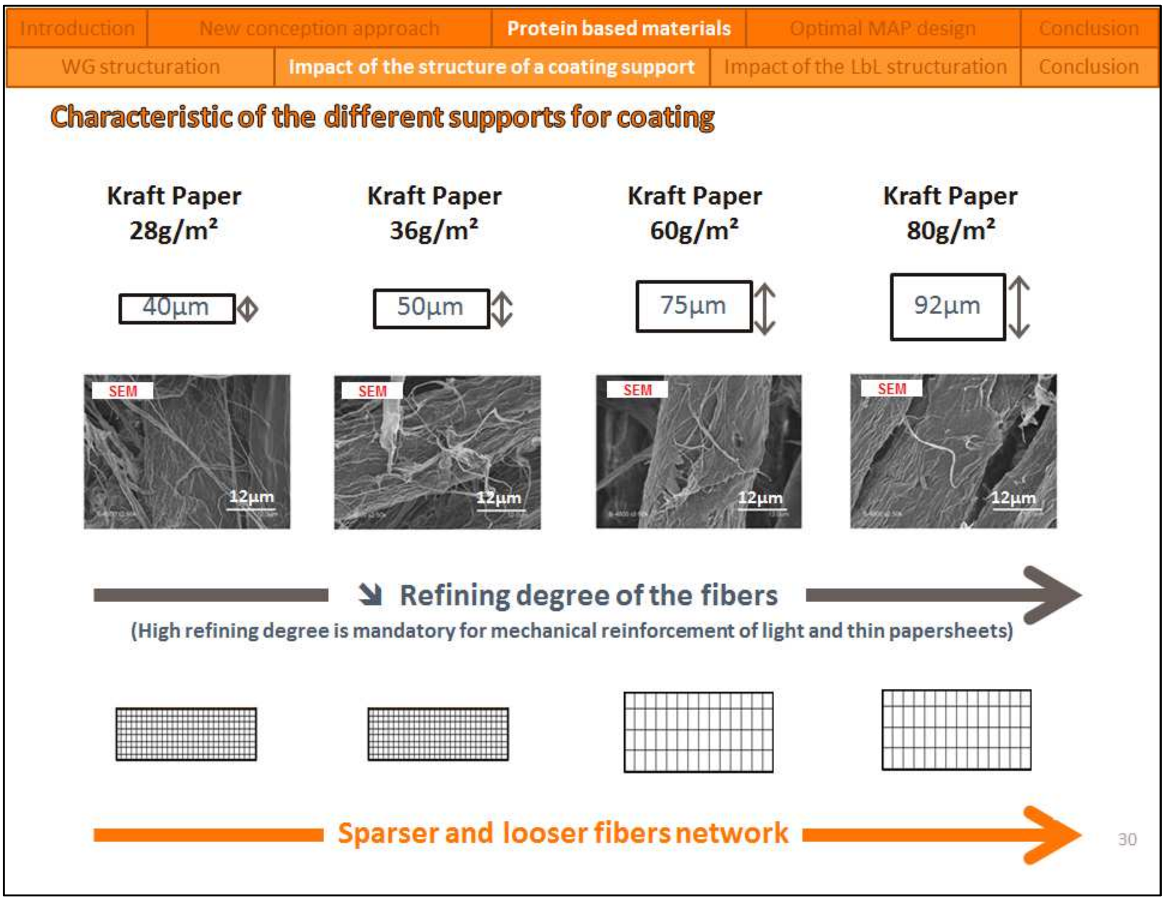
Many coating support related variables have already been studied:
surface roughness, surface treatment, chemical composition, etc…
(Gallstedt et al. 2005, Guillaume et al. 2010, Trezza et al. 1994)

No study on the in-bulk structure of the support

Penetration of the coating solution
→**Impregnation (%)**

4 kraft support papers:
-Identical chemical composition and surface properties
-Different in-bulk structure

Impact on coated papers:
-Structure
-Transfer properties



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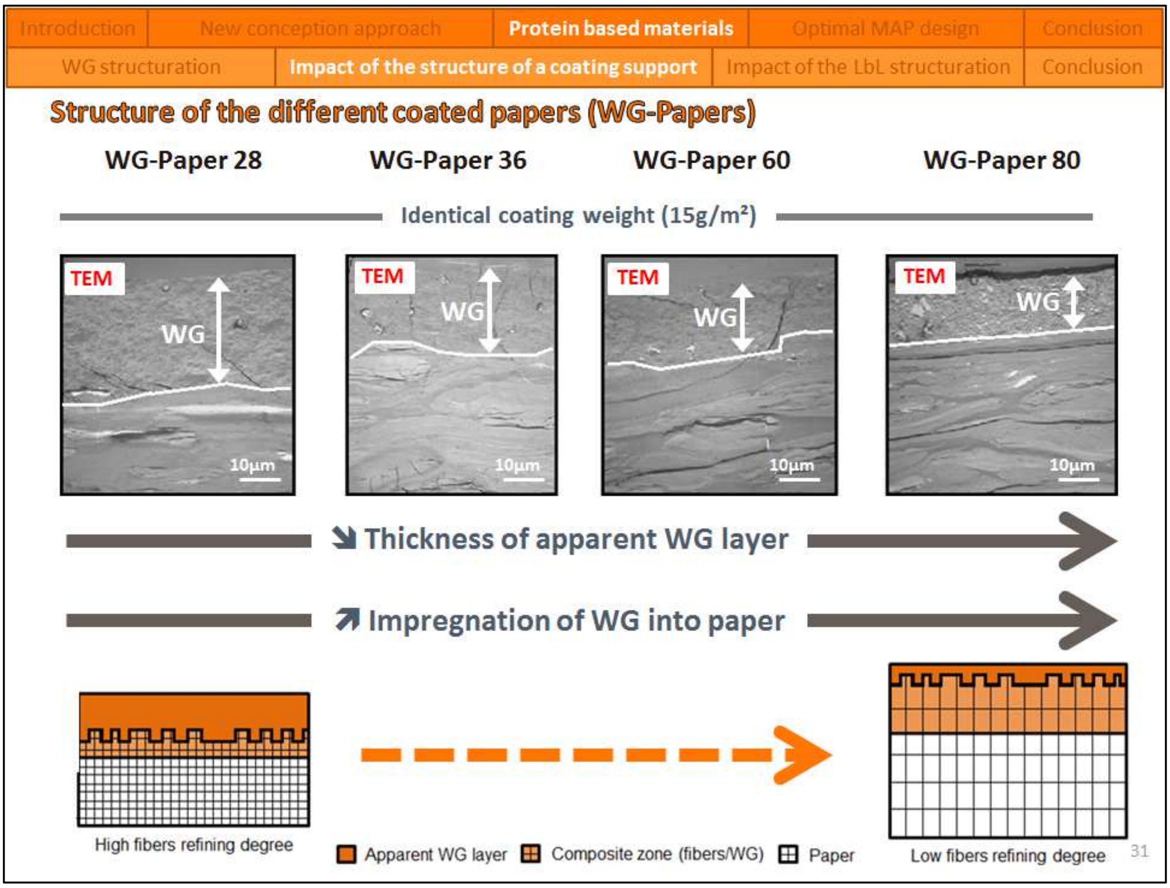
WG structuration | **Impact of the structure of a coating support** | Impact of the LbL structuration | Conclusion

Characteristic of the different supports for coating

Kraft Paper 28g/m²	Kraft Paper 36g/m²	Kraft Paper 60g/m²	Kraft Paper 80g/m²
40µm	50µm	75µm	92µm

↘ **Refining degree of the fibers**
(High refining degree is mandatory for mechanical reinforcement of light and thin papersheets)

Sparser and looser fibers network



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Structure of the different coated papers (WG-Papers)

WG-Paper 28 **WG-Paper 36** **WG-Paper 60** **WG-Paper 80**

Identical coating weight (15g/m²)

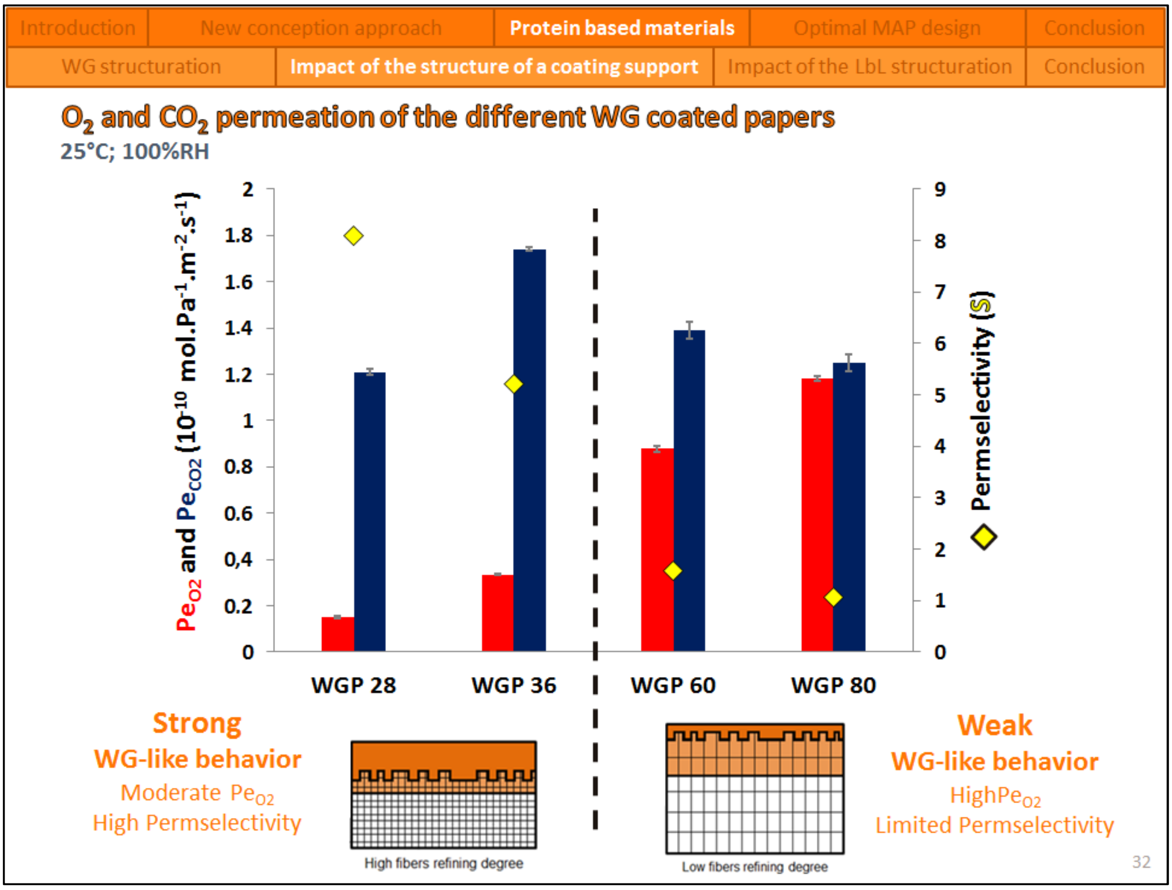
↘ Thickness of apparent WG layer

↗ Impregnation of WG into paper

High fibers refining degree

Low fibers refining degree

▨ Apparent WG layer ⊞ Composite zone (fibers/WG) ⊞ Paper



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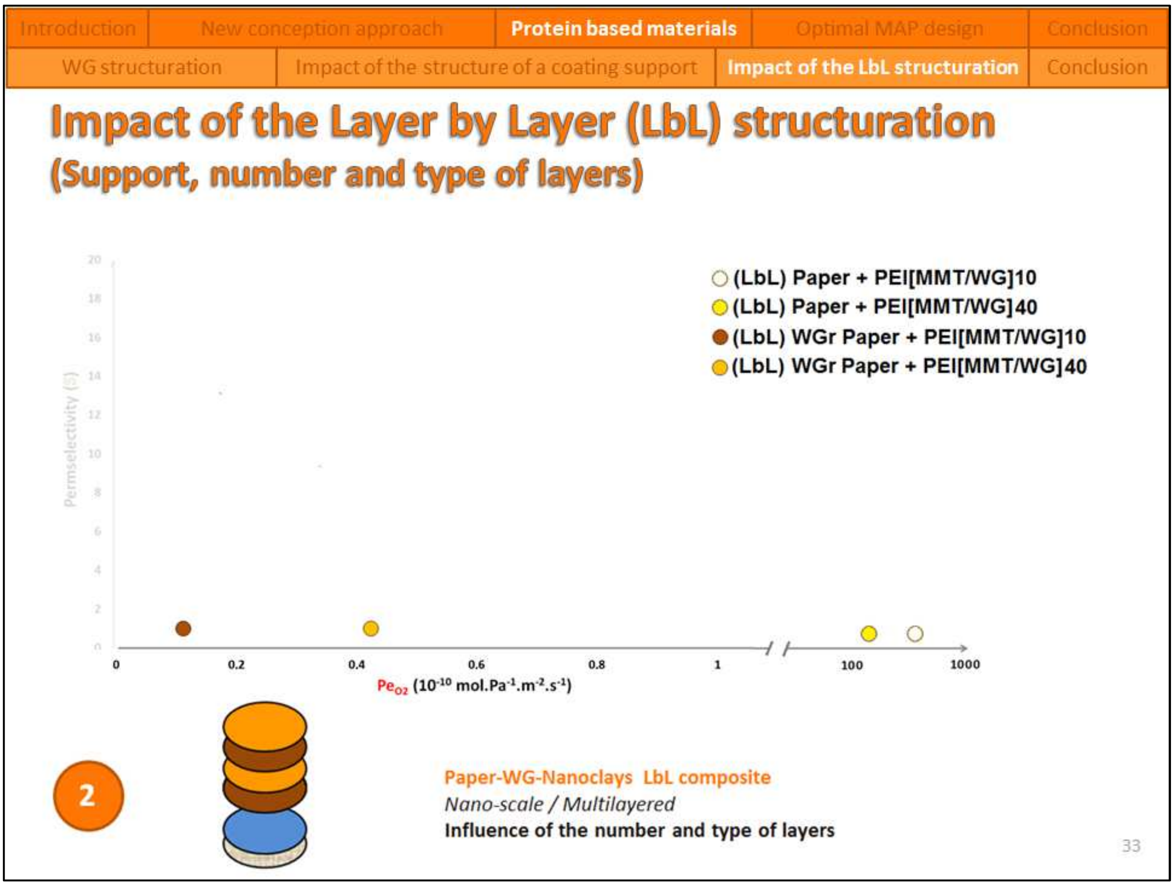
O_2 and CO_2 permeation of the different WG coated papers

25°C; 100%RH

Strong
WG-like behavior
Moderate Pe_{O2}
High Permselectivity

Weak
WG-like behavior
HighPe_{O2}
Limited Permselectivity



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WG structuration | Impact of the structure of a coating support | **Impact of the LbL structuration** | Conclusion

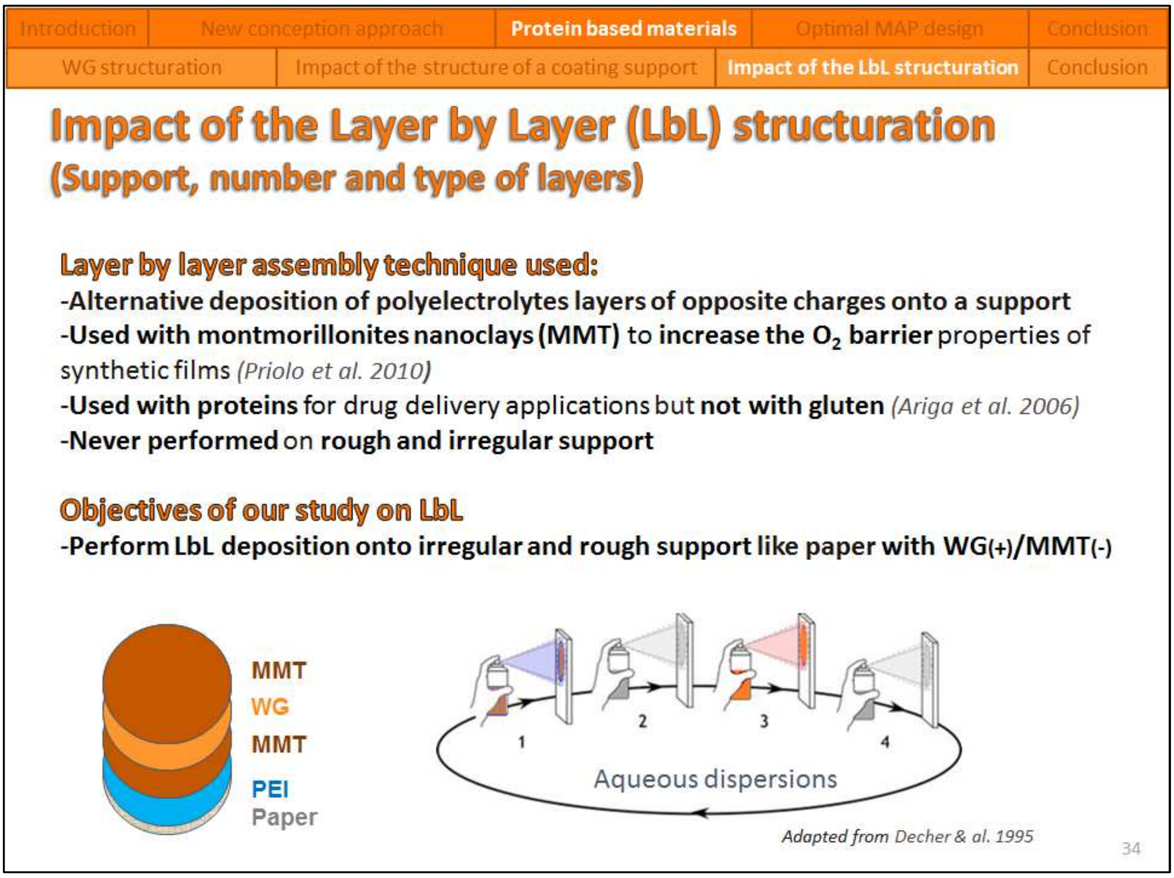
Impact of the Layer by Layer (LbL) structuration

(Support, number and type of layers)

Paper-WG-Nanoclays LbL composite

Nano-scale / Multilayered

Influence of the number and type of layers



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Impact of the Layer by Layer (LbL) structuration

(Support, number and type of layers)

Layer by layer assembly technique used:

-**Alternative deposition of polyelectrolytes layers of opposite charges onto a support**

-**Used with montmorillonites nanoclays (MMT)** to **increase the O_2 barrier** properties of synthetic films *(Priolo et al. 2010)*

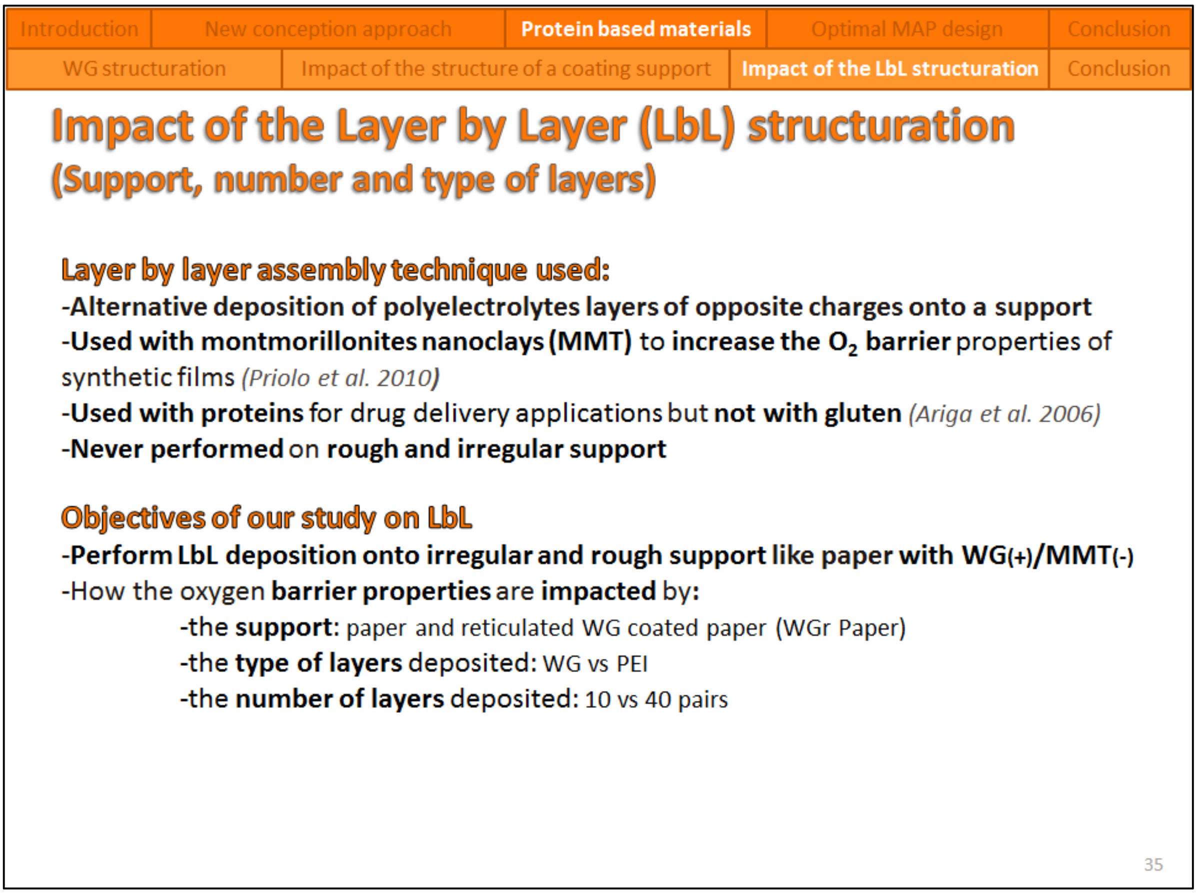
-**Used with proteins** for drug delivery applications but **not with gluten** *(Ariga et al. 2006)*

-**Never performed** on **rough and irregular support**

Objectives of our study on LbL

-**Perform LbL deposition onto irregular and rough support like paper with WG(+)/MMT(-)**

Adapted from Decher & al. 1995



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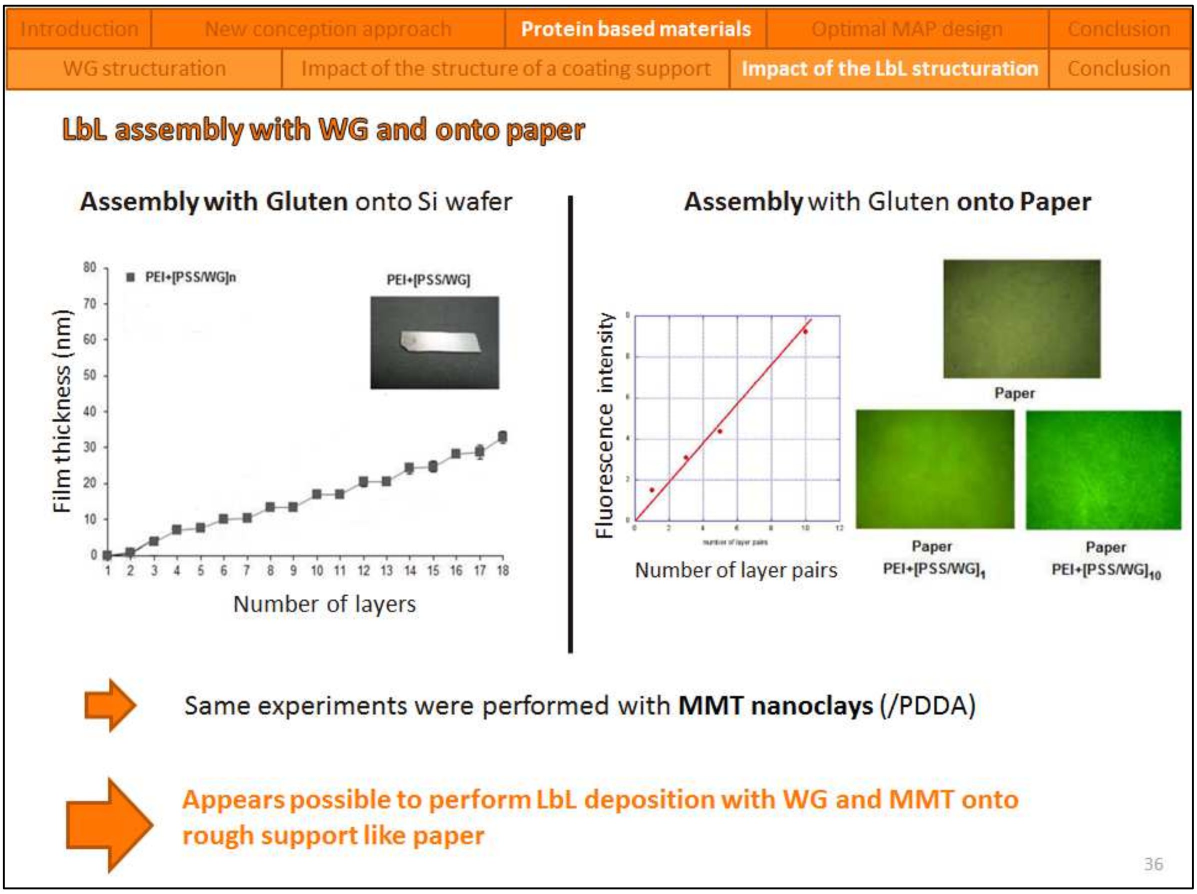
Objectives of our study on LbL

-**Perform LbL deposition onto irregular and rough support like paper with WG(+)/MMT(-)**

-How the oxygen **barrier properties** are **impacted** by:

- -the **support:** paper and reticulated WG coated paper (WGr Paper)
- -the **type of layers** deposited: WG vs PEI
- -the **number of layers** deposited: 10 vs 40 pairs



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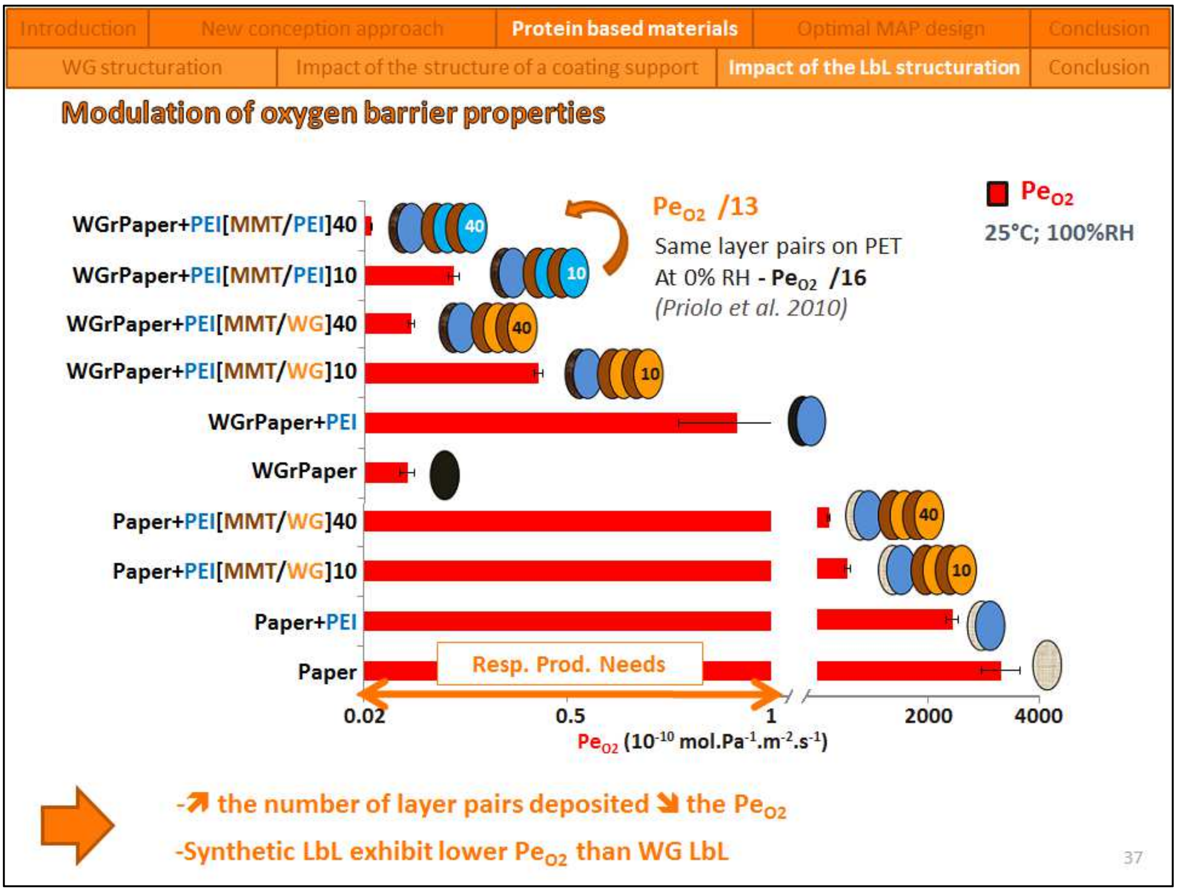
LbL assembly with WG and onto paper

Assembly with Gluten onto Si wafer

Assembly with Gluten **onto Paper**

Same experiments were performed with **MMT nanoclays** (/PDDA)

Appears possible to perform LbL deposition with WG and MMT onto rough support like paper



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Modulation of oxygen barrier properties

Pe$_{O2}$ /13

Same layer pairs on PET

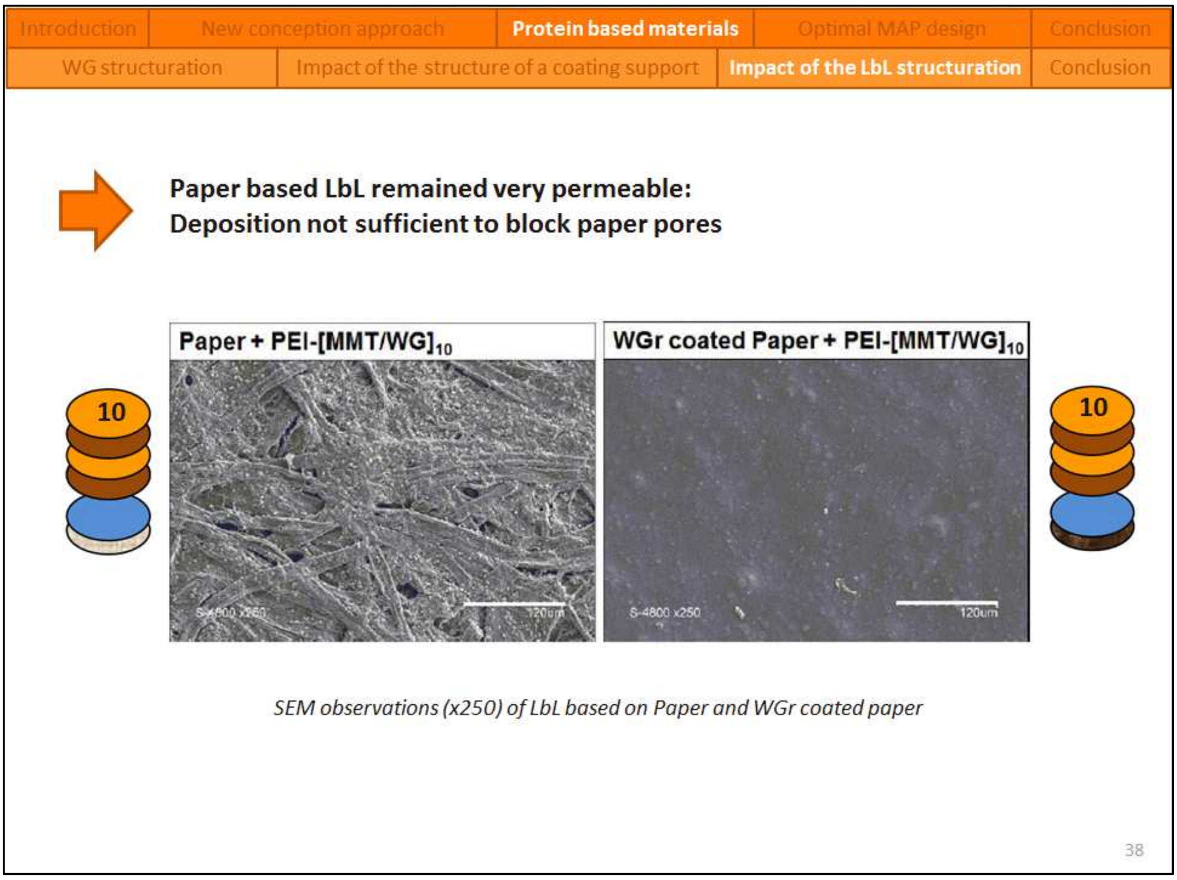
At 0% RH - **Pe$_{O2}$ /16**

(Priolo et al. 2010)

-↗ the number of layer pairs deposited ↘ the Pe$_{O2}$

-Synthetic LbL exhibit lower Pe$_{O2}$ than WG LbL

37



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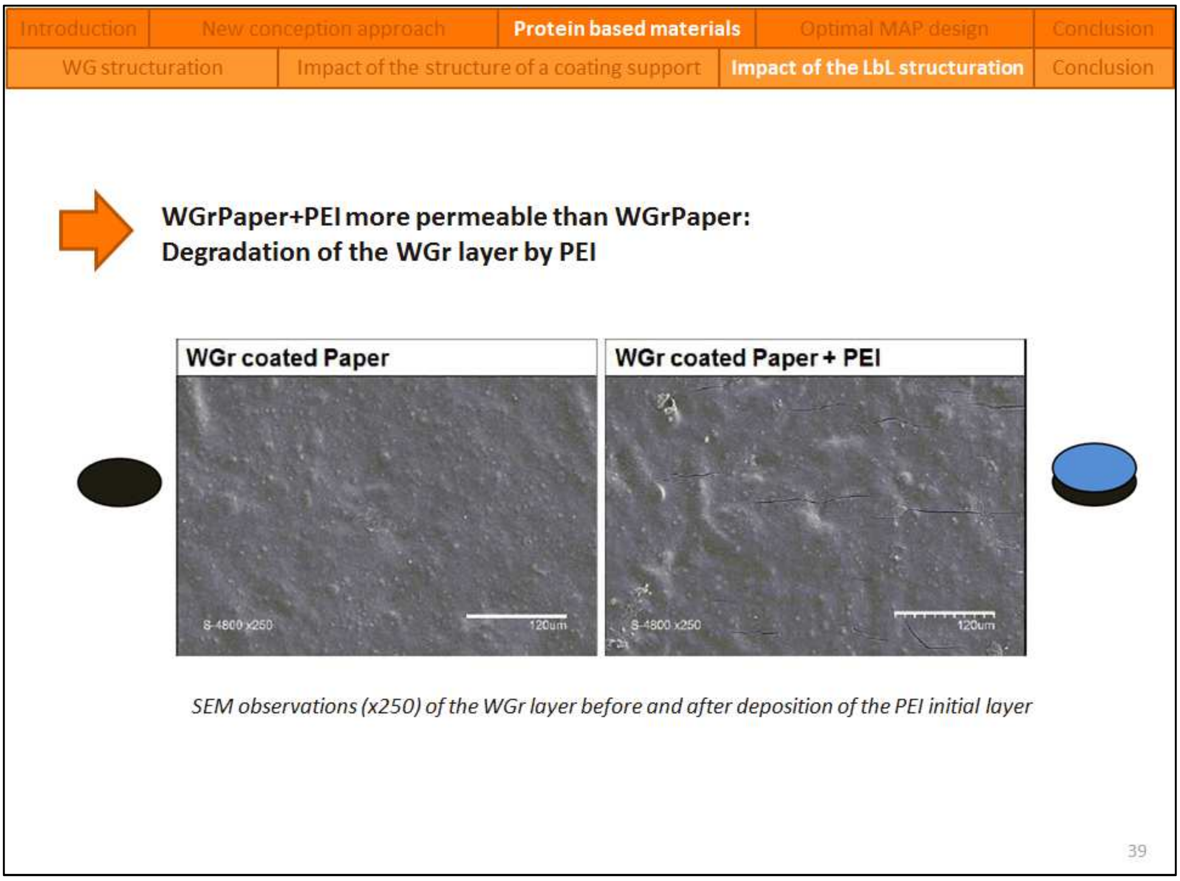
Paper based LbL remained very permeable:
Deposition not sufficient to block paper pores

SEM observations (x250) of LbL based on Paper and WGr coated paper

38

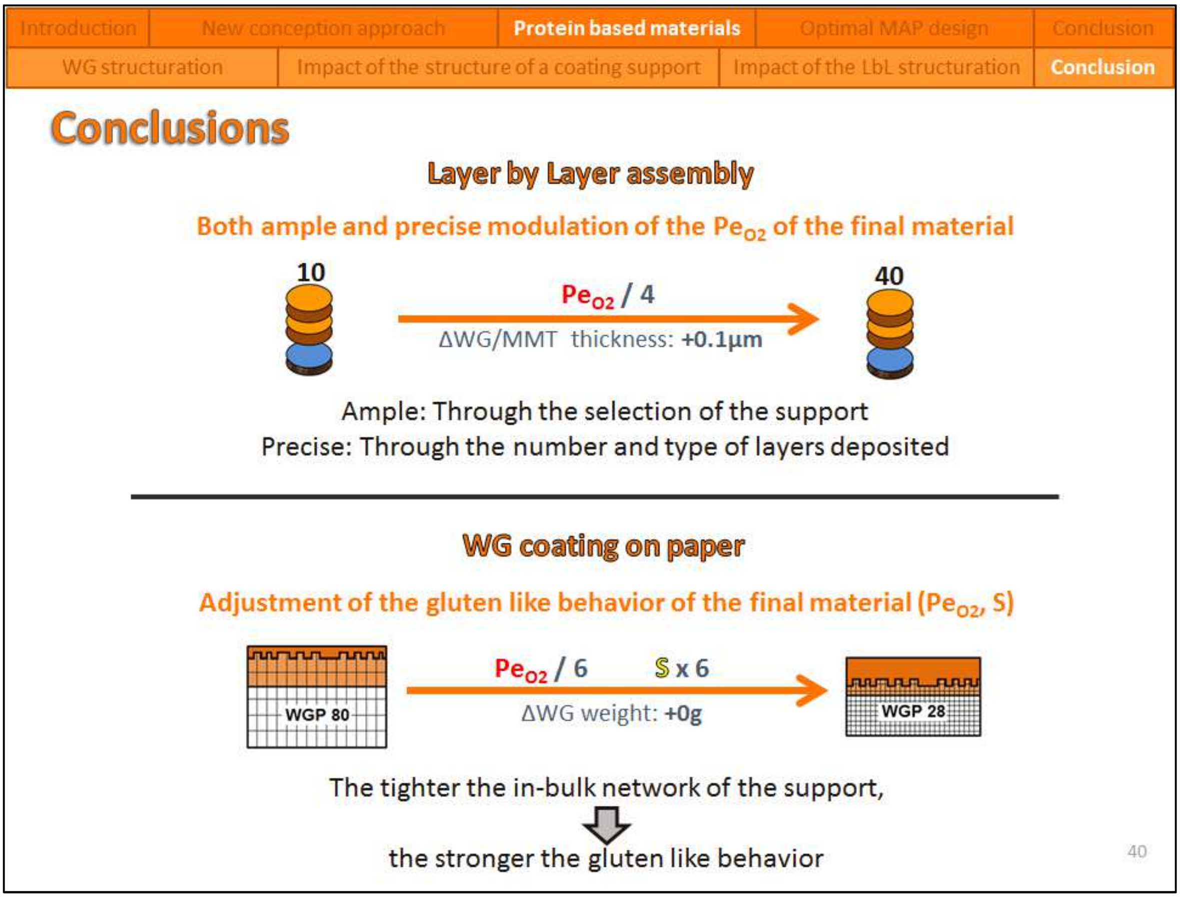


WGrPaper+PEI more permeable than WGrPaper:
Degradation of the WGr layer by PEI

WGr coated Paper

WGr coated Paper + PEI

SEM observations (x250) of the WGr layer before and after deposition of the PEI initial layer



Conclusions

Layer by Layer assembly

Both ample and precise modulation of the Pe_{O2} of the final material

Pe_{O2} / 4

ΔWG/MMT thickness: **+0.1µm**

Ample: Through the selection of the support
Precise: Through the number and type of layers deposited

WG coating on paper

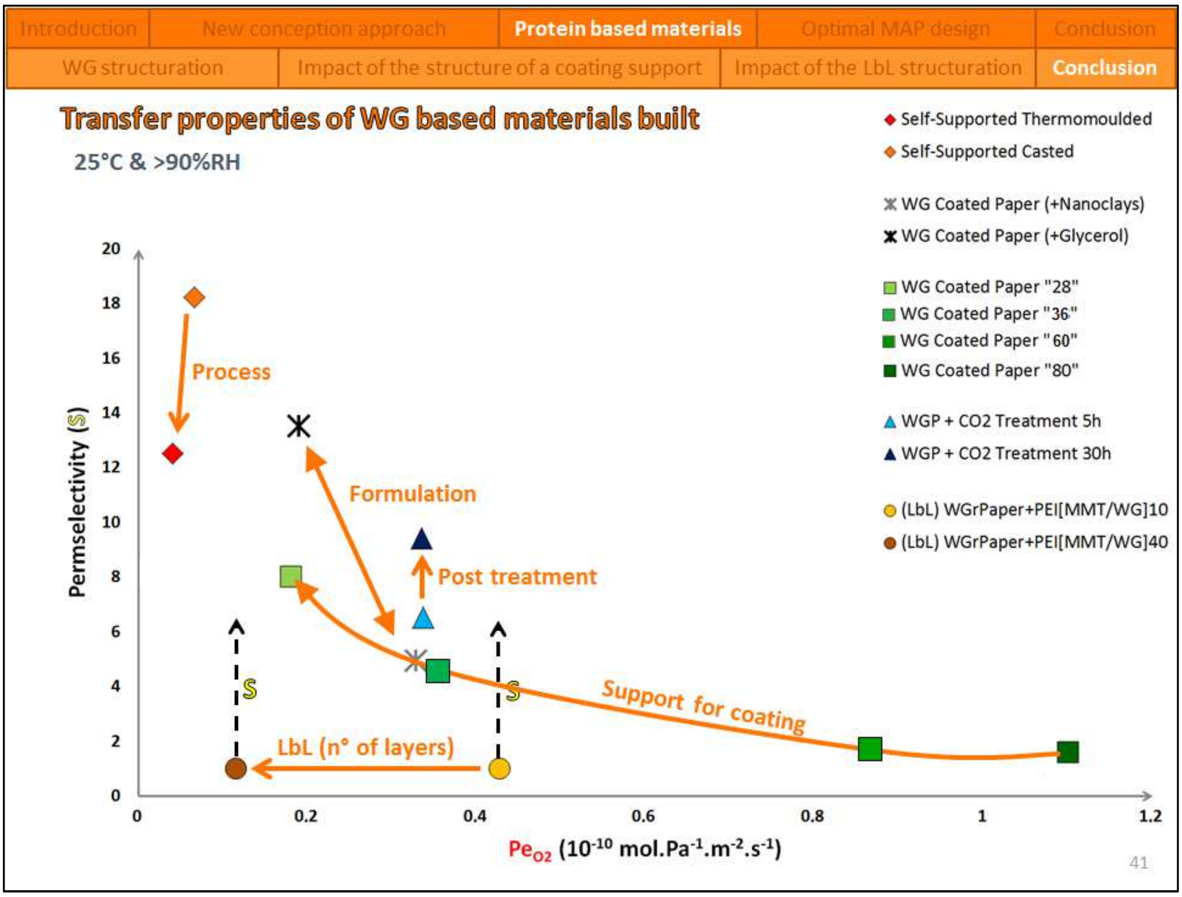
Adjustment of the gluten like behavior of the final material (Pe_{O2}, S)

Pe_{O2} / 6 S x 6

ΔWG weight: **+0g**

The tighter the in-bulk network of the support,

the stronger the gluten like behavior



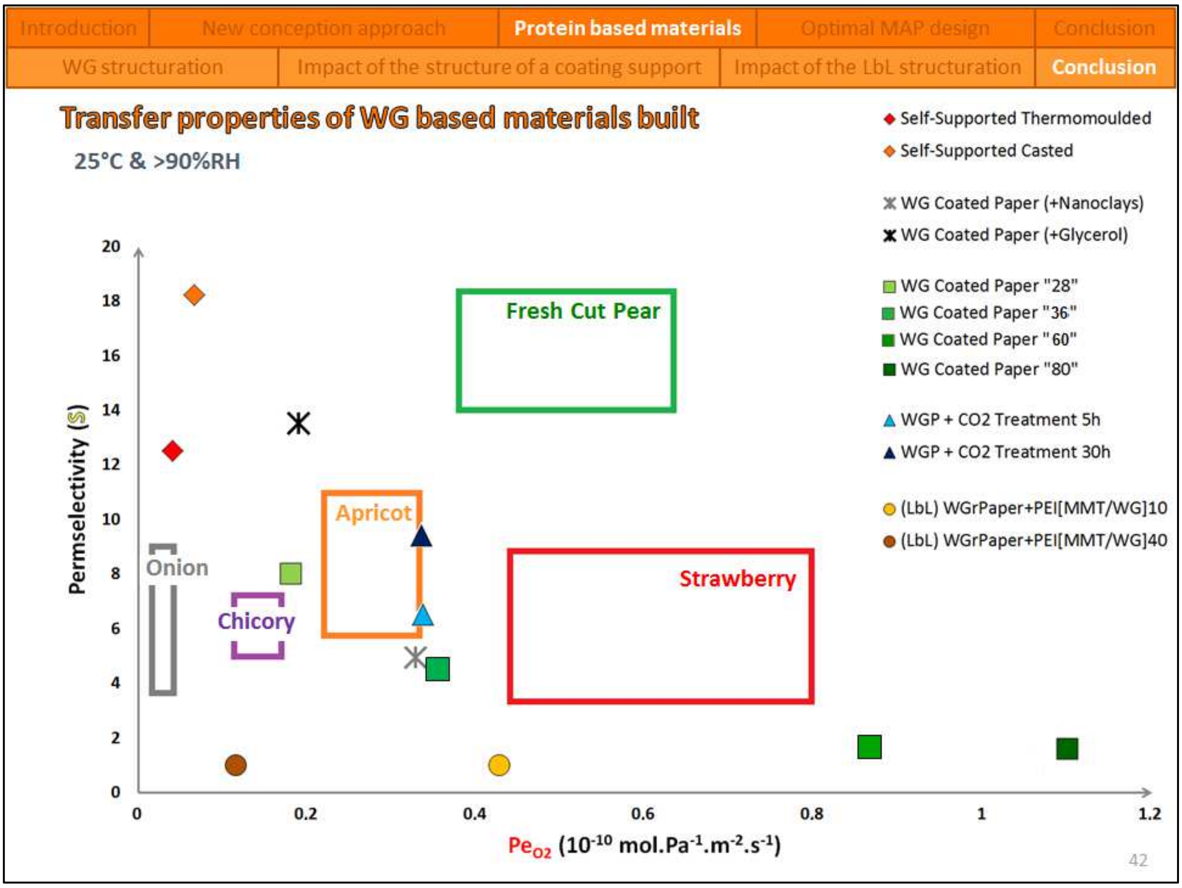
Introduction | New conception approach | **Protein based materials** | Optimal MAP design | Conclusion

WG structuration | Impact of the structure of a coating support | Impact of the LbL structuration | **Conclusion**

Transfer properties of WG based materials built

25°C & >90%RH

- ◆ Self-Supported Thermomoulded
- ◆ Self-Supported Casted
- ✱ WG Coated Paper (+Nanoclays)
- ✱ WG Coated Paper (+Glycerol)
- ■ WG Coated Paper "28"
- ■ WG Coated Paper "36"
- ■ WG Coated Paper "60"
- ■ WG Coated Paper "80"
- ▲ WGP + CO2 Treatment 5h
- ▲ WGP + CO2 Treatment 30h
- ● (LbL) WGrPaper+PEI[MMT/WG]10
- ● (LbL) WGrPaper+PEI[MMT/WG]40



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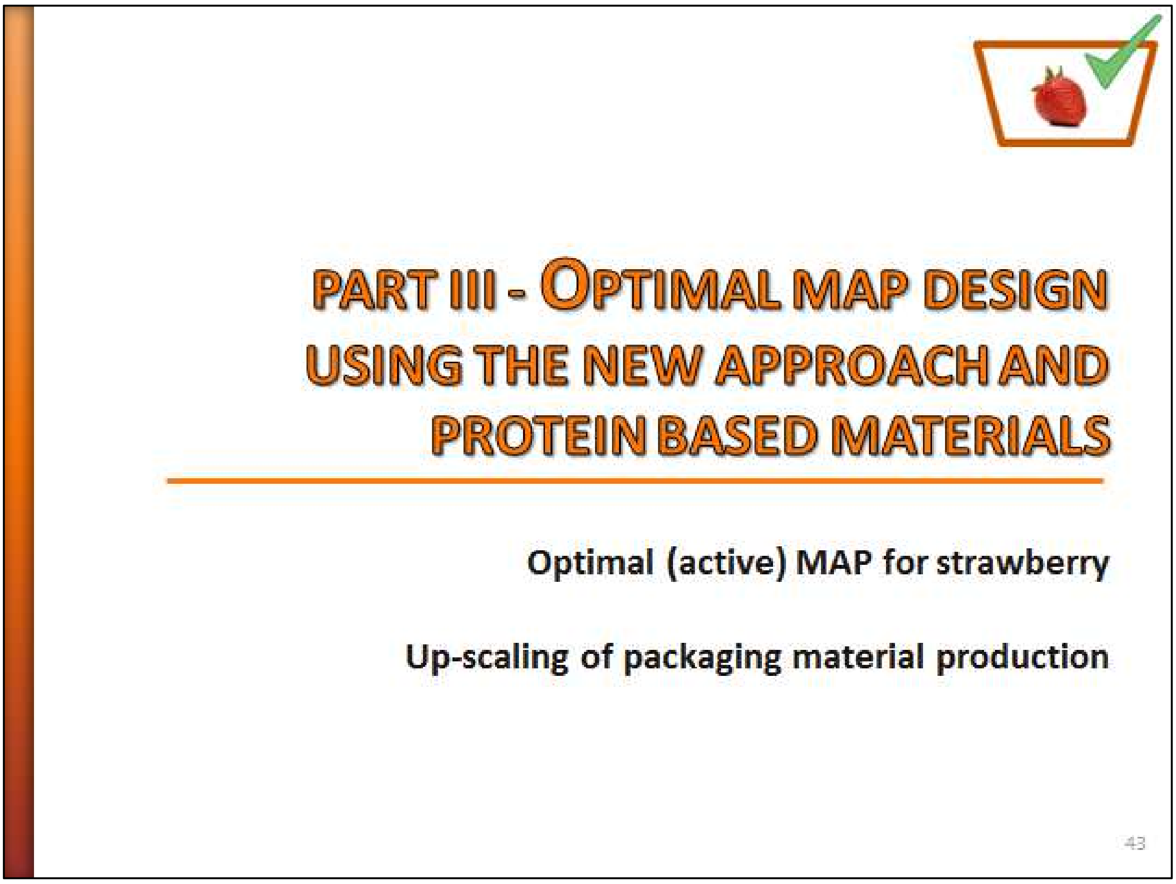
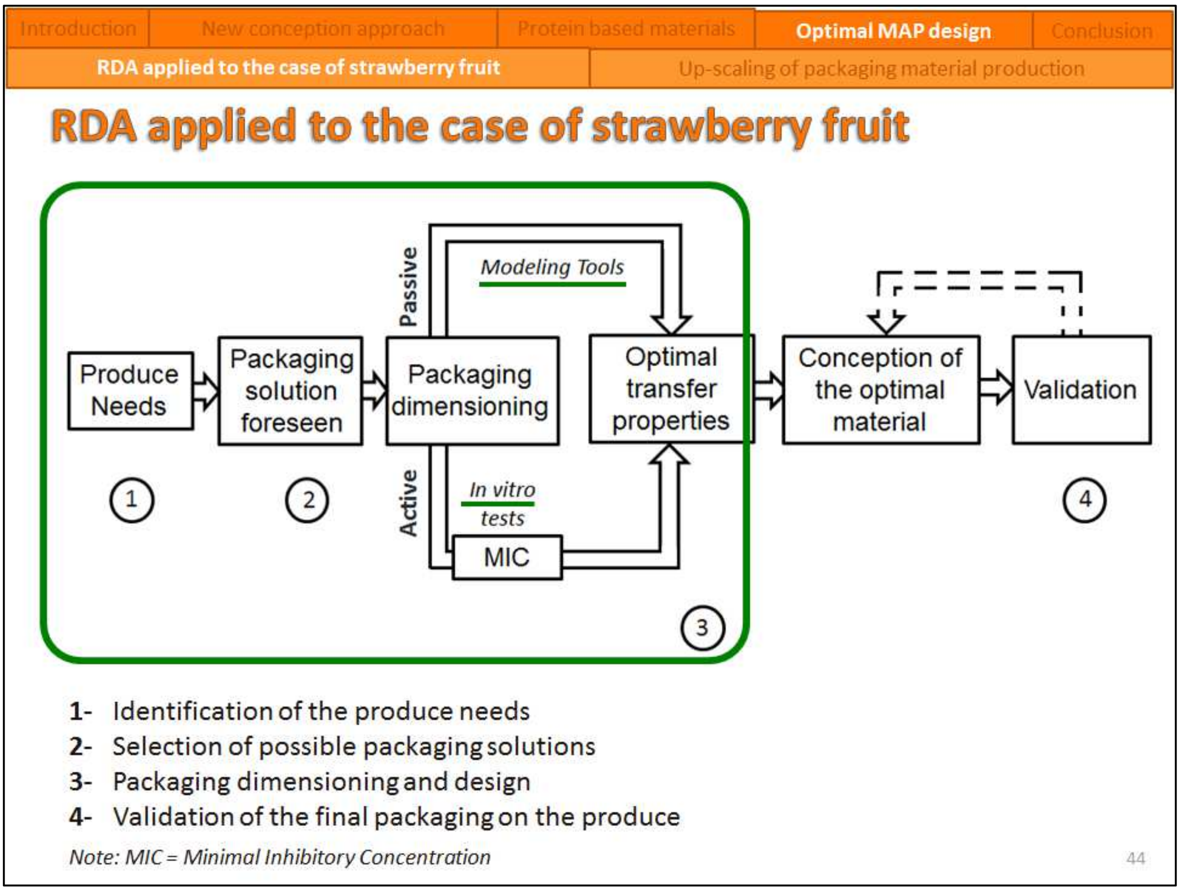
- ✱ WG Coated Paper (+Nanoclays)
- ✱ WG Coated Paper (+Glycerol)
- ■ WG Coated Paper "28"
- ■ WG Coated Paper "36"
- ■ WG Coated Paper "60"
- ■ WG Coated Paper "80"
- ▲ WGP + CO2 Treatment 5h
- ▲ WGP + CO2 Treatment 30h
- ● (LbL) WGrPaper+PEI[MMT/WG]10
- ● (LbL) WGrPaper+PEI[MMT/WG]40



PART III - OPTIMAL MAP DESIGN USING THE NEW APPROACH AND PROTEIN BASED MATERIALS

Optimal (active) MAP for strawberry

Up-scaling of packaging material production

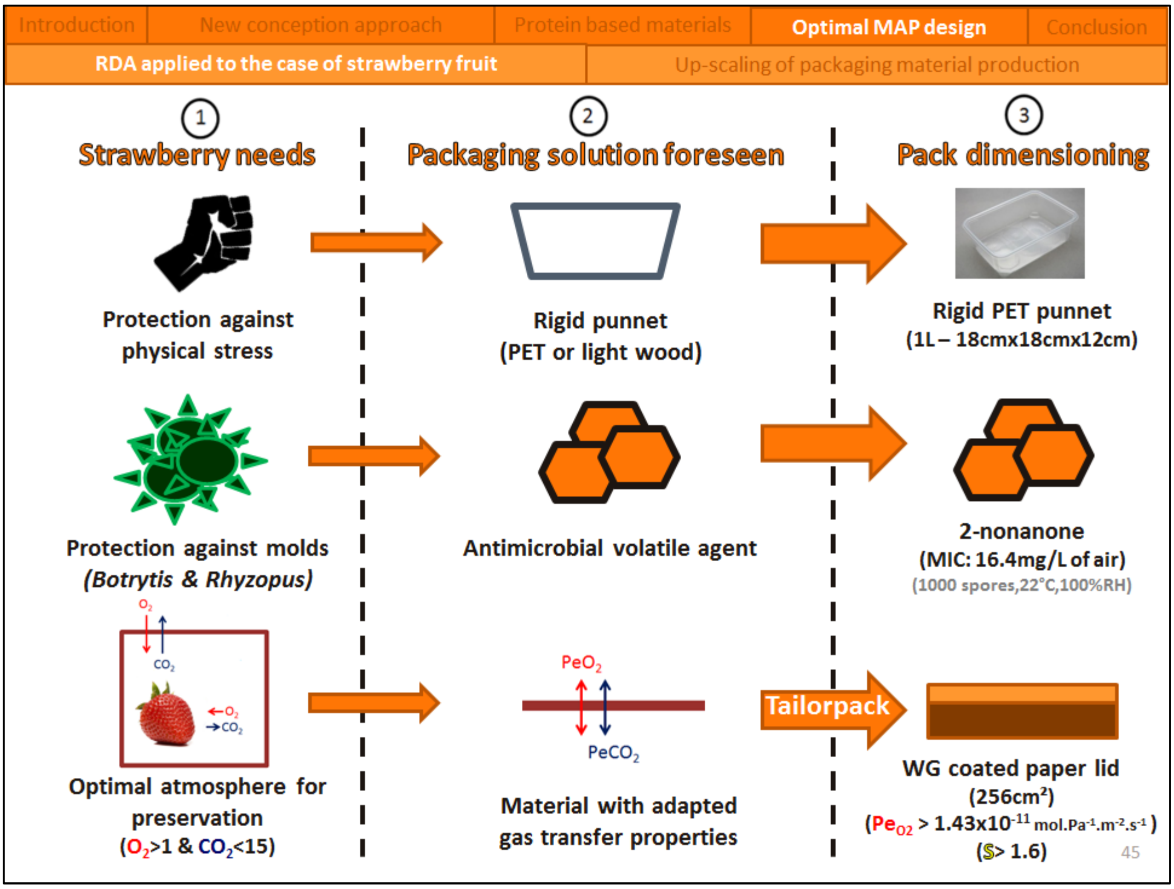


RDA applied to the case of strawberry fruit

Produce Needs
①
Packaging solution foreseen
②
Passive
Modeling Tools
Packaging dimensioning
Optimal transfer properties
Active
In vitro tests
MIC
③
Conception of the optimal material
Validation
④

1- Identification of the produce needs
2- Selection of possible packaging solutions
3- Packaging dimensioning and design
4- Validation of the final packaging on the produce

Note: MIC = Minimal Inhibitory Concentration



Introduction | New conception approach | Protein based materials | **Optimal MAP design** | Conclusion

RDA applied to the case of strawberry fruit | Up-scaling of packaging material production

① **Strawberry needs**

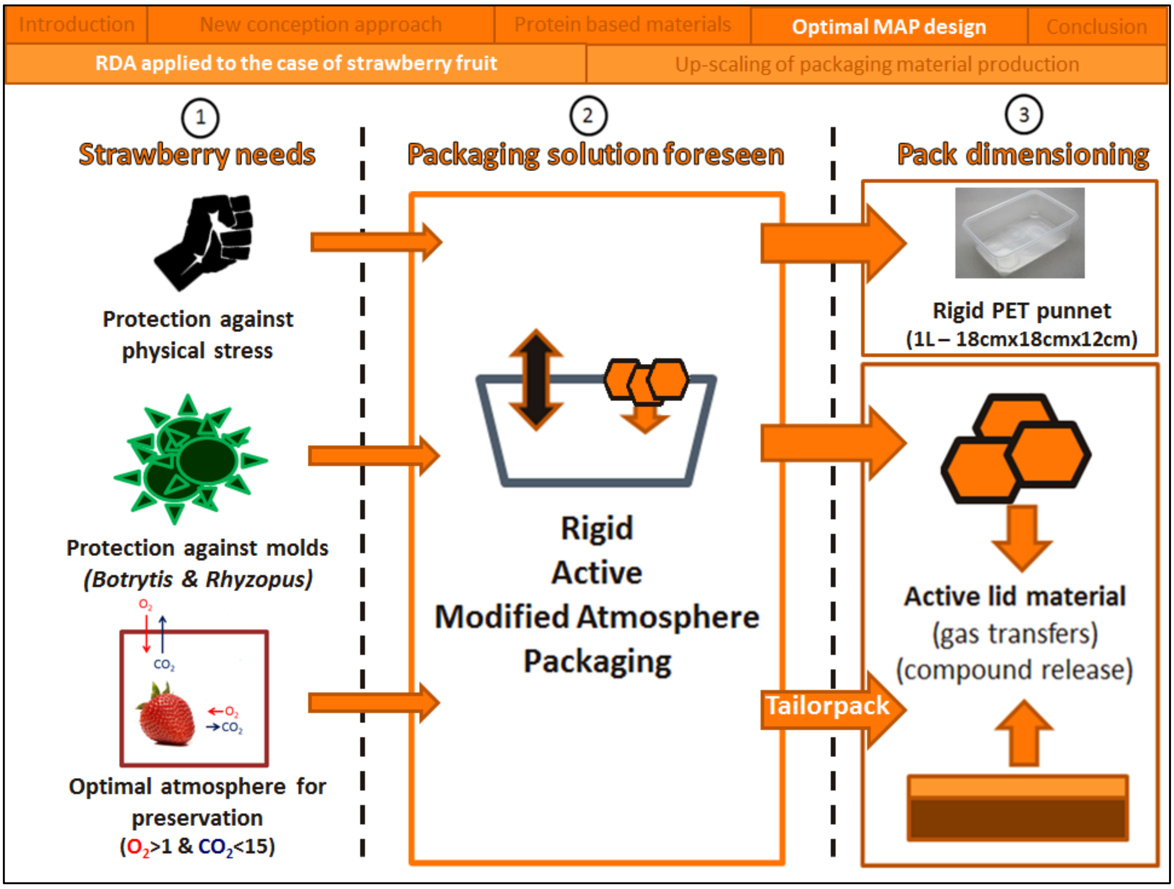
② **Packaging solution foreseen**

③ **Pack dimensioning**

Protection against physical stress → **Rigid punnet (PET or light wood)** → **Rigid PET punnet (1L – 18cmx18cmx12cm)**

Protection against molds *(Botrytis & Rhyzopus)* → **Antimicrobial volatile agent** → **2-nonanone (MIC: 16.4mg/L of air)** (1000 spores, 22°C, 100%RH)

Optimal atmosphere for preservation (O_2>1 & CO_2<15) → **Material with adapted gas transfer properties** → Tailorpack → **WG coated paper lid ($256 cm^2$) ($Pe_{O2} > 1.43 \times 10^{-11}$ mol.Pa^{-1}.m^{-2}.s^{-1}) (S > 1.6)**



Introduction | New conception approach | Protein based materials | **Optimal MAP design** | Conclusion

RDA applied to the case of strawberry fruit | Up-scaling of packaging material production

① **Strawberry needs**

② **Packaging solution foreseen**

③ **Pack dimensioning**

Protection against physical stress

Protection against molds *(Botrytis & Rhyzopus)*

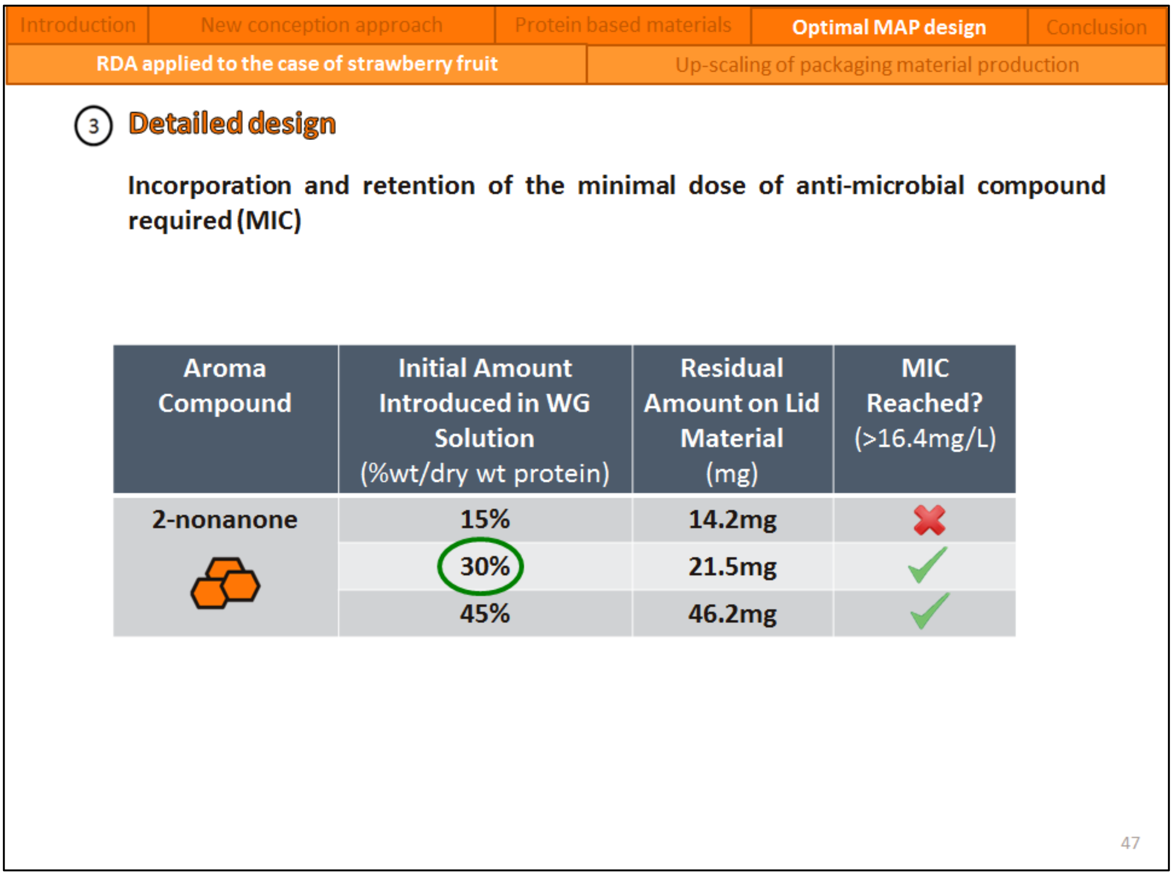
Optimal atmosphere for preservation (O_2>1 & CO_2<15)

Rigid Active Modified Atmosphere Packaging

Rigid PET punnet (1L – 18cmx18cmx12cm)

Tailorpack

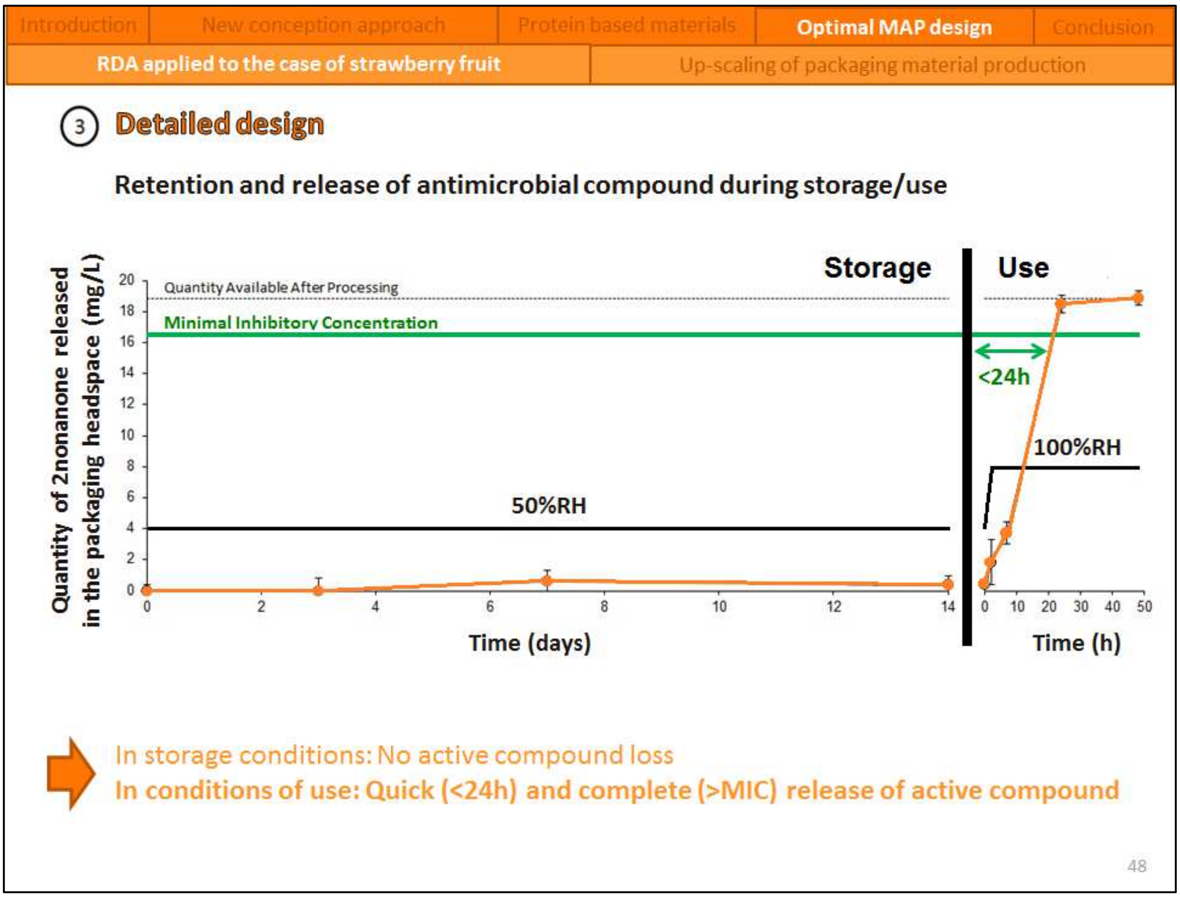
Active lid material (gas transfers) (compound release)



③ Detailed design

Incorporation and retention of the minimal dose of anti-microbial compound required (MIC)

Aroma Compound	Initial Amount Introduced in WG Solution (%wt/dry wt protein)	Residual Amount on Lid Material (mg)	MIC Reached? (>16.4mg/L)
2-nonanone	15%	14.2mg	✗
	30%	21.5mg	✓
	45%	46.2mg	✓

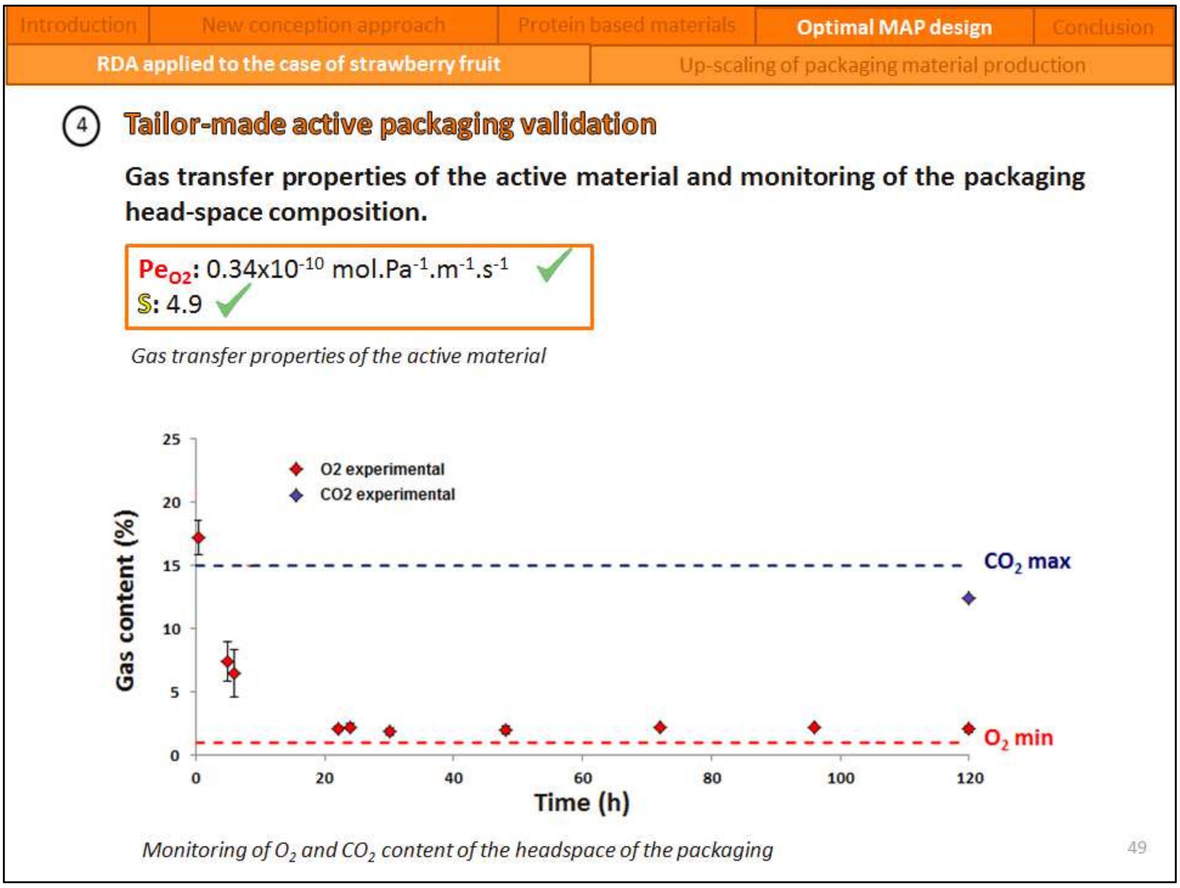


③ Detailed design

Retention and release of antimicrobial compound during storage/use

In storage conditions: No active compound loss

In conditions of use: Quick (<24h) and complete (>MIC) release of active compound



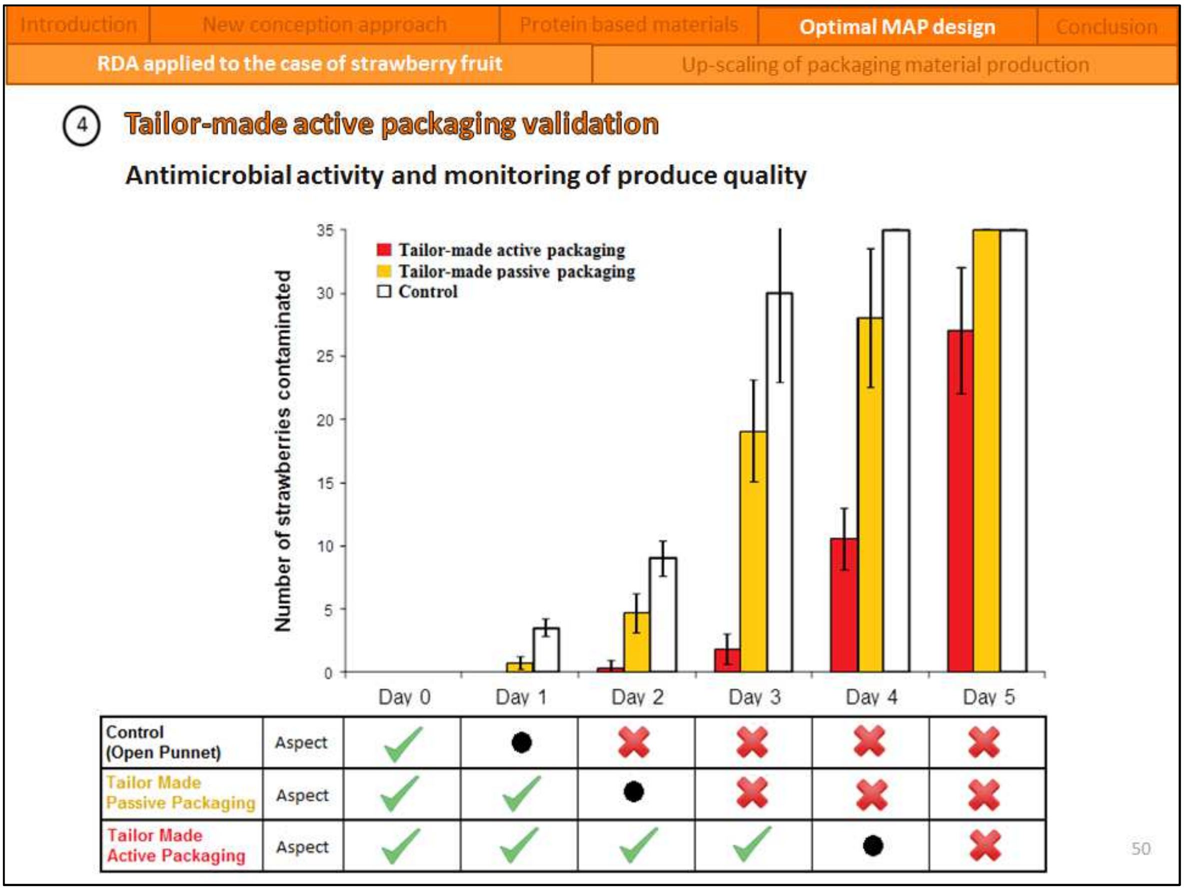
④ Tailor-made active packaging validation

Gas transfer properties of the active material and monitoring of the packaging head-space composition.

Pe_{O2}: 0.34×10^{-10} mol.Pa^{-1}.m^{-1}.s^{-1} ✓
S: 4.9 ✓

Gas transfer properties of the active material

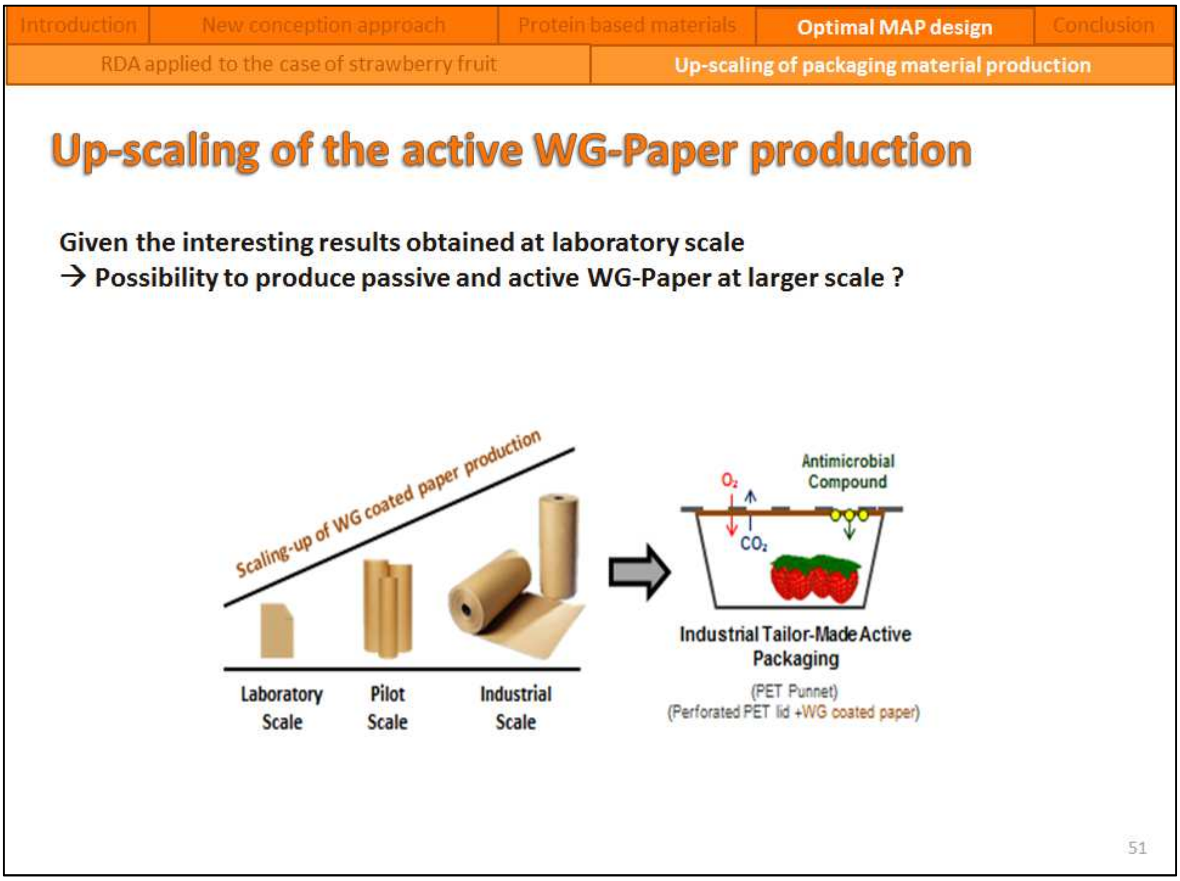
Monitoring of O_2 and CO_2 content of the headspace of the packaging



④ Tailor-made active packaging validation

Antimicrobial activity and monitoring of produce quality

		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Control (Open Punnet)	Aspect	✓	●	✗	✗	✗	✗
Tailor Made Passive Packaging	Aspect	✓	✓	●	✗	✗	✗
Tailor Made Active Packaging	Aspect	✓	✓	✓	✓	●	✗

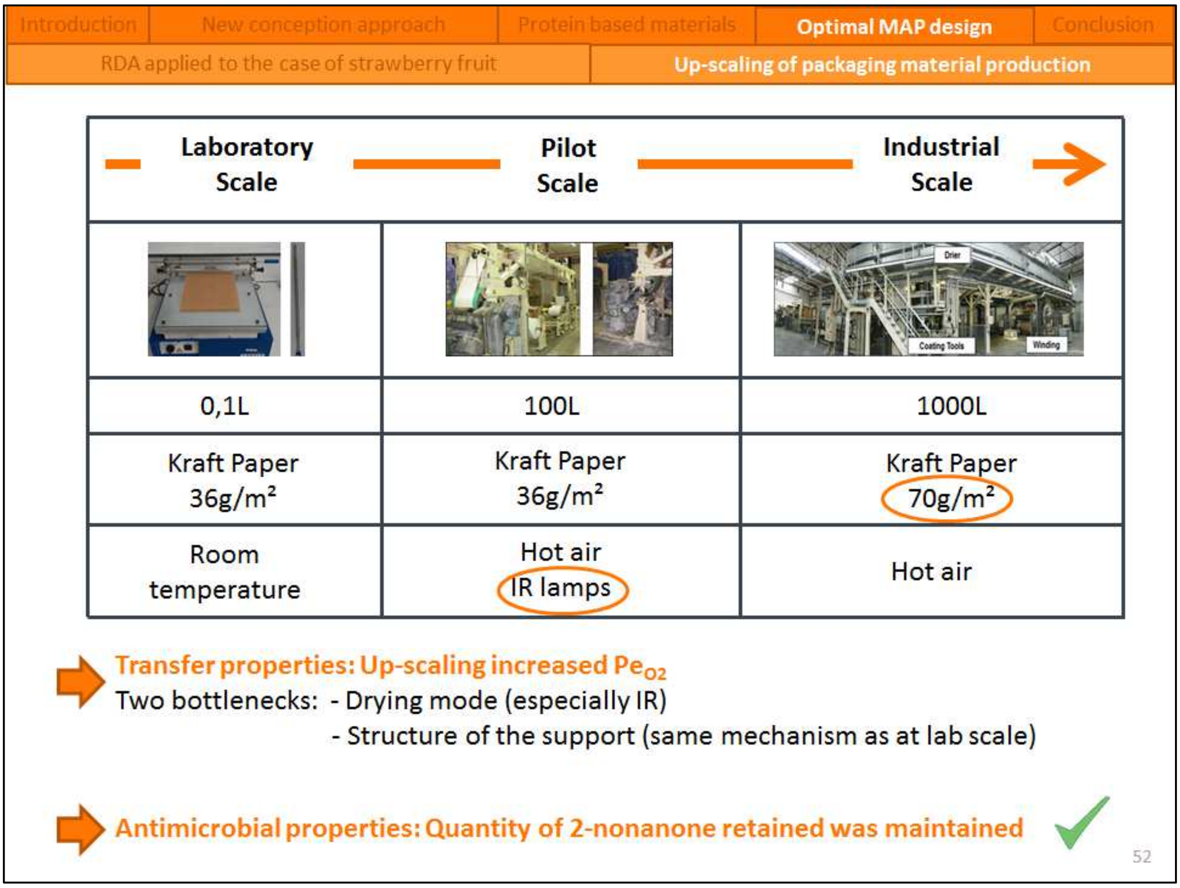


Introduction | New conception approach | Protein based materials | **Optimal MAP design** | Conclusion

RDA applied to the case of strawberry fruit | **Up-scaling of packaging material production**

Up-scaling of the active WG-Paper production

Given the interesting results obtained at laboratory scale
→ Possibility to produce passive and active WG-Paper at larger scale ?



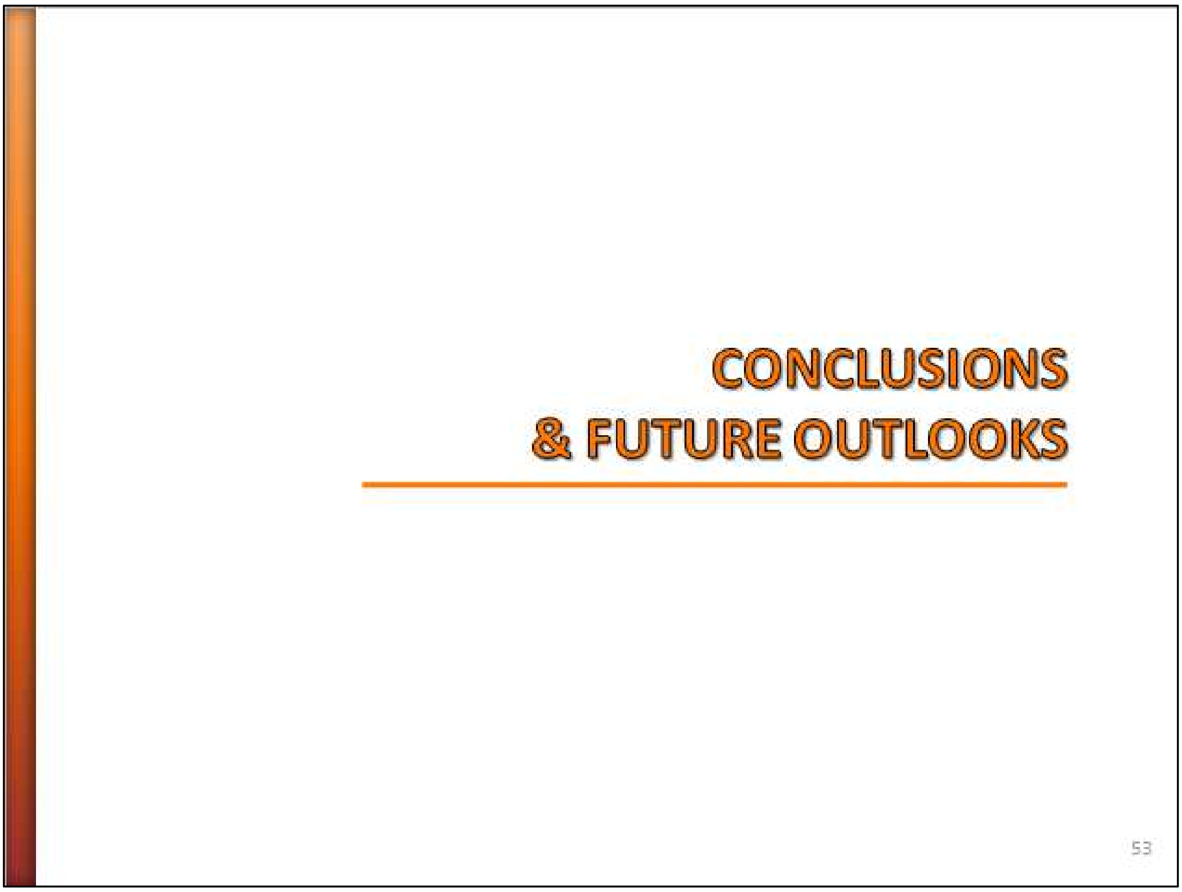
Introduction | New conception approach | Protein based materials | **Optimal MAP design** | Conclusion

RDA applied to the case of strawberry fruit | **Up-scaling of packaging material production**

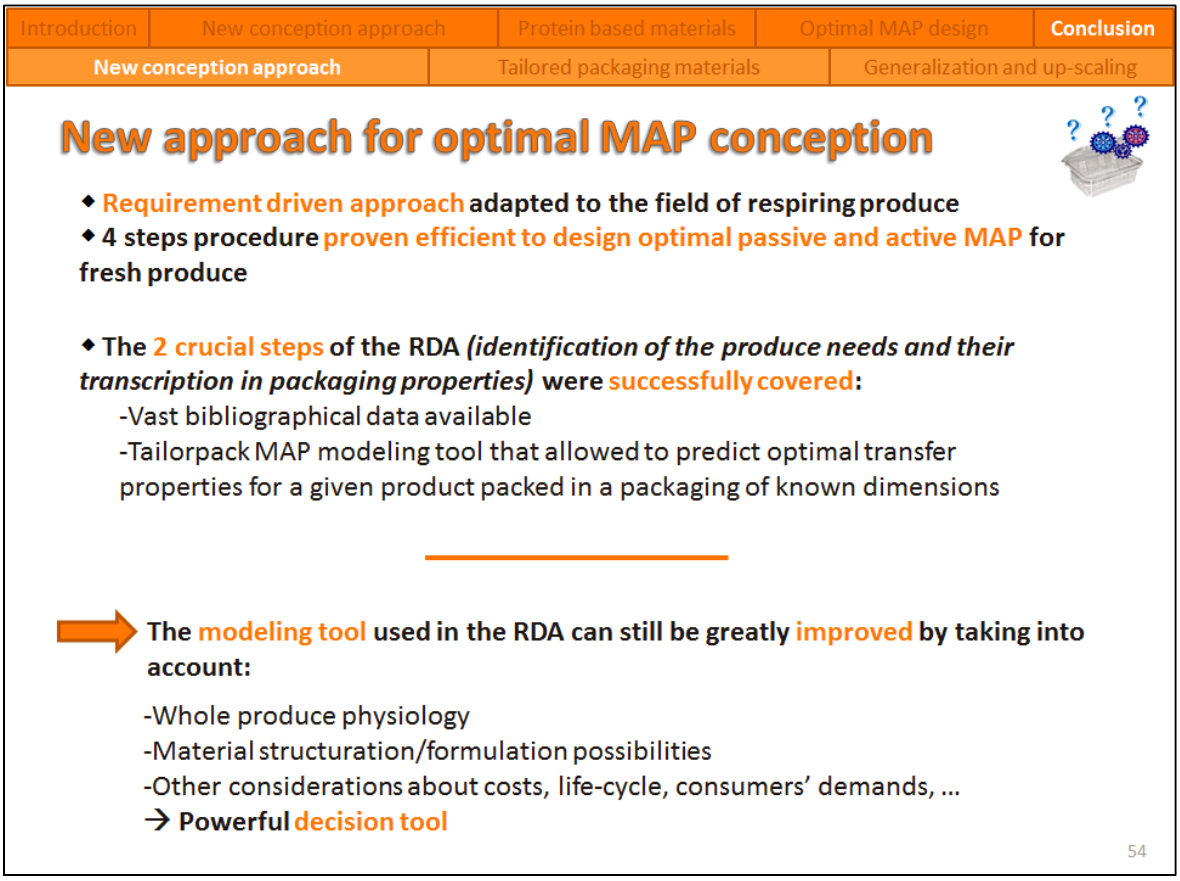
Laboratory Scale	**Pilot Scale**	**Industrial Scale**
0,1L	100L	1000L
Kraft Paper 36g/m²	Kraft Paper 36g/m²	Kraft Paper 70g/m²
Room temperature	Hot air IR lamps	Hot air

Transfer properties: Up-scaling increased Pe_{O2}
Two bottlenecks: - Drying mode (especially IR)
- Structure of the support (same mechanism as at lab scale)

Antimicrobial properties: Quantity of 2-nonanone retained was maintained ✓



CONCLUSIONS
& FUTURE OUTLOOKS



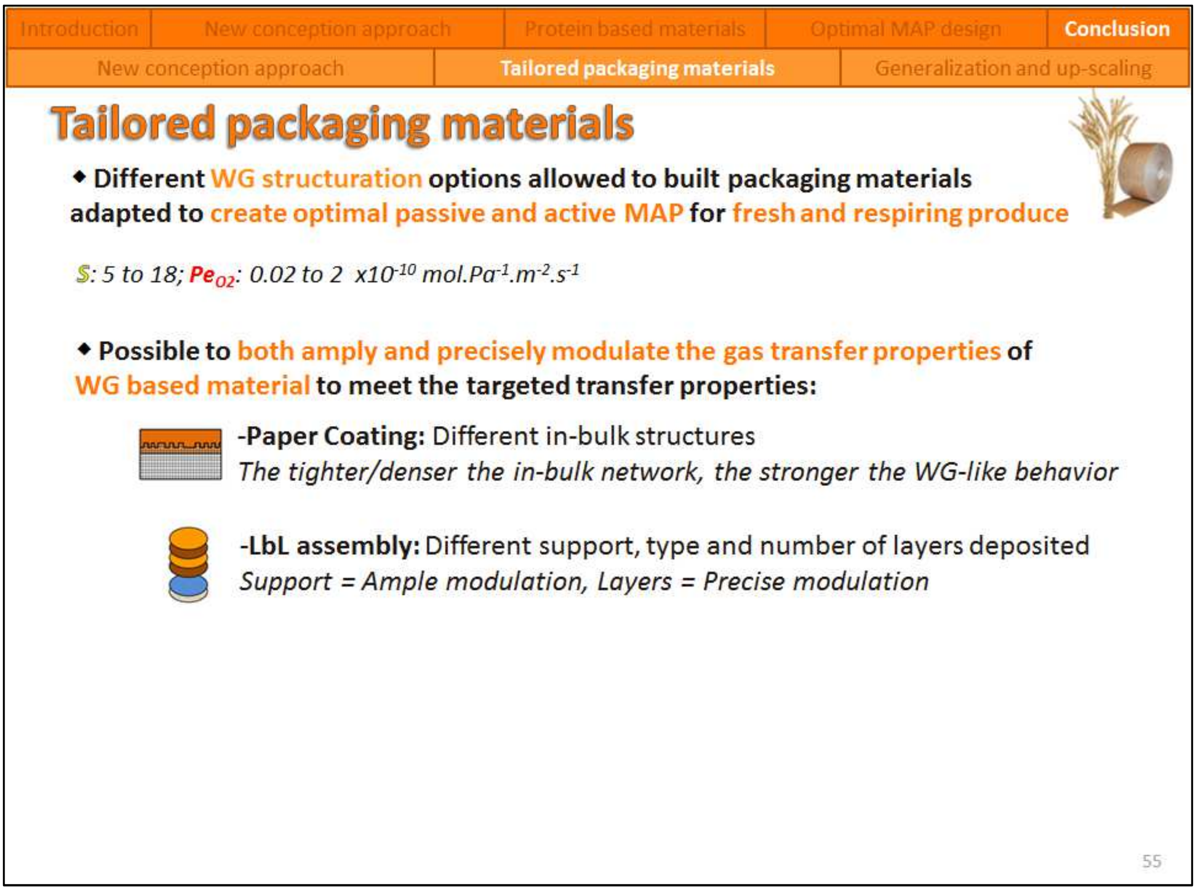
New approach for optimal MAP conception

- **Requirement driven approach adapted to the field of respiring produce**
- **4 steps procedure proven efficient to design optimal passive and active MAP for fresh produce**

- **The 2 crucial steps of the RDA *(identification of the produce needs and their transcription in packaging properties)* were successfully covered:**
  - -Vast bibliographical data available
  - -Tailorpack MAP modeling tool that allowed to predict optimal transfer properties for a given product packed in a packaging of known dimensions

The modeling tool used in the RDA can still be greatly improved by taking into account:

-Whole produce physiology
-Material structuration/formulation possibilities
-Other considerations about costs, life-cycle, consumers' demands, …
→ **Powerful decision tool**



Introduction | New conception approach | Protein based materials | Optimal MAP design | **Conclusion**

New conception approach | **Tailored packaging materials** | Generalization and up-scaling

Tailored packaging materials

◆ **Different WG structuration options allowed to built packaging materials adapted to create optimal passive and active MAP for fresh and respiring produce**

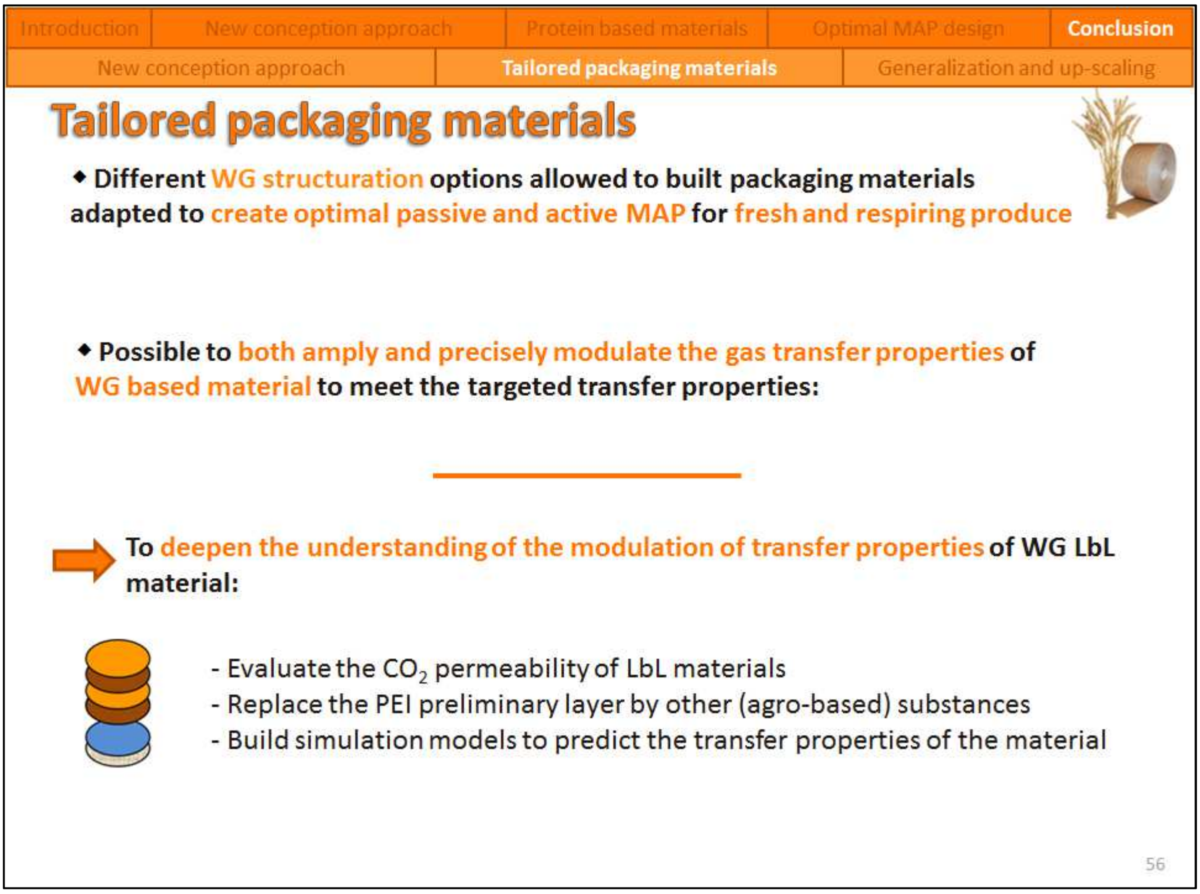
S: 5 to 18; Pe_{O2}: 0.02 to 2 $x10^{-10}$ mol.Pa^{-1}.m^{-2}.s^{-1}

◆ **Possible to both amply and precisely modulate the gas transfer properties of WG based material to meet the targeted transfer properties:**

-Paper Coating: Different in-bulk structures
The tighter/denser the in-bulk network, the stronger the WG-like behavior

-LbL assembly: Different support, type and number of layers deposited
Support = Ample modulation, Layers = Precise modulation

55



Introduction | New conception approach | Protein based materials | Optimal MAP design | **Conclusion**

New conception approach | **Tailored packaging materials** | Generalization and up-scaling

Tailored packaging materials

◆ **Different WG structuration options allowed to built packaging materials adapted to create optimal passive and active MAP for fresh and respiring produce**

◆ **Possible to both amply and precisely modulate the gas transfer properties of WG based material to meet the targeted transfer properties:**

➡ **To deepen the understanding of the modulation of transfer properties of WG LbL material:**

- Evaluate the CO_2 permeability of LbL materials
- Replace the PEI preliminary layer by other (agro-based) substances
- Build simulation models to predict the transfer properties of the material

56

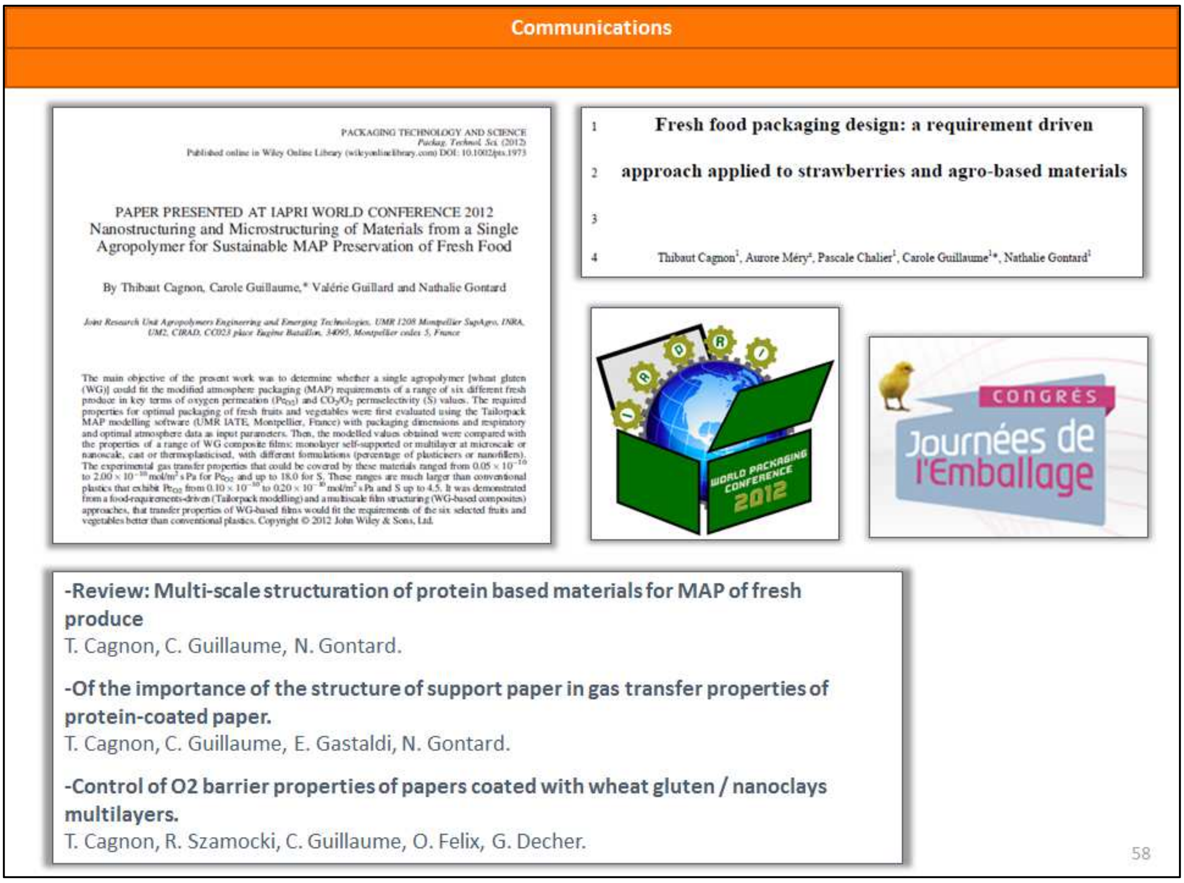
Introduction | New conception approach | Protein based materials | Optimal MAP design | **Conclusion**

New conception approach | Tailored packaging materials | **Generalization and up-scaling**

Generalization of the RDA and Up-scaling of new materials production

- Principles of the RDA appear easy to adapt at an industrial level
- Lack of powerful and user-friendly "MAP design - decision tool" to help select the best material / structuration given not only scientific but also industrial and cost considerations

- Up-scaling of the WG based material (WG-Papers) was proven possible
- Necessity to take into account the different processing conditions at industrial scale from the beginning of the packaging conception



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PAPER PRESENTED AT IAPRI WORLD CONFERENCE 2012
Nanostructuring and Microstructuring of Materials from a Single Agropolymer for Sustainable MAP Preservation of Fresh Food

By Thibaut Cagnon, Carole Guillaume,* Valérie Guillard and Nathalie Gontard

Joint Research Unit Agropolymers Engineering and Emerging Technologies, UMR 1208 Montpellier SupAgro, INRA, UM2, CIRAD, CC023 place Eugène Bataillon, 34095, Montpellier cedex 5, France

The main objective of the present work was to determine whether a single agropolymer [wheat gluten (WG)] could fit the modified atmosphere packaging (MAP) requirements of a range of six different fresh produce in key terms of oxygen permeation (Pe_{O2}) and CO_2/O_2 permselectivity (S) values. The required properties for optimal packaging of fresh fruits and vegetables were first evaluated using the Tailorpack MAP modelling software (UMR IATE, Montpellier, France) with packaging dimensions and respiratory and optimal atmosphere data as input parameters. Then, the modelled values obtained were compared with the properties of a range of WG composite films: monolayer self-supported or multilayer at microscale or nanoscale, cast or thermoplasticised, with different formulations (percentage of plasticisers or nanofillers). The experimental gas transfer properties that could be covered by these materials ranged from 0.05×10^{-10} to 2.00×10^{-10} mol/m² s Pa for Pe_{O2} and up to 18.0 for S. These ranges are much larger than conventional plastics that exhibit Pe_{O2} from 0.10×10^{-10} to 0.20×10^{-10} mol/m² s Pa and S up to 4.5. It was demonstrated from a food-requirements-driven (Tailorpack modelling) and a multiscale film structuring (WG-based composites) approaches, that transfer properties of WG-based films would fit the requirements of the six selected fruits and vegetables better than conventional plastics. Copyright © 2012 John Wiley & Sons, Ltd.

Fresh food packaging design: a requirement driven approach applied to strawberries and agro-based materials

Thibaut Cagnon[1], Aurore Méry[1], Pascale Chalier[1], Carole Guillaume[1*], Nathalie Gontard[1]

-Review: Multi-scale structuration of protein based materials for MAP of fresh produce
T. Cagnon, C. Guillaume, N. Gontard.

-Of the importance of the structure of support paper in gas transfer properties of protein-coated paper.
T. Cagnon, C. Guillaume, E. Gastaldi, N. Gontard.

-Control of O2 barrier properties of papers coated with wheat gluten / nanoclays multilayers.
T. Cagnon, R. Szamocki, C. Guillaume, O. Felix, G. Decher.

Many Thanks to

The members of the Tailorpack Project…

F. Rambert

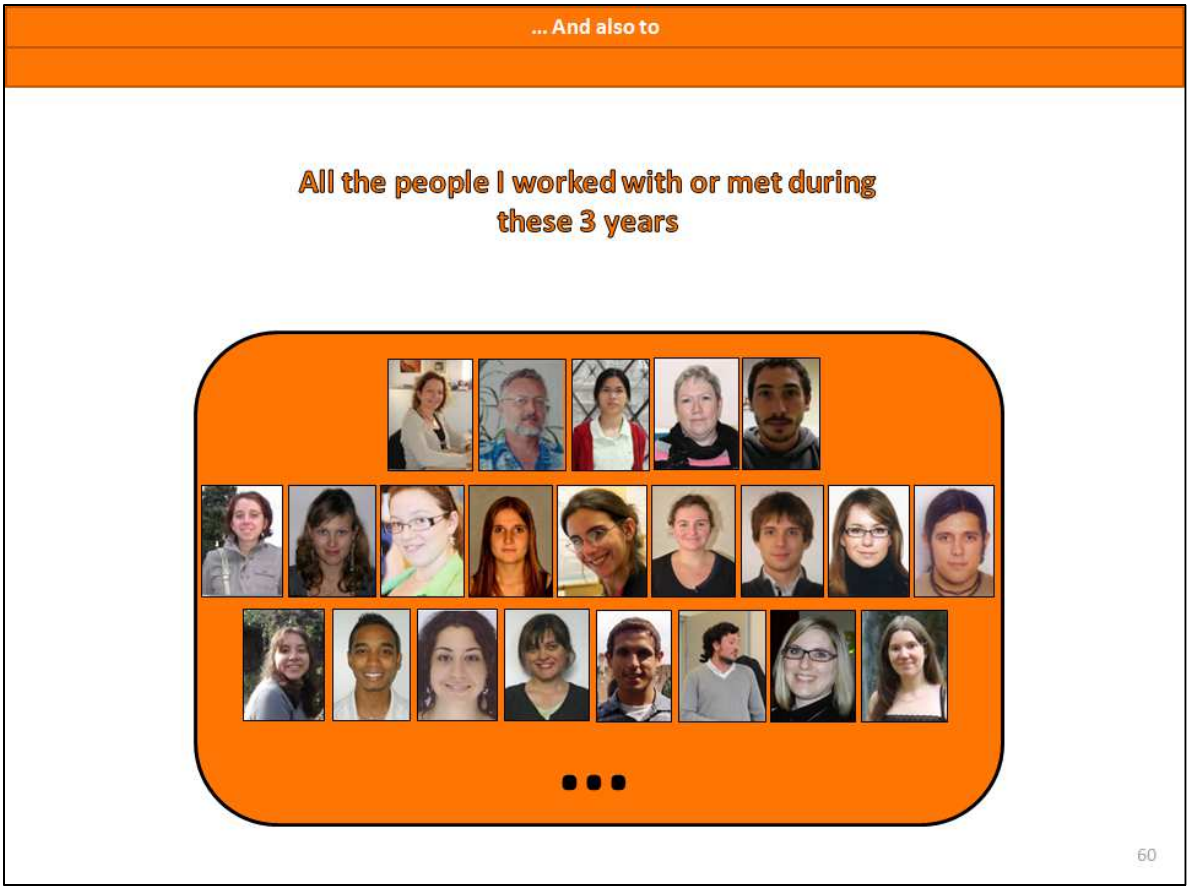
T. Roick

B. Gouble

P. Reling

O. Bongrand

TAILORPACK



… And also to

All the people I worked with or met during these 3 years

…

Annexe 2

Valorisation des compétences des docteurs : Développement d’agro-matériaux adaptés à la conservation optimale des fruits et légumes frais

Thibaut Cagnon, Nathalie Camus

Recueil : Valorisation des compétences «NCT», MED de Montpellier, 2012

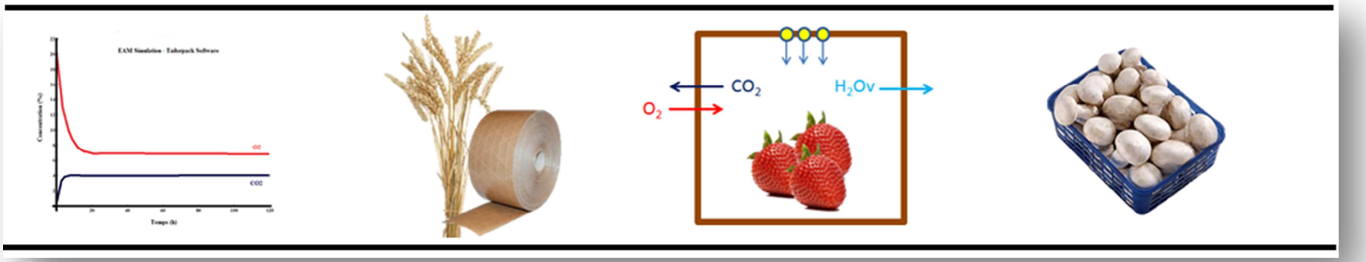
QU’EST-CE QUE LE NCT : Afin d’aider les doctorants à mieux valoriser les compétences scientifiques et transversales acquises durant la thèse ainsi que la portée socio-économique de leur travaux, la Maison des Ecoles Doctorales de Montpellier (MED) et l’Association Bernard Gregory (ABG) proposent une formation individuelle encadrée par un mentor: Le Nouveau Chapitre de la Thèse.

Cette formation aboutit à un rapport qui est présenté dans les pages suivantes.

Valorisation des compétences des docteurs, NCT ®

Thibaut Cagnon

DEVELOPPEMENT D'AGRO-MATERIAUX ADAPTES A LA CONSERVATION OPTIMALE DES FRUITS ET LEGUMES FRAIS



Mentor : Nathalie Camus

Ecole Doctorale : Science des Procédés, Science des Aliments
Unité de Recherche : UMR IATE
Montpellier Supagro / Université Montpellier II

Sujet académique de la thèse : Caractérisation et modélisation des transferts de matière dans des systèmes emballage/aliments - Structuration à façon de matériaux multicouches pour la conservation des fruits et légumes

Directrice de Thèse : Nathalie Gontard
Encadrante Principale : Carole Guillaume

Date de soutenance prévue : Novembre 2012

ORGANISATION DU RAPPORT

LA THESE

Présentation

Contexte Sociétal et Environnemental. Encore aujourd’hui une part trop importante des fruits et légumes récoltés ne sont jamais consommés du fait de leur détérioration entre leur récolte et le moment de leur consommation. Qu’elle se produise lors de leur transport, sur le point de vente ou chez le consommateur. Ces pertes ont évidemment un impact économique important mais génèrent aussi une image de gâchis de moins en moins acceptable.

A côté de ce problème, se trouve celui des emballages et leur mauvaise ou sur-utilisation. Malgré leur caractère très majoritairement recyclable, les plastiques utilisés pour l’emballage des fruits et légumes (barquettes, films, sachets) sont source de pollution et surtout, sont issus de ressources fossiles non renouvelables.

La combinaison de ces 2 problèmes crée un fort intérêt pour des matériaux d’emballage non polluants et issus de ressources renouvelables capables de prolonger la conservation des fruits et légumes.

Contexte Scientifique. Les différents fruits et légumes frais présentent chacun des besoins spécifiques pour une conservation optimale (régulation de leur respiration, protection contre les chocs et les micro-organismes par exemple). Pour répondre à ces besoins il est nécessaire de conditionner ces produits dans des emballages à atmosphère modifiée créant les conditions de conservation optimale.

Cependant, peu de matériaux d’emballage sont conçus dès le départ dans cette optique. Et les matériaux plastiques utilisés habituellement ne sont en général pas adaptés, ce qui peut contribuer aux pertes de marchandises évoquées précédemment.

Tout ceci plaide pour la création de matériaux conçus dès le départ dans le but de répondre aux besoins des produits frais au travers de propriétés mécaniques et de transfert bien spécifiques.

Les agro-matériaux et en particulier les matériaux à base de protéines comme le gluten de blé s’avèrent être particulièrement adaptés à cette tache de par la vaste gamme de perméabilités à l’oxygène et au dioxyde de carbone qu’ils couvrent. Ces propriétés leur permettant de créer des atmosphères à teneur en O_2 modérée et pauvre en CO_2 adaptées aux fruits et légumes. Leurs faibles propriétés mécaniques pouvant elle être compensées par divers types de renforts et notamment l’utilisation de supports naturels tels que le papier.

Résumé de la Thèse, Objectifs et Enjeux. Ma thèse est donc axée sur le développement de matériaux d'emballage à base de gluten de blé et de papier pouvant améliorer la conservation de fruits et légumes frais tels que les champignons ou les fraises.

Elle comporte deux objectifs principaux. D'abord, structurer (fabriquer) par différentes techniques des bio-composites papier/gluten capables de répondre aux besoins des fruits et légumes sélectionnés. Et ensuite, analyser et comprendre les facteurs permettant de moduler les propriétés de ces matériaux afin de répondre aux besoins d'une plus large gamme de produits.

L'enjeu principal de cette thèse réside dans le fait de partir des besoins du produit pour entièrement concevoir et fabriquer l'emballage à l'aide d'outils de simulation (principe de l'ingénierie reverse) plutôt que simplement tester un à un les emballages plastiques disponibles sur le marché. En résumé, de passer d'une approche essai erreur à une approche de type ingénierie reverse (très peu utilisée actuellement dans le domaine) pour le développement d'emballages pour fruits et légumes afin d'engendrer un gain de temps et de créer des matériaux réellement optimaux

Elle présente également des enjeux annexes importants. Le premier économique du à la réduction des pertes de marchandise et au coût modéré des matières premières utilisées. Et le second écologique à court et long terme du à l'utilisation de matériaux non polluants et issus de sources renouvelables.

La Thèse et son Contexte de Travail

Intégration dans le Programme Tailorpack. Ma thèse faisait partie du programme Tailorpack (ANR-07-PNRA-029) qui s'est terminé en Décembre 2011et avait pour but d'étudier tous les aspects de l'ingénierie reverse appliquée à l'emballage des fruits et légumes. Depuis la détermination de leurs paramètres vitaux (respiration, murissement, etc…) et de leur atmosphère optimal de conservation, jusqu'au test des matériaux d'emballage conçus en passant par la mise au point d'un outil de simulation / aide à la création d'emballage à atmosphère modifiée. Le programme avait également pour but d'explorer des moyens novateurs de structuration (mise en forme) des agro-polymères (plastiques provenant de ressources agronomiques comme le blé ou le soja) tels que l'introduction de nano-argiles ou la dépose dite couche par couche.

Au travers de la thèse j’ai pris part à presque toutes les étapes du procédé d’ingénierie reverse qui constituait la colonne vertébrale du programme Tailorpack, depuis l’utilisation de l’outil de simulation jusqu’au test des emballages en conditions réelles. Mais le cœur de mon travail a consisté en la conception et la caractérisation des matériaux à base de gluten.

En dehors du cadre de la thèse, j’ai également participé aux étapes de production pilote des matériaux et également réalisé des analyses sur les solutions d’enduction pour une future production à échelle industrielle.

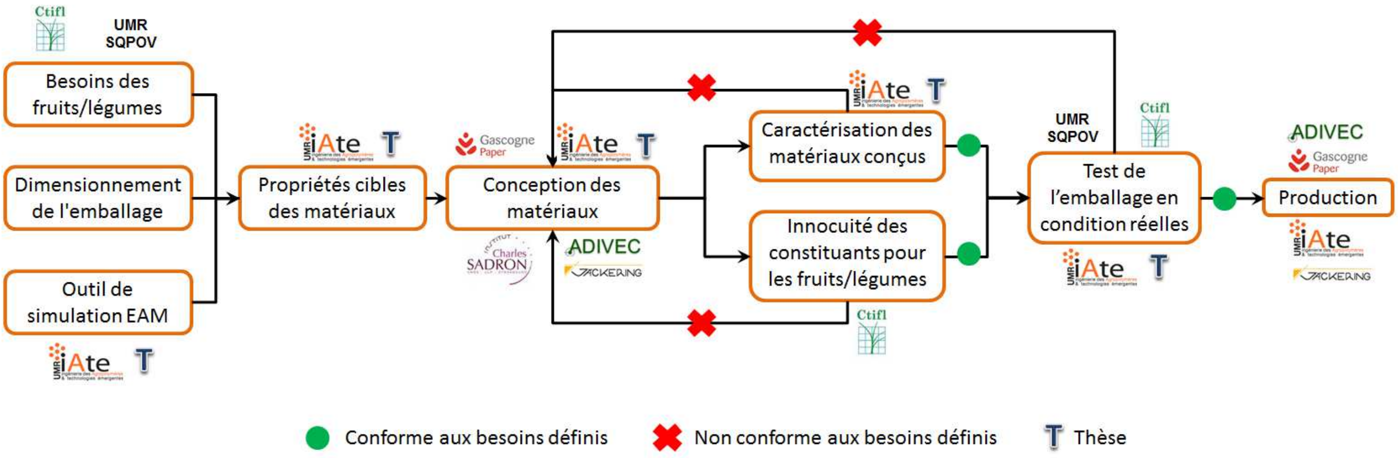


Figure 1. Principe de l’ingénierie reverse appliquée à l’emballage des fruits et légume – Implication des partenaires de la thèse et du programme Tailorpack

L’UMR IATE. Ma thèse, tout comme le programme Tailorpack, s’inscrit pleinement dans le thème de prédilection de l’unité de recherche qui m’a accueilli (UMR Ingénierie des Agro-polymères et Technologies Emergentes, axe 3) : l’étude des transferts (gaz, graisses, composés volatiles, …) aux travers d’agro-matériaux destinés à l’emballage. Elle présentait de plus l’intérêt de solliciter tous les pôles de compétence du laboratoire : modélisation d’emballage à atmosphère modifiée, structuration de matériaux à base de gluten, incorporation d’agents antimicrobiens, caractérisation des matériaux réalisés…

Implication et Motivation Personnelles

Le Sujet. Le thème de la thèse et du programme européen Tailorpack a été le facteur déterminant dans ma décision de me lancer dans ce projet. En effet, c'était pour moi l'occasion de mettre en pratique les connaissances théoriques et pratiques acquises lors de mon cursus d'ingénieur matériaux (spécialité polymères/céramiques). Mais également, de me former à ce nouveau type de matériau, dans un nouvel environnement de travail. La présence de partenaires industriels, tout comme son côté « ingénierie » bien développé furent également des facteurs déterminant dans mon choix car je n'envisageais pas de me couper totalement du monde de l'industrie/ingénierie pour me consacrer uniquement à de la recherche que l'on pourrait qualifier de fondamentale.

Le Doctorat. Le doctorat était surtout pour moi l'occasion de perfectionner mes connaissances scientifiques chose qu'il aurait été plus difficile de faire « sur le tas » au sein d'une entreprise. Acquérir toute la rigueur et la clarté nécessaires à la communication scientifique (orale ou écrite) me semblait également très intéressant pour la suite de ma carrière. Et enfin il s'agissait d'une opportunité de pouvoir directement gérer un projet, mon projet, et prouver mes capacité d'organisation et de planification.

Insertion dans le Programme Tailorpack. Mon implication dans le programme au travers de la thèse a commencé un an après le début de celui-ci (remplacement d'un départ). Je n'ai donc participé ni à son montage, ni à la définition des objectifs et le travail des différents partenaires était déjà bien avancé à mon arrivé. Je devais également partir des bases posées par mon prédécesseur. Mais j'ai toute de même réussi à construire mon projet de thèse et ses objectifs de façon bien plus personnelle en accord avec mes encadrants. Mon souhait était de trouver une balance favorisant autant que possible l'aspect développement (conception de matériaux pleinement fonctionnels) sans négliger bien sur l'aspect science fondamentale obligatoire dans tout travail de thèse.

GESTION DU PROJET DE THESE

Préparation du Projet de Thèse

Montage du Projet de Thèse. Un poste de thèse était prévu et financé sur 3 ans dès le montage du programme Tailorpack en 2007. Cette thèse devait se dérouler au sein de l'équipe IATE et était intitulée *« Conception à façon de matériaux antimicrobiens visant à l'amélioration de la durée de conservation des fruits et légumes via modélisation et approche intégrée »*. Elle visait donc au développement de l'outil de modélisation précédemment évoqué, à son utilisation (à l'aide des données fruits et légumes collectées par des partenaires, voir ci-après) et enfin à la réalisation et l'étude des matériaux répondant aux propriétés cibles déterminées par la simulation.

Ce poste a été occupé pendant un an par un premier ingénieur doctorant qui s'est occupé de toute la partie modélisation avant de quitter le projet. La thèse a donc été reprise en recentrant le sujet sur l'utilisation de l'outil de simulation et la réalisation/étude des matériaux d'emballage. Le tout avec pour premier objectif à court termes la fabrication de matériaux fonctionnels

.

Partenaires Impliqués. Les partenaires impliqués dans le projet de Thèse sont les mêmes que ceux impliqués dans le programme Tailorpack.

Du coté scientifique, l'UMR Sécurité et Qualité des Produits d'Origine Végétale et le Centre Technique des Fruits et Légumes d'Avignon travaillent principalement sur les fruits et légumes, la détermination de leur paramètres vitaux et leur sensibilité aux micro-organismes. L'Institut Charles Sadron Strasbourg est également impliqué au niveau du développement de matériaux novateurs crée par dépose couche par couche.

Du coté industriel, Arjowiggins puis Gascogne Paper ont assuré l'approvisionnement en support papier et apportent, avec Adivec toutes leurs connaissances relatives à ce matériau. Les entreprises Amylum puis Jackering ont quant à elles été sollicitées pour l'approvisionnement en gluten de blé en vue des expériences de laboratoire, comme des essais pilote.

Facteurs de Succès/Risques

Table 1. Facteurs de succès et de risques du projet de thèse

● Facteurs de Succès

*Une grande majorité des compétences/expertise requises au sujet du développement de matériaux bio-sourcés est disponible directement au sein de la structure d'accueil (IATE)
*Les partenaires du programme Tailorpack apportent leur maitrise des autres domaines
*Accès à de nombreux moyens d'analyse dans le laboratoire d'accueil et sur les sites universitaires de Montpellier
*Financement assuré

● Facteurs de risques

*Interdépendance des partenaires pour l'avancement des recherches
*Décalage temporel sur les objectifs initiaux Thèse/Tailorpack
*Absence de technicien (expérimentations) dans le laboratoire
*Intégration dans un programme déjà lancé, pas de temps d'adaptation au sujet, nécessité de résultats expérimentaux immédiats

Afin de contenir les facteurs de risques et d'éviter qu'ils ne perturbent le bon déroulement de ma thèse, j'ai mis en place des stratégies de réponse. Pour éviter que l'interdépendance entre les partenaires ne perturbe l'avancement de mes travaux ou de ceux des dits partenaires, j'ai maintenu un contact régulier tout au long du projet et l'ai renforcé lors des périodes d'objectifs communs. Afin de palier à l'absence de temps d'adaptation au sujet de thèse, j'ai commencé par un survol des grandes notions nécessaires à sa compréhension globale puis réparti l'étude bibliographique approfondie tout au long des 3ans en me concentrant à chaque fois sur le domaine précis sur lequel je travaillais (structuration des matériaux ou emballage à atmosphère modifié par exemple). J'ai également pris le parti de me former sur les techniques d'analyses et le matériel expérimental utilisé sur le tas et en prenant des informations auprès des utilisateurs réguliers plutôt qu'en passant par la voie écrite afin de gagner un maximum de temps et d'efficacité. Cela m'a également permis de mieux connaitre les systèmes expérimentaux et de mesure, et donc de palier a l'absence de technicien spécialiste au sein du laboratoire. Enfin, j'ai maitrisé le facteur de risque le plus important, le décalage temporel entre les objectifs de la thèse et du programme Tailorpack en essayant au maximum de les regrouper et d'obtenir des résultats pertinents et valorisables dans le cadre des 2 projets. Quand ce n'était pas possible, je me suis appuyé sur une organisation précise et bien planifiée pour rester dans les temps.

Suivi du Projet de Thèse

Une fois la thèse lancée, j'ai activement participé à son suivi au moyen de 3 leviers. Tout d'abord je participais aux réunions du programme Tailorpack qui se déroulaient tous les 6 mois et permettaient de faire le point aussi bien sur mes travaux de thèse que sur les résultats des autres partenaires. Lors de ses réunions, les objectifs du programme pour les 6 mois suivants étaient également définis. Ensuite j'ai activement participé à l'organisation de mes comités de thèse qui ont eu lieu à la fin de la première et de la seconde année et qui furent l'occasion de faire un point plus approfondi sur la thèse, ses orientations et ses résultats. Lors de ces comités, les publications servant à la valorisation de mes travaux ont été décidées et discutées. Enfin j'ai organisé tout au long de ma thèse des réunions thésard/encadrants afin de régler au plus vite tout problème interne au déroulement de ma thèse ou de finaliser les publications.

De plus, tout au long de la thèse et du programme Tailorpack, j'ai maintenu un contact avec les partenaires du programme via e-mail, téléphone ou visite sur site afin de maintenir une bonne synergie entre toutes les équipes, de renforcer les liens entre les personnes et d'éviter des blocages. Dans cette optique, une visite à l'usine papetière de Gascogne Paper, des échanges de « services techniques » (tels que la caractérisation de solutions de gluten industrielles) ou encore des campagnes d'expérience simultanées avec l'UMR SQPOV ont également été mises en place.

Il est également important de noter que le fait d'avoir eu pour encadrante la coordinatrice du programme Tailorpack, Carole Guillaume, aura grandement facilité les choses afin de faire coïncider les objectifs de la thèse et du programme. En effet, selon moi, rien ne remplace des réunion/conversation en direct pour mettre à plat les problèmes ou organiser le travail.

Coût Financier, Humain et Matériel

Coût du Projet de Thèse. Le coût total de cette thèse a été évalué à 263k€. Les ressources humaines auront constitué le principal poste de dépense avec plus de 70% du budget alloué suivi de très loin par le matériel (13%) et les infrastructures (7%). Concernant les sources de financement, il était équitablement réparti entre l'Agence Nationale pour la Recherche et les structures locales (UM2 et Sup'Agro). *(Voir Annexe page 313 pour le calcul détaillé).*

On peut également remarquer que le programme Tailorpack dont faisait partie ma thèse affichait un budget total de plus de 1 500k€.

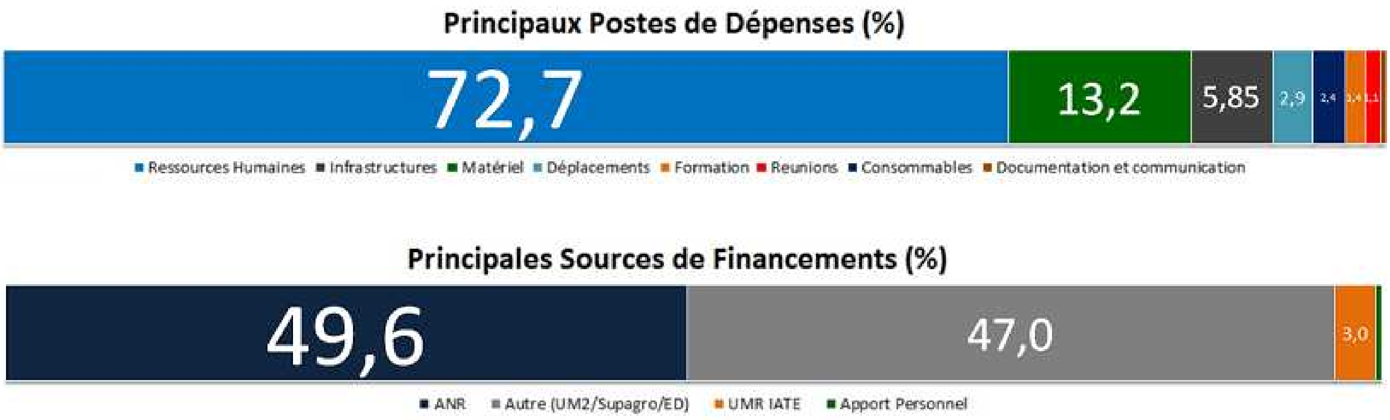


Figure 2. Principaux postes de dépenses et principales sources de financement exprimées en pourcentages du budget total de la thèse

Moyens Humains. En plus de mon investissement à temps complet sur le programme et la thèse, il faut ajouter le suivi au quotidien assuré par mon encadrante principale, Carole Guillaume et le suivi/contrôle au moment des étapes importantes par ma directrice de thèse, Nathalie Gontard. Il faut aussi ajouter l’investissement conséquent des autres professeurs et maitres de conférences du laboratoire quand leur expertise était requise, en particulier Pascale Chalier et Emmanuelle Gastaldi, ainsi que l’aide précieuse de M. Muller et M. Macouire quand des interventions/réparations sur certains appareillages du laboratoire s’avéraient nécessaires. Enfin, le temps passé par les partenaires du programme Tailorpack à obtenir les résultats dont je me suis servi par la suite sont évidemment à prendre en compte.

Il est à noter que le laboratoire de l’axe 3 de l’UMR IATE dans lequel j’ai conduit ma thèse ne possédait pas de technicien chargé d’opérer et de suivre les différents montages expérimentaux ou appareillages. Ma formation a donc été assurée par les membres du laboratoire ou les thésards déjà en place et j’ai moi-même transmis ces connaissances par la suite aux nouveaux arrivants.

Matériel Mobilisé. Enfin, sur le plan matériel, cette thèse aura mobilisé des installations internes à l'UMR IATE, universitaires et industrielles. Pour la conception et la caractérisation des matériaux à l'échelle laboratoire, presque tous les équipements de l'Axe 3 de l'UMR IATE auront été sollicités (outils d'enduction, chromatographie à phase gazeuse, système de mesure de perméabilité, digidrop pour mesure d'angle de contact…) ainsi que les microscopes électroniques de l'Institut européen des membranes (IEM) et du CRIC à Montpellier. Pour les essais pilotes de production, la location pour 2 jours d'une coucheuse à papier pilote du Centre technique du papier (CTP) de Grenoble aura été nécessaire, tout comme le recours à la halle de technologie de l'IUT de Montpellier pour la préparation des solutions de gluten en grande quantité (100L).

COMPETENCES MISES EN ŒUVRE

Connaissances et Compétences Scientifiques et Techniques

J'ai, au cours de ma thèse, pu développer de nombreux savoirs et savoir-faire scientifiques venant compléter ceux que je possédais déjà au travers de mes précédentes expériences professionnelles. Certaines de ces compétences sont spécifiques au domaine étudié lors de la thèse, mais une majorité est facilement adaptable et transférable à toute mission à caractères scientifique/ingénierie.

- Capacité à assurer une vieille scientifique et technologique au travers des études bibliographiques et de la rédaction d'une review tout au long de la thèse
- Intégration et mise en pratique du concept d'ingénierie reverse aboutissant au développement d'emballages optimaux pour fruits et légumes
- Maitrise des procédés de mise en forme des matériaux plastiques (synthétiques et bio-sourcés) au travers de la fabrication de matériaux viables et fonctionnels.
- Maitrise des techniques de mesure de la perméabilité des matériaux et des nombreuses normes d'unités existantes
- Connaissance et expérience des contraintes d'un passage d'une échelle laboratoire à une échelle de production pilote grâce à la réalisation d'une fabrication de matériaux d'emballage pilotes (100L de solution d'enduction, 4 bobines de papier).

- Apprentissage et maitrise des bases de la programmation en langage scientifique (Matlab®) via l'optimisation et l'utilisation sous sa forme initiale (hors-ligne) du logiciel Tailorpack
- Acquisition de nombreux savoirs sur les emballages pour produits frais, les emballages actifs/intelligents et les agro-matériaux qui viennent compléter mon bagage général sur les polymères et leur mise en forme.

Compétences Transversales

Au-delà des compétences et savoirs purement scientifiques, j'ai également développé de nombreuses compétences transversales qui me seront utiles quel que soit le poste que j'occuperai dans le futur.

- Aptitude à la gestion de projet démontrée au jour le jour durant les 3ans de thèse et par l'accomplissement, dans le temps et malgré les facteurs de risques, des objectifs fixés aussi bien pour la thèse que pour le programme Tailorpack (Outils : Définition d'objectifs, GANT, Réunions de suivi/réorientation, Contact permanent entre les membres du projet)
- Forte aptitude au travail en équipe grâce à des qualités d'écoute, de synthèse et d'organisation qui furent nécessaire pour gérer en bonne intelligence le partage des équipements (mise en place d'un planning de réservation) et organiser collégialement les réunions d'équipe à l'échelle du laboratoire ou des doctorants de celui-ci (sondage *doodle*, etc…)
- Capacité à gérer les contraintes et optimiser les points forts d'un travail coopératif inter-équipes mise en évidence tout au long du programme Tailorpack et qui aura abouti à 3 valorisations scientifiques communes (2 articles, 1 poster)
- Capacité à transmettre les savoirs et savoir-faire acquis, au travers d'enseignements (Cours et TD) et de la formation de nouveaux arrivants (stagiaires/doctorants) sur les montages expérimentaux du laboratoire.
- Aisance dans la communication grâce aux nombreuses présentations de mes résultats que ce soit à mes encadrants, aux partenaires du programme Tailorpack ou à un public expert lors d'un congrès.
- Maitrise de la langue Anglaise à l'écrit comme à l'oral via la rédaction d'articles scientifiques et la participation à des congrès en langue anglaise (auditeur et conférencier)

- Compétences Organisationnelles de par les nombreuses démarches administratives nécessaires à l'inscription en thèse, à des congrès ou à la mise en place de missions en dehors du laboratoire (en France ou à l'étranger).
- Compétences de gestion/achat via la demande, la comparaison et le choix de devis pour des réparations ou achats de matériels ou la commande de produits.
- Capacité de management démontrée au travers de l'encadrement technique de deux stagiaires (délégation de mon encadrante), leur formation sur les appareils expérimentaux et un suivi de leurs résultats. J'ai également su gérer de façon efficace une situation de conflit avec l'une des stagiaire, en posant le diagnostic du problème (manque d'organisation, non suivi de certaines règles de sécurité), organisant un entretient de recadrage et finalement en prenant la décision qui s'imposait suite à l'absence d'améliorations.
- Constitution d'un réseau composé des collaborateurs du programme Tailorpack, des fournisseurs et prestataires de services contactés pour la thèse et également d'autres chercheurs sans lien avec le projet (participation à l'impulsion d'un nouveau projet entre l'UMR IATE et l'UMR IES). Et également entretient du réseau existant, composé de contacts (professeurs, anciens collègues, fournisseurs/clients, …) rencontrés lors de mon cursus et de mes précédentes expériences professionnelles.

Qualités Personnelles

Les savoir faires et connaissances acquis et développés au cours des 3 ans de thèse vont de pair avec des qualités personnelles qui auront été indispensable à la réussite de ce projet.

- Tenace, motivé et efficace pour mener à bien un projet de 3ans, surmonter les difficultés et tenir les délais.
- Autonome afin de dépendre le moins possible des autres dans mon travail personnel et d'être capable de progresser avec une aide minimale si besoin.
- Proactif, force de proposition sur la thèse (sur l'orientation, sur les publications, le choix des congrès et formations) et capable d'anticiper des besoins dans le cadre du programme Tailorpack.
- Adaptable et ingénieux pour m'habituer au plus vite à un nouvel environnement de travail et pour m'accommoder au mieux de changements de planning intempestifs ou répondre rapidement à des problèmes techniques.

- Rigoureux, organisé et appliqué dans la collecte et l’analyse des résultats.
- Responsable, du suivi des appareils que j’utilisais pendant ma thèse et d’une des salles de laboratoire (approvisionnement, état général, sécurité,…)
- Logique, critique et objectif pour toujours vérifier et assurer la qualité de mon travail et peser mes décisions.
- Ouvert et Altruiste afin d’échanger avec et aider au mieux les autres membres de l’équipe et de créer une bonne dynamique de groupe. Mais aussi de maintenir une bonne synergie dans la coopération inter-équipe au sein du programme Tailorpack.
- Synthétique et Clair dans la valorisation écrite comme orale de mes résultats.
- Pédagogue et Capable de vulgariser dans la transmission de mes connaissances aux élèves auxquels j’ai enseigné et de mes savoir-faire aux stagiaires que j’ai formés.

RESULTATS ET IMPACT

Impact Scientifique et Technique

Impact Scientifique. Sur le plan scientifique, cette thèse a permis de pousser plus loin la compréhension des différentes structurations possibles pour un agro-polymère (seul, enduit, déposé couche par couche…). Leurs impacts respectifs sur les propriétés mécaniques et de transfert des matériaux crees ont également été mieux définis et compris.

L’étude des différents matériaux créés a prouvé qu’il était possible de renforcer efficacement les matériaux à base de gluten tout en conservant les propriétés de transfert qui les rendent si particuliers et intéressants. Et enfin la totale adaptation des matériaux à base de gluten à une utilisation en tant qu’emballage pour fruits et légumes frais a pu être constatée au travers de tests en conditions réelles.

On peut également noter que cette thèse a mis en lumière un nouveau champ d’application pour les matériaux couche par couche qui étaient jusqu’à présent cantonnés à des domaines ultra pointus tels que les bio-matériaux à application médicale ou les semi-conducteurs

Enfin, ces résultats ont été valorisés par 5 publications (1er auteur) dans des journaux scientifiques internationaux et la participation à un congrès international sur l’emballage (IAPRI 2012).

Impact Technique. Sur le plan technique, la principale réussite consiste en la validation de la démarche de l'ingénierie reverse sur le cas précis des fraises. Une bonne connaissance du produit combiné à l'utilisation du logiciel de simulation d'emballage Tailorpack (www.tailorpack.com), crée dans le cadre du programme du même nom, a bien permis de définir les propriétés nécessaires des matériaux (transfert de gaz, vecteur d'agent antimicrobien, solidité suffisante pour utilisation « emballage »). Ceux-ci ont été conçus à partir des connaissances évoquées dans les paragraphes précédents, puis ils ont été testés avec succès en conditions réelles (résultat : 2 jours de conservation en plus).

A côté de cela, une production à échelle pilote a elle aussi été testée et a abouti à la fabrication de bobines de papier enduit viables mais présentant des performances moins intéressantes que celles des matériaux à échelle laboratoire. Cependant les conditions drastiques imposées par l'essai pilote par rapport aux conditions industrielles réelles permettent d'envisager avec une certaine confiance une production industrielle test. Il est important de noter que les partenaires Gascogne Paper et Adivec, étaient très intéressés par l'aspect barrière aux graisses des matériaux développés (étudiés pour le programme Tailorpack seulement) et que des développements futurs dans cette direction son sérieusement envisagés par ces 2 entreprises pour remplacer les résines d'enduction actuelles dans les boites à hamburger notamment. Il est à noter que dans ce cas (emballage de produits alimentaires contenant du gluten), l'usage de matériaux à base de gluten ne pose pas de problèmes. Cependant dans le cas d'une industrialisation de ces matériaux pour l'emballage fruit et légumes, se poserait la question des allergies au gluten. Le problème pourrait toutefois être contourné en ayant recours à d'autres protéines pour concevoir les matériaux, moyennant bien sur des mises au point et ajustements.

Au final, il est maintenant du ressort des producteurs de fruits et légumes et/ou des autres acteurs de ce secteur (des fabricants d'emballage à la grande distribution) d'utiliser la démarche de l'ingénierie reverse. Soit « seule » pour déterminer le meilleur matériau disponible sur le marché pour le produit choisi et ainsi gagner en temps et en coût par rapport à la démarche essai/erreur. Soit combinée à la fabrication de matériaux réalisés sur-mesure (si possible bio-sourcés pour des raisons écologiques) afin d'obtenir des matériaux réellement optimaux. Dans les 2 cas un allongement substantiel de la durée de vie du produit pourra être obtenu par rapport à la situation actuelle, limitant les pertes entre la récolte et la consommation du fruit ou du légume considéré.

Impact Personnel

De façon plus personnelle cette thèse a été l'occasion pour moi d'approfondir ma connaissance des matériaux et d'ajouter les agro-polymères à mes domaines de compétence. Elle m'aura aussi permis de maitriser de nouvelles techniques d'analyses scientifiques telles que les mesures de perméabilité aux gaz ou le suivi de composés volatils par chromatographie. Cette thèse m'a aussi permis de grandement améliorer mes capacités de rédaction et de communication de mes résultats à destination de tous types de publics.

Sur un plan non scientifique, j'ai pu mettre en pratique mes capacités d'organisation et de gestion du travail et du temps avec succès et prouver ainsi ma capacité à gérer un projet à l'organisation et au timing souvent complexe. J'ai également pu développer mes compétences de formation et de transmission des savoirs au travers d'une cinquantaine d'heures d'enseignements, de la formation technique de 2 stagiaires et des échanges quotidiens avec les autres membres du laboratoires (thésards, stagiaires, ingénieurs recherche…).

Enfin, cette expérience m'a permis de confirmer les orientations que je souhaitais donner à ma carrière. Je me suis rendu compte que j'étais très attaché au développement et à la conception ce qui m'incite à me tourner vers une carrière à dominante « développement » plutôt que « recherche pure ». Et je suis au final confiant quant à l'utilité des acquis de ma thèse pour les postes que j'envisage à l'avenir. En effet ils me permettent de compléter un profil ingénieur « développement/utilisation matériaux » construit lors de mon cursus et de mes stages par la connaissance de matériaux innovants et encore trop peu utilisés industriellement (agro-matériaux), la maitrise d'une approche importante dans beaucoup d'industries et novatrice dans la conception des emballages (ingénierie reverse) et par une plus grande expérience de toutes les composantes de la gestion de projet.

POURSUITE DE CARRIERE

Pour la suite de ma carrière j’envisage principalement 2 types de postes :

- Chef de projet R&D en plasturgie. Pour monter et gérer des projets basés sur des plastiques novateurs, bio-sourcés et/ou actifs
- Ingénieur conseil matières plastiques. Soit au sein d’une entreprise de plasturgie pour faire le lien entre les fournisseurs et les différents services internes. Soit au sein d’un groupe fournisseur de matières premières pour conseiller les clients.

Ces postes se basent sur des connaissances techniques et scientifiques similaires de par leur domaine mais demandent des compétences transversales et proposent des missions assez différentes. Dans le tableau page 16, sont présentés les missions, les compétences requises et mes atouts pour chacun de ces 2 postes.

Entre ces 2 métiers, j’ai une préférence pour le poste d’Ingénieur conseil, en particulier si il prend place au sein d’une entreprise de plasturgie et a pour objectif de faire le lien entre fournisseurs, service R&D, service production et finalement, service relation clients. J’ai déjà eu l’occasion d’occuper ces fonctions durant 6 mois lors de mon stage d’ingénieur et je m’y étais senti particulièrement à l’aise sur les plans techniques et organisationnels. La variété du travail et des interlocuteurs rencontrés m’avait également beaucoup plus.

J’aimerai également, à plus ou moins long terme, avoir l’occasion de transmettre mes acquis et mon savoir. Soit dans le cadre de mes futures fonctions, soit au travers de vacations dans le cadre d’une université ou d’une école d’ingénieur.

Quoi qu’il en soit, mon expérience de thèse me permettra d’apporter une vraie valeur ajoutée par rapport à un profil ingénieur classique de même expérience sur le plan de l’autonomie, des connaissances et savoir-faire scientifiques et surtout du réseau scientifique et technique (expertise, analyse d’échantillons, etc…). Il me restera cependant à développer plus amplement mon réseau dans le secteur de la plasturgie industrielle, au travers de rencontres lors de congrès et salons, via les réseaux sociaux professionnels (ex : linkedin, viadeo) ou au cours de mes futures activités. Je pense également que des formations sur les logiciels de conception/production assistées par ordinateur pour le domaine de la plasturgie seraient bénéfiques pour compléter mon profil.

Table 2. Détail des postes envisagé pour la poursuite de carrière.

Poste	Missions	Compétences Requises	Atouts
Chef de Projet R&D	-Proposer des innovations ou répondre à des demandes d'optimisation -Définir et planifier (risques, ressources, chiffrage) -Suivre et encadrer (coordonner les services, synthétiser et analyser les résultats, animer les réunions, recadrer le projet…) -Finaliser et communiquer (reporting final, communication des informations techniques aux autres services,…)	-Connaissances des technologies impliquées dans le projet -Compréhension des besoins des clients ou du marché -Maitrise des principes et outils de gestion de projet -Gestion/Coordination d'équipes -Capacité de synthèse	-Connaissances techniques et scientifiques -Au fait des besoins et impératifs du domaine -Bon sens du relationnel et de la communication -Bonne capacité à transmettre l'information -Capacité d'adaptation -Capacité de Management -Autonomie, esprit d'initiative -Rigueur
Ingénieur Conseil	-Veille technologique constante -Diagnostiquer et/ou répondre aux problèmes techniques soulevés par les clients ou les autres services de l'entreprise -Suivre l'évolution des solutions appliquées avec le client -Etre force de proposition pour d'éventuelles innovations sur les gammes de produits des clients ou de l'entreprise	-Connaissance très pointue du domaine -Bonne connaissance des différents services de l'entreprise ou des clients -Capacité à recueillir et synthétiser l'information -Réactivité/Efficacité -Sens de l'écoute	-Connaissances techniques et scientifiques -Au fait des besoins et impératifs du domaine -Sens de l'écoute -Aisance dans la communication -Capacité à vite cerner et comprendre les problèmes pour émettre un diagnostic -Capacités d'adaptation et de réaction -Force de proposition

ANNEXE – CALCUL DU COUT DE LA THESE

	Nature de la dépense	Détails		Coûts totaux (euros TTC)				Origine des financements (euros TTC)			
				Nombre d'unités	Coût unitaire moyen	Quote-part utilisation	Total	ANR	UMR IATE	Autre (UM2/Supagro/ED)	Apport Personnel
1	**Ressources Humaines**										
1.1	Doctorant			36 (m)	2416	100%	87000	87000			
1.2	Encadrant 1 (tutrice principale)			720(h)	52	100%	37440			37440	
1.3	Encadrant 2 (directrice)			360(h)	70	100%	25200			25200	
1.4	Encadrant 3			360(h)	52	100%	18720			18720	
1.5	Encadrant 4			360(h)	52	100%	18720			18720	
1.6	Autre personnel (hors sous-traitance)	Stages					2400	2400			
1.7	Sous-traitance (Microscopie)						2000	2000			
	Sous-total Ressources Humaines						191480	91400		100080	
2	**Consommables**										
2.1	Fournitures expérimentales						6000	6000			
2.2	Fournitures de bureau						250		250		
2.3	Autres achats						0				
	Sous-total Consommables						6250	6000	250		
3	**Infrastructures**										
3.1	Secrétariat			3 (a)	11400	4%	1400			1400	
3.2	Loyers des locaux			3 (a)	116000	4%	14000			14000	
3.3	Electricité, eau, chauffage, …	Inclus dans loyer					0				
3.4	Autres						0				
	Sous-total Infrastructures						15400			15400	
4	**Matériel (amortissements)**										
4.1	Matériel d'expérimentation (dont les ordinateurs et logiciels spécialisés)	Achat: Aw-metre	Entretient: Chambre climatique				27400	24000	3400		
4.2	Ordinateur de bureau	PC portable et Ecran fixe					2500	1250	1250		
4.3	Logiciels de bureau	Pack Office et Licence Matlab					1000		1000		
4.4	Autre (Location machine pilote)						4000	4000			
	Sous-total Matériel						34900	29250	5650		
5	**Déplacements**	Trajet	Hébergement								
5.1	Missions en France	600	800				1400	1000	400		
5.2	Missions à l'étranger	0	0				0				
5.3	Congrès en France *(Matbim 2012 Dijon)*	200	800				1000		350	650	
5.4	Congrès à l'étranger *(Iapri 2012 USA)*	3000	2200				5200			5200	
	Sous-total Déplacements						7600	1000	750	5850	
6	**Formation**										
6.1	Formations	130h (MED)	40h (Labo)	170 (h)			2500			2500	
6.2	Autres frais (Inscription à l'Université, Sécurité Sociale étudiante, etc.)						1170				1170
	Sous-total Formation						3670			2500	1170
7	**Documentation et communication**										
7.1	Affranchissements, Internet, téléphone						500		599		
7.2	Publicité, communication, impressions						50		50		
7.3	Documentation (périodiques, livres, bases de données, bibliothèque, etc.)						600		600		
7.4	Autres						0				
	Sous-total Documentation et communication						1150		1249		
8	**Reunions (Thèse, Programme, etc.)**						3000				
	Sous-total Réunions						3000	3000			
9	**Charges financières**						0				
	Sous-total Charges financières						0				
10	**Charges exceptionnelles**						0				
	Sous-total Charges exceptionnelles						0				
	TOTAL						**263450**	**130650**	**7899**	**123830**	**1170**

TRANSFERTS DANS LES SYSTEMES EMBALLAGE/ALIMENTS: STRUCTURATION A FACON DE MATERIAUX MULTICOUCHES POUR L'EMBALLAGE SOUS ATMOSPHERE MODIFIEE DES PRODUITS FRAIS

RESUME: Malgré l'ampleur que prend la technologie sous atmosphère modifiée pour le conditionnement des fruits et légumes frais, l'adéquation des matériaux d'emballage avec les besoins des produits est insuffisante. D'une part parce que la conception de ces emballages repose encore sur des approches empiriques de type essai-erreur, et d'autre part parce que la plupart des matériaux disponibles sur le marché présentent des propriétés de transfert aux gaz trop restreintes compte tenu de la gamme de propriétés nécessaire pour couvrir les besoins de ces produits. En vue d'apporter des solutions à ces deux verrous, les travaux de thèse ont porté sur :

- la mise en place et la validation d'une approche basée sur l'ingénierie reverse visant à identifier les propriétés de transfert requises ou cibles pour un végétal donné ;
- l'étude des procédés de structuration à différentes échelles pour moduler les propriétés de transferts de papiers enduits de protéines de blé, issus de ressources renouvelables.

Une démarche d'ingénierie reverse organisée en 5 étapes dont l'identification des besoins des produits et la prédiction des propriétés de transferts requises a été proposée et validée à travers la conception d'un papier enduit actif pour l'emballage sous atmosphère modifiée adapté à la conservation de fraises. Les différents procédés de structuration étudiés ont permis de produire des matériaux couvrant des gammes de permsélectivité allant de 5 à 18 et de perméance à l'oxygène allant de $0,02x10^{-10}$ à $2x10^{-10}$ $mol.Pa^{-1}.m^{-2}.s^{-2}$, ce qui répond parfaitement aux besoins d'une sélection représentative de produits frais respirant. Que ce soit des papiers enduits par des procédés conventionnels (couche épaisse) ou des procédés innovants (nano-structuration couche par couche en présence de feuillets d'argile, montmorillonites), il est possible de moduler les propriétés de transferts aux gaz des papiers enduits soit en jouant sur le raffinage des papiers supports, le nombre de paires de couche (gluten/montmorillonite), ou encore en soumettant les matériaux ainsi obtenus à un balayage gazeux de CO_2.

Cette nouvelle approche et l'efficacité des matériaux protéiques à couvrir les besoins des fruits et légumes ouvrent la voie à la conception de nouveaux emballages mieux adaptés aux produits respirant.

MOTS CLES: EAM, Transferts de gaz, Approche intégrée, Gluten de blé, Relation structure/propriétés

MASS TRANSFERS IN FOOD/PACKAGING SYSTEMS: STRUCTURING TAILOR-MADE MULTILAYER MATERIALS FOR MODIFIED ATMOSPHERE PACKAGING OF RESPIRING PRODUCE

ABSTRACT: Despite the growing importance of the modified atmosphere packaging (MAP) technology for fresh fruits and vegetable preservation, the adequacy of the packaging materials with the produce needs remains a problem. On one hand because the packaging development approaches are still based on empirical trial and error methods, and on the other hand, because of the non-adequate gas transfer properties (too restricted to cover the large game of gas transfer properties required) of the vast majority of conventional synthetic plastics currently used for fresh food packaging. In order to overcome these hindrances, the thesis work was split on two parts:

- the establishment and the validation of a new approach based on reverse engineering aiming to identify the optimal (targeted) properties for optimal preservation of a selected produce;
- the study of the various structuration processes at different scales to modulated the gas transfer properties of bio-sourced wheat gluten coated papers.

The new reverse engineering approach for MAP conception consisted in 5 steps including the definition of the produce needs and the prediction of the optimal gas transfer properties of the packaging, and was validated through the conception of an active optimal packaging for strawberry preservation. The different structuration processes studied allowed production of a gluten based materials able to cover ranges of permselectivity and oxygen permeations going from 5 to 18 and $0.02x10^{-10}$ à $2x10^{-10}$ $mol.Pa^{-1}.m^{-2}.s^{-2}$, which perfectly matched the needs of a representative selection of fresh produce. Be it for conventional coating techniques (thick layer) or innovative processes (layer-by-layer nano-structuring of gluten/montmorillionite layers), it was possible to modulate the transfer properties of coated papers by changing the refining degree of the support paper, the number of layer pairs (gluten/montmorillionite) deposited, or even by submitting such materials to a CO_2 treatment.

This new approach and the efficiency of the protein-based materials for covering the fresh fruits and vegetable needs open the way for conception of new optimal packaging for respiring produces.

KEY WORDS: MAP, Mass transfer, Integrated approach, Wheat gluten, Structure/properties relationship