Incorporation and release of organic volatile compounds in a bio-based matrix by twin-screw extrusion

Natalia Castro Gutierrez

To cite this version:

Natalia Castro Gutierrez. Incorporation and release of organic volatile compounds in a bio-based matrix by twin-screw extrusion. Agricultural sciences. Institut National Polytechnique de Toulouse - INPT, 2016. English. NNT : 2016INPT0014 . tel-02796218v2

HAL Id: tel-02796218
https://hal.inrae.fr/tel-02796218v2

Submitted on 11 Oct 2023

THÈSE

En vue de l'obtention du

DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE

Délivré par :

Institut National Polytechnique de Toulouse (INP Toulouse)

Discipline ou spécialité :

Sciences des Agroressources

Présentée et soutenue par :

Mme NATALIA CASTRO GUTIERREZ
le jeudi 18 février 2016

Titre :

INCORPORATION ET LIBERATION DE COMPOSES ORGANIQUES VOLATILS DANS UNE MATRICE D'AGROMATERIAUX PAR EXTRUSION BI-VIS

Ecole doctorale :
Sciences de la Matière (SDM)

Unité de recherche :
Laboratoire de Chimie Agro-Industrielle (L.C.A.)
Directeur(s) de Thèse :
MME CHRISTINE RAYNAUD
MME VANESSA DURRIEU

Rapporteurs :
M. ALAIN DUFRESNE, INP DE GRENOBLE
Mme ELISABETH GUICHARD, INRA DIJON

Membre(s) du jury :
M. CHRISTIAN CHERVIN, INP TOULOUSE, Président
M. ADEM GHARSALLAOUI, UNIVERSITE LYON 1, Membre
Mme CHRISTINE RAYNAUD, INP TOULOUSE, Membre
Mme STEPHANIE KOLB, GIVAUDAN FRANCE SAS, Membre

"Muere lentamente quien no viaja,

Quien no lee, quien no escucha música,

Quien no halla encanto en si mismo.

Muere lentamente quien destruye su amor propio, quien no se deja ayudar.

[...]

¡Vive hoy! - ¡Haz hoy!

¡Arriesga hoy!

¡No te dejes morir lentamente!

¡No te olvides de ser Feliz!

Pablo Neruda

" A mi me gusta comer de verdad,

beber de verdad, besar de verdad,

Charlar con los amigos de verdad,

Enamorarme de verdad, y cuándo pones tando

En todas esas cosas lo mas normal es que salgas lleno de cicatrices."

Joaquín Sabina

Remerciements

Cette thèse a été effectuée au sein du Laboratoire de Chimie Agro-Industrielle (LCA, UMR 1010 INRA/INP-ENCIASET) dans le cadre d'un partenariat industriel avec Givaudan France SAS.

Je tiens tout d'abord à remercier M. Carlos Vaca Garcia et Gérard Vilarem, directeurs du LCA, de m'avoir accueillie au sein du laboratoire. Merci pour vos remarques et conseils avisés, ainsi que pour votre aide et soutien lors des démarches administratives.

Je souhaite aussi remercier Givaudan Frances SAS et plus précisément l'équipe de Delivery and Emitting systems, pour leur accueil, toujours chaleureux, les échanges et discussions que l'on a eu lors de mes visites. Merci à Lucille, Ian Harisson, Stéphane Bone, Nelly, Carol et Guy-Emmanuelle pour leurs conseils et leur aide précieuse (surtout pour la partie application).

J'adresse mes plus sincères remerciements à mes quatre encadrants (Vanessa, Stéphanie, Christine et Antoine) qui m'ont donné leur confiance en me permettant d'effectuer ces travaux de recherche et avec qui cette thèse n'aurait pu être aboutie. Merci d'avoir toujours laissé la porte ouverte pour n'importe quelle question et pour la liberté que vous m'avez octroyée. Cela m'a permis de gagner confiance en moi et de développer mon esprit scientifique. Un grand merci pour votre aide, vos encouragements, vos conseils durant ces années, mais surtout merci pour votre investissement lors de la rédaction. Vous avez contribué à ma formation en tant que scientifique et personne ; vous m'avez appris à mettre de la rigueur au coeur de mon travail et à prendre plaisir à faire de la recherche. Nos discussions furent toujours un vrai plaisir pour moi, car chacune d'elle a contribué au maintien de ma passion pour la recherche (malgré les essais infructueux en extrusion) et grâce à cela je garderai un doux souvenir de cette période. Vanessa et Stéphanie, merci de m'avoir appris que la qualité et la rigueur ne sont pas seulement des caractéristiques allemandes. Antoine, merci pour ta bonne humeur et ton esprit décontracté dans cette équipe à forte majorité féminine. Christine, merci pour ta confiance, merci d'être toujours à mon écoute, et d'avoir été là pendant ma convalescence (je ne te remercierai jamais assez). Vanessa, merci pour ton soutien inconditionnel et tes qualités humaines inégalables. Et bien sûr Christian Quellet, merci pour votre expertise scientifique et vos précieux conseils et bien évidement, pour votre fine touche de bonne humeur !

Je tiens à exprimer ma gratitude aux membres du jury pour avoir accepté d'évaluer mes travaux de recherche. Madame Guichard et Monsieur Dufresne, c'est un honneur pour moi de vous avoir eu comme rapporteurs de mon manuscrit. Merci pour l'intérêt que vous avez montré pour mes travaux, merci pour vos remarques constructives et vos conseils qui m'ont permis d'améliorer la qualité scientifique de ce manuscrit. Vous avez su me guider vers une nouvelle perspective pour mieux juger mes travaux. Monsieur Gharsallaoui merci pour vos remarques qui m'ont donné une nouvelle façon d'interpréter mon travail de thèse. Je fais part de toute ma gratitude à M. Christian Chervin d'avoir présidé mon jury avec enthousiasme et bonne humeur, ce qui m'a permis de me sentir dans un environnement bienveillant.

Je remercie chaleureusement l'ensemble des membres du laboratoire (Didier Dangla, Gilles Humbert, Ingrid Pelletier, Vincent, Emmanuelle Dao, Anne Lung, Didier Nicolas, Céline Mathieu, Géraldine Giacinti, Romain Armengau, Karine Tremauville, Elodie Gon…) qui a contribué de près ou de loin à la réalisation de cette thèse dans une ambiance conviviale. William (membre de la Extrusion Team) merci pour ton aide inconditionnelle et ton optimisme permanent, surtout lors des longues campagnes d'extrusion bi-vis. Domi, vraiment merci pour toute ton aide au niveau de l'analytique; tu es le génie de l'analytique et surtout merci d'avoir presque perdu un œil lorsque tu cherchais l'erreur dans mes matrices Excel ! Sophie, le deuxième génie de l'analytique, merci pour ta patience et ta douceur lorsque tu expliques quelque chose. Merci Philippe Evon et Laurent Labonne, l'équipe de Tarbes, pour votre sympathie, votre aide et nos discussions sur l'extrusion, mais aussi merci d'être venu me chercher à la gare.

Merci à mes collègues de bureau qui ont dû supporter mes chants et mes danses et mes mélanges de langues (fragnol ou spanglish). Les anciens, Mr. Brault, Monseigneur Pujol, Leslie, Alla, Alexandre, Thibaud, Carl, Mathieu, merci pour vos conseils et les bons moments passés ensemble. A Pablo pour les excellents repas mexicains et les dégustations de bières. Evelien pour les moments passés dans les salons de thé et bars à travailler et à discuter de pâtisserie, sitcom et gin. Marian, gracias por tu ayuda, tu apoyo, tus consejos, nuestras platicas en la oficina y las fiestas! Jesus, camarada, algún día Maduro madurara y se irá ! Jeff, Mike, Eric et Jeronimo, merci pour votre gentillesse et sympathie. Sabina et Hien merci de nous avoir fait découvrir vos pays à travers la gastronomie et la musique. Benji, merci pour ton soutien, nos discussions sur «la vie », les eaux thermales de Colombie et les raclettes. Chris merci pour les cours de tennis et nos discussions au marché du dimanche.

Je tiens spécialement à remercier Houssein, le chef de «l'Extrusion Team », merci pour tout ce que tu m'as appris et transmis. Merci pour ton soutient, nos discussions, nos taquineries, pour tout, je t'apprécie énormément. Mon Gabonais, merci pour ton sens de l'humour un peu dérisoire et mal compris. La Colombie est prête pour tes «attaques à la latérale». ! Mi negrito bailarin, merci pour tous les bons moments passés ensembles (au labo, Rio Loco, où à el Do), j'espère que tu garderas toujours ton rythme tropical. Cécile, que dire…merci pour les balades en vélos, las rumbas, les tours gastro de Toulouse, les cours de français... merci simplement d'avoir été là! Lupita, mi querida lupita muchas gracias por tu apoyo, tus consejos laborales y extra-laborales y por siempre haber dejado la puerta abierta para mi, pues sabes muy bien como hacerme sonreir de nuevo. Alice, qui aurait pensé qu'après notre stage de fin d'étude on finirait au LCA en train de faire une thèse? Merci pour de m'avoir accompagné tout au long de cette péripétie ! Les cours de chansons françaises au bureau et nos magnifiques prestations, le stress avant le congrès et le shopping après! Merci pour ton amitié.

A ma famille, ma sœur et mes parents, ma famille française (Choco, Françoise, Julien, François, Tata, Matt, Ju, Vincent, Djemba, Vicky, Marie) merci pour votre soutien, votre amour et votre patience. ! Los quiero!

! Gracias a todos por haber hecho esto posible !

A mi familia : por su amor y apoyo incondicional

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ABREVATIONS

DMA: Dynamic Mechanical Analysis

DVS: Dynamic Vapor Sorption

TGA: Thermogravimetrical Analysis

DSC: Differential Scanning Calorimetry

SEC: Size Exclusion Chromatography

GPC-FID: Gas Phase Chromatography – Flame Ionization Detector

MS: Mass Spectroscopy

ASE: Accelerated Solvent Extraction

SEM: Scanning Electron Microscope

TSE: Twin-screw Extrusion

T_g: Glass transition temperature

DE: Dextrose Equivalent

DP: Degree of Polymerization

M_n: Number average molecular weight

M_w: Weight average molecular weight

I_p: Polydispersity Index

OSAN: Octenyl Succinic Anhydride

HPMC: Hydroxypropyl Methylcellulose

SME: Specific Mechanical Energy

RH: Relative Humidity

MCT: Medium Chain Triglycerides

PPI: Pea Protein Isolate

EE: Encapsulation Efficiency

ER: Encapsulation Rate

IR: Incorporation Rate

ROI: Region of Interest

RSD: Relative Standard Deviation

PREFACE

As the world's leading flavors and fragrances company, Givaudan designs and manufactures aromas and perfumes for large varieties of applications from luxury perfumery to home products. So far, the incorporation of perfumes in readily biodegradable and hydrosoluble bio-based materials is a key challenge in industrial applications such as indoor deodorizers or cleaning/washing products.

The "Fractionation and Transformation of Agro resources" research group of the Laboratory of Agro-Industrial Chemistry has been studying for more than 15 years different materials coming from renewable agricultural resources (i.e. maize, corn, plum, pea…) The thermo mechanical transformation of these products and by-products allows to conceive many materials among which some are nowadays industrialized. Knowledge and know-how on the physicochemical properties and the processing of natural polymers have already let to foresee a various range of materials and structures in which some aromatic compositions can be incorporated.

Furthermore, in the same research group, a specific research axis has been dedicated to the development of new natural encapsulating materials and the "Analytical chemistry and the transformation of agro-constituents and their environmental impact" research group of the very same lab possesses analytical devices and expertise to qualify and quantify the incorporation and the release of volatile organic compounds in any kind of matrices.

The compatibility between the partners was then optimal to study the incorporation and the release of model perfumes in a bio-based matrix and thus develop new encapsulating agro-materials. However they had to meet and this encounter was possible thanks to the work of a French designer.

Gilles Belley led in 2009 a brainstorm on a project called "The Vegetal Factory", in collaboration with Stephanie Sagot, head of the art center "La Cuisine" and with the Laboratory of Agro-Industrial Chemistry. In this project, he offered the possibility to integrate natural materials, biodegradable and sensitive to environmental conditions, inside our domestic habitat. He drew, in particular, two objects called “The twig” and “The branch”. These were supposed to liberate perfumes upon humidification inside the household. The whole project was later awarded from the Agora Grant for design and noticed by Givaudan.

*Figure 1. **Twig perfume on its stone support, courtesy of*** **La fabrique Végétale*****. Photography Felipe Ribon & Gilles Belley***

*Figure 2. **Branch perfume and its fragrance diffuser, courtesy of*** **La fabrique Végétale*****. Photography Felipe Ribon & Gilles Belley***

From these product concepts has been build a more tangible research project about the twin-screw extrusion encapsulation of perfumes in a bio-based matrix, with a precise requirement specification: producing a water-soluble and glassy delivery system containing at least 10% (w/w) of perfume. This project was the framework of this PhD thesis.

The present manuscript is structured in four chapters.

The first chapter presents an overview of the evolution of flavor encapsulation by extrusion in the past six decades.

It presents the key issues of melt extrusion when compared to other encapsulation technologies especially in terms of environmental footprint, operating conditions, encapsulating and encapsulated materials used and final properties of the delivery systems.

The second one describes the physicochemical properties of different maltodextrins according to their DE-values, focusing on their melt viscosity and glass transition temperatures. The effect of two plasticizers is also discussed.

The goal of this preliminary study was to define suitable materials and operating conditions before starting the twin-screw experiments.

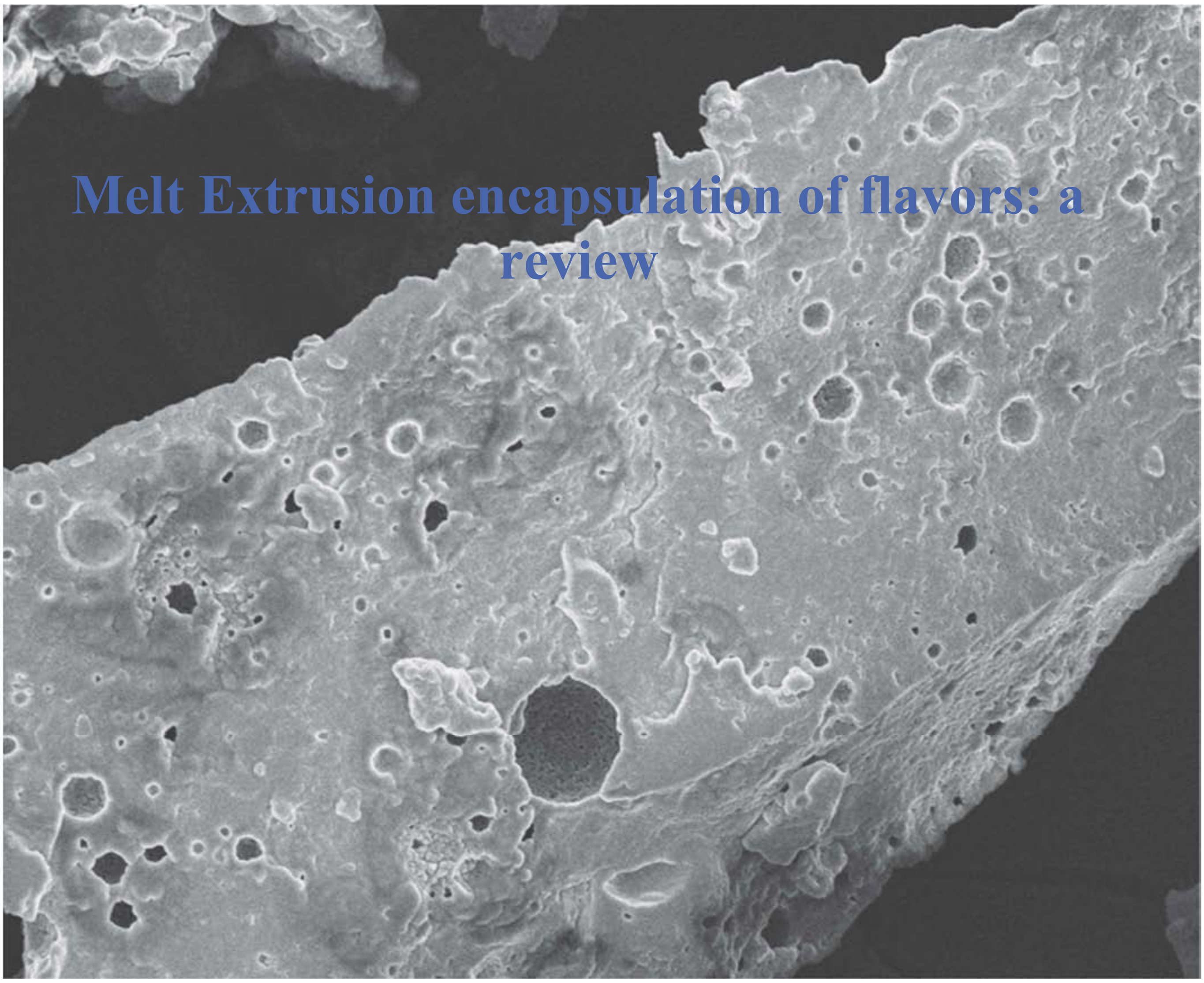
The third chapter is related to the determination of the extrusion encapsulation conditions using a model hydrophobic active compound, MCT-oil. Before using a complex perfume it was necessary to start with a simpler product to set up the extrusion processing. To facilitate the incorporation of the hydrophobic compound in the hydrophilic maltodextrin-based matrix, two kinds of compatibilizing biopolymers are compared: pea protein isolate and a modified starch hydrolyzate (Hi-Cap 100). Investigations of the physicochemical characteristics (morphology and thermal properties) of these new bio-based delivery systems are characterized.

The last chapter is composed of two parts; the first part corresponds to the extrusion encapsulation of a technical perfume and characterization of the final properties of the obtained delivery systems. The second part, deals with the study of the encapsulation efficiency of the process and the kinetic release profile of the perfume under different environments. Additionally, sensory assessments regarding a potential application in the fragrance field are conducted.

Each of these chapters is written in the form of a scientific publication.

Chapter 1

Melt Extrusion encapsulation of flavors: a review



Melt Extrusion encapsulation of flavors: a review

Natalia Castro[a, b], Vanessa Durrieu[a, b], Christine Raynaud[a, b], Antoine Rouilly[a, b], Luc Rigal[a, b] Christian Quellet[c]

[a] *Université de Toulouse, INP-ENSIACET, LCA (Laboratoire de Chimie Agro-industrielle), F-31030 Toulouse, France*

[b] *INRA, UMR-1010-CAI, F-31030 Toulouse, France*

[c] *Givaudan Schweiz AG, Ueberlandstrasse 138, CH-8600 Dubendorf, Switzerland*

Abstract

Encapsulation of flavor and aroma compounds has been largely explored in order to meet appraisal demands from consumers by improving the impact of flavor during the consumption of food products. Even though several techniques have been used for encapsulating volatile compounds, i.e. spray drying, fluidized bed coating, coacervation and melt extrusion, those most frequently used in the food industry are spray drying and melt extrusion. In this article, the different techniques of encapsulation of flavors and fragrances in polymer-based matrices by extrusion are reviewed and partly re-defined, emphasizing the differences between the various techniques reported so far and the role of matrix types, additives and operative conditions. Also, the role of water as a key parameter for controlled release and shelf stability of the delivery system will be discussed.

Keywords: *Microencapsulation, melt extrusion, ram extrusion, melt injection, fragrances, flavors*

Remark: published in Polymer Reviews, Volume 56, issue 1, 2016, doi:10.1080/15583724.2015.1091776

1. INTRODUCTION

Flavors, fragrances and bioactive food compounds (employed in the nutraceutical and the pharmaceutical domains) are often supplied in powder or granulated form for better handling and more accurate dosing in final product. Over the last decades, encapsulation technologies have added new functionalities to these forms, such as protection against evaporation, oxidation, moisture and other aggressive environmental agents to provide extended shelf life, or controlled release under pre-determined conditions [1–6].

The most common encapsulation technologies used in the flavor industry comprise spray drying, spray coating and extrusion [6–12], and earlier variants of extrusion also known as melt injection, have been known since the late 50's. A common feature to all these technologies is the dispersion of the active substance (or encapsulated material) in a matrix that is impervious to both active substance and external deleterious agents.

Extrusion cooking is widely used in the food industry since the seventies. This highly versatile processing technology allows the combination of many unit operations (i.e. mixing, grinding, cooking, extraction…), among which encapsulation has recently been investigated. As encapsulation technique, melt extrusion is promising on economic and environmental points of view, as it is a one pot process (formation of the wall material, dispersion of the active principle, and forming of the encapsulated material), without any use of organic solvent and a reduced energy and water consumption (especially when compared to spray-drying).

This review focuses on the state of the art on melt extrusion encapsulation of flavors and fragrances. This technology was first reported in industrial patents, and is now becoming an academic research topic.

Section 2 covers the basics of flavor encapsulation, emphasizing the influence of matrix materials, compatibilizers and morphology on encapsulation efficiency and release behavior.

Section 3 focuses on the matrix itself, emphasizing the influence of humidity on its encapsulating properties. Usually, this matrix is built up of carbohydrates and is in the glassy state, although other matrices have also been used. The way this matrix is formulated and processed and how the core material is incorporated into this matrix during extrusion are key parameters that are discussed in detail.

Finally, Section 4 deals with extrusion itself, emphasizing the importance of process parameters on the overall quality of the product.

2. MICROENCAPSULATION OF FRAGRANCES AND ODOR ACTIVE COMPOUNDS IN SOLID FORMS

Flavors and fragrances are highly complex chemical compositions of sensitive volatile organic compounds with different physicochemical properties (i.e. volatility, water solubility) and an average molecular weight from about 50 to 300 Da. The capture, the retention and the rendition of such complex compositions in their integrity, combining with low losses during encapsulation, are the key objectives of encapsulation [5,6,13–15]. In the last two decades, the volume of encapsulated oils has grown significantly. In 2001 it was estimated that 20% to 25% of all flavors commercialized in the world, were in an encapsulated form, and between 10% and 20% of these could not be encapsulated by spray-drying [16]. In order to satisfy this important demand, melt extrusion appeared as a suitable and flexible technology to produce such encapsulates in large volumes. Furthermore, extrusion had a number of advantages over spray drying, such as lower energy consumption during operation and minimal emission of odor-contaminated exhaust air. Finally, the conditions of extrusion allow a better control of the state of the matrix, especially if carbohydrates glasses are considered [17]. Benczcedi et al., [18] have demonstrated that lemon, lime and tangerine flavors had better stability (no oxidation was observed) and longer shelf life when encapsulated by melt extrusion compared to spray-drying (4 years at 20°C compared to 2 years at 20°C). The drawbacks of extrusion are the limited loading, usually not exceeding 15% to 20% and the coalescence of the droplets of active compounds [19,20].

The two first studies of flavor encapsulation by "extrusion" used a carbohydrate–matrix comprising sucrose and corn syrup to entrap the essential oil [21,22]. Following these pioneering works, the development and research of new materials and procedures for the encapsulation of flavors increased significantly in the food industry. Encapsulation of aroma compounds in a carbohydrate polymer in a glassy state, also known as "glass encapsulation"[16] became very popular, and numerous patents were submitted by companies like Griffith Laboratories, Sunkist Growers, Nabisco Brands and McCormick & Company [23–27]. All processes described herein involve the entrapment of the flavor in a carbohydrate matrix (starch, modified starch, corn syrup, sucrose, gums, maltodextrins…). Patents disclosing the use of biopolymers-based matrix by melt-extrusion instead of low molecular weight sugars, dextrins and maltodextrins were mainly published in the 1990's (Fig.1) [28–30], by industrial companies. Details about the encapsulation process and the key process parameters are usually scarce in such documents.

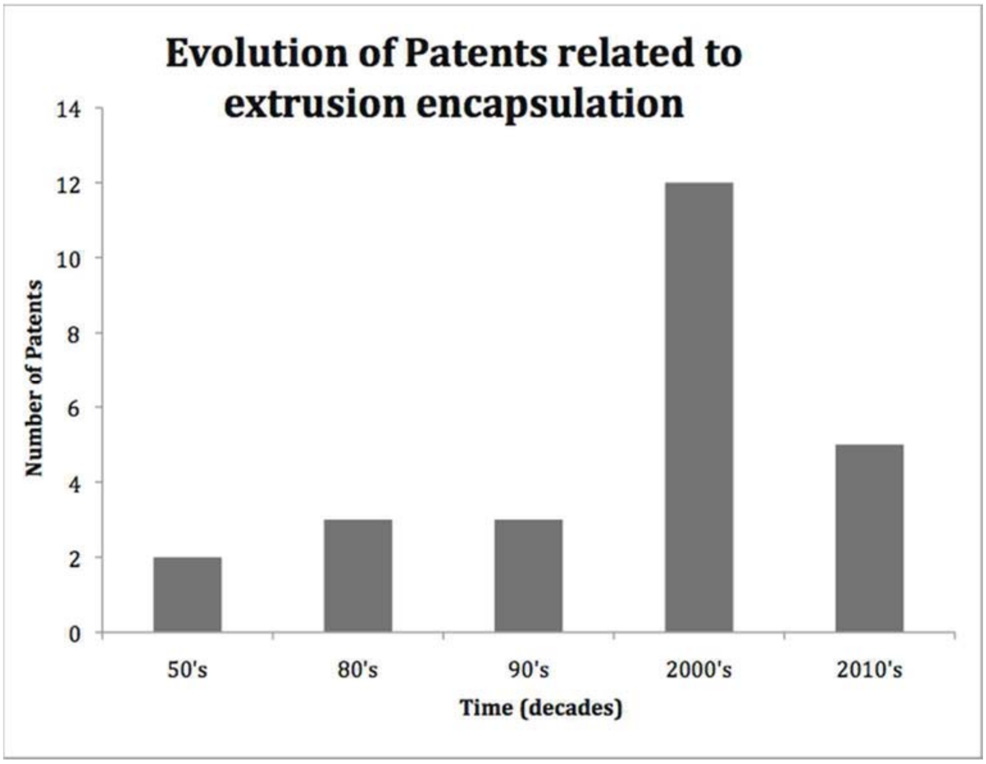


Figure 1. Trends in extrusion encapsulation technologies in the last decades.

All of these methods are, however, based on the same process steps:

(i) Incorporating a volatile compound (flavor, fragrance or other sensitive molecules) in a thermoplastic matrix and

(ii) Forcing this mass through an orifice or die to shape the encapsulated material.

The release mechanism involved in these technologies is essentially dissolution in water, which may be immediate or delayed in time. Temperature may also be used as a trigger, but more occasionally. Alternative mechanisms, such as diffusion or mechanical breakage of core-shell capsules [6] are not discussed in this review.

The efficiency and quality of the overall encapsulation process is the result of the combination of system morphology, i.e. the way the encapsulated material is dispersed in the matrix, and the physical state of this matrix.

Classically, the system is viewed as a dispersion of the oil phase in the form of small inclusions in the matrix. Different morphologies have been postulated for different release profiles (Fig. 2):

(i) Coarse dispersion in the matrix [23,30–33],
(ii) Fine dispersion using emulsifying and/or compatibilizing agents [34,35],
(iii) Film coating of the core material [36–43],
(iv) Fine dispersion and external film coating,
(v) Fine dispersion and coating of the core and the matrix [32].

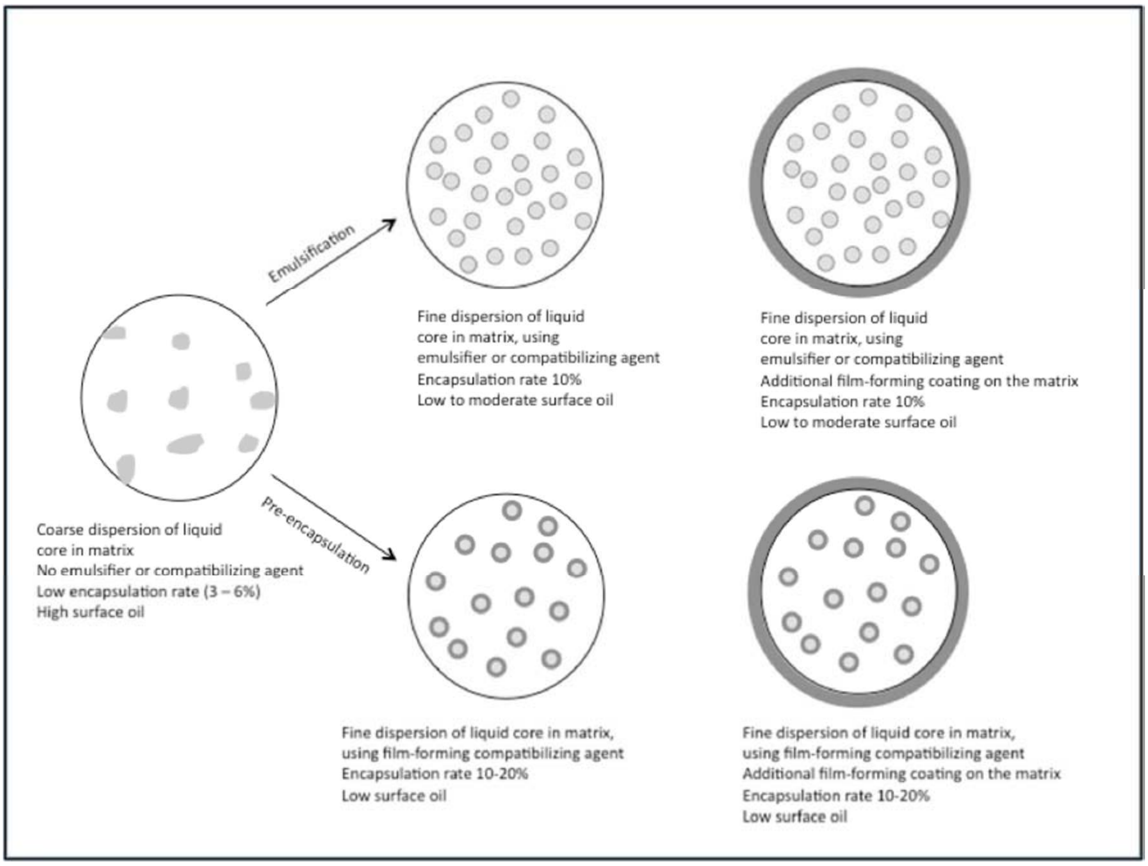


Figure 2. Schematic view of possible encapsulation morphologies obtained using extrusion microencapsulation.

The most appropriate matrix physical state for encapsulation is the glassy state, where both free volume and molecular mobility are minimized [30,44–47]. Hence, the glass transition temperature of the matrix is a key parameter for encapsulation in solid matrices. This will be discussed more thoroughly in the next sections. The polarity of the matrix is another key factor that controls both encapsulation and retention of the volatiles. Hence, for hydrophobic ingredients, such as flavor and especially fragrances, the highest retention is obtained in hydrophilic matrices, which can be easily explained in terms of solubility. Besides, hydrophilic matrices have low permeability with respect to oxygen (Section 3.2). Hence, carbohydrates in the glassy state show better retention of volatile compounds and extended shelf life stability [13,16,46,48–50]. The encapsulation in glassy carbohydrates is also referred to glassy microencapsulation.

However, the situation is rendered more complex by the fact that individual molecules may interact with the matrix itself, which in turn affects the properties of this matrix and the release profile of the flavor. The chemical diversity of encapsulated ingredients is therefore another key feature, which must retain attention. The most recurrent flavors used in the food industry and reported in the literature are diacetyl, terpenes, such as d-limonene, terpene alcohols, such as geraniol, menthol and thymol, terpene ketones, such as camphor and menthone, short chain esters, such as ethyl propionate and isoamyl butyrate, aldehydes, such as acetaldehyde and hexanal, lactones, such as heptalactone and nonalactone, sulfur-containing ingredients, such as 3-methylthiohexanol, thiolactones and the like, and nitrogene- and nitrogene-sulfur heterocycles, such as pyrazines and thiazoles. Flavor (and perfume) ingredients are therefore characterized by a broad range of physico-chemical properties, whereas the most relevant of these properties as far as encapsulation is concerned are the presence of chemical functions, molecular weight and steric hindrance, vapor pressure and relative solubility in both oil and matrix phase. All of these parameters control the interactions between the ingredients and the matrix, their diffusion through this matrix, the encapsulation yield, the storage stability of the dry product and the release profile [7,10].

The interactions of flavor ingredients and the matrix and their effects on flavor encapsulation and release have been extensively investigated in the literature. In particular, the formation of flavor complex with starch has attracted much interest [51–54]. Most of these studies have, however, been performed in solution, i.e. under conditions where starch is fully plasticized and amylose has a sufficient conformational flexibility to accommodate guest molecules and form the inclusion complexes. It has been proven by DCS and X-ray diffraction measurements, that complexation proceeds through amylose helix formation to form reversible inclusion complexes. Such conditions are not met in extrusion, due to the low water content of the extrudate, unless higher processing temperatures are applied. The formation of flavor-cyclodextrin complex is also well documented [7,38,42,55]. In all cases, the inclusion complexation constant has been found to depend strongly on the molecular shape and polarity of the guest molecule. The encapsulation and release of flavors in and from low molecular weight carbohydrates and carbohydrate oligomers, such as corn syrup solids and maltodextrins, has been extensively reviewed in the case of spray drying Goubet et al. [7] as well as in the case extrusion-mediated glass encapsulation [17,48,56]. It appears from the above studies, that the entrapment of flavor ingredients at the molecular level in the matrix can occur, providing suitable interactions that can lead to flavor-carbohydrate complex with reduced diffusion. Such interactions have been investigated by inverse gas chromatography, providing a better understanding on how retention and release work in these systems. Owing to the complexity and diversity of these oils, the study and quantification of retention or release of volatile compounds remain difficult, and few standardized methods are known. In some studies, polymer-flavor complexes in solution have been analyzed in order to determine the type of interactions involved and how flavors are released [7,57–59]. Other studies focused their attention on determining the type of interactions existing between the solid matrix and two or three specific flavor compounds, thanks to inverse gas chromatography [60–62]. Hence, contradictory results are frequent. For example, Gunning et al. [63] observed that the percentage of flavor release from a low water content sucrose/maltodextrin matrix into the headspace increased when temperature raised above 60°C, while the contrary was found in other maltodextrin matrix [64]. In the latter case, thermally enhanced retention was attributed to a change in the polarity of the matrix with increasing temperature [64]. As documented later in this review, such discrepancies are certainly linked to different level of moisture in the matrix.

Direct entrapment or solubilization of the flavor ingredients in the matrix has been considered as a convenient way to encapsulate volatile substances. The quality of such encapsulation process depends, however, on the flavor-matrix interactions mentioned above. For example, lactones are better retained in starch-based matrices, while alcohols are better encapsulated by carbohydrates, whereas flavors ingredients having similar chemical functions but different molecular weights or topologies may show different entrapment behavior [7]. This selectivity may lead to strong distortion of the flavor release profile. Furthermore, the flavor loading that can be reached by this method is limited to 3 to 6 (%w/w) of the extrudate. Above this limit, a flavor exudation occurs during the extrusion process, leading to the formation of separated liquid phase, which may flow out of the extrudate or disperse in the matrix in the form of irregularly shaped liquid inclusion (Fig 2, case (i)). Such a coarse dispersion leads to the formation of large amounts of surface oil, which is deleterious to the quality of the product. The presence of surface oil is indeed highly undesirable, because

(i) Surface oil is not encapsulated and therefore its release is not controlled and

(ii) Such oil is readily oxidized, which leads to loss of product organoleptic quality, and contributes to powder caking. High quality encapsulates must have low surface oil levels.

An intensive development work has been done in the last decades to increase the flavor load in encapsulates, while keeping the surface oil at the lowest possible level and extending the storage stability of the product. This is usually achieved by providing a fine dispersion of the encapsulated oil in the matrix by using suitable combinations of mixing powder and solubilizing or compatibilizing agents [18] (Section 3). The resulting product morphology after drying is that of a “dry emulsion” (Fig 2, case (ii)).

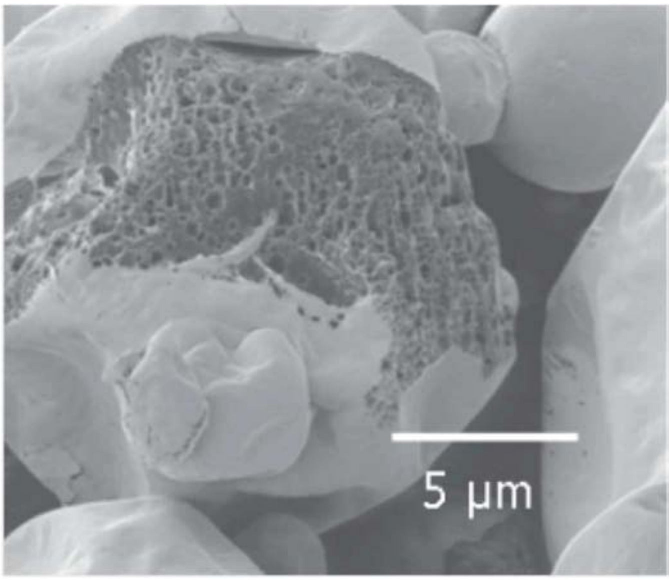


Figure 3. Spray dried particles with “dry emulsion” morphology (courtesy of Givaudan Schweiz AG).

Figure 3 shows such morphology in the case of a spray-dried powder with oil inclusions having a diameter of less than one micron. The matrix was obtained by spray drying a high internal phase fragrance emulsion comprising maltodextrins and modified starch. This method allows increasing the payload more than 40% [8]. Another way to reduce surface oil of the extrudate involves preparing an emulsion of the active compound, comprising a film-forming agent, and injecting this emulsion directly into the extruder (Fig 2, case (iii)). This pretreatment effectively decreases flavor losses during the process and allows co-encapsulation of different flavors in the same encapsulate [40].

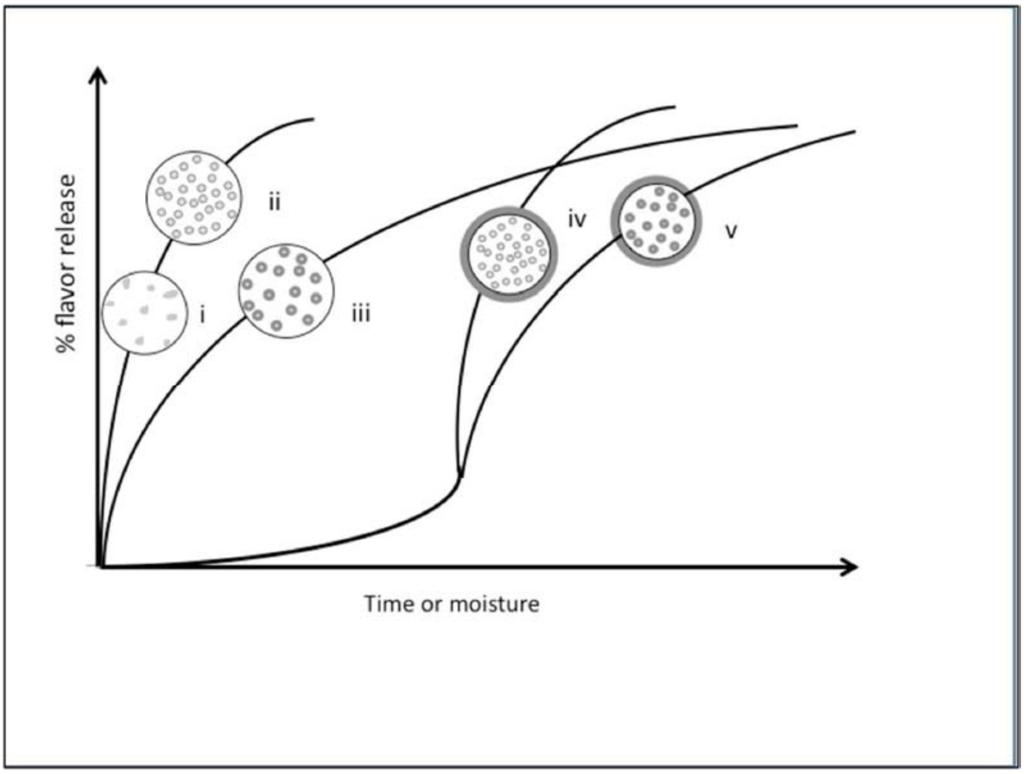


Figure 4. Scheme of release profile depending on the morphology of the delivery system obtained by extrusion.

The release of the encapsulated actives from systems based on carbohydrates is triggered by exposure to moisture. Critical water-induced plasticization of the matrix, which is marked by a decrease of the carbohydrate glass transition to sub-ambient temperatures, starts at 50% to 70% relative humidity [56]. Ultimately, the matrix dissolves and the full flavor is released. Figure 4 schematizes the expected influence of the product morphology on the release rate of the active compound over time if the encapsulate is exposed to moisture. *Note that, in the present case, increasing time (at constant moisture) is equivalent to increasing the moisture content or the water activity in the system.* This is, however, an idealized view, which is shown here for guiding the reader through this review. The fast and early release corresponds to encapsulation of the active compound without any pre-encapsulation or coating treatment prior to extrusion (curves (i) & (ii)). Release of these kinds of structure is more known as a burst-like release, all the compounds are liberated at the same time. Incorporating a film-forming agent at the oil/matrix interface increases the resistance to moisture and may result in a more gradual flavor release (curve (iii)). Finally, combining above morphologies with an external coating leads to similar release profiles, but delayed in time (or occurring at a higher moisture level)(curves (iv) & (v)). For example, Menzi et al. [65] have proposed to apply a vegetable fat coating on granulated materials obtained by spray coating of a flavor/water emulsion on a sugar carrier material, which was shown to improve the storage stability of encapsulated flavors and to delay their release in chewing gums. Alternatively, Leusner et al. [66], Bouquerand [36], and Chang et al. [35] have employed Miglyol as an additive to their formulation, in order to provide extra protection to their active principle, as well as to reduce the release rate. Others techniques and other wall materials have also been explored in order to lower the production cost and to target other application areas [28,67,68].

It must be stressed, however, that the loss of volatiles from encapsulates during handling and storage starts at water levels much below than the plasticization limit. This leakage involves at least three mechanisms:

(i) Evaporation of the surface,
(ii) Diffusion of the subsurface flavor oil droplets to the surface of the matrix, and
(iii) Exudation of the flavor compounds through the matrix fractures or cracking [63].

Controlling the hygroscopicity and the physical integrity (absence of capillary cracks and other defects) of the matrix is therefore a crucial aspect of flavor and fragrance encapsulation. For example, high molecular weight carbohydrates offer longer shelf live and high stability to active compounds compared to short molecular weight sugars and maltodextrins [14,69]. On the other hand, starch, modified starches, proteins or gums give delivery systems with thermoplastic behaviors, which gradually swell in presence of moist environments and slow down the release of the active principle [16].

If the aim of the final product is to enhance the release rate, low molecular weight polymers such as maltodextrins having a high dextrose equivalent (DE) must be used. For example, Swisher [22] and Schultz and Calif [21] have used in their formulations low molecular weight carbohydrates (e.g. sucrose, corn syrup). The same effect can be obtained by using plasticizer, such as glycerin and other polyols (Section 3.4.4), which are less volatile than water, and therefore are better retained in the matrix.

The next section provides an overview of the carriers and additives used in extrusion encapsulation.

3. MATRIX MATERIALS

3.1. Introduction

Matrix materials used in extrusion encapsulation must combine good processability and good barrier properties. This is achieved by using certain biopolymers or mixtures of biopolymers and low molecular weight molecules, such as sugars and sugar alcohols (Table 1). The most frequently used matrix materials, or *carriers*, are carbohydrates. The choice of encapsulating materials is based on five criteria: natural origin, barrier properties with respect to gas and small volatile molecules, large scale and low cost availability [12,70,71]. The advantage of using such biomaterials for microencapsulation is on one hand the simplicity of the release mechanism, mainly triggered by moisture or heat, and on the other hand their biodegradability. Flavor encapsulation additionally requires food industry authorized materials. However, the major drawback of these natural carriers, when used without additives, is the low flavor load of about 5 to 6% (w/w) [72]. The use of lipids and proteins has also been reported (Table 1).

Many researchers have focused their attention on two aspects of biomaterial-mediated encapsulation:

(i) the physicochemical properties of the matrix, such as molecular weight, viscosity, solubility, film forming properties, degree of polymerization and chemical functional groups, which can significantly affect the retention and release of aroma compounds [7] and

(ii) (ii) the physical state of the carrier, which, as mentioned above, is a key parameter to successful encapsulation.

Carbohydrate oligomers, starch and proteins, like most non cross-linked, thermoplastic polymers, can be found in two physical states: a viscoelastic or “plastic”-state, where the polymers are characterized by a high chain mobility, and where the active materials can be dispersed in the matrix; and a glassy, brittle state, where active materials have a very low mobility and are therefore entrapped in the carrier (or matrix) [7,12,29,69].

A major prerequisite for stable encapsulation of volatile materials is that the matrix is below its glass transition temperature T_g. Indeed, below T_g, diffusion processes slow down dramatically, due to the abrupt decrease of the mobility of the polymer chains and the concomitant decrease of the matrix free volume in the matrix [56,63]. However it has been demonstrated that diffusion is more important in the vicinity of the polymer matrix glass transition temperature, than above this temperature, because the free volume below glass transition is higher, so diffusion of solutes is enhanced. A key feature of most biomaterials and especially of carbohydrates is the fact that the level of water included in the matrix controls T_g. The relationship between water activity and T_g in carbohydrate has been extensively discussed by Slade and Levine [73] and is still the most important factor influencing processability and volatile retention[14]. Water molecules insert between the polymeric chains, opening the three dimensional structure of the polymer and breaking interactions between chains. Low energy interactions between water molecules and polymeric chains are thus established, and so the polymeric structure becomes soft and flexible, i.e. the polymer goes from a brittle, glassy state to a plastic, rubbery state (Section 3.4.4). Product stability is governed by the amount of water, both added and already existing inside the system; water is the key factor controlling the stability of biopolymers [74–77]. Kollengode and Hanna [38,55] have demonstrated that a delivery system with low moisture content (9%) showed higher retention of volatiles like cinnamic aldehyde, eugenol, nonanoic acid and 3-octanone, than delivery system with high moisture content (17%). Gunning et al. [63] have demonstrated in their studies that retention of volatiles is correlated to the glass transition temperature of the system. For instance, in a low water content matrix composed of maltodextrin and sucrose, the highest amounts of volatiles were released when the matrix was above its glass transition temperature. However, water may be a handicap because all the volatile compounds are flashed off when water evaporates during processing. High temperature and pressure inside the extruder barrel make the steam rapidly blow off out to the surface of the matrix dragging the volatiles with it [78]. This is one of the major causes of flavor and fragrance loss during processing. For all of these reasons, controlling the exact formulation prior to extrusion process can be crucial for the final product[16,69].

Table 1 Review of all the different wall materials and additives used in extrusion microencapsulation (melt injection and melt extrusion) in chronological order

Reference	Wall material
(Swisher, 1957)	Corn syrup
(Schultz and Calif, 1958)	Corn syrup, sucrose, dextrose, maltose, mannose, galactose
(Sair and Sair, 1980)	Casein, sodium hydroxide
(Miller and Mutka, 1987)	Carbohydrates, i.e starch, modified starch, sucrose, maltose, corn syrup, fructose, dextrose, glycerol, maltodextrins (DE2-DE20)
(Saleeb and Pickup, 1989)	Maltose monohydrate, maltodextrins, mannose,
(Carr et al., 1991)	Native corn starch
(Kollengode and Hanna, 1997a)	Corn starch+ β-cyclodextrins
(Kollengode and Hanna, 1997b)	Corn starch
(Black et al., 1998)	Whey protein, lipids, modified starch, maltodextrins, dextrose, sucrose, lactose,
(Hau et al., 1998)	Wheat starch
(Porzio and Popplewell, 1999)	Maltodextrins (DE5-DE15), corn syrup (DE24-DE42), starch, modified starch, gum, gelatine
(Saleeb and Arora, 1999)	Maltose, glucose, maltotriose, mannose, sucrose, dextrose, xylitol, arabinol, sorbitol, mannitol, corn syrup,
(Reifsteck and Jeon, 2000)	Corn syrup, flour, starch
(Ubbink et al., 2001)	Potato, corn starch, modified starch, proteins, glycerol
(Benczedi and Bouquerand, 2001)	Sucrose, glucose, lactose, maltose, fructose, ribose, dextrose, sorbitol, mannitol, xylitol, lactitol, pentatol, arabinose, pentose, xylose, galactose, maize syrup, maltodextrins (DE8-

	DE10), gums
(Porzio and Popplewell, 2001)	Maltodextrins (DE10-DE15), corn syrup (DE24-DE42), gums, starch, modified starch, methoxypectin, ribose, glucose, fructose, galactose, xylose, sucrose, maltose, proteins (casein)
(Porzio and Zasypkin, 2010)	Modified starches, maltodextrins (DE10-DE20), sucrose, maltose, glucose, xylose, fructose, trehalose corn syrup (DE24-DE42), fatty acids, gums, proteins (casein)
(Bhandari et al., 2001)	Soy flour, corn flour, corn starch, β-cyclodextrins
(Benczedi and Bouquerand, 2001)	Sucrose, maltose, fructose, mannitol, glucose, ribose, dextrose, arabinose, sorbitol, xylose, galactose, starch, maltodextrins, gums, modified starch, proteins
(Lengerich, 2002)	Starch, modified corn starches, cyclodextrins, cellulose, polyvinyl alcohol, dextrins, corn syrup, gelatin, sorbitol, casein, carrageenan, alginates, pectins, xanthan, gum arabic, guar gum, fat, chitosan
(Benczedi and Bouquerand, 2003)	Sucrose, maltose, fructose, mannitol, glucose, ribose, dextrose, arabinose, sorbitol, xylose, galactose, starch, maltodextrins, gums, modified starch, protein
(Leusner et al., 2002)	Oligosaccharides (oligofructose), inulin, fructose, sucrose, dextrose, maltose, lactose, medium chain triglycerides
(Kohlus and Pacha, 2004)	Sucrose, fructose, maltose, ribose, mannitol, maltodextrins, Xylitol, polybutyl-methacrylate
(Yuliani et al., 2004)	Sucrose, maltose, glucose syrup, glycerine, glucose, β cyclodextrin
(Yuliani et al., 2006)	Corn starch, β cyclodextrin
(Gouin, 2004)	Maltodextrins, starches, fat

(Porzio and Zasypkin, 2010; Zasypkin and Porzio, 2004; Zasypkin, 2011; Zasypkin et al., 2013)	Modified starch, lactose, dextrose, maltodextrins
(Bohn et al., 2005)	Sucrose, maltodextrins
(Valentinotti et al., 2006)	Sucrose, maltose, glucose, lactose, levulose, ribose, dextrose, isomalt, sorbitol, mannitol, xylitol, lactitol, pentatol, arabinose, maltodextrins, gums, hydrogenated starch, cyclodextrins, cellulose
(Bouquerand, 2007)	Maltodextrins (DE8-DE10), lactose, dextrins, pre-gelatinized starch, medium chain triglycerides
(Chang et al., 2010)	Maltodextrins (DE8-DE10), medium chain triglycerides
(Lengerich et al., 2010)	Caseinates, wheat proteins isolates, pre-gelatinized starch, low molecular weight carbohydrates, durum flour
(Benczedi et al., 2011)	Mono and di-saccharides, citric acid, hydrogenated corn syrup, polysaccharides, gums, maltodextrins, modified starch
(Gregson and Sillick, 2012a)	Erythritol mannitol, sorbitol, maltodextrins (DE10-DE20), gum acacia, alginates, pectins, proteins, hydrogenated starch hydrolysates
(Gregson and Sillick, 2012b)	Maltodextrins (DE10-DE20), modified starch, sucrose, maltose, trehalose, soy lecithin, antioxidants
(M. A. Emin and H. P. Schuchmann, 2013)	Native maize starch
(T. M. Goss Milani et al., 2014)	Soy protein isolate

(Chang et al., 2014)	Modified starch/ matodextrin/lecithin
(Tackenberg et al., 2015)	Maltodextrins (DE-12 and DE-17)/ sucrose

3.2. Carbohydrates and polysaccharides

Carbohydrates were the first polymers used for flavor encapsulation, and are still being used because of their good physicochemical properties (low viscosity, good solubility in water and excellent barrier properties with respect to volatile organic compounds, at least under dry conditions) [7,79]. Presently, starches, modified starches and sugars, either in a glassy or crystalline state, are considered to be the best hydrophilic matrices for entrapment and protection of volatiles. This can be explained by the low solubility of oxygen and volatiles in the matrix and, by the low free volume available for molecular transport [13]. The advantages of amorphous carbohydrate matrices in a glassy state are illustrated in the review by Ubbink and Krüger [69]. Amorphous food powders present great barrier properties against flavor losses and oxidation and are therefore often used for encapsulation and stabilization of complex flavor mixtures.

However, as already mentioned above, the quality of the protection against oxidation and leakage depends strongly on the glass transition temperature, which in turn depends on the water activity in the carbohydrate matrix, and on the surface to volume ratio of the extruded materials, since changing the granule morphology can impact the rate of water uptake and volatile losses [69].

3.2.1. Maltodextrins

Maltodextrins are obtained by acid or enzymatic hydrolysis of starch and, depending on how they are produced, may differ in their dextrose equivalent (DE) (relative to the degree of hydrolysis, a higher DE means greater hydrolysis), which ranges from 0 (corresponding to long-chain glucose polymers) to 100 (corresponding to pure glucose). The DE is inversely proportional to the average molecular weight of the polysaccharide, and the maltodextrins normally found in microencapsulation have a DE varying from 3 to 20 [80]. Maltodextrins are the reference wall material in extrusion entrapment of food ingredients due to their film-forming properties, high water solubility, low oxygen solubility, binding characteristics, good protection against oxidation and low cost [12].

The influence of the molecular weight and DE of maltodextrins on the behavior of the carriers has been extensively discussed in the so-called “food polymer science” approach, [14,71,73]. In fact, molecular weight is the one parameter that is directly linked to physicochemical properties (viscosity, glass transition temperature, solubility…) even though DE can also be correlated to some physicochemical properties, e.g solubility of the polysaccharide increases when DE increases. Some authors have reported that the retention of flavors decreases with increasing DE [3,7,67,81] and this has been attributed to the fact that, when DE increases, the maltodextrins become more hygroscopic and their solubility in water increases, which does not favor the retention of volatiles. Conversely, when DE decreases, hygroscopicity also decreases, while the molecular weight, the apparent viscosity, the cohesiveness, the glass transition temperature and the film-forming properties increase, with all of these properties favoring of a good encapsulation.

However, maltodextrins have low emulsifying properties and for this reason some emulsifiers are needed in order to improve the incorporation of the active material, as well as to lower the viscosity and to enhance the flow of the melt inside the extruder. Moreover, by lowering the surface tension of the extrudate, the emulsifiers help to give products with a less sticky and less porous surface [36] which is beneficial to a better encapsulation of volatiles.

Examples of different formulations, as well as some encapsulation rates and efficiency are given in Table 2. In general, compositions of the delivery system are almost the same, the moisture content of the extrudate is similar for all studies covered, no matter whether the active compounds are flavors, fragrances or bioactive food compounds.

Table 2 Composition of delivery systems using only maltodextrins as the main ingredient (nd=not determined)

Reference	Initial formulation composition (% w/w)	Active core (%w/w)	Extrudate moisture content (%w/w)	Encapsulation efficiency (% w/w)	Encapsulation Rate (% w/w)	Technology
(Porzio and Popplewell, 1999)	Maltodextrin DE-10 /water (85.6/5.3)	Diacetyl (9.1)	8.3	nd	4.9	Melt Extrusion
(Porzio and Popplewell, 2001)	Maltodextrin DE-10 /water(81.4/10)	Diacetyl (8.6)	7.6	nd	4.4	Melt Extrusion
(Benczedi and Bouquerand, 2001)	Maltodextrin DE-19/water/lecithin (90/6/1)	Strawberry flavor (3)	nd	nd	nd	Melt Extrusion
(Benczedi and Bouquerand, 2003)	Maldotextrin DE-19/water/silicon dioxide/lecithin(87/7 /2/1)	Fragrance (3)	nd	nd	nd	Melt Extrusion
(Bouquerand, 2007)	Maltodextrin DE-10/miglyol/lecithin (77.6/1/0.5)	Ascorbic acid (20.8)	nd	nd	18.9	Melt Extrusion

Reference	**Initial formulation composition (% w/w)**	**Active core (%w/w)**	**Extrudate moisture content (%w/w)**	**Encapsulation efficiency (% w/w)**	**Encapsulation Rate (% w/w)**	**Technology**
(Chang et al., 2010)	Maltodextrin/lecithin /miglyol (75.6/1/0.5) ; (88.4/1/0.4)	Ascorbic acid (18.9) ;(16.1)	(9.2) ;(7.9)	(97.2) ;(97.9)	(18.6) ;(15.3)	Melt Extrusion
(Benczedi et al., 2011)	Maltodextrin DE-19 ;DE-12 ;DE-6 (83)	Orange oil (nd)	nd	nd	(8.3) ; (8.1) ; (7.9)	Melt Extrusion

3.2.2. Starch

Starch is a polysaccharide, consisting of D-glucose chains. It is a mixture of two homopolymers, amylose, which is a linear polymer (10-20%) and amylopectine, which is a crosslinked polymer (80-90%). Amylose and amylopectin are interconnected by 1,4-α and 1,6-α glycosidic bonds, which are part of the ramifications in the molecule's structure. For this reason, the supra-molecular structure of starch is in a semi-crystalline form. Amylopectin is organized in the form of sheets giving the crystalline portion and amylose is in an amorphous form. Under normal conditions of temperature and pressure, starch is insoluble in aqueous solvents [12,71,82].

Several studies have been conducted to better understand the thermal transitions, and the changes of physical state of starch. The theory mentioned by Donovan [83] allowed a better understanding of what is happening during the changes in the physical state of this material, and particularly for determining in which states the polymeric matrix is when water and temperature are in excess [84,85]. Swelling of the amorphous regions is observed when water is in excess (the hydrogen bonds between the polysaccharide chains are cut, and the starch granules absorb water and swell). This phenomenon is associated with the initiation of the gelatinization temperature (60°C-85C°, depending on the type of starch). The crystalline regions are degraded (dissociation and opening of the amylopectin "propellers") and starch is converted into a gel.

When starch is in a gelatinized state, the phenomenon of retrogradation (reorganization of its crystalline structure) is observed. In this case, the gel is more rigid and tends to expel water included between the polysaccharides chains (a phenomenon known as syneresis). Starch rearranges itself into a more crystalline and stiff structure. The glass transition of the sample depends on the rate of hydration. Actually pre-gelatinized starch is used for the entrapment of volatiles, due to enhanced diffusion of the latter, and in fact, pre-gelatinized starch is soluble in cold water, which facilitates processing conditions for encapsulation.

Since starch is a more complex molecule than maltodextrins (greater physicochemical properties) more interactions can be established with active compounds. Starch has often been used in extrusion encapsulation (Table 3) due to the stable inclusion complexes of starch forms with flavor [51]. Indeed these inclusion complexes proved to be stable at high temperatures and showed great stability when stored for longer periods of time. Forming these complexes requires however conformational changes of the amylose moieties, which require in turn high processing temperatures to counterbalance the relatively low level of water in the extrudate.

Regarding the physicochemical properties of starch, it has been demonstrated that amylose content can affect expansion and in fact, it increases with the amylose content (this is without taking into account temperature and moisture content). It has been found that the expansion ratio increases from 8.3 to 16.4 as the amylose content of native starch increases from 0 to 50%. Above 50% of amylose content, the expansion ratio decreases [86]. As expansion is related to volatile losses, it is assumed that for better retention, starch with low amylose content should be chosen. In addition, Hau et al. [31] have shown that for starch with an amylose/amylopectin ratio of 27/73, water content influences the binding of volatiles. In fact, volatile uptake increases as the water content of the delivery system increases from 19% to 43%, and this could be due to the decrease of viscosity of the melt allowing the volatile compound to be better dispersed inside the carrier. However, when the moisture content increases, the expansion ratio decreases and this tendency is the same for starches with different amylose contents (amylose content varied from 0 to 70%). The maximal expansion ratio of various starches was obtained with a moisture content of 14% [86].

The extrusion of starch involves its gelatinization, at least partially, with water or a water/plasticizer mixture before or during the initial steps of the extrusion, and the water content ranges typically from 10 to 45%. In some cases part of the water is added together with the encapsulated oil in the form of an emulsion [40].

Table 3 Composition of the delivery systems using only starch as the main ingredient (nd=not determined)

Reference	Initial formulation composition (% w/w)	Active core (%w/w)	Extrudate moisture content (%w/w)	Encapsulation efficiency (% w/w)	Encapsulation Rate (% w/w)	Technology
(Carr et al., 1991)	Corn starch/water (80-95/10)	Atrazine (5-20)	8-25	73 - 96	nd	Melt Extrusion
(Kollengode and Hanna, 1997b)	Corn starch/water (95/nd)	Cinnamaldehyde (5)	nd	24.1	nd	Melt Extrusion (direct injection)
		Eugenol (5)		20.5		
		nonanoic acid (5)		15.1		
		3-octanone (5)		25.8		
(Hau et al., 1998)	Wheat starch/water (67.3-84.1/15.90-32.7)	Diacetyl, 3-methylbutanal, heptane (nd)	19-43	nd	nd	Melt Extrusion
(Ubbink et al., 2001)	Potato starch/capsule/glycerol/water	Orange oil (5.8)	nd	nd	nd	Melt Extrusion

(64.1/2.4/2.7/25)

Reference	**Initial formulation composition (% w/w)**	**Active core (%w/w)**	**Extrudate moisture content (%w/w)**	**Encapsulation efficiency (% w/w)**	**Encapsulation Rate (% w/w)**	**Technology**
(Lengerich, 2002)	Semolina/wheat gluten/wheat starch/vegetable oil /water (25.1/18/25.1/7.9/1.3)	Various materials (22.3)	nd	nd	nd	Melt Extrusion
(M. A. Emin and H.P. Schuchmann, 2013)	Native Maize starch (nd)	Medium chain triglyceride (4)	18	nd	nd	Melt extrusion

3.2.3. Modified starch

In the context of encapsulation, the term “modified starch” actually includes dextrins, on which octenyl succinate groups have been grafted by esterification of the hydroxyl groups with mono octenyl succinic acid. These products are obtained by acidic or enzymatic degradation of starch and subsequent chemical treatment with the succinic derivate. The modifications are made in order to improve the chemical and physical properties of the dextrin to meet specific needs. The advantage of the so-called starch octenylsuccinate, also known as OSAN (octenyl succinic anhydride), lies in its remarkable emulsifying properties, which are related to the presence of the hydrophobic octenyl moieties that allow better interactions with aroma compounds [12,82]. The roles of these materials on the retention of volatiles in carbohydrate matrices have been discussed in the study made by Zasypkin and Porzio [30]. Clear benefits were found in terms of oil droplet dispersion, viscoelastic properties and surface oil.

It should be noted that there are other ways to modified starches, for example by oxidation in the presence of sodium hypochloride in order to decrease its viscosity. Or, on the other hand, to improve its viscosity, starch can be modified with propylene oxide. In general, starch properties can be modified according to the final application (i.e. thickening agent, emulsifier, texturizing agent).

3.2.4. Carbohydrate mixtures

Mixtures of oligosaccharides are often used either in spray-drying or extrusion encapsulation of flavors because they offer wall material with better barrier properties [14,15,50]. As mentioned above, the physicochemical properties of carbohydrates are a key parameter that needs to be taken into account during formulation and processing of delivery systems. In particular, high molecular weight polysaccharide matrices have higher residual porosity, which enhances oxygen uptake and is detrimental to final product shelf life. However, high molecular weight polysaccharides may be easier to process owing to their higher viscosity. As usual in formulation, a trade-off between both performance indicators can be reached by mixing different molecular weight polysaccharides. And in fact, most of the formulations used for encapsulation of volatiles or other sensitive active materials found in the literature used a mixture of different molecular weight carbohydrates, see Table 4 below (starch, maltodextrins and mono- or di-saccharides e.g. sucrose, mannose, lactose…).

The most recurrent formulations found in the literature are those employing a mixture of high molecular weight polysaccharides (molecular weight greater than 2000 Da) and low molecular weight polysaccharides (molecular weight less than 1000 Da), for example a mixture of maltodextrin and glucose syrup or maltodextrin and gums, or starch and maltodextrins or disaccharides [14]. Such mixtures (Table 4) allow adjustment of the glass transition temperature, the hygroscopicity and porosity of the matrix.

Cyclodextrins, and more particularly β-cyclodextrins, are cyclic oligosaccharides which have also been considered as wall material in combination with other oligosaccharides. These materials are obtained from starch by enzymatic conversion and are very resistant to high temperatures (100 to 300°C). Cyclodextrins have a toroid structure, with the inner core less hydrophilic than the surface of the molecule. The advantage of this arrangement is that the inner core can establish inclusion complexes with various hydrophobic substances, while remaining water-soluble. β-cyclodextrins have been used in melt extrusion microencapsulation to pre-encapsulate flavors prior to extrusion; either by forming a flavors/β-cyclodextrins emulsion or by spray-drying the flavors with β-cyclodextrins [12,38,41,42,78].

Table 4 Composition of delivery systems of oligosaccharides as the main ingredient (nd= not determined), for melt extrusion, examples listed are calculated for one hour of production

Reference	Initial formulation composition (% w/w)	Active core (%w/w)	Extrudate moisture content (%w/w)	Encapsulation efficiency (% w/w)	Encapsulation Rate (% w/w)	Technology
(Schultz and Calif, 1958)	Sucrose/corn syrup/water (53.8/26.9/14)	Orange oil (5.4)	nd	nd	nd	Melt Injection
(Miller and Mutka, 1987)	Corn syrup/sugar /water (48/33/nd)	Orange oil (17.5)	5	nd	16.7	Melt Injection
(Saleeb and Pickup, 1989)	Maltose monohydrate/maltodextrin (24.1/72.5); mannose/maltodextrin (24/72.2)	Ethyl butyrate (3.4); lemon oil (3.8)	3-6	nd	(3.4) ; (3.3)	Melt Extrusion (single screw)
(Kollengode and Hanna, 1997a)	Corn starch/β-cyclodextrin (nd)	Cinnamaldehyde, eugenol, nonanoic acid, 3-octanone (nd)	nd	(42) ; (46) ; (26) ; (36)	nd	Melt Extrusion (direct injection)

Reference	Initial formulation composition (% w/w)	Active core (%w/w)	Extrudate moisture content (%w/w)	Encapsulation efficiency (% w/w)	Encapsulation Rate (% w/w)	Technology
(Black et al., 1998)	Whey protein/sucrose/maltodextrin/water (50/25/25/excess)	Cinnamic aldehyde (nd)	nd	nd	nd	Melt Extrusion
(Porzio and Popplewell, 1999)	Maltodextrin/corn syrup/methyl cellulose (72.5/20/7.5)	Orange oil (nd)	nd	nd	8.3	Melt Extrusion
(Gunning et al., 1999)	Sucrose/maltodextrins (52.8/47.2)	Cherry, pepper mint flavors (nd)	(3.5) ;(5.2)	nd	(10) ; (7.4)	Melt Injection
(Reifsteck and Jeon, 2000)	Corn syrup/sugar/flour/starch (nd)	Flavors (nd)	nd	nd	nd	Melt Extrusion
(Zasypkin and Porzio, 2004)	Hi-Cap100/EmCap12639/lactose (40/30/30); EmCap12634/Hi-	Lemon flavor (10-20)	(7.7) ;(7.6)	nd	nd	Melt Extrusion

	Cap100/lactose (40/20/40)					
(Bohn et al., 2005)	Sucrose/maltodextrin (nd)	Benzaldehyde (nd)	(4-5.4)	nd	nd	Melt Injection
(Yuliani et al., 2006)	Native corn starch/β-cyclodextrin (nd)	d-Limonene (nd)	nd	nd	nd	Melt Extrusion (pre- encapsulation by spray drying)
(Gregson and Sillick, 2012b)	Maltodextrin/trehalose/leci-thin/water (35.8/35.8/0.8/19.3)	Orange oil (8.3)	5.8	nd	nd	Melt Injection
(Chang et al., 2014)	Modified starch/maltodextrin/lecithin	Vitamine E (5-8)	nd	nd	93	Melt Extrusion
(Tackenberg et al., 2015)	Maltodextrine DE12 and Maltodextrine DE-17/sucrose (nd)	Orange terpenes and tocopherol (nd)	nd	nd	67	Melt Extrusion

3.3. Proteins

Due to their amphiphilic, emulsifying, film forming and solubility properties, proteins are now used as an innovative raw material for microencapsulation, and those most often employed are sodium caseinate, soy and pea protein isolates. Whey proteins and soy proteins make good wall materials for flavor and essential oil encapsulation due to their good gel-forming, emulsifying and surfactant properties. Indeed, these protein isolates have been widely used, mainly by spray-drying [87], in microencapsulation of different types of active materials (i.e. essential oils, flavors, tocopherols, oils rich in polyunsaturated fatty acids, etc) [71,88].

The major problem related to proteins is that they are not as chemically inert as polysaccharides, and side reactions can take place (Schiff base formation and Maillard reactions). This may result in browning (oxidation reaction between amino-acid groups of proteins and aldehyde groups of the flavor molecule) of the final product [87,89,90]. As a result, interactions between proteins and flavors may cause a loss of flavor perception in the final product or the production of off-flavors. The latter are the result of the reaction of aldehydes with the amino, disulfide, sulphydryl or thiol groups of the proteins through Van der Waal interactions or hydrogen bonds [78].

In fact, the determination and understanding of the type of interactions between flavors or fragrances and proteins have raised considerable interest among academic researchers. Landy et al.,[59] have investigated the interactions between aroma compounds and proteins (sodium caseinate) by measuring the vapor-liquid partition equilibrium (by headspace analysis or exponential dilution) in order to understand how these volatiles are retained. They were able to establish that, depending on the concentration of protein and the type of chemical group of volatile compounds, retention can be affected. In some cases, the liberation of active compounds is slowed down or inhibited due to irreversible interactions between some flavor compounds and the protein support (i.e. aldehydes and ketones interact with the amino acids) [91]. These interactions result in flavor loss or modification. There are two major problems related to proteins as encapsulating agent; (i) proteins are highly reactive compounds that can bind irreversibly to flavor molecules inducing loss or modification of the flavor, (ii) proteins are molecules with different types of chemical groups and structure, thus they can have different interaction sites (i.e. hydrophobic and/or hydrophilic binding sites). For example, soy proteins do not retain some volatile compounds such as alcohols but they do retain aldehydes and ketones through irreversible interactions and consequently the release rate of these volatiles is very low.

Furthermore, hydrophobic core materials may be more soluble in proteins, because of the presence of hydrophobic moieties in this material, which may lead to enhanced diffusion and leakage of the encapsulated active, especially if the latter is volatile.

Mixtures of proteins and fats, or proteins and oligosaccharides have also been proposed [23,90]. For example, Lengerich et al. [39] used a mixture of protein and flour (durum flour and whey protein) as matrix (Table 5). Alternatively, the pre-encapsulation of the active compound in a water-in-oil emulsion has been proposed in order to improve the barrier properties of the delivery system [40,92]. In this case, the aqueous phase was composed of a solution of sodium caseinate, and the emulsion was injected directly into the second barrel of a seven-section barrel extruder. This latter preparation gave higher encapsulation efficiency than the encapsulation efficiency found in Black's work [23].

In addition, Black et al. [23] have also determined the release rate behavior of different proteins (gluten, soy protein, egg albumin, acid casein, whey protein concentrate) mixed with a mixture of polysaccharides (e.g. sucrose and maltodextrins) and glycerin as plasticizer. They determined that for cinnamic aldehyde (principal component of cinnamon flavor) gluten had the highest release rate compared to the other proteins, and indeed gluten has better viscoelastic properties than the other proteins cited above.

These results confirm that proteins admixed with polysaccharides or fat are better carriers than a matrix composed only of proteins. As mentioned in Guichard's paper [90], a solution of sodium caseinate (0,1% in water) and egg albumin decreases the activity coefficient of flavor compounds and ensures better retention. In these types of mixtures, proteins act more as a compatibilizing rather than encapsulating agent; they help to decrease the surface tension of the flavors and the polymer matrix in order to obtain homogeneous blends.

Table 5 Composition of delivery systems using proteins or mixture of proteins and oligosaccharides as the main ingredient (nd=not determined)

Reference	Initial formulation composition (% w/w)	Active core (%w/w)	Extrudate moisture content (%w/w)	Encapsulation efficiency (% w/w)	Encapsulation Rate (% w/w)	Technology
(Sair and Sair, 1980)	Casein/water (43.2/45.8)	Orange oil (7.6)	6	92	nd	Silent cutter Autoclave
(Black et al., 1998)	Whey protein/ maltodextrins (lodex-10)/ sucrose/water (nd/nd/nd/excess)	Cinnamic aldehyde (nd)	nd	nd	nd	Extrusion (not specified if melt injection or melt extrusion)
(Lengerich et al., 2010)	Durum flour/wheat protein/sodium caseinate/glycerol/erythrobic/ water (35.3/8.8/2.3/12/2.4/27)	Oil rich in polyunsaturated fatty acids/vanilla (11.9/0.3)	nd	nd	nd	Melt extrusion

3.4. Additives

3.4.1. Introduction

Additives can be considered as components, which impart specific properties to the final product. For instance hydrophobic coating compounds like waxes or oils are normally employed to enhance oxidative stability and lower releasing rates. Plasticizers are employed to decrease the processing temperature and thus avoiding thermal degradation [68]. Carboxylic acids like ascorbic acid, citric acid, erythorbic acid and other components such as lecithin, caseinate and gelatin are used as food preservatives and/or antioxidants [50,93]. However, the most important additives in terms of encapsulation performances are certainly emulsifiers which can be added either to the feed emulsion or to the carrier itself in order to ensure small oil droplets of aroma or fragrance compounds inside the matrix, thereby providing better dispersion in the carrier and higher protection of the core material [16]. Table 1 gives a perfect overview of all the “raw materials” used.

3.4.2. Emulsifiers and other compatibilizing agents

Emulsifiers are used in encapsulation principally to increase the compatibility between the matrix and the active materials. Besides the OSAN modified starch mentioned above, a number of emulsifiers could be used.

Gums, such as gum Arabic, have been proposed and used either alone or in combination with maltodextrins. For example, Jacquot et al. [79] found efficient flavor encapsulation by spray drying a flavor /gum Acacia/maltodextrin (DE18) emulsions, although gums are also claimed to delay water uptake and thereby enhancing the controlled release of the encapsulate [32]. However, these high molecular weight compounds are detrimental to the matrix barrier properties against oxygen and to the protection against oxidation [94]. Therefore, due to these two limitations and also to the fact that gums are rather expensive and suffer from irregular market availability and variable quality, they are preferred as additives and not as a polymer matrix.

Another solubilizer widely employed in microencapsulation is lecithin, soy lecithin being the most used of the range. Lecithin has the additional advantage of acting as a lubricant, thereby improving the flowability of the melt. It helps to decrease the stickiness (especially for maltodextrins and starches) and the structural surface defaults on the surface of the delivery system [35,36,50,92,93]. An additional benefit is the lowering of the extrudate surface tension, which in turn decreases the porosity of the product and provides a better protection against oxygen permeability.

Medium chain triglycerides (MCT) are less well known than gums or lecithin and are synthesized from glycerol and fatty carboxylic acids (i.e caproic, caprylic, capric, and lauric acids). There are different types of MCT depending on the length of the major fatty acid chain (from C6 to C12) and all are colorless, tasteless and odorless, hence their use in the food and cosmetics industry. In microencapsulation, they are used as lubricants providing better flowing materials and easier shaping of the molten mixture at the die exit. In addition, they offer protection to the active ingredient by acting as a coating material, thus slowing down release of the active compound [35,36]. Moreover, in some studies, MCT have been used as “solvent vector” in which the flavors or fragrances are dissolved in order to facilitate their handling or to offer an extra protection (formation of an oil/flavor droplets) prior to processing [16]. In recent studies, MCT is employed as a model active-oil compound allowing to determine the dispersion and the mixing efficiency of twin-screw extrusion processing [19,20]. Additionally, they can also act as antioxidant because they are able to reduce the vapor pressure of the active material [40]. According to the references cited before, MCT can be used with any type of carriers, but here they are specially employed with maltodextrins, starch and oligosaccharides (oligofructose), as emulsifiers and vectors to enhance the adsorption of the active compound.

Hydroxypropyl methylcellulose (HPMC) is a semi-synthetic polymer employed in some formulations to control the release of flavors when the delivery systems have to be solubilized in water. Porzio and Popplewell [95] have suggested that, when dissolved in water, HPMC rehydrates, thereby increasing the viscosity of the medium and slowing down the diffusion of the flavor in the medium. HPMC is less commonly used than OSAN-modified starch, lecithin or gums.

Finally, ethyl cellulose is commonly found in the food industry as a colloidal stabilizing agent (E462). In microencapsulation it is also used as a viscosity modifier because it allows decreasing the interfacial tension between the core material and the encapsulating carrier to be lowered, along with the energy required.

3.4.3. Antioxidants

The second group of additives is antioxidants. These are usually employed in the case of microencapsulation of sensitive and readily oxidizable active compounds e.g. oils rich in polyunsaturated fatty acids, bioactive food compounds like polyunsaturated fatty acids (omega-3 oils), fragrances and flavor compounds [25,41,66,96–99]. For example, the antioxidants most commonly employed for protection of volatiles or high sensitive core compounds are ascorbic acid, citric acid, erythorbic acid, and mixed tocopherols [99].

3.4.4. Plasticizers

In the polymer industry, plasticizers are an important class of low molecular weight compounds, whose role is to modify the mechanical properties of polymers, by lowering down the glass transition temperature. Plasticizers reduce the density, the viscosity, the hardness, and the strength of a polymer. And at the same time they render the system more flexible and resistant to fractures [100] and improve the processability of the polymer.

In the case of melt extrusion microencapsulation, plasticizers are required to ensure formation of the melt inside the extruder's barrel. If the carrier employed is in a solid-state, a plasticizer may be necessary. However for some carrier, depending on the physical state of the core material, the use of a plasticizer may be optional [23]. In general two groups of plasticizers are distinguished in this area, water and polyols (also known as low molecular weight alcohols).

As already mentioned out in the preceding sections, water is the most frequently used plasticizer for carbohydrates and is also a key process parameter (Table 6). However, other plasticizers, such as sugar alcohols, polyols, glycols, polyglycols, linear alcohols, glycerin, etc., have been proposed to avoid early losses of volatile by water (or flash) distillation during the process. The sugar alcohols are synthesized from carbohydrates whose carbonyl groups have been reduced to a primary or secondary hydroxyl group. Polyols are low molecular weight plasticizers, characterized by their significant impact on the mechanical properties. They are often employed in the fabrication of biopolymeric films because they improved the mechanical properties of these films in terms of flexibility and elasticity [101,102]. For example sorbitol, glycerol, erythritol, xylitol, and aqueous-based compositions such as alcoholic solutions of polypropylene glycol, polyethylene glycol, pentanol and hexanol are used for plasticization of the biopolymer matrix in extrusion microencapsulation [23,40,99,103]. All of these alternative plasticizers are bulkier than water and are supposed to provide matrix materials more ductile and homogeneous in the extruder barrel. One drawback is that such matrices are also more permeable to volatiles, and another is that such alternative plasticizers are not easily removed from the final product. Both drawbacks are detrimental to encapsulation.

[23] evaluated the release rate of cinnamic aldehyde using the same type of carrier but changing the nature of the plasticizer: glycerin or water. Modified starch, whey protein, soy protein and egg albumin were tested for the same amounts of plasticizers. Results showed that flavor release was more important for extrudates plasticized with water than extrudates plasticized with glycerin (and this is true for all the matrices except for the modified starch matrix). Besides, Porzio and Popplewell [95] have used water as a plasticizer, setting very low initial water content around 3 to 5% (w/w), in order to obtain an extrudate glass transition temperature of equal or higher than 40°C.

Table 6 Composition of delivery system and its initial and final moisture content in relation with the glass transition temperature (nd=not determined)

Reference	Initial formulation composition (%w/w)	Core material (%w/w)	Initial moisture content (%w/w)	Extrudate moisture content (%w/w)	Tg (°C)	Technology
(Swisher, 1957)	Corn syrup/brominated vegetable oil/emargol (88/4.1/0.8)	Orange oil (7.1)	3-8.5	nd	nd	Melt Injection
(Schultz and Calif, 1958)	Sucrose/corn syrup (53.8/26.9)	Orange oil (5.4)	14	nd	nd	Melt Injection
(Sair and Sair, 1980)	Casein (43.2)	Orange oil (7.6)	45.8	6	nd	Silent cutter Autoclave
(Miller and Mutka, 1987)	Corn syrup/sugar (48/33)	Orange oil (17.5)	nd	5	nd	Melt Injection
(Saleeb and Pickup, 1989)	Maltose monohydrate/maltodextrin (24.1/72.5); mannose/maltodextrin (24/72.2)	Ethyl butyrate (3.4); lemon oil (3.8)	nd	3-6	50-80	Melt Extrusion (single screw)
(Kollengode and Hanna, 1997b)	Starch (95,2)	Cinnamaldehyde, eugenol, nonanoic acid, 3-octanone: (nd)	15	nd	nd	Melt Extrusion (direct injection)
(Black M., Popplewell L., and Porzio M. 1998)	Whey protein/maltodextrins (DE10)/ sucrose (50/25/25)	Cinnamic aldehyde (nd)	Excess	nd	nd	Extrusion (not specified if melt extrusion or melt injection)

(Porzio M. and Popplewell L. 1999)	Maltodextrin (85.6)	Diacetyl (9.1)	5.3	8.3	35-50	Melt Extrusion
(Hau M., Gray D., and Taylor A. 1998)	Wheat starch (67.3-84.1)	Diacetyl, 3-methylbutanal, heptane (nd)	15.9-32.7	19-43	nd	Melt Extrusion
(Gunning et al., 1999)	Sucrose/maltodextrins (52,8/47,2)	Cherry, pepper mint flavors (nd)	nd	(3.5); (5.2)	nd	Melt Injection
(Reifsteck and Jeon 2000)	Corn syrup/sugar/flour/starch (nd)	Flavors (nd)	nd	nd	nd	Melt Extrusion
(Porzio and Popplewell, 2001)	Maltodextrin DE10 (81.4)	Diacetyl (8.6)	10	7.6	51	Melt Extrusion
(Benczedi and Bouquerand, 2001)	Maltodextrin DE19/lecithin (90/1)	Strawberry flavor (3)	6	nd	<40	Melt Extrusion
(Ubbink et al., 2001)	Potato starch/capsule E/glycerol (64.1/2.4/2.7)	Orange oil (5.8)	25	nd	nd	Melt Extrusion
(Leusner et al., 2002)	Fructooligosaccharide/ Miglyol (65.8/4.9)	Calcium (28)	1.3	nd	nd	Melt Extrusion
(Benczedi and Bouquerand, 2003)	Maldotextrin DE19/silicon dioxide/lecithin	Fragrance (3)	7	nd	40	Melt Extrusion

	(87/2/1)					
(Zasypkin and Porzio, 2004)	Hi-Cap100/EmCap12639/lactose (40/30/30); EmCap12634/Hi-Cap100/lactose (40/20/40)	Lemon flavor (10-20)	nd	(7.7);(7.6)	(13); (15)	Melt Extrusion
(Bohn et al., 2005)	Sucrose/maltodextrin (nd)	Benzaldehyde (nd)	nd	4-5.4	38- 54	Melt Injection
(Yuliani et al., 2006)	Native corn starch/β-cyclodextrin (nd)	d-Limonene (nd)	nd	nd	nd	Melt Extrusion (pre- encpasulation by spray srying)
(Bouquerand, 2007)	Maltodextrin DE10/miglyol/lecithin (77.6/1/0.5)	Ascorbic acid (20.8)	nd	nd	35.8	Melt Extrusion
(Chang et al., 2010)	Maltodextrin/lecithin/miglyol (75.6/1/0,5);maltodextrin/lecithin/miglyol (88.4/1/0,5)	Ascorbic acid (18.9);(16.1)	(4);(2)	(9.2);(7.9)	<35	Melt Extrusion
(Zasypkin, 2011)	OSAN starch/gournd oregano/lactose/dextrose monohydrate (43.88/18.62/25.27/2.65)	Flavor (6.6)	2.4	7.6	44.9	Melt Extrusion
(Benczedi et al., 2011)	Maltodextrin DE19, DE12, DE6 (83)	Orange oil (nd)	25	7.5-15.1	49-54	Melt Extrusion

(Gregson and Sillick, 2012a,b)	Maltodextrin/trehalose/lecithin (35.8/35.8/0.8)	Orange oil (8,3)	19.3	5.8	51	Melt Injection
(M.A. Emin and H.P. Schuchmann, 2013)	Native maize starch	Medium chain triglycerides (4)	18	nd	nd	Melt Extrusion
(Chang et al., 2014)	Modified starch/maltodextrin/lecithin	Vitamin E (5-8)	nd	nd	30	Melt Extrusion
(Tackenberg et al., 2015)	Maltodextrin DE12 or Maltodextrin DE17/ sucrose	Orange terpenes and tocopherols (nd)	4-5.7	2-12	54-58	Melt Extrusion

4. EXTRUSION MICROENCAPSULATION TECHNOLOGIES

4.1. Introduction

A categorization of the extrusion technologies for microencapsulation has been made in recent works [29,67,68,104], leading to a clear distinction between *ram extrusion* (also called melt injection) and *screw extrusion* (also called melt extrusion).

Swisher [22], Schultz and Calif [21] defined *ram extrusion* as a process consisting of a rotating screw inside a heated cylindrical barrel, where the raw materials are introduced in order to be melted. Next, a piston (here called a ram) pressurizes the molten mixtures through a die and transforms them into the desired shape. The main advantage of ram extrusion is the simplicity of the set-up. The major inconvenience is the limited melting capacity of the apparatus, producing poor temperature and composition uniformity in the extrudate [68]. The resulting material has the consistence of a hard candy entrapping the active.

In screw extrusion, the apparatus is composed of a single screw or two co-rotating screws inside a multiple heated barrel section, with inlets in each barrel where the raw material or additives can be introduced. The design of the apparatus allows controlled shear stress and controlled temperature depending on the conditions desired. In addition, according to the screw profile, different conveying, mixing and shearing zones can be established to treat the materials. The raw materials are then mixed, melted and transported to a die system where the molten mass is shaped. The advantage of screw extrusion is its versatility in terms of operating conditions. The major disadvantage is the difficulty of accurately controlling the parameters of this complex set-up to ensure the good and constant quality of the final product. On the other hand, to achieve a high quality material trough using an extrusion process, it is important to have a solid background knowledge in the materials science, so that the adequate formulation and the process variables can accurately be chosen [16,30,67–69].

Co-extrusion consists of a dual fluid stream of immiscible liquid core and shell materials. Coating and core materials are pumped separately through concentric feed tubes and exit through the concentric orifices of the nozzles as a fluid rod or drop under the action of mechanical or sonic vibrations. Thanks to the action of surface tension, the wall material entraps the core material. The wall material is further solidified by a temperature drop or cross-linked in a bath containing suitable cross-linking agents. This technology does not need a pre-treatment of the carriers nor the active compounds [2,8,105,106]. This type of process will not be further addressed in this review.

The process steps of the three technologies used for microencapsulation of volatile organic compounds referred as extrusion encapsulation are described in Table 7. The term extrusion is used here to designate the exiting of a molten mass through an orifice under pressure (either by a mechanical piston, as for melt injection, or forced by an endless screw as in melt extrusion). The three technologies employ similar carrier materials and almost the same operational conditions. However melt extrusion differs from the two others because it does not involve the cooling step using a dehydrating solvent and in general the melting of the coating material and the injection of the core are made in-situ. The product delivered by the three methods is a matrix where the active compounds are dispersed inside, usually in the form of droplets.

Melt extrusion is considered nowadays as one of the most promising techniques for microencapsulation of flavors and bioactive compounds because it is a highly flexible process, economical and environmentally friendly. The versatility of the twin-screw extruder allows adapting the conditions and parameters depending on the carrier, core material and the product desired.

Table 7 Overview of extrusion microencapsulation processes: melt injection, co-extrusion and melt extrusion (Zuidam and Shimoni, 2010)

Technology	Melt Injection or Durarome	Co-Extrusion	Melt Extrusion
Process steps	1. Melting of the coating material	1. Dissolution or dispersion of the active compound in oil (emulsion)	1. Melting of the coating material inside a twin-screw extruder
	2. Dispersion or dissolution of the active compound in the coating material	2. Preparation of the aqueous or fat coating material	2. Direct introduction of the active compound (pure or in a pre-encapsulated form)
	3. Extrusion of the molten mixture through filter	3. Using of a concentric nozzle, and simultaneously pressing the oil phase through the outer one	3. Dispersion of the active compound into the coating material
	4. Coating and dehydration of the extrudate (cooling solvent)	4. Dehydration of the extrudate by dropping it into a gelling or cooling bath (cooling solvent)	4. Cooling and shaping of the extrudate (ambient temperature)
Morphology	Matrix	Reservoir	Matrix
Load rate (%)	5-20	70- 90	5-40

Particle size (µm)	200-2000	150- 8000	300-5000

4.2. Melt injection or Durarome® or Ram extrusion

The first technologies that were developed to encapsulate sensitive and volatile organic compounds relied on the preparation of an emulsion where flavors were finely dispersed in the coating material. Next, the dough was forced to exit through an orifice, and the high mechanical stress and shear allowed a homogeneous, finely dispersed emulsion and a semi-solid matrix to be produced. Finally a cooling step was required to obtain a solid glassy extrudate, and thus the mixture was cooled down in an isopropanol bath (also called a bath of dehydrating liquid) and then shaped into granulates [10,107,108]. These steps are shown in Figure 5. The aim was only to protect the sensitive active ingredient. These methods were called "extrusion encapsulation" since they involved in their process the use of a screw (as a stirrer or as a shear stress tool) in order to force a molten carbohydrate mixture to exit through a die or a series of dies [14,16,21,22,25,41,56,106,107,109].

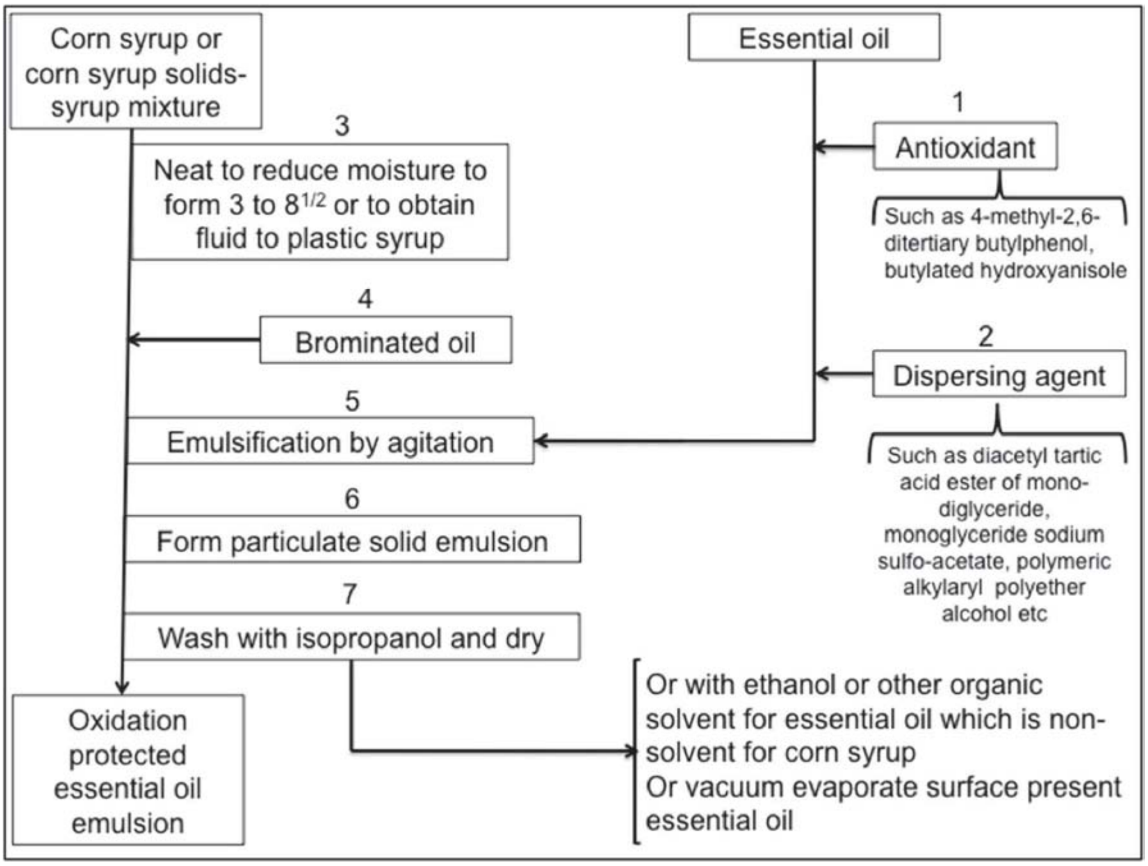


Figure 5. Scheme of extrusion encapsulation method adapted from Swisher [22].

The process of encapsulation is divided here into three steps; the first step consists of melting the carbohydrate matrix in the presence of a plasticizer (generally water or glycerol),–if required. Usually, the melting temperatures do not exceed 140°C in order to avoid thermal degradation of the active compound. Typical melting temperatures lie between 110 and 140°C. The second step corresponds to the addition of the active ingredient into the melt. In cases where the core ingredient is sensitive to oxidation, this step is carried out under an inert atmosphere. From the literature, the active compound is added to the carrier mixture, directly or as an oil-in-water emulsion, and the mixture is strongly stirred so as to disperse the flavor into the melting carbohydrate matrix. The third step is exiting and cooling of the dough. The mixture is forced to exit through a die, which results in the formation of a homogeneous product where the flavor is finely dispersed. The matrix is still in the rubbery state, but it is directly cooled down and dehydrated in isopropanol to induce transition to the glassy state. As shown in Table 8, microencapsulation by melt injection can be carried out in various types of apparatus (steam jacket mixer, tank reactor with an orifice or multiple nozzles). Depending on the type of device, different forms can be obtained (rods, droplets).

The drying step is mandatory every time the extrudate contains high levels of plasticizers and cold isopropanol is often used as a drying agent at this stage. Concomitantly, the extrudate is transformed into a glassy matrix by the dual effect of desiccation and cooling. This drastic shift from a paste to a glassy state fosters the entrapment of the active compound.

The Durarome® process named after the trade name of the first commercially available line of encapsulated flavors made by Firmenich S.A. [17,48,56], is based on this method and involves the dispersion of the flavor into a sucrose and maltodextrin candy matrix.

However, the cooling step is more considered as a counterproductive stage rather than being an advantage, because it is an extra step in the whole process and increases both the time and the cost of production. This is not to mention the costs due to solvents like isopropanol and the fact that such a process is not compliant with today’s food regulations, which tend to limit the use of organic solvent in food production processes.

Nonetheless, it is important to stress that the aim of encapsulation in these early days, was to protect the flavor compounds against oxidation and evaporation, in order to extend the flavored product’s shelf life [21,22,25,26]. The controlled release of the active compound is a more recent property that can be adjusted by modifying either the formulation of the microcapsules or the processing conditions [69].

Different apparatus have been developed since the pioneering work of Swisher to perform ram extrusion. These go from vertical screw-less extruders to multiple needle droplet-generators (also known as a nozzle encapsulation technology), and those mentioned in Uhlemann and Reiss' review [14]. Actually there are six other methods ((i) simple dripping, (ii) electrostatic extrusion, (iii) coaxial airflow, (iv) vibrating jet/nozzle, (v) jet cutting, (vi) spinning disk atomization), which have been recently described in the literature as extrusion encapsulation technologies. However they correspond more to a co-extrusion encapsulation technology because they are based on the same principles as melt injection [4,5,11].

Table 8 Extrusion microencapsulation by melt injection or ram extrusion technology: description of all the processing conditions

Type of apparatus	Emulsification conditions	Cooling conditions	Reference
Steam Jacketed Lenhart mixer	1. Emulsification of the carrier material 2. Emulsification of the core material and additives 3. Melting and mixing between 85 and 125°C	Isopropanol bath (-20°C) and vacuum dried	(Swisher, 1957)
Mixer and heater reactor, with an orifice	1. Emulsification of the carrier material 2. Emulsification of the core material and additives, 3. Melting and mixing between 130 and 150°C	Cold air tunnel	(Schultz and Calif, 1958)
Steam Jacketed stainless vessel with an agitator equipped with a plate with multiple orifices of 0,762 mm of diameter	1. Emulsification of the carrier material 2. Emulsification of the core	Isopropanol bath (-20°C) and vacuum dried	(Miller and Mutka, 1987)

	material and additives 3. Melting and mixing between 125 and 130°C		
nd	1. Melting of the carriers 2. Melting of the core material and additives 3. Emulsification step	Isopropanol bath (-20°C)	(Bohn et al., 2005)
Tank reactor with a stirrer and outlet valve with die holes	1.Melting and emulsification of the carriers and additives 2. Emulsification of core material and additives 3. Emulsification of carriers and core material at 70°C	Dehydrating solvent isopropanol or hexane (-4°C)	(Valentinotti et al., 2006)

4.3. Melt extrusion or extrusion microencapsulation

Melt extrusion encapsulation differs from melt injection encapsulation, not only because the apparatus employed is different but also because no pre- and/or post-treatment is applied to the materials (carriers, active compounds and extrudate). The major difference between these two processes is the moisture content: in melt injection high levels of water are required so that the slurry can be extruded; while in melt extrusion the melt can take place at low water content levels. The advantage of working at low moisture content is that no post-extrusion drying process after is required, thus the material obtained is more homogenous (has less fractures on the surface). Therefore, melt extrusion encapsulation corresponds to a process allowing a glassy delivery system to be obtained, by melting matrix components and mixing them with the active compounds under specific conditions.

The technology applied is generally a twin-screw extruder, whose flexible configuration allows the melting, addition, mixing and cooling of the carbohydrate mixture in a continuous system. According to the configuration of the extruder, different barrel temperatures, various inlet ports for liquid injection or solid feed, and screw profiles (conveyance, mixing and nest against) can be set up depending on the active ingredient and the biopolymer matrix [15,19,20,49]. The process is usually divided into three steps (Figure 6): first of all, the introduction of powder mixture of the carbohydrate into the extruder's first barrel section, plus a plasticizer or additives can be added if required into the barrel next to the solid feed section. Then the heating and mixing zone are established upstream in order to form a rubbery, viscous and homogeneous mass before the introduction of flavors. Finally these flavors can be finely dispersed into the molten mass in the last sections of the extruder, via a pump [16].

The liquid aroma compounds are generally introduced into the extruder's first barrel sections or right at the end. Also, depending on the product's final application, a pre-encapsulation step and/or post-coating of the delivery system can be made in order to increase final product performance.

Single-screw extrusion can also be used here, but the mechanical shear exerted on the molten mass is lower than in twin-screw extrusion, due to the fact that only a conveying screw is used, and this is filled-up all along the extruder barrel. Saleeb and Pickup [27] have employed a single-screw extruder for the encapsulation of orange oil flavor in a maltodextrin matrix. Extrusion temperature was ranged between 98 and 105°C and screw speed was set around 60 rpm. The flavor load obtained in this example was similar to the values found in twin-extrusion encapsulation that are around 5 and 40% (Table 9).

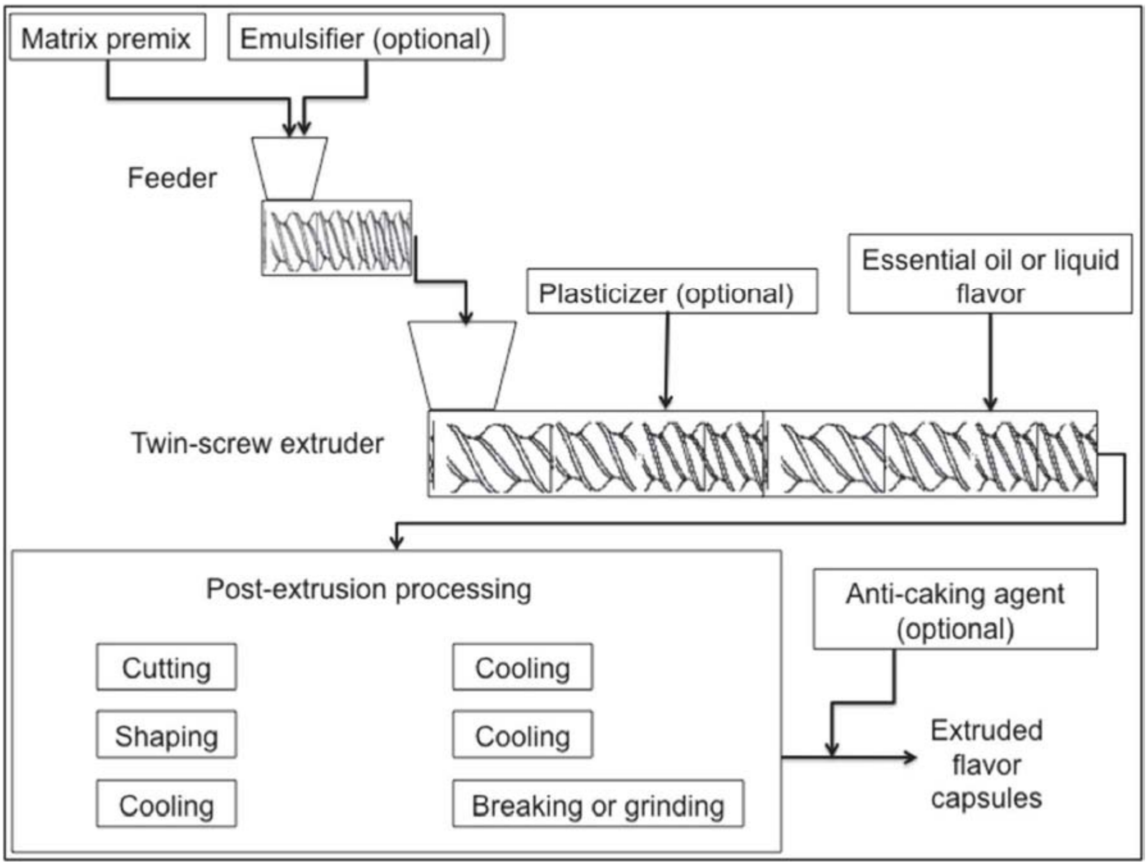


Figure 6. Scheme of flavor encapsulation by melt extrusion using a Twin-screw extruder adapted from Ubbink and Schoonman [16].

Table 9 Processing parameters for melt twin-screw extrusion microencapsulation:*extruder has 4 temperatures zones, the numbers in brackets correspond to a range of values. SME (=specific mechanical energy)

Type of extruder (ratio L/D)	Feeder rate solid (kg/h)	Feeder rate liquid (kg/h)	Screw speed (rpm)	Temperature profile (°C)	Die diameter (mm)	Pressure die (Pa)	SME (kJ/kg)	Reference
(Coperion) ZSK30 &ZSK57-16:1& 34:1	5.4	nd	[50- 500]	nd	nd	nd	nd	(Carr et al., 1991)
(Plasticorder) 2803 - 20:1	nd	nd	100	*60,120,110, 100	7	nd	[360- 540]	(Kollengode and Hanna, 1997a, 1997b)
(Clextral) BC 21- 16:1	3.7	[0.7 -1.81]	nd	*50, 100, 120, 80	10	nd	nd	(Hau et al., 1998)
nd	6.8	[0.18-0.42]	nd	[93- 121]	nd	nd	nd	(Porzio and Popplewell, 2001)
(Clextral) BC 21& BC45-16:1& 20:1	[5- 50]	nd	nd	[90-130]	[0.7- 2]	[1x10^{5} -50x10^{5}]	< 10	(Benczedi and Bouquerand, 2001)(Benczedi and Bouquerand, 2001)(Benczedi and Bouquerand, 2003)(Benczedi et al., 2011)
(Berstorff) ZE25- 40:1	3.5	nd	nd	[70-120] [a](die 50-70)	7	2.1x10^{6}	nd	(Ubbink et al., 2001)
(Clextral) BC45- 20:1	[25-35]	[0.10- 0.481]	175	[140-160] [a](die 185-195)	4	nd	nd	(Boutboul et al., 2002a)
(Coperion) ZSK25&Buhler44-40:1	4 9.6	[0.4-1.1] [3.2-6.8]	[150-200] 80	[15-120]	[0.5-1] 0.5	nd	< 180	(B. H. Van Lengerich, 2002)(Lengerich et al., 2010)
(Buhler) DNDL44- 40:1	9	0.18	nd	[30-160]	[0.25- 1]	nd	< 180	(Leusner et al., 2002)
40:1 & 50:1	[2.16-115]	nd	[50-700]	nd	[0.8-1]	nd	< 180	(Kohlus and Pacha, 2004)
nd	[6-15]	[0.12-0.72]	100	<121	0.79	6.86x10^{6}	nd	(Zasypkin and Porzio, 2004; Zasypkin, 2011; Zasypkin et al., 2013)
(Prism Eurolab) KX16- 40:1	0.96	0.4	[158- 242]	[50-167]	2	nd	nd	(Yuliani et al., 2006)
(Clextral) BC 21- 16:1	nd	nd	nd	nd	[1-3]	[1x10^{5} -10x10^{5}]	nd	(Bouquerand, 2007)

(Haake Polylab System)- 24:1	3	nd	80	*80, 105, 115, 95	3	[1x10^6 -3.5x10^6]	nd	(Chang et al., 2010)
ZSK 26 Mc Coperion 29	10-30	1-3	300-800	140	3	nd	nd	(M. A. Emin and H.P. Schuchmann, 2013)
LTW 26 HB-Feinmechanik GmbH&Co (25:1))	1.50-3.00	nd	[248-748]	105-145	1.25	nd	[920-2115]	(Tackenberg et al., 2015)

4.4. Key process parameters

The processing conditions in extruders are strongly determined by the chemical stability and physical properties of the coating and matrix material (molecular weight, desired glass transition of the final product, melt viscosity and melting point). All these properties should be taken into account to establish adequate processing conditions [68]. However, depending on the technology applied (ram extrusion, Durarome® or melt extrusion) process variables are also very important and can directly affect the macroscopic characteristics of the final product, e.g. the texture, aspect, and release properties. Figure 6 shows the different independent processing variables, for melt extrusion, influencing the properties of the final material [110].

As already mentioned, carbohydrates constitute the mostly used matrix materials in melt extrusion and extrusion encapsulation [69], and since these materials are essentially glassy and brittle, polar plasticizers are necessary to insure homogeneous melting of the carrier under appropriate thermo-mechanical stress and shear conditions. The preferred plasticizer is water.

The glass transition temperature of the delivery system depends on two important process parameters: extrusion temperature and moisture content. In addition, extrusion temperature and moisture content are directly related to viscosity and in the same way the volatile retention relies on viscosity. For this reason, temperature and moisture content are considered the most important factors affecting volatile retention. Therefore controlling viscosity is critical and thus, measurements of exit die pressure are always made [94]. The examples given in Table 9 show that, in general, pressure at the die exit is in of the same range for the studies presented ($1x10^5$ and $7x10^6$ Pa) and glass transition temperatures for these delivery systems are around 30°C and 50°C. This implies that the moisture contents employed for these formulations are of the same order. And in fact, moisture content of the examples shown in Table 6, are in agreement with the pressure values given in Table 9. Zasypkin and Porzio [30], Chang et al. [35] and Benczedi et al. [34] measured pressures at the die's exit of $6{,}86x10^6$ Pa, $3{,}5x10^6$ Pa and $1x10^5$ to $50x10^5$ Pa and a moisture content of 7,5% (w/w), 9,3% (w/w) and 12,3% (w/w) respectively.

As mentioned earlier, water is the key parameter governing the stability of biopolymers. It influences the crystalline and amorphous structures, the glass transition temperature and consequently the thermoplastic properties of biopolymers. Increasing the moisture content of a biopolymer increases the chain mobility and the heat capacity, but it decreases the viscosity and the system glass transition temperature. All these physico-chemical properties can be explained by how water interacts with the biopolymer [74]. Understanding water-biopolymer interactions gives a better insight thermo-mechanical processing. Similarly, knowing water-biopolymer interactions allows the processing conditions to be determined in order to better target the final properties of the material.

Glass transition temperature of the biopolymer is correlated to water-biopolymer interactions. Thanks to a combination of mechanical spectrometry and differential scanning calorimetry data, the glass transition temperature of a protein-based matrix can be determined. The results provide a better understanding of the phase transition behavior of amorphous biopolymers at different moisture contents. For instance, Kokini and co-workers [111] determined protein state diagrams, in order to predict physical states and phase transitions of the material during processing conditions (e.g. extrusion or baking). Figure 7 shows the state diagram of proteins under different physical conditions (i.e. cooling, heating, drying, wetting) during extrusion cooking processing. As mentioned above, this diagram demonstrates the importance of moisture content and the temperature conditions that are required to obtain the desired polymeric matrix during a thermo-mechanical process (melt extrusion, melt injection, thermo-molding…).

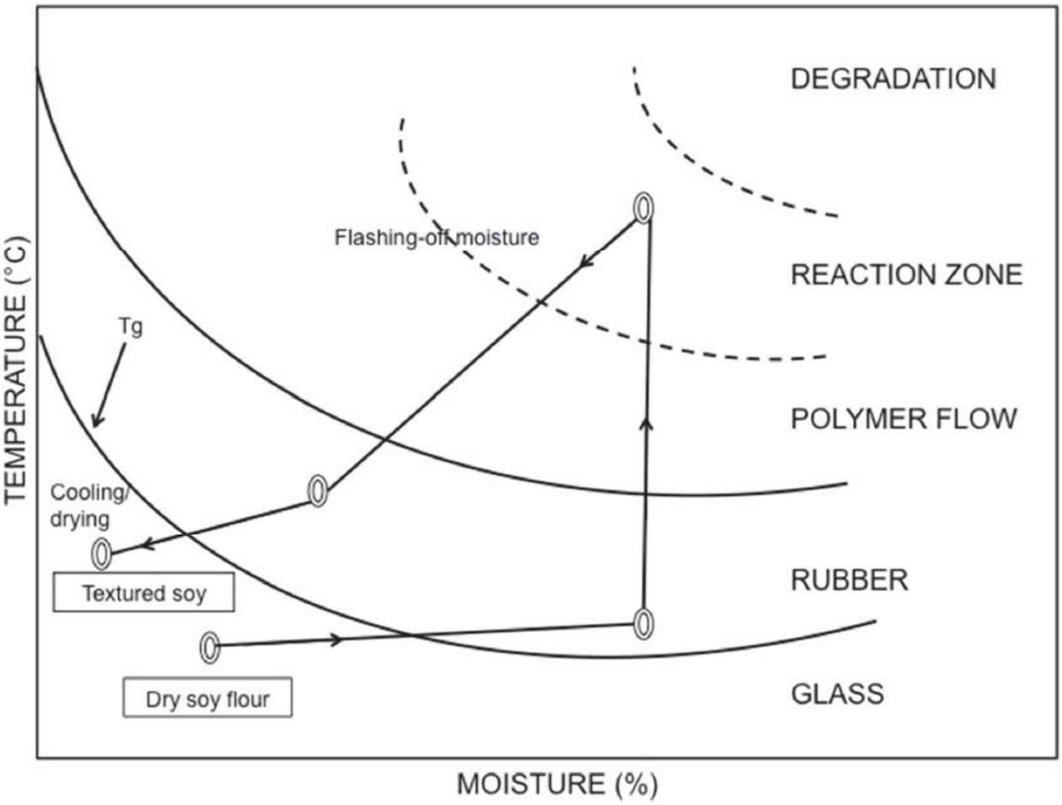


Figure 7. State diagram showing transformations of proteins during the wetting, heating, cooling, and drying stages of extrusion cooking adapted from Kobini *et al.* [111].

The other main process variables in melt extrusion are the temperature profile, screw profile and geometry, screw speed, feed flow rate, moisture content and feed composition [19,20,49]. The influence of these variables can be evaluated by measuring the mechanical or the thermal energy, the residence time, or other properties of the extrudate like product expansion (axial and radial expansion), breaking strength, encapsulation efficiency and release rate [35,112,113]. The process of melt extrusion encapsulation has different independent variables and measurable responses that must be taken into account (Figure 8). The most frequently measured responses are encapsulation rate or encapsulation efficiency, and both can be used for discussion.

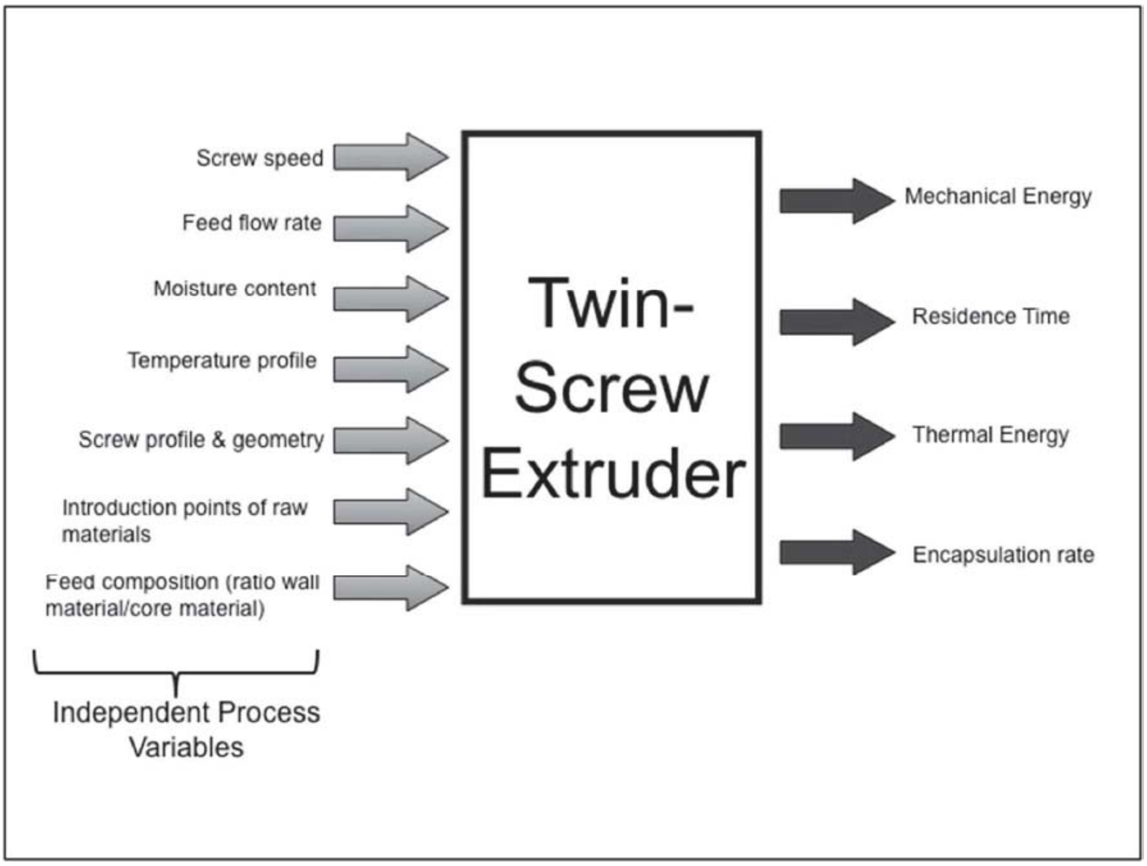


Figure 8. Scheme of Twin-screw extrusion microencapsulation processing variables; on the left the independent process variables and, on the right the measurable responses.

The processing conditions described in both patents and academic works (Table 9) are quite similar. The temperature profile is more or less the same, but the choice of extrusion temperature depends on the type of matrix and active material. In the case of active materials like sensitive oils rich in polyunsaturated fatty acid, extrusion temperature does not exceed 120°C [39,98]. In fact, the temperature profile retained for melt extrusion does not exceed 160°C in order to avoid thermal degradation of the compounds to be encapsulated (fragrances, flavors, bioactive food compounds…). However, not only the core material, but also the carrier material may be affected by the temperature profile. For example, mixtures of oligosaccharides are more resistant to temperature than maltodextrins, which begin to break down at around 180°C. Hence, Leusner et al. [66] have applied an extrusion temperature of about 160 °C to entrap ascorbic acid and calcium in a mixture of oligosaccharides, while Chang et al. [35] applied a temperature no higher than 115° to entrap ascorbic acid, just in maltodextrins.

Although the temperature profile has a great impact on flavors' stability during processing, screw speed is also important to control in order to avoid degradation of the flavors by mechanical shear stress. Indeed, screw speed is crucial because it exerts shear stress into the polymer/active core mixture, modifying its viscosity by involving self-heating through viscous friction, and also determining the residence time of the mixture inside the extruder. Usually a long residence time and high shear stress can cause thermal degradation not only of the active core (flavors, fragrances, bioactive food compounds) but also of the carriers (degradation, polymerization or offside reactions). Similarly, viscosity decreases when the shear stress exerted increases. For this reason, when the compound to be entrapped is very sensitive to temperature or shear stress, mild extrusion conditions are required. In the example quoted above, Chang et al., [35,50] have employed gentle temperatures not above 115°C and a screw speed of 80 rpm for the encapsulation of ascorbic acid in maltodextrin. In the case of proteins used as carriers, screw speed is around 150 rpm, in order to avoid their degradation by mechanical shear.

The screw profile, along with the temperature profile or screw speed, is the major parameter governing the structural transformation of the polymeric matrix (viscosity, expansion, physical changes). Hence, the screw profile can play a central role influencing the residence time inside the extruder. Nonetheless in some papers, this parameter is not described or studied. In general the screw profile chosen for most of the examples found in the literature consisted of conveying and mixing elements. Reverse pitch screw elements are avoided in order to reduce both shear stress and residence time. Recent works have focused on the influence of screw profile on the polymer-based matrix, but not on the effect that it could have on volatile's retention [19,20].

The parameter that has been given the most of attention in the literature is the location of the flavor injection port. In fact, depending on the position where the flavor is introduced in the extruder barrel, the retention of volatiles can change, possibly leading to significant losses during the process [15,55,78,94]. Indeed, the location of the injection port directly influences four key factors in the extrusion process, which in turn may impact the retention of volatiles. These factors are: (i) the pressure drop when the extrudate exits the die, (ii) the relative volatility and diffusion (thermodynamic parameters) of the active compounds in the system, (iii) the interactions between the active compounds and the matrix, and (iv) the degradation reactions (oxidation, thermal degradation, polymerization). For example, Lengerich [98] has demonstrated that changing the point of introduction can reduce the losses of active compounds. The highest loss (72,3%) was obtained when active compound was introduced in the first barrel section, whereas when it was introduced into the seventh barrel section of the extruder, losses were about 12,2%. This is because when the active compound is injected into the first barrel, it is exposed to temperatures around 120°C and 140°C for a longer period of time. Conversely, if the active compound is introduced in barrel seven, it is only exposed briefly to high temperatures.

There are three methods to introduce flavors into an extruder. The first consists of pre-incorporation of the flavors into the feed material prior to extrusion, either by preparing an emulsion of carrier/active core, or by spray-drying the flavors with a part of solid carrier and then mixing this with all carrier material. The disadvantage of this method is that since the active compound is added at the beginning of the extrusion process, volatile molecules are more likely to be degraded because of the harsh conditions at the beginning of the extrusion process. The second method is the direct injection of flavors into the extruder, into the last or the middle barrel section. The problem with this procedure is that, even though it leads to better retention rates, flavors are lost due to expansion at the extruder's exit die, due to the pressure increase linked to the reduction in size of the exit die. As a consequence the volatiles are flashed-off at the die level. Finally, the last method is a combination of pre-incorporation and post-coating of the delivery system, this method is highly cost-intensive but it improves the quantity of flavor retained in the matrix and allows the release of the active compounds to be slowed down.

A principal difficulty encountered when encapsulating liquids by extrusion processes is solid-liquid separation, which leads to oil exudation from the extrudate mass and is due to filtration of the phase having the highest mobility through the less mobile phase (for a comprehensive overview of solid-liquid separation in extruder, see Bouvier and Campanella [114]).

Hence, rare are the papers where flavors are introduced directly into the extruder without any pre-encapsulation treatment. Kollengode and Hanna [38,55] were the first to inject pre-encapsulated the flavors directly into the end barrel section of an extruder. In their case, the flavors were pre-encapsulated with β-cyclodextrin and then injected into the extruder. Even though this technique of pre-encapsulation of the core material before extrusion allows having a better protection of flavors against losses, the pre-encapsulation step raises the cost, and even more so if β-cyclodextrin is employed. Using β-cyclodextrin and spray drying are very highly expensive pre-encapsulation techniques.

In more recent industrial patents, direct injection of flavors in the form of an emulsion (i.e. direct injection of flavors in a pre-encapsulated form) has become more and more common in order to minimize losses of volatiles during the extrusion process, vary the release rate and reduce production costs. Core materials are introduced into the extruder as an emulsion of active and additive (plasticizers, compatibilizing agents and antioxidants compounds). Or in other cases, core materials are mixed with a part of the matrix compounds that are in a liquid state (i.e. corn syrup) [35,40,55,66,115].

The use of extrusion as a microencapsulation technology is relatively new and comprehensive engineering models adapted to the behavior of paste-like oil-in-matrix “emulsions” are missing. In this context, the comprehensive exposition of the engineering principles of extrusion technology in food and non-food materials, recently published by Bouvier and Capanella [114], can be considered as an inspiring source for further work in this area.

5. CONCLUSION AND FUTURE PROSPECTS

Encapsulation of flavors and fragrances, as well as other active compounds (nutraceutics and bioactive food components, pesticides, dyes, enzymes) is a domain that is still in expansion due to the increasing consumer's demands for better quality from the raw materials to the final products.

It is a must to reduce energy consumption, waste production and pollution. Therefore, global policies are focused on leading research and industrial development, into a more environmentally friendly and sustainable domains [116] .

Twin-screw extrusion can be seen as versatile technology that can be employed in different industrial domains, and can contribute with great benefits to sustainable development i.e. for green extraction of raw materials [117–119].

As an encapsulation process, twin-screw extrusion technology can be categorized as a green process (if compared with other encapsulation technologies: interfacial polycondesation, suspension and emulsion polymerization, and fluidized bed coating among others). Twin-screw extrusion encapsulation, as it was mentioned before, is a one-pot encapsulation technique, which combines: the formation of the wall material, the dispersion of the active principle, and at the exit of the die the forming of the encapsulated material. All these three different stages take place inside the barrel or the die of the extruder [30]. To compare, spray-drying encapsulation technology needs the prior preparation of the liquid formulation [8,105,107,120,121]. The reducing number of steps in an industrial process contributes to reduce energy consumption. Moreover, encapsulation by twin-screw extrusion does not require a pre- or post-treatment after extrusion unlike most of the other encapsulation methods.

Two other remarkable assets of twin-screw extrusion are the absence use of organic solvents (contrary to the polymerization techniques[122] and fluidized bed coating[123]) and the low amount of water (20% water content) as compared with spray-drying technology (that requires more than 80% of water). Both of these points involve the reduction of pollution and production costs during the manufacturing process.

To counteract the strong dominance of spray drying in the microencapsulation area, apart from reducing production costs, extrusion encapsulation appears as a versatile and sustainable technique for glassy microencapsulation. Extrusion microencapsulation is presented as a pioneering technology allowing the creation of new delivery systems, providing not only protection of the active compound but also, in some extent, its controlled release.

There remain, however, clear areas of improvement that would help extrusion encapsulation to become a more universal tool. In first rank, increasing the internal, encapsulated liquid phase (payload) in the extrudate would help reducing the material cost and make the technology more affordable for other applications, such as laundry products or agro-formulations. Secondly, there is a strong need for matrices having simultaneously a high encapsulation power and a low hygroscopicity, e.g. for better stability under moist environment. Finally, much remains to be done in the area of triggered release of volatile materials under pre-defined conditions.

Among the different technologies employed in extrusion encapsulation, with all the processing parameters that have to be controlled in order to obtain "the perfect delivery system" with the specific characteristics (T_g, moisture content, encapsulation efficiency…), a lack of understanding of the phenomena occurring during microencapsulation seems to be the major limitation in this domain. For this reason a concerted approach to food material science, materials science, flavor and fragrances chemistry and physical chemistry are required for further progress in this area. Emphasis must be given to determine the type of interactions between the matrix and the encapsulated materials, the state of this matrix, and the extrudate morphology, so as to establish the mechanisms, which control the release of volatile active compounds when and where they are desired.

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Transition Chapter 1 to 2

Thanks to this overview about the different types of extrusion processes employed for the encapsulation of active compounds in the food, fragrance, agriculture and pharmaceutical domains, twin-screw extrusion has been demonstrated to be a versatile, cost efficient and green technology. The large number of patents denoted its great performance on the industrial scale.

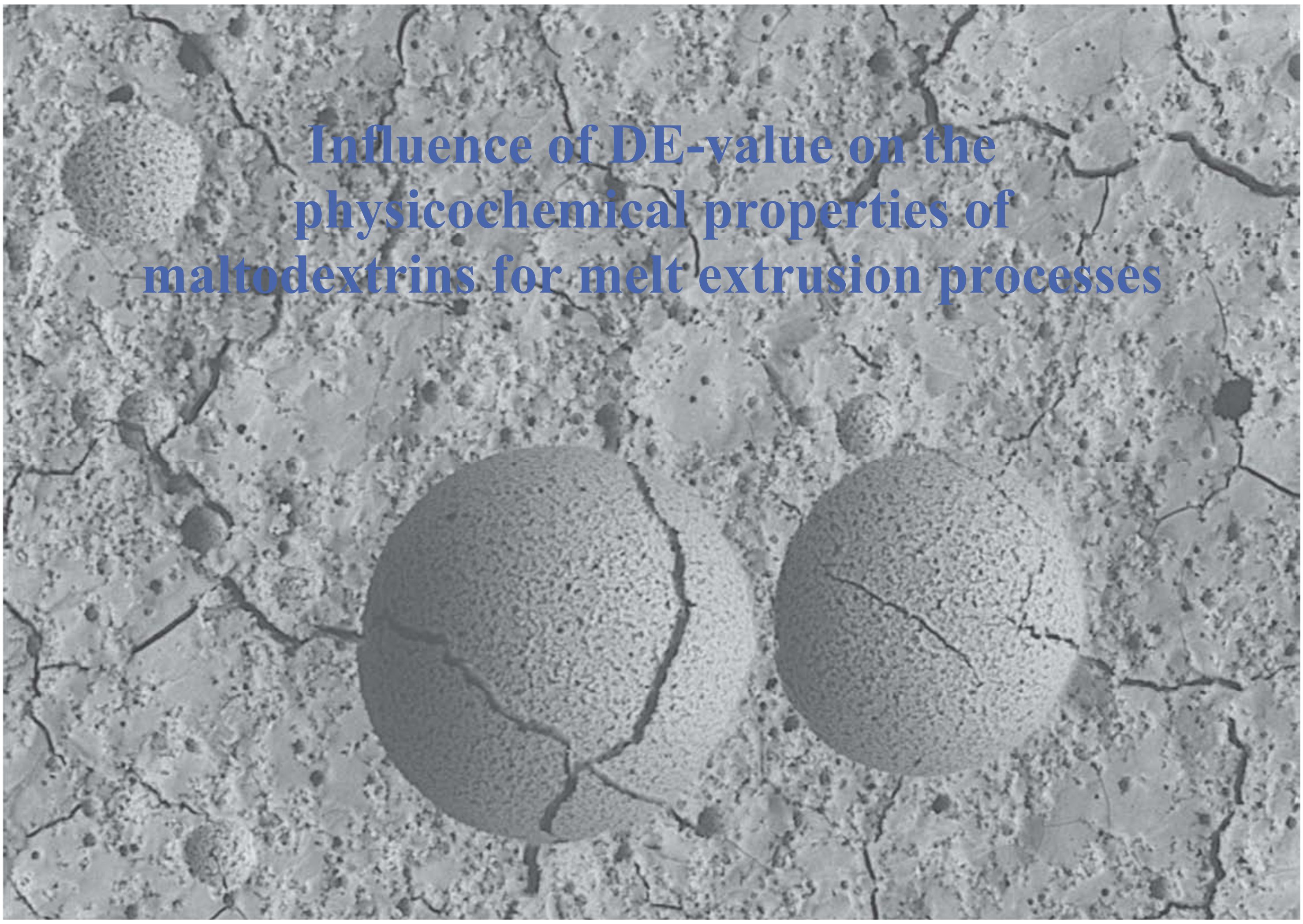
Carbohydrates appear as excellent wall material for the encapsulation of flavors and active ingredients for pharmaceutical applications. And since the encapsulation of various active compounds in starch-based matrix using this process has been studied in detail, maltodextrins seem a good alternative for new bio-based material.

The Laboratory of Agro-Industrial Chemistry (LCA) has great knowledge on twin-screw extrusion; not only on the transformation/fractionation of by-products from the food and agriculture, but also on the elaboration of bio-composites. Relying on this know-how, it was obvious that this technology could be used for the elaboration of new maltodextrin-based matrices for the incorporation of organic volatile compounds.

Our investigations were then oriented on the determination and analysis of the physicochemical properties of different types of maltodextrins. The aim of these characterizations was to predict the behavior of the raw materials before exploring extrusion encapsulation conditions.

For this reason in the next chapter, a series of elementary physicochemical analysis (DVS, TGA, DMA, SEC, rheology measurements…) were run so as to identify the adequate wall material.

Chapter 2



Influence of DE-value on the physicochemical properties of maltodextrins for melt extrusion processes

Influence of DE-value on the physicochemical properties of maltodextrins for melt extrusion processes

Natalia Castro[a, b], Vanessa Durrieu[a, b], Christine Raynaud[a, b], Antoine Rouilly[a, b]

[a] *Université de Toulouse, INP-ENSIACET, LCA (Laboratoire de Chimie Agro-industrielle), F-31030 Toulouse, France*

[b] *INRA, UMR-1010-CAI, F-31030 Toulouse, France*

e-mail: Antoine.rouilly@ensiacet.fr

Phone number: (+33) 5 34 32 35 09

fax: (+33) 5 34 32 35 97

Abstract

In this study, five different types of maltodextrins (DE-2, DE-6, DE-12, DE-17 and DE-19) were characterized for the physic-chemical properties. TGA, DVS and SEC analyses were carried out and additionally apparent melt-viscosity (in a micro-extruder) and the glass transition temperature (analyzed by DMA) of maltodextrin/plasticizer mixtures were also measured in order to evaluate both the effect of plasticizer nature and content and the effect of the DE-value. For this, three plasticizing agents were compared: water, D-sorbitol and glycerin. The adsorption isotherms showed that depending on the DE-value and the relative humidity they were exposed to, different behavior could be obtained. For example, for relative humidities below 60% RH maltodextrin DE-2 was the least hygroscopic. And on the contrary for relative humidities above 75% RH maltodextrin DE-2 was the most hygroscopic. The rheology measurements showed that the viscosity decreased with the increase of the DE-value and the plasticizer content, as expected. On the contrary, no direct correlation could be established between the DE-value and the glass transition temperature. These results demonstrated that to predict maltodextrins behavior and to better adapt the process conditions, combined analyses are mandatory as the DE-value alone is not sufficient. The most compelling evidence was obtained by size exclusion chromatography, which pointed out that maltodextrins had a bimodal molecular weight distribution composed of high and low, molecular weight oligo-saccharides. Indeed, maltodextrins are highly *polydisperse materials (i.e. polydispersity index ranging from 5 to 12) and that should be the reason why such distinct behaviors were observed in some of the physico-chemical analyses that were preformed.*

Keywords: Maltodextrins, dextrose equivalent value, apparent viscosity, glass transition temperature, water sorption isotherm, molecular weight distribution

Remark: in press Carbohydrate Polymers, doi:10.1016/j.carbpol.2016.03.004

1. INTRODUCTION

Maltodextrins are obtained from the acid and/or enzymatic controlled hydrolysis of starch. Maltodextrins are composed of D-glucose units connected by (1-4) glucosidic linkage to give D-glucose polymers of variable length and therefore different molecular weight. The number of the reducing sugar content is defined by the dextrose equivalent value (DE-value), which is calculated on a dry weight basis. Maltodextrins are a mixture of saccharides with a DE-value ranging from 3 to 20. Starch is associated to a DE-value of zero, and glucose to a DE-value of 100 [1,2].

Maltodextrins are one of the most common compounds used in the cosmetic, food and pharmaceutical domain. It can be employed as the main ingredient of a formulation or as an additive. Maltodextrins are great film forming and texturizing agents, as they can increase viscosity, retard crystallization or decrease stickiness and hygroscopicity of a mixture but also improve shelf-life stability of food matrices (Roos & Karel, 1991). Maltodextrins are popular in the food industry not only for all the previous reasons but also because they are highly soluble in water and non-sweet compared to classical sugars [3,4]. Not to mention that maltodextrins are odor-, color- and tasteless so they appear as the best option to be employed as encapsulating agents either by spray-drying or twin-screw extrusion. Nowadays, maltodextrins are used as the main ingredient rather than additive for the elaboration of bio-based materials by melt extrusion [5–7].

The key for a successful encapsulation of an active compound is based on the understanding of the physicochemical properties of the wall material employed and therefore the adaptability of the process conditions and of the technology to be used. For maltodextrins, the main problem is the lack of experimental data concerning the physicochemical properties of these raw materials. Actually, there are more mathematical models allowing predicting the behavior of some of the physicochemical properties than studies measuring them because of the rigidity and brittleness of these carbohydrates-based materials.

Therefore in order to better understand maltodextrins, the aim of this paper was to determine in the first place the molecular characteristics (molecular weight distribution, sorption isotherm, apparent viscosity, and glass transition temperature) of five different grades of pure maltodextrins; and in a second place, to analyze the effect of the type and the amount of plasticizer on the apparent viscosity and glass transition temperature of these mixtures. Ergo, the formulations herein studied can be adapted to the principal encapsulation technologies. Thus, tuning the formulation upstream can for instance improve the flowability of the mixture inside an extruder, and control the properties of the final maltodextrin-based products.

2. MATERIALS AND METHODS

2.1. Raw materials

Roquettes Frères (Lestrem, France) supplied maltodextrins with different dextrose equivalent (Glucidex-2, Glucidex-6, Glucidex IT-12, Glucidex IT-19 and Glucidex 17). These maltodextrins are obtained by controlled hydrolysis of native corn-starch. The main difference between these two ranges of product is based on the powder particle size. Glucidex-IT has bigger particle size, providing a better solubilization and free-flowing properties. Two plasticizers were employed, glycerin (CAS: 56-81-5, MW=92 g.mol^{-1}) and D-sorbitol (CAS: 50-70-4, MW=182 g.mol^{-1}) both supplied by Sigma Aldrich (St Quentin Fallavier, France). Reagents used for the dextrose titration and for size exclusion chromatography were also provided by Sigma Aldrich: Copper (II) sulphate pentahydrate (CAS: 7758-99-8, MW=249.69 g.mol^{-1}), Methylene blue (CAS: 61-73-4, MW=319.85 g.mol^{-1}), potassium sodium tartrate tetrahydrate (CAS: 6381-59-5, MW=282.22 g.mol^{-1}), sodium hydroxide (CAS: 1310-73-2, MW: 40 g.mol^{-1}), Di-sodium hydrogen phosphate (CAS: 10028-24-7, MW=177.99 g.mol^{-1}), sodium phosphate (CAS: 10049-21-5, MW=138.0 g.mol^{-1}) and sodium chloride (CAS: 7647-14-5, MW=58.44).

2.2. Determination of the dextrose equivalent value of maltodextrins

The DE-value were measured by the Hagedorn-Jensen method [8] in order to confirm the dextrose equivalent value established by the manufacturer. The DE-values obtained for the five different types of maltodextrins are in agreement with the DE-values indicated by the supplier (Table 1). “Theoretical” degree of polymerization and number average molecular weight were determined by the following equations [1] and are summarized in Table 1:

$$DPtheo = \frac{111.11}{\mathrm{DE}} \tag{1}$$

$$\bar{M}ntheo = 162DP + 18 \tag{2}$$

The molecular characteristics presented in Table 1 are used as references to compare with the experimental values obtained in this study in section 3.1.

Table 1. Measured DE-value of different types of maltodextrins (triplicates). DE-values were experimentally measured. DP_{theo} and Mnt_{heo} were calculated from equations 1 and 2.

	Maltodextrin DE-X				
Molecular Characteristics	**DE-2**	**DE-6**	**DE-12**	**DE-17**	**DE-19**
DE-value	2.1±0.0	6.29±0.02	12.31±0.02	17.7±0.04	19.04±0.06
DP theo	52.9	17.7	9.0	6.3	5.8
$\bar{M}n$ theo (g.mol^{-1})	8589	2880	1480	1035	963

2.3. Size exclusion Chromatography

SEC analyses were performed using a Dionex (Voisins le Bretonneux, France) size exclusion chromatography (SEC) equipped with a high-sensitivity inverse refractive index detector Prostar 350/352 from Varian Analytical Instruments (Walnut, C.A., USA).

The average molecular weights of maltodextrins were determined by gel permeation chromatography (GPC) on a PL aquagel-OH 50 columns. The column system was composed of three columns; 2 Agilent PL aquagel-OH 30 8 µm, 7.5 x 300 mm (p/n 1120-6830 Polymer Laboratories Ltd. Church Stretton, UK) and a PLgel precolumn. The column oven temperature was set at 30°C. The eluents were 0.02 M NaCl in 0.005 M sodium phosphate buffer Sigma Aldrich (St Quentin Fallavier, France), at pH 7 and prepared as the protocol described by [9].

External calibration was made with Pullulan standards, from Polymer Laboratories (Marseille, France), with specific average molecular weights ranging from 360 and 380,000 Da, dissolved in 0.005 M sodium phosphate buffer with 0.02 M NaCl, pH 7.5.

The results were treated by Chromeleon software in order to obtain the number average (M_n), the weight average (M_w) molecular weights and the polydispersity index (Ip) of each analyzed sample. All samples were run in triplicates.

2.4. Determination of the moisture content

Moisture content of the samples was determined by gravimetric method (NF-V-ISO03-921). One gram of each sample was weighted and left to dry in an oven at 103±2°C for 24 hours until there were no mass variations of the sample. Measures were run in triplicates for each sample.

2.5. Dynamic vapor sorption analyses (DVS)

Water sorption isotherms were performed on a Dynamic Vapor Sorption (DVS) Advantage System from Surface Measurement Systems (Alperton, UK). This machine is equipped with a very accurate recording microbalance, able to measure changes in the sample mass as low as 0.1µg. Samples were exposed to a constant temperature (25°C) and programmed relative humidities varying from 0 to 90% divided into 15% increments (14 steps). A mixture of dry and moisture-saturated nitrogen flowing over the samples assured the changes in the relative humidity of the DVS-chamber. Ten milligrams of the sample were placed inside the chamber and before starting the data acquisition, all the samples were dried for 300 minutes under a stream of dry nitrogen (0% RH) at 103°C in order to obtain the dry weight. Equilibrium was achieved, when the changes in the mass of the sample were lower than 5.10^{-3} % min^{-1}.

2.6. Thermogravimetric Analysis (TGA)

Thermogravimetric analyses were performed on a SETSYS-Evolution TGA-SETARAM Instrumentation KEP Technologies (Caluire et Curie, France) in order to establish the thermal stability of each compound. The temperature of analysis was set from 25°C to 600°C at a ramp rate of 7.5 °C/minute, under inert Argon atmosphere. The samples weighted between 13 and 25 mg.

The onset temperature was mathematically determined by the intersection between the ray parallel to the plateau of the mass weight of the sample after dehydration and the ray going through the vertex of the DTG plot. The onset temperature corresponds to the start of the major sample degradation. All the graphics were plotted thanks to Origin software (OriginLab Corporation, Northampton, MA, USA).

2.7. Rheology: apparent viscosity

The apparent viscosity measurements of different maltodextrin/plasticizer mixtures, at constant temperature (80°C), were performed on a Haakee MiniLab Micro Rheology Compounder (Thermo Fisher Scientific, USA), equipped with a back flow channel, designed as a slit capillary, with two pressure transducers; one in the entrance and another one at the exit of the capillary zone. The apparent viscosity is deduced from the capillary geometry and the calculated apparent shear rate $\dot{\gamma}$, which in turn was determined from the volume flow $\dot{V}$ and the pressure drop. Around 7 and 10 g of the maltodextrin/plasticizer mixture was introduced manually and measurements were made from 219 to 821 s^{-1} shear rate gradient (corresponding to a screw speed varying from 50 to 250 rpm).

Viscosity measurements were obtained for the following compositions, maltodextrin/water 88/12 (% (w/w) and maltodextrin/plasticizer 80/20 (% (w/w). However viscosity measurements for the formulations containing 10 (%, w/w) of plasticizer were impossible because the torque alarm of the apparatus was triggered.

2.8. Dynamic Mechanical Analysis

Glass transition temperature (T_g) of maltodextrin/plasticizer films (film preparation is described below) were measured on a Triton Technology Dynamic Mechanical Analysis apparatus (Triton Technology, UK), by thermal scans in the simple geometry of the single cantilever-bending mode. The amplitude (25μm) and the multi-frequency (1 and 10 Hz) modes were kept constant during the analysis. Samples were placed in an aluminum pocket and the temperature range of analysis was set from -100°C to 200°C at a scanning rate of 2°C/minute. Therefore the mechanical properties of the samples cannot be considered but all thermal relaxations in the temperature range studied, are related to the sample inside the pocket.

Maltodextrins films were prepared by casting method. They were prepared by dissolving 20g of maltodextrin/plasticizer mixture 90/10 and 80/20 (% (w/w)) in 100 mL of tap water at room temperature and stirred with a magnetic stirrer at 1200 rpm for 10 minutes. Films were stabilized in a controlled humidity chamber set up at 60% of relative humidity and 25°C for two weeks before analysis. Experiments were run in duplicates.

3. RESULTS AND DISCUSSION

3.1. Molecular characterization of pure maltodextrins

3.1.1. DE-value assessment and molecular weight distribution

The average number molecular weight and degree of polymerization of the different types of maltodextrins used in this study were calculated thanks to the measured DE-value and were compared to the results obtained by size exclusion chromatography (Table 2).

Table 2. Molecular characteristics of different grads of maltodextrins (triplicates). Mn_{theo} = theoretical average molecular weight determined by equation (2) and DP_{theo}= theoretical degree of polymerization determined by equation (1). M_n corresponds to the number average molecular weight and M_w corresponds to the weight average molecular weight; boths were experimentally measured by SEC (n=3)

Maltodextrin DE-X	Mn (g.mol^{-1})	Mw (g.mol^{-1})	Ip	Mn_{theo} (g.mol^{-1})	DP_{theo}
DE-2	-	-	-	8589	52.9
DE-6	2225±57	25847±583	12±1	2879	17.7
DE-12	1507±24	15400±265	10±1	1479	9.0
DE-17	983±31	5672±431	6±1	1035	6.3

DE-19	937±59	4978±785	5±1	962	5.8

The molecular weight distribution of maltodextrins is crucial because it allows to get a better understanding of their behavior in terms of structural and functional properties [10]. For that reason, the determination of the molecular weight distribution of the four types of maltodextrins is at the bottom of this study. The molecular weight distribution of the analyzed maltodextrin became narrower as the DE-value increased, since the polymer chains were shorter.

It can be noticed, in Figure 1, that all maltodextrins had a bimodal molecular weight distribution; they were composed of high and low molecular weight oligo-saccharides. The first peak corresponded to the higher molecular weight polysaccharides. For maltodextrins with high DE-value, the retention time was more or less the same. However, maltodextrin DE-12 presented a larger population of high molecular weight polysaccharides than DE-17 and DE-19 maltodextrins. On the contrary, the peak of maltodextrin DE-6 presented an interesting shape.

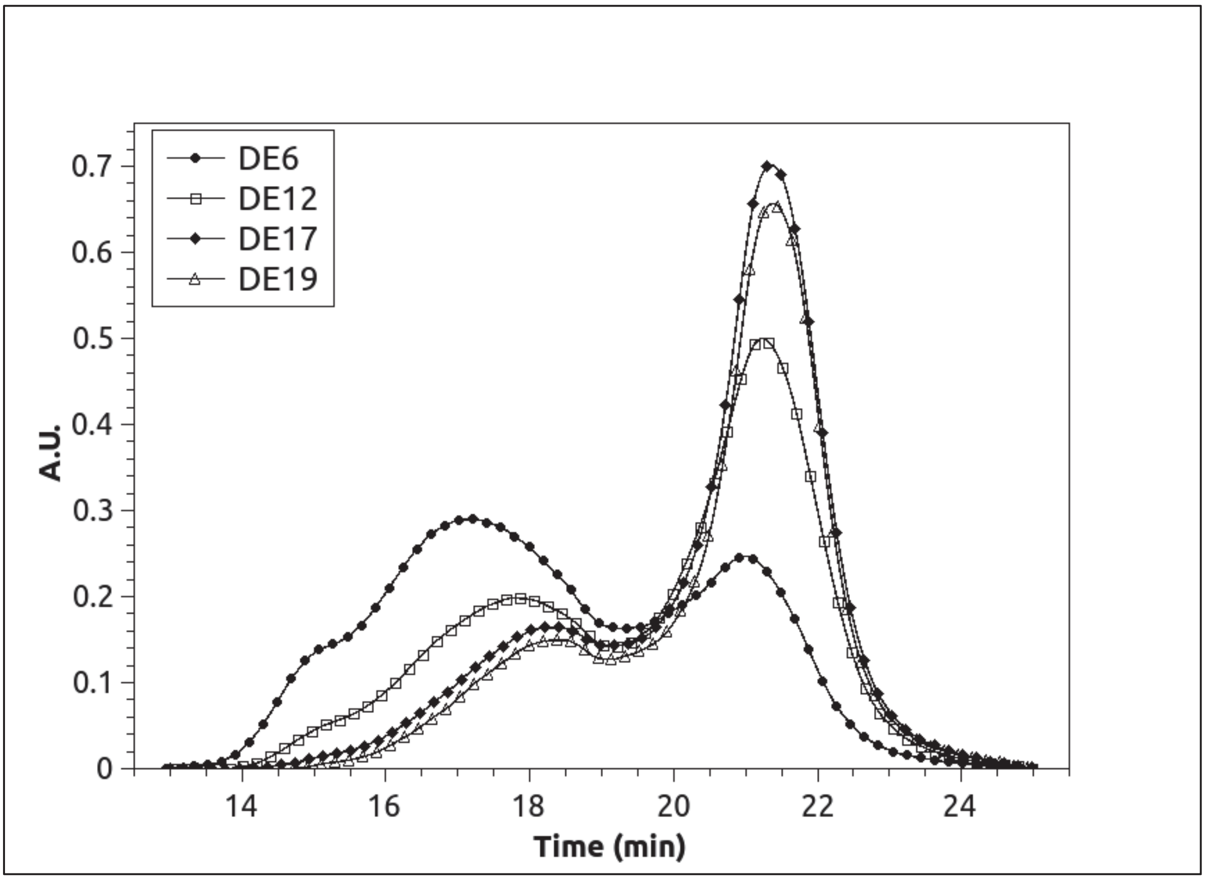


Figure 1. SEC Chromatogram of different grads of maltodextrins.

In fact, the first peak presented the shortest retention time and a shoulder peak can be appreciated at 14.5 minutes. This shoulder peak indicates the presence of even higher molecular weight polysaccharides. Thus maltodextrin DE-6 had the most important and largest population of high molecular weight polysaccharides.

On the opposite, for the second peak, representing low molecular weight oligo-saccharides, the order was almost inverted. Maltodextrin DE-6 presented a small population of low molecular weight polysaccharides. Whereas, maltodextrins DE-17 and 19 were composed of a considerable population of low molecular weight oligo-saccharides. However, maltodextrin DE-12 was the one having the lowest molecular weight oligo-saccharides, even though this population was restricted. Therefore maltodextrin DE-6 and DE-12 were the ones having the largest molecular weight distribution among all the others, confirmed by the high values of the polydispersitiy index (respectively 12 and 10) and degree of polymerization (respectively 17.7 and 9.0).

The results obtained in our study are in agreement with the literature. These commercial maltodextrins found in the market have a broad molecular weight distribution as demonstrated in the study of Dokic et al. (1998)[1]. And acid hydrolysis is known to give mixture of saccharides with a wide molecular weight range varying sometimes from monomer to polymers of the same size than starch [11].

The molecular weight distributions of maltodextrins DE-6, 12 and 19 are in agreement with the values found in the literature [1,5,10–16].

3.1.2. Hygroscopicity

In the literature some theories concerning the DE-value and the hygroscopic character of maltodextrins are exposed. In general, maltodextrins are considered to by polysaccharides with a low hygroscopic character [17]. Looking more closely at the DE-value of maltodextrins, it is expected that, when the DE-value increases, the hygroscopic character of maltodextrin is also increased, since the surface of contact is increased. This characteristic was confirmed by [11], where high molecular weight maltodextrins presented low moisture content and conversely low molecular weight maltodextrins had high moisture content. However, our results seemed to indicate two different behaviors depending on the relative humidity on which maltodextrins were exposed to and the transition zone where the behavior change occurred is comprised between 60 and 75% RH (Figure 2). Indeed in this region it appeared that the adsorption behavior of all maltodextrins changed. As a matter of fact, is in this area that any maltodextrins changed behavior. On the one hand maltodextrins DE-6 remains the most hygroscopic followed by maltodextrins DE-19. On the other hand, at 68% RH, interestingly, the three other maltodextrins intersected themselves.

In general the sorption-desorption isotherms of all maltodextrins presented a sigmoidal shape and showed a pronounced hysteresis (in this paper only sorption is represented). They were all associated to a type II sorption isotherm except for maltodextrin DE-2 that was more like a type III sorption isotherm and presented a very unique hygroscopic behavior, and will be discussed later on this paper. The transition zone ($60\leq\%RH\leq75$) marked a clear difference between the “bonded-water” bound onto the surface of the sample and the free-water (i.e. microcapillary water). Type II isotherms correspond to the general isotherm found in all food products. Meaning that for maltodextrins having a type II isotherm, water is first bond to the most polar groups onto the surface of maltodextrins, and then water is adsorbed on all the free hydroxyl groups corresponding to the hydration monolayer. Then, when all the polar sites are occupied, water molecules are bound to the monolayer through hydrogen bonding and/or Van der Waals interactions. This corresponds to the multilayer formation.

On the contrary, maltodextrin DE-2 seemed more like a type III isotherm, because in the curve there was no flattish part indicating the formation of the monolayer. In other words, for maltodextrin DE-2 there was an immediate formation of a multilayer system, with strong adsorption of “free water” observed at higher relative humidities. Perhaps, because maltodextrin DE-2 is longer and consequently has a high molecular weight, it is possible that the polymeric chain tends to entangle. Therefore absorption of water occurs in a “disorganized” way. The multilayer starts to form even though there are still hydroxyl groups available on the surface but not of easy access for the water molecules. Further information will be required to prove the organization of the polymeric chain of maltodextrins DE-2 compared to high DE-value maltodextrins.

On the contrary, for the maltodextrins of high DE-value, the multilayer starts to form once all the hydroxyl groups on the surface are occupied since they are easy to access (there is no entanglement of the polymeric chains).

For all these reasons, and to sum up, two tendencies emerge from this study.

On one hand and for relative humidities below 60% RH, moisture content increases as the molecular weight of maltodextrins increases. Herein hygroscopicity increases as the DE-value decreases. Meaning that the longer the polymeric chains are, the more hydroxyl functions are available to adsorb water. Thus maltodextrin DE-6 is the most hygroscopic. The same tendency was observed for starch and hydrolysate starch products of different molecular weight studied by [18,19] due to the fact that the longer the polymeric chain is, the more important is the affinity for water and thus, higher is the water retention in the material. When the polymeric chains are longer, there is a high probability that chains begin to entangle with each other giving rise to a disordered system and therefore increasing the free-volume.

On the other hand, and for relative humidities above 60% RH, moisture content increases as the molecular weight of maltodextrins decreases. Therefore maltodextrin DE-19 presented the most hygroscopic character among the high DE-value maltodextrins. This is in agreement with the results obtained by [11] were high DE-value maltodextrins had higher moisture content than low DE-value maltodextrins. This allied what was mentioned before, that is to say that the shorter the polymer chains are, more important is the surface area of exchange.

Maltodextrins DE-2 and DE-12 have particular sorption isotherm depending on the relative humidity of the environment meaning perhaps significant changes on their macromolecular structure. Maltodextrin DE-2 behaves more as long-chain than as a small-chain polymer. In fact, its sorption isotherm is very much a like as the ones obtained for starches [20,21].

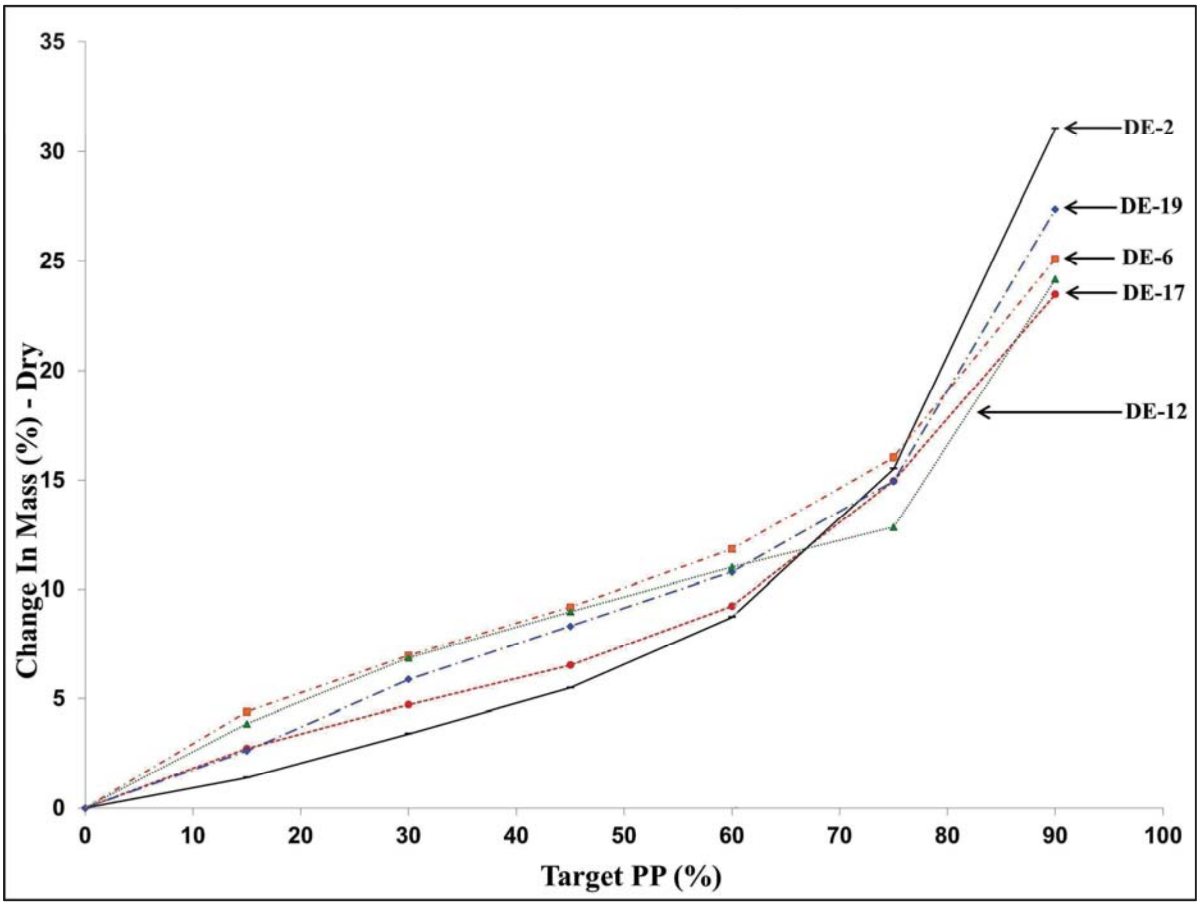


Figure 2. Vapor sorption isotherm of the various types of maltodextrins.

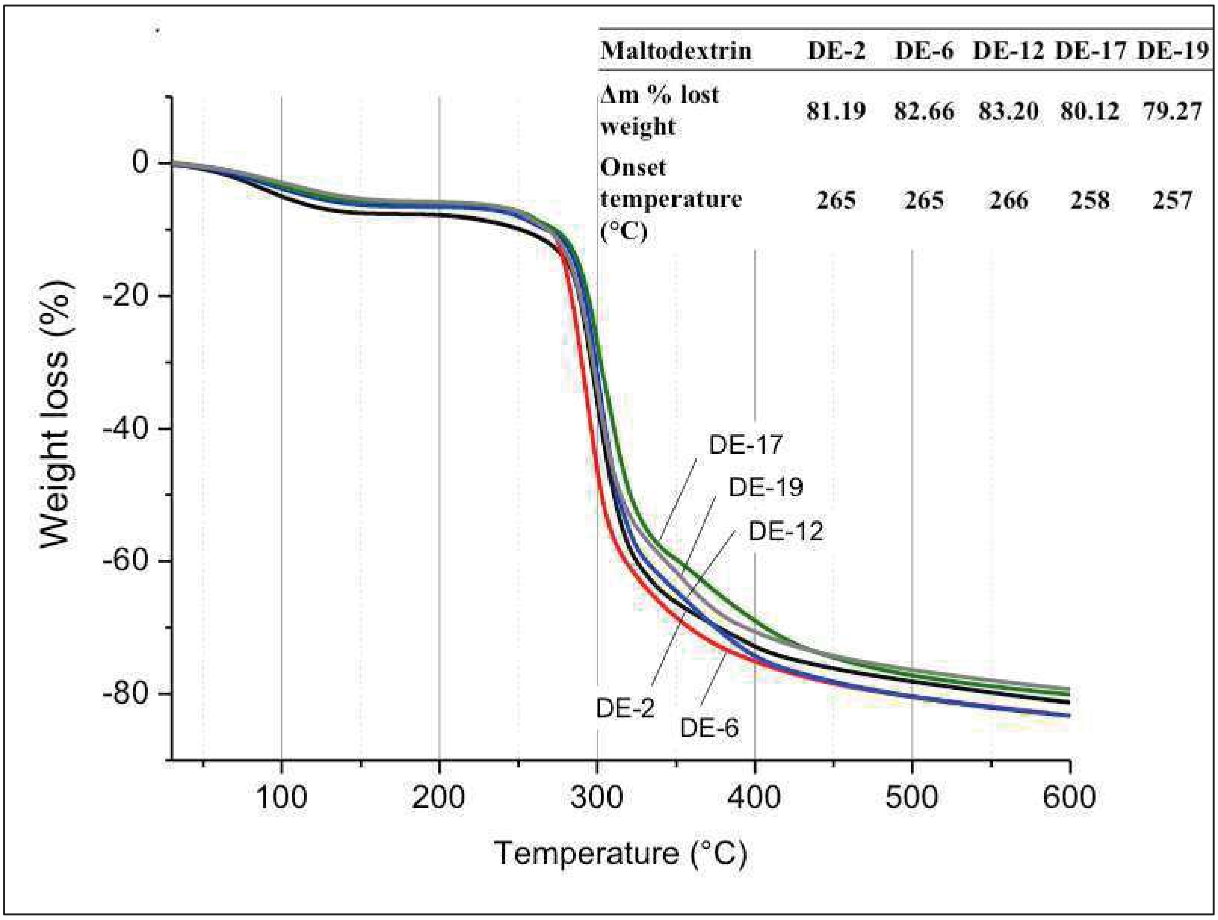
The sorption isotherm permits to have a general idea of the hygroscopic character of the material and thus predict the shelf-life stability of the material under specific environmental or process conditions [12]. In this case for example, maltodextrin DE-12 will be more stable than maltodextrin DE-2 when exposed to environment above 60% RH. In addition if the final application is targeting a delivery system that must be highly hygroscopic, maltodextrin DE-6 and DE-12 will be privileged over others (off course for relative humidities below 60% RH). The same remarks was pointed out in the study of [3] where high molecular weight oligosaccharides lead to an increased moisture absorption. However it was reported in other study that when maltodextrins were exposed to high relative humidities (90% RH), the affinity to water for low DE-value maltodextrin increased. Maltodextrin DE-4 had a moisture content of 6% (w/w) opposed to maltodextrin DE-15 which had a moisture content of 3% (w/w) [11]. Special attention must be given when comparing all these different studies because the botanical origin of maltodextrin and the type of hydrolysis are crucial parameters affecting the final physicochemical properties of maltodextrins. This can be one of the reasons explaining such differences disclosed in the literature.

3.1.3. TGA

Thermogravimetric analysis allows determining the thermal stability of each maltodextrin. In general, all the maltodextrins presented the same dehydration and decomposition phases corresponding to the classical thermogravimetric profile of carbohydrates [22,23]. As it is represented in Figure 3, the first stage (temperature range from 25°C to 150°C) is associated to the dehydration of maltodextrins, which is a small weight loss. For the five analyzed maltodextrins the weight loss fitted perfectly to the moisture content measured in the sorption isotherms (Figure 2). The second stage corresponds to the region where decomposition reactions take place and where the major weight loss of the samples occurred. Since the analyses where run on an inert atmosphere, the total degradation of maltodextrins to the ash content was not completed (this part being related to the third stage, the completely degradation of the sample).

Maltodextrin DE-6 showed a more slightly rapid decomposition than the other types of maltodextrins, meaning that in this particular case the lower the DE-value is, the more rapid the decomposition will be. At the end of the pyrolysis reactions maltodextrin DE-19 had lost 79.27% of the initial weight and maltodextrin DE-6 and DE-12 have lost around 80 to 83% of their initial weight respectively.

As stated in the thermogram above, the five maltodextrins could withstand temperatures up to 250°C without fearing their degradation. This implies that temperature can be one of the process parameter, in twin-screw extrusion for example, to be modified in order to regulate the viscosity or the glass transition temperature of the system. Off course, special attention must be given when extrapolating the temperature conditions to extrusion because this thermal analysis was made at inert atmosphere.



Maltodextrin	DE-2	DE-6	DE-12	DE-17	DE-19
Δm % lost weight	81.19	82.66	83.20	80.12	79.27
Onset temperature (°C)	265	265	266	258	257

Figure 3. TGA of different grades of maltodextrins.

3.2. Rheology and DMA analysis of maltodextrin/plasticizer mixtures

3.2.1. Rheology: apparent viscosity measurements

Maltodextrins are highly soluble in water and thus measurement of their viscosity is classically performed in solution but the characterization of their apparent melt viscosity was quite a challenge. Small amounts of water and plasticizer (not exceeding more than 20 (%, w/w)) were employed. After several trials, the chosen process temperature was 80°C to keep it as low as possible for future encapsulation of thermolabile active compounds. Also to allow measurements of the viscosity of high molecular weight maltodextrins, because for temperatures below 80°C their viscosity was too high. Indeed, for high molecular weight maltodextrins 80°C was the perfect temperature to have a non-Newtonian fluid behavior, whereas at higher temperature there was no viscosity at all (for the same amount of plasticizer). For these reasons the temperature 80°C was found to be ideal because allowing viscosity measurements for this wide range of maltodextrins. These results accentuated the fact that maltodextrins can be extruded at low temperature. D-sorbitol and glycerin were used at two different ratios 10 and 20 % (w/w). And water constituted the third plasticizer added at a ratio of 12 (%, w/w). It was not possible to test D-sorbitol, glycerin, and water at the same ratios because the viscosity behavior for each plasticizer was not the same. For example, viscosity measurements were not possible for mixtures of maltodextrins containing 10% (w/w) of water only. Besides, in the case of water at 20% (w/w) the mixtures behaved as a Newtonian fluid thus no viscosity was able to be measure.

The apparent viscosity (η) of the different maltodextrin/plasticizer mixtures was determined by the Power-law or Ostwald-de Waele equation:

$$\eta = K.\dot{\gamma}^{m-1} \tag{3}$$

The apparent viscosity η (Pa.s), K is the flow consistency index (Pa.s^{m}), $\dot{\gamma}$ shear rate (s^{-1}) and m is the power-law index. The flow consistency index (K) corresponds to the value of the viscosity for a 1 s^{-1}shear rate equals. And the power-law index, m, indicates the type of fluid based on their flow behavior with respect to a Newtonian fluid. Therefore, for m values below 1, samples are considered as pseudoplastic or shear thinning fluids. According to this, all the maltodextrins/plasticizer formulations tested behaved as shear thinning fluids. In general, the results showed that the pseudoplastic index varied conversely to the flow consistency index Table 3. Moreover, the values of the apparent viscosity of all formulations were more or less in the same order of magnitude (Figure 4).

Table 3. Flow consistency and power-law indexes of the maltodextrins mixtures with 12% (w/w) of water and 20% (w/w) of plasticizer.

Formulation	K (Pa.s)	m	R²
Maltodextrine DE-2 + water 12% (w/w)	39875	0.06	0.99
Maltodextrine DE-6 + water 12% (w/w)	22636	0.07	0.94
Maltodextrine DE-12 + water 12% (w/w)	33838	0.09	0.99
Maltodextrine DE-17 + water 12% (w/w)	334	0.64	0.98
Maltodextrine DE-19 + water 12% (w/w)	942	0.53	0.95
Maltodextrine DE-2 + glycerol 20% (w/w)	24300	0.13	0.99
Maltodextrine DE-6 + glycerol 20% (w/w)	23950	0.13	0.98
Maltodextrine DE-12 + glycerol 20% (w/w)	132	0.76	0.92
Maltodextrine DE-17 + glycerol 20% (w/w)	61	0.85	0.79
Maltodextrine DE-12 + D-sorbitol 20% (w/w)	18059	0.15	0.96
Maltodextrine DE-17 + D-sorbitol 20% (w/w)	20649	0.11	0.96
Maltodextrine DE-19 + D-sorbitol 20% (w/w)	270	0.43	0.84

When exposed to the same moisture content 12% (w/w) and at the same temperature 80°C maltodextrin DE-2 had the highest viscosity among the other maltodextrins (Figure 4). The flow consistency index of maltodextrin DE-6 was 22636 Pa.s and was 33838 Pa.s for maltodextrin DE-12, which indicates that viscosity of maltodextrin DE-6 was lower than the viscosity of maltodextrin DE-12. Though, maltodextrin DE-12 was supposed to have a lower viscosity since its average molecular weight is smaller than the average molecular weight of maltodextrin DE-6 (respectively 15400g.mol^{-1} and 25847 g.mol^{-1}). Maltodextrins with low DE-value are expected to have a higher viscosity [10]. For instance, [1] also found unexpected behavior related to the viscosity of concentrated maltodextrin solutions. In fact, maltodextrin DE-25 had a viscosity higher than maltodextrin DE-15. This discrepancy is explained due to the higher percentage of longer linear chains and broader molecular mass distribution of maltodextrin DE-25 compared to maltodextrin DE-15. In our case, even though maltodextrin DE-6 had a more important part of high molecular weight polysaccharides than maltodextrin DE-12, the polydispersity indexes of both were about of the same range (respectively 12 and 10). Meaning that both maltodextrins had broader molecular mass distributions. Based on the power law index, maltodextrin DE-2, DE-6 and

DE-12 had similar values (Table 3) very closed to 0, revealing an important entanglement of the polymeric chains due to high molecular weight oligomers.

Related to maltodextrin DE-17 and DE19, the power-law index indicates that they were the ones presenting a more plastic character among the other three maltodextrins and very low entanglement of the polymeric chains. Additionally, interesting results were obtained for maltodextrin DE-19 at 20% (w/w) of glycerin. In fact glycerin is such a good plasticizer that for maltodextrin DE-19 the mixture behaved as a Newtonian fluid (viscosity did not decrease when increasing shear rate).

Glycerin appeared to be a better plasticizer than D-sorbitol since it decreased considerably the viscosity for the same type of maltodextrin. For example, considering the same amount of plasticizer, the values of the flow consistency and the power-law indexes for maltodextrin DE-12 were much lower for glycerin (K=132 Pa.s, m=0.76) than for D-sorbitol (K=18059 Pa.s, m=0.15). Another argument demonstrating that glycerin was a better plasticizer than D-sorbitol is the fact that for high molecular weight maltodextrins (e.g. maltodextrin DE-2 and DE-6) viscosity measurements were possible only for mixtures containing 20 (%, w/w) of glycerin.

With regard to the DE-value and viscosity (no matter how it is measured) there is a linear dependency [1,2,10]. The viscosity of the mixture decreases as the DE-value increases. Based in our results, this linear dependency between DE-value and viscosity is respected for the mixtures containing 20% (w/w) of plasticizer. However in the case of water at 12% (w/w) this linearity is not respected. Indeed maltodextrin DE-19 presented a higher viscosity than maltodextrin DE-17 maybe because it has a more important population of high molecular weight polysaccharides than maltodextrin DE-17.

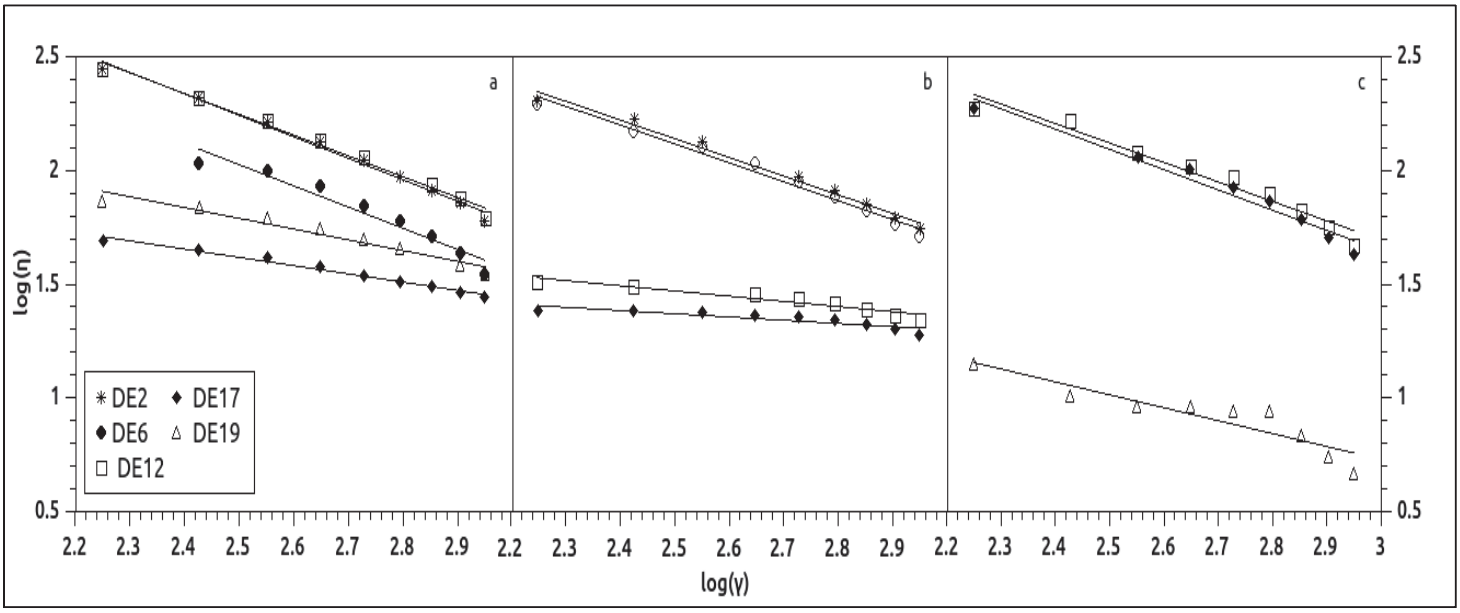


Figure 4. Apparent viscosity of the different mixtures of maltodextrin/plasticizer tested at 80°C:

a) Maltodextrins + water12% (w/w), b) Maltodextrins + glycerin 20% (w/w), c) Maltodextrin + D-sorbitol 20% (w/w)

3.2.2. DMA

Glass transition temperature (T_g) has been longtime used as an accurate indicator for food matrices stability [24]. Indeed, T_g behavior influences the properties of food materials like texture, taste and off course shelf-life stability during the stocking conditions and, last but not least, it served as an indicator to determine the melt extrusion process parameters (Sablani, Kasapis, & Rahman, 2007). Molecular weight of the material, water content and process temperature are the main parameters affecting the T_g. For those reasons, for the last 80 years, the T_g of mono- and oligosaccharides have been exhaustively studied since they are the main ingredient of food products [26–30,30].

Maltodextrins are very rigid and brittle materials and therefore their characterization and workability have been a challenge. Even though several thermodynamic models have been developed [16,28,31,32] in order to determine by extrapolation the glass transition temperature of pure and dry maltodextrins, there's still a gap between the experimental values found and those determined by the existing mathematical models [2,13,15,24,26,27]. After all, the determination of the glass transition temperature has always been a challenge in material science, specially the glass transition temperature of carbohydrates. Recent studies have pointed out the importance of the mass transfer involving the polymer and its plasticizer, and the impact of the transient moisture content of the broadening of the measured glass transition [16]. Based on the literature, the glass transition temperature of maltodextrins decreases when the DE-value increases, since the length of the polymer chain is lower (DP is lower). In this part, special attention is given to the glass transition temperature of the maltodextrin/plasticizer mixture to investigate the influence of the DE-value and of the plasticizer nature.

Figure 5 represents the classical thermogram obtained by DMA. In this chart, each plot is associated respectively to a mixture of a specific maltodextrin/plasticizer, in this case D-sorbitol at 10% (w/w).

The thermograms of all the maltodextrin/plasticizer films presented the same shape; especially two relaxations, α and β were observed (Figure 5). In this paper only the thermogram for maltodextrin/D-sorbitol 10 (%, w/w) is illustrated. The first relaxation, noted α, is associated to polymer rich region and corresponds to the relaxation on the right of the thermogram. And the second relaxation, β, is associated to plasticizer rich phase and is the one found on the left side of the thermogram [10,33].

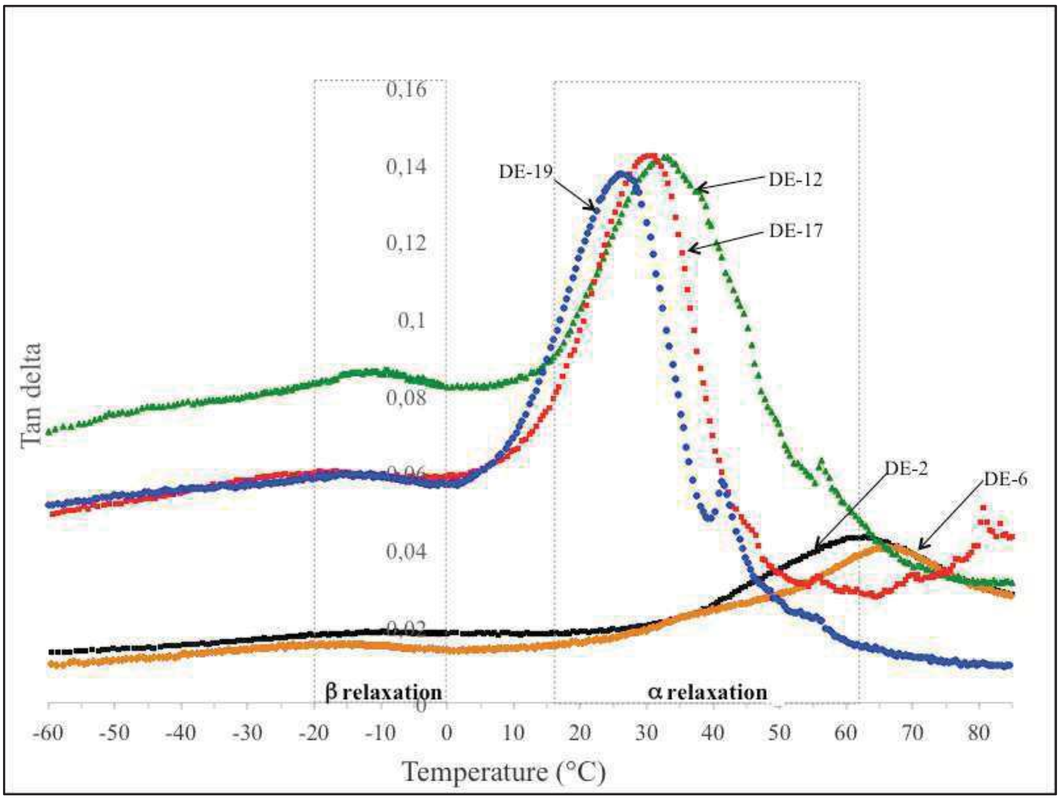


Figure 5. Thermogram obtained by Dynamic Mechanical Analysis for different grades of maltodextrins with 10% (w/w) of D-sorbitol.

In our case, the classical model of Couchman-Karaz [31] used for the determination of the glass transition temperature of an homogeneous blend constituted of two components, does not fit our experimental results. The values of the β relaxation temperature measured were in agreement with the thermic relaxation associated to pure sorbitol -3°C, pure glycerin -52°C and pure water -137°C [33]. The relaxation on the right corresponded to a relaxation proper to the polymer and known to be representative of the glass transition temperature. Herein, noted as relaxation α (Figure 5). In this study particular attention is given to the α relaxation because it controls the product final properties.

The role of plasticizers is to increase the mobility of the polymeric chains by introducing themselves between the polymeric chain, and creating H-bond interactions plasticizer/polymer [34]. This allows opening the polymeric chains and increasing the mobility and thus the free volume of the polymer. When the free volume of the polymer is increased, the viscosity of the polymer is then reduced and hence the glass transition temperature is also decreased. So, a plasticizer is classified as an excellent plasticizer when the free volume of the polymer is increased and thus the viscosity and the glass transition temperature are reduced.

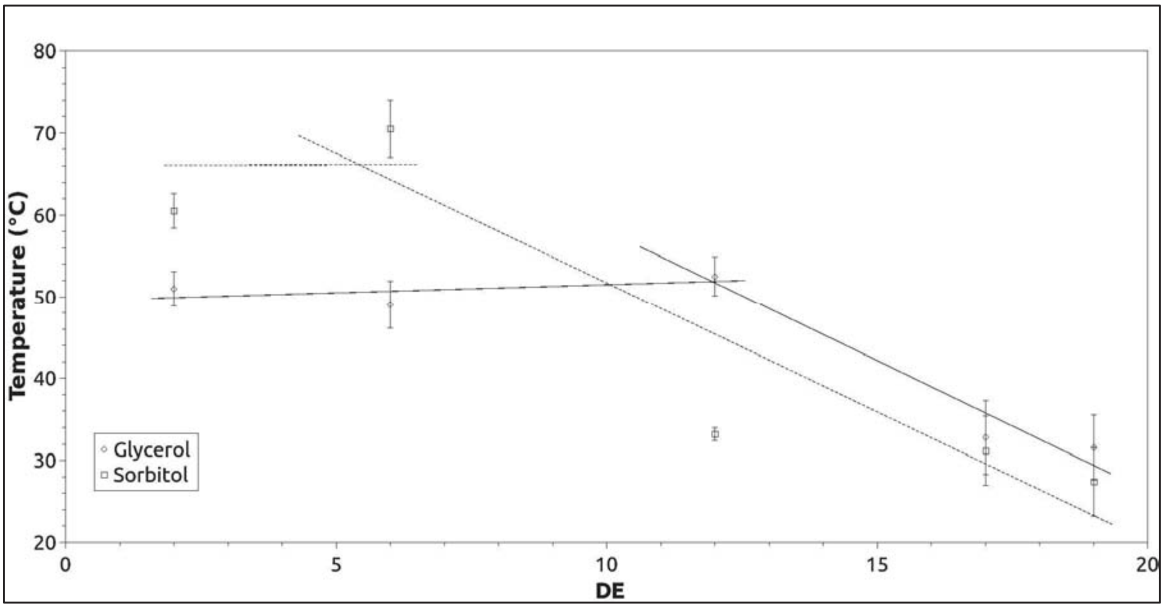


Figure 6. Linear dependencies Tg-DE-value; Tg measured for maltodextrins/plasticizer mixtures 10% (w/w) of plasticizer (n=2).

For all the compositions containing 10 or 20% (w/w) of plasticizer, it is clear that there was a trend depending on the DE-value. Maltodextrin DE-12 could be considered as the hinge element of the two tendencies observed. For low DE-value, glycerin appeared to be a better plasticizer than D-sorbitol since both of the relaxation temperatures were decreased (Table 4 and 5). However, for high DE-value maltodextrins, D-sorbitol was a better plasticizer than glycerin since the α relaxation is slightly lower than for those with glycerin. This trend can clearly be observed thanks to Figure 6, representing only the glass transition temperatures of the systems containing 10% (w/w) of plasticizer.

Table 4. Relaxation temperatures and moisture content of the mixtures maltodextrin/plastizicer at 10% (w/w).

Maltodextrin DE-value	Plasticizer (10 %, w/w)	T_α (1Hz) °C	T_β (1Hz) °C	Moisture content % (w/w)
Maltodextrin DE-2	glycerin	51	-59	10.6±0.2
	sorbitol	62	-10	10.0±0.2
Maltodextrin DE-6	glycerin	49	-58	9.4±0.5
	sorbitol	68	-17	10.0±0.2
Maltodextrin DE-12	glycerin	55	-55	8.7±0.4
	sorbitol	33	-12	8.7±0.2
Maltodextrin DE-17	glycerin	35	-55	8.6±0.7
	sorbitol	30	-15	12.3±0.5
Maltodextrin DE-19	glycerin	30	-55	9.3±0.3
	sorbitol	26	-15	9.8±1.4

Table 5. Relaxation temperatures and moisture content of the mixtures maltodextrin/plastizicer at 20% (w/w).

Maltodextrin DE-value	Plasticizer 20% (w/w)	T_α (1Hz) °C	T_β (1Hz) °C	Moisture content % (w/w)
Maltodextrin DE-2	glycerin	15	-51	12.9±0.1
	sorbitol	39	-12	9.8±0.2
Maltodextrin DE-6	glycerin	10	-52	10.3±0.1
	sorbitol	41	-12	10.3±0.1
Maltodextrin DE-12	glycerin	13	-30	10.6±0.0
	sorbitol	33	-12	8.9±0.2
Maltodextrin DE-17	glycerin	38	-40	10.8±0.3
	sorbitol	30	-15	8.8±0.3
Maltodextrin DE-19	glycerin	26	-45	10.5±0.1
	sorbitol	26	-15	9.0±0.0

In the presence of glycerin at 10% (w/w) the α relaxation decreased when the DE-value increased except for DE-12. The same tendency was observed for D-sorbitol, the α relaxation decreased when the DE-value increased except for maltodextrin DE-6. For both plasticizers, the measured values for the β relaxation temperature were more or less of the same range and did not change much when the DE-value or the amount of plasticizer was increased.

However, for maltodextrins films with 20% (w/w) of glycerin, results were unexpected, since the glass transition temperature increased with the DE-value. Indeed, the α relaxation increased and the β relaxation decreased as the DE-value increased. This behavior was associated to a segregation phenomenon. The blend was more like a heterogeneous mixture and thus glycerin acted as antiplasticizer agent. This plasticizer/antiplasticizer behavior has already been noticed for D-sorbitol used in starch-based films. For example, at low sorbitol content (below 27 (%, w/w)) sorbitol acts as an antiplasticizer by increasing the glass transition temperatures of the films [33]. For example, in the case of maltodextrin DE-17, the α relaxation increased when the amount of glycerin increased demonstrating that segregation occurs and thus the antiplasticizing effect of glycerin.

Moisture content of films containing 10 (%, w/w) of plasticizer (Table 4) and films containing 20 (%, w/w) of plasticizer (Table 5) were about the same range (from 8.6 to 12.9 (%, w/w)). Glycerin films presented higher moisture content than sorbitol films. Clearly because glycerin is more hygroscopic than sorbitol and as a consequence it has a higher capacity to adsorb water than sorbitol films [35]. This was in agreement with other studies, where glycerin plasticized films of starch, gluten and whey protein presented higher moisture content than sorbitol films [17,35–37]. Two tendencies were observed; for low DE-value maltodextrins in the case of glycerin the moisture content decreased as the DE-value increased. On the contrary for sorbitol, the moisture content increases as the DE-value increases. As for the high DE-value maltodextrin there was no relevant behavior to stress out since the moisture content remained constant as the DE-value increased.

3.3. Global outline of maltodextrins behavior

The first important thing to remember of this study, before going further on the discussion, is that the use of the DE-value as a predicting tool for description of the physicochemical properties of maltodextrins is not completely the most appropriate. The degree of dextrose is a necessary parameter but not sufficient to predict the physicochemical properties of maltodextrins, given that it does not take into account the bimodal molecular weight distribution of these materials and their polydispersity (Figure 1).

There were clearly two different behaviors observed depending on the DE-value. The gap seems to be marked by maltodextrin DE-12, in the case of hygroscopicity, glass transition temperature and viscosity. Maltodextrin DE-6 and DE-12 had similar viscosities behaviors that could be explained by the their very dispersive molecular weight profile, both had the highest dispersive index and degree of polymerization. Conversely, the similarities of the molecular weight profile of maltodextrin DE-17 and DE-19 were the basis of unexpected glass transition and viscosity behaviors.

Maltodextrin DE-2 was taken apart from the other four maltodextrins since it presented a completely different behavior relating to hygroscopicity and degradation. Its behavior recalled the behavior of long chain polysaccharides more like amylose or amylopectin.

Rheological and DMA results have demonstrated that glycerin is a better plasticizer than D-sorbitol for all types of maltodextrins. For DMA tests, both the α and β relaxations presented lower values for films containing glycerin than for those containing D-sorbitol. Besides, the formulations containing glycerin presented the lowest viscosity value compared to the formulations made with D-sorbitol. Indeed, D-sorbitol has a more significant steric hindrance than glycerin, thus it is more difficult to fit within the polymer chains. Besides, for the same mass of plasticizer weighted, as D-sorbitol has a higher molecular weight than glycerin, less molecules of D-sorbitol were present in the mixture and so its plasticizer effect was lower. Our findings confirm previous results where glycerin was also found as a better plasticizer than D-sorbitol for starch-based materials [17].

It is hard to establish a direct correlation between the DE-value and the glass transition temperature even though, for some studies, there is a linear correlation [2,10,15]. The difficulty to establish this correlation is based in on the fact that the DE-value does not take into account that maltodextrins are composed of a bimodal molecular weight distribution. Expressed differently, maltodextrins are composed of a mixture of different DP-fractions and the DE-value omits this fact. Also, the DE-value neglects the molecular structure of maltodextrins, and it has been demonstrated that linear chains give rise to a higher glass transition temperature than branched chains of the same weight average molecular weight [2]. Not to mention, that related to viscosity, linear chains have a lower viscosity than branched chains. In line with what was just mentioned, maltodextrin DE-12 had a significant proportion of high molecular weight polysaccharides that could be branched and therefore be responsible of the important viscosity. Or, in terms of molecular weight distribution maltodextrin DE-19 had a more important population of high molecular weigh polysaccharides and therefore its viscosity was more important than maltodextrin DE-17's viscosity at 12% (w/w) of moisture content (Figure 4). For this reason, it is more rigorous to correlate the whole molecular weight distribution of maltodextrins to the viscosity and the glass transition temperature.

For example, the glass transition temperature of maltodextrin DE-12 (55°C) was higher than the glass transition temperature of maltodextrin DE-17 (35°C) at the same amount of glycerin at 10% (w/w) (Table 4). However increasing the plasticizer amount of 20% (w/w) completely changed the trend, glass transition temperature of maltodextrin DE-12 (13°C) was lower than glass transition temperature of maltodextrin DE-17 (38°C) (Table 5). Taking a deeper look inside the others maltodextrins, it is imperative to remark that there was an optimal plasticizer content for each maltodextrin. For example in the case of glycerin; for maltodextrin DE-2, increasing the plasticizer content allowed to decreased the α relaxation temperature, acting as a plasticizing agent. However for maltodextrin DE-17, increasing the glycerin content induced an increase of the α relaxation temperature acting as antiplasticizing agent (Figure 6). For D-sorbitol, increasing the amount of plasticizer did not influence the α relaxation, for high DE-value maltodextrins. This seems to imply, that for each DE-value maltodextrins there was an optimal amount of plasticizer.

For the sorption isotherm maltodextrins DE-12 and DE-6 were the most hygroscopic among the other maltodextrins for relative humidities below 60% RH because they were the ones having a more important population of high molecular weight polysaccharides. This was expected as the higher the degree of polymerization is, the higher the amount of bounded water is. In addition, maltodextrin DE-19 was more hygroscopic than maltodextrin DE-17 because it had a more important population of high molecular weight polysaccharides. It seems that for relative humidities below 60% RH, high molecular weigh polysaccharides are responsible for the water adsorption. In contrast, for relative humidities above 60% RH low molecular weigh polysaccharides are responsible of water adsorption, reveling a structural change in the macroscopic organization when the threshold humidity is past. Additional experiments, as X-ray diffraction, will be interesting to verify the organization of such carbohydrates according to their molecular weight and moisture content.

4. CONCLUSION

To better understand the behavior of maltodextrins and hence adapt the extrusion process conditions, the DE-value is not the only parameter to take into account because in some particular cases it does not predict the behavior of Tg or the viscosity in specific environments. It is better to trust the molecular weight distribution or the sorption isotherm in order to get a better understanding.

It is very important not underestimate the botanical origin of starch, as well as the amylose/amylopectin ratio since both parameters directly affect the molecular weight distribution of maltodextrins, and thus their physicochemical properties. Special attention needs also to be given to the type of hydrolysis since it will determine the molecular weight distribution of maltodextrins. An acid hydrolysis will give a broader molecular weight distribution conversely to the enzymatic hydrolysis. Nowadays enzymatic cocktails (amylase and pullulanase enzymes) combine with acid hydrolysis are employed in order to obtained maltodextrins with a more accurate molecular weight profile.

The role of a plasticizer in general is to improve the processability of a mixture, in both ways by allowing setting up softer processing conditions (in terms of temperatures or mechanical strength by decreasing the viscosity) and also by enhancing the incorporation and dispersion of active agents. As a consequence, the flowability of the mixture being extruded is increased.

The most important thing worth noting in this study, based on the rheological measurements, moisture content and off course, the DMA relaxations temperatures, is that glycerin is a better plasticizer than D-sorbitol. This is because glycerin is a very low molecular weight molecule and highly hygroscopic therefore, it binds easily to water and to the polymeric chains by H-bond interactions. As a consequence, glycerin allows a better disentanglement of the polymeric chains, enhancing water adsorption.

However maltodextrins are very complex materials and their window of processability is very narrow. If the plasticizer content is to below 10% (w/w) maltodextrins are brittle and unstable. And if the plasticizer content is above 20% (w/w) maltodextrins are too ductile to be handle. We want to bring the attention to the fact that maltodextrins can be extruded at low temperature, which changes operating conditions that are commonly used. This brings a large range of applications without fearing degradation of thermolabile active compounds and reducing Maillard's reaction from taking place in carbohydrates-based materials. And last but not the least, extrusion of maltodextrins at low temperature is an appealing economical argument. Under these circumstances, maltodextrin DE-12 for example, seems to be an efficient raw material for melt extrusion applications. While maltodextrin DE-19 is more appropriate to be used for spray drying due to its lower viscosity and its hydrophilic character at high relative humidities[38,39].

Acknowledgments

Special thanks to Christophe Drouet from the Cirimat for the TGA analysis and Guadalupe Vaca-Medina for the DVS analysis and the precious advises.

Givaudans S.A.S France, for supporting this research project.

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Transition chapter 2 to chapter 3

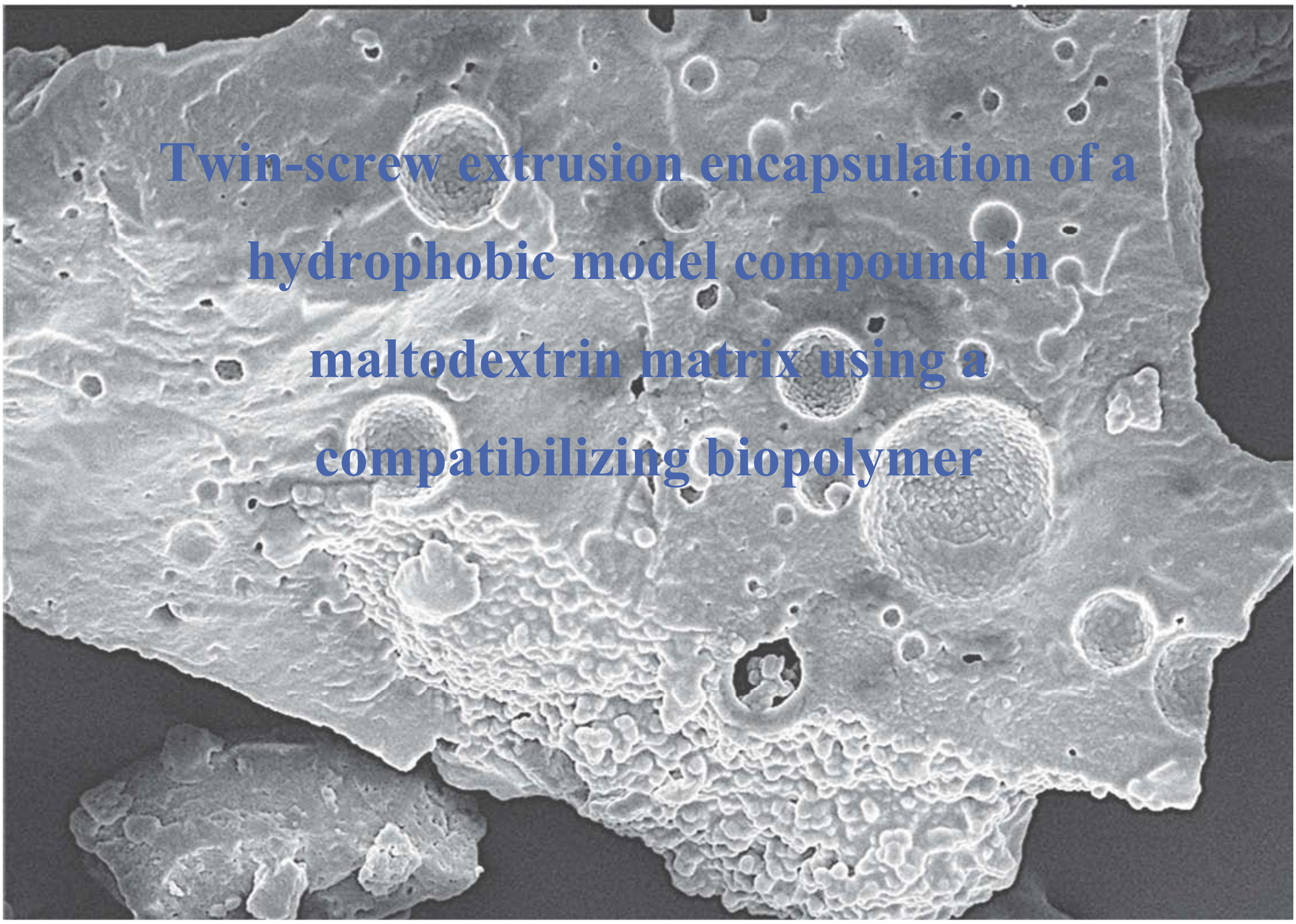
Maltodextrins were selected as the wall material for the new bio-based delivery systems. According to the specifications of the final product, which were a highly hygroscopic and glassy matrix at room temperature, and based on the results obtained on the viscosity, thermal and sorption isotherm measurements, maltodextrin DE-12 was selected as the best suited for this application.

Indeed, maltodextrin DE-12 presented an adequate viscosity at 12% (w/w) moisture content at 80°C and presented the highest hygroscopic character when exposed at relative humidities comprised between 30-75% RH. Besides, at the two distinct amounts of plasticizers, maltodextrins, in general, were at a glassy state at room temperature.

In the next chapter, the determinations of the extrusion processing conditions were addressed. In one hand, the temperature and screw profile and additionally, the moisture content were established so as to have the best compromise between the viscosity and the texture of the blend. And in another hand, the maximum amount of active ingredient that could be incorporated was also determined. The selected encapsulated material was MCT-oil, a model hydrophobic compound used to optimize and validate the extrusion and encapsulation process. Once these four parameters were optimized, characterizations of the obtained bio-based delivery systems were examined.

Furthermore, comparison between a classical compatibilizing agent, such as a modified starch, and a non-common one, pea protein isolate (=PPI) was evaluated notably regarding the maximal incorporation of the MCT-oil and the flowability of the mixture during extrusion.

CHAPTER 3



Twin-screw extrusion encapsulation of a hydrophobic model compound in maltodextrin matrix using a compatibilizing biopolymer

Twin-screw extrusion encapsulation of a hydrophobic model compound in maltodextrin matrix using a compatibilizing biopolymer

Natalia Castro[a, b], Christine Raynaud[a, b], Antoine Rouilly[a, b] , Vanessa Durrieu[a, b]

[a] *Université de Toulouse, INP-ENSIACET, LCA (Laboratoire de Chimie Agro-industrielle), F-31030 Toulouse, France*

[b] *INRA, UMR-1010-CAI, F-31030 Toulouse, France*

Email: Antoine.rouilly@ensiacet.fr

Phone number: (+33) 5 34 32 35 09

Fax: (+33) 5 34 32 35 97

Abstract

In the last decades, the incorporation of phytochemical or nutraceutical components into carbohydrate-based matrix using twin-screw extrusion has become a promising encapsulation technology. Carbohydrates (e.g. starch, maltodextrins) are the most recurrent materials employed in the food and pharmaceutical domains for the encapsulation of active components. However, the influence of all the process parameters on the properties of the final product remains a challenge and is quite difficult to estimate. Even though, there are now several groups of research interested in the elucidation of the effect of extrusion parameters (e.g. screw speed, screw geometry, and feed rates…) on the functional properties and microstructure of the final product, there is still a lot of things to explore and discover in this field. Therefore, in this paper, special attention has been given to the incorporation of a hydrophobic model compound (medium chain triglyceride oil = ***MCT-oil****), in a maltodextrin matrix with a compatibilizing biopolymer. A comparison between four different formulations and the influence of the MCT-oil content have also been investigated.*

Promising results were obtained comparing pea protein to a commonly used compatibilizer (octenyl succinate hydrolysate starch = ***Hi-cap100****) especially concerning the physicochemical properties (i.e. hygroscopicity, glass transition temperature) of the delivery* *system and the encapsulation rates of the MCT-oil (up to 90% of encapsulation efficiency for a MCT-oil load of 12%). Moreover, twin-screw extrusion has proved to be a very suitable and versatile technology to be employed in this encapsulation domain.*

Key words: *Twin-screw extrusion, medium chain triglycerides oil, maltodextrin, encapsulation, pea protein isolate*

1. INTRODUCTION

As mentioned in Chapter 1, extrusion has proved its great performances in different domains since it is a versatile and low cost technology [1,2]. In particular, in the food and pharmaceutical industries, where it has demonstrated its efficiency for encapsulation applications [3,4]. Even though the encapsulation rates of extrusion is claimed to be around 12% [5], that compared to spray-drying, is still a low encapsulation efficiency, the number of extruded delivery systems continues to grow [6]

Indeed, spray drying has been for more than five decades the most employed technology, at an industrial scale, for the encapsulation of nutraceutical, flavors, pigments or pharmaceutical active compounds as it is a versatile and low-cost technology, allowing high load of actives [7–11].

However, extrusion encapsulation seems a good alternative to counter the hegemony of spray drying. As mentioned above, extrusion is a versatile technology that can be easily adapted to a large variety of wall and active ingredients, in order to obtain in one step process, a diverse range of products with different textures and shapes [3]. In fact, recent studies have reported that, in the flavor industry for example, around 2-3% of their products were elaborated by melt extrusion. This demonstrates that extrusion has gained ground in the encapsulation field, and revealed itself, as a promising technology [3,12,13].

For this reason, and based on the know-how earned in the food industry and the bio-based materials from starch produced by melt extrusion [14–16], we decided to employ this technology to elaborate a new bio-based delivery system.

Several studies have reported carbohydrates as the most recurrent wall material employed in flavor encapsulation [13,17–19]. The benefits of using carbohydrates as wall materials are based on the following properties:

- Limit the molecular diffusion of labile molecules so as to increase their stability during storage [4,20] ;
- Lower viscosity values (for starch hydrolysate derivatives [21]) ;
- Excellent protection against oxidation (e.g. for encapsulation of oils [22,23]) ;
- Good bulk properties;
- Inexpensive.

The objective of this study was to propose an innovative bio-based delivery system elaborated by twin-screw extrusion to encapsulate hydrophobic compounds. This new delivery system had to be highly hygroscopic, in order to easily release the active compounds when exposed to different relative humidities. And obviously, the delivery system had to be at a glassy state at room temperature, so the active compound can be protected from external degradations.

According to this, MCT-oil and maltodextrins were respectively chosen as model hydrophobic compound and main wall material for this new bio-based delivery system. Four mixtures were selected as the new bio-based delivery systems:

- **A-10:** Maltodextrin I-12/pea protein (90/10): containing 10% (w/w) of PPI;
- **B-15:** Maltodextrin I-12/pea protein (85/15): containing 15 % (w/w) of PPI;
- **C-1:** Maltodextrin I-12/pea protein/ modified starch (89/10/1): containing 10% (w/w) of PPI and 1% (w/w) of Hi-cap100;
- **D-5:** Maltodextrin I-12/pea protein/modified starch (85/10/5): containing 10%of PPI and 5% (w/w) of Hi-cap100

However, the major draw back of carbohydrates is their poor emulsifying properties, which induce low flavor load. Hence, two biopolymers (pea protein or modified starch) were tested as compatibilizing agent. To differentiate our work from other studies, we decided not to use any additional emulsifying agents. Under these circumstances, we could focus on the influence of the formulation on the extrusion conditions.

The purpose of this work was to evaluate the influence of these additives (pea protein isolate and Hicap100) and the MCT-oil load on process parameters (extrusion operating conditions: screw configuration, temperature profile) and product final properties (hygroscopicity, thermal properties, encapsulation efficiency). Determinations of the extrusion parameters were conducted in favor to establish mild processing conditions and the least number of steps (no pre- or post-treatment, so as to reduce costs).

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Maltodextrin

Based on our preliminary study on maltodextrins, maltodextrins [24] DE-12 (Glucidex IT-12), supplied by Roquettes Frères (Lestrem, France), revealed itself as the best candidate to be employed as the main ingredient of the bio-based material.

In fact, maltodextrin DE-12 presented the highest viscosity values compared to maltodextrin DE-17 and DE-19 in presence of water, sorbitol or glycerol. Related to the hygroscopic character, maltodextrin DE-12 was the one having the highest hygroscopicity for a large range of humidities comprised between 10 and 60% RH.

Consequently, maltodextrin DE-12 suited perfectly the specifications of the final product and met all the requirements for the extrusion process.

2.1.2. Modified starch: Hi-cap100

Hi-cap100® was provided by Ingredion GmbH (Hamburg, Germany). Hicap100 belongs to the group of modified starches derived from waxy maize, more known as the OSA-starches (for Octenyl-Succinate Acid starches)

The OSA-starch was patented by [25]. It is obtained by the esterification of starch in presence of octenyl succinic anhydrides. This modification confers to the OSA-starch greater stability against oxidation, an active-surface due to the carboxyl group (that can be negatively charged), and an amphiphilic nature thanks to the octenyl moiety.

All these qualities make Hicap100 an excellent emulsifier and/or compatibilizer agent to be used for the encapsulation of flavors, vitamins, and oils (cf. Chapter 1, § 3.2.3).

2.1.3. *Pea protein isolate*

Pea protein isolate (PPI), SFP-87, was supplied by Roquettes Frères (Lestrem, France).

PPI was analysed for proximate composition (moisture, lipid, ash and protein contents) and amino acid profile using the same protocols described by [26] for plant protein isolate.

PPI is composed of 5.33 ± 0.01 % (w/w) of ashes, 0.47 ± 0.01% (w/w) of lipids, 7.03 ± 0.52 % (w/w) of polysaccharides and, 80.22 ± 0.01 of proteins.

The amino acid profile of pea protein isolate was performed with Biochrom30 (Cambridge, UK) and its composition is shown in Table 1. It can be seen that Aspartic, Glutamic and Leucine acids are the principal amino acids composing the pea protein isolate. It is very important to know the composition and the chemical structure of the amino acids that make up pea protein isolate, in order to understand the possible interactions that can be set with the other ingredients of the matrix (e.g. maltodextrins and water) and the active compound.

Table 1. Composition of pea protein isolate: Amino acid distribution

Amino acid	% (w/w)
Aspartic acid	11.67
Threonine	4.35
Serine	6.86
Glutamic acid	17.27
Glycine	7.40
Alanine	6.56
Cysteine	0.61
Valine	5.41
Methionine	1.17
Isoleucine	4.42
Leucine	8.44
Tyrosine	2.66
Phenylalanine	4.21

Histidine	2.32
Lysine	6.67
Arginine	5.52
Proline	6.16

Herein PPI is employed as an innovative compatibilizer agent, to be compared to the “classical” compatibilizer Hicap100. Indeed, PPI have proved to be a performant additive in spray-drying encapsulation, thanks to its good film-forming and emulsifying properties, and also because it is non-allergenic [27].

2.1.4. MCT-oil: Medium-chain triglycerides

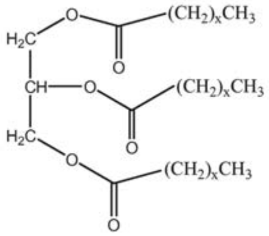
Miglyol812N Neutraloel (CAS=65381-09-1), was supplied by CREMER Oleo, GmbH & Co. KG (Hamburg, Germany).

Miglyol812N, also known as medium-chain triglycerides (=MCT-oil), is composed of a glycerol backbone and three fatty acids, in this case caprylic and capric fatty acids (Table 2). The fatty acids come from palm kernel oil and coconut oil.

This oil is largely used in the pharmaceutical and food industry, as an excipient to improve the texture and viscosity of a product. Also, it is used as solvent in the food industry for the production of dietary food.

MCT-oil is odorless, tasteless, colorless and stable at high temperature (its boiling point is 456°C).

Table 2. Composition of the MCT-oil; x=8 or/and x=10.

Name	Structure	Molecular Weight (g/mol^{-1})
Caprylic and capric triglyceride		372.54

MCT-oil has been chosen as the model hydrophobic compound to be encapsulated thanks to its well defined functional properties, its thermal stability, and its suitable viscosity. Besides, it does not form lipid-amylose complexes when mixed with starch or other starch derivatives[28].

2.2. Twin-screw extrusion encapsulation

As mentioned in the introduction, four formulations were chosen as new bio-based delivery systems:

- **A-10:** Maltodextrin I-12/pea protein (90/10): containing 10% (w/w) of PPI;
- **B-15:** Maltodextrin I-12/pea protein (85/15): containing 15 % (w/w) of PPI;
- **C-1:** Maltodextrin I-12/pea protein/ modified starch (89/10/1): containing 10% (w/w) of PPI and 1% (w/w) of Hi-cap100;
- **D-5:** Maltodextrin I-12/pea protein/modified starch (85/10/5): containing 10%of PPI and 5% (w/w) of Hi-cap100

All the different blends were conducted using a co-rotating and co-penetrating twin-screw extruder Evolum HT25 from Clextral (Firminy, France). The extruder is composed of ten thermo-regulated modules of 10 cm length each (Table 3). The screws have a 25 mm diameter and a distance between the shafts of 21 mm.

Table 3. Technical characteristics of the Evolum HT25 extruder.

Characteristics	**HT25 Evolum (Clextral, France)**
L/D ratio	40
Barrel internal diameter (mm)	25
Barrel D_{ext}/D_{int}	3.32
Screw diameter (mm)	24.8
Lenght of a module (mm)	100
Numbre of modules	10

The screw profile was divided in four zones: conveying, plasticization and two mixing zones (Figure 1). Plasticization and mixing zones were only composed of kneading and mixing elements (BL22/+45° and BL22/90°). In the first part of the extruder, the carbohydrate mixture was fed at a rate of 4.3-4.4 kg/h with gravimetric feeder K-Tron KML KT20, Coperion K-Tron Pitman, Inc. (Sewell, USA) composed of two concave screws. Then, it was mixed with water (1.8 kg/h), directly pumped into the second barrel with a magnetic centrifugal pump VERDERmag GLMD, VERDER Sarl (Eragny-sur-Oise, France), which enhanced plasticization of the blend under the mechanical shear of the screws. MCT-oil was introduced at barrel five after the plasticization of the blend thanks to peristaltic pump PM600 JOUAN S.A. (Saint-Herblain, France) as illustrated in Figure 1. External calibration of both water and MCT-oil pumps, were realized before extrusion, so as to determine their exact feed rate. No die at the end of the extruder barrel was employed in order to avoid the flash-off of water due to the high pressure generated on the extrudate by the size reduction from the barrel to the die.

All the samples were stabilized in a controlled climatic chamber at 60% of relative humidity and 25°C, more than three weeks before analysis.

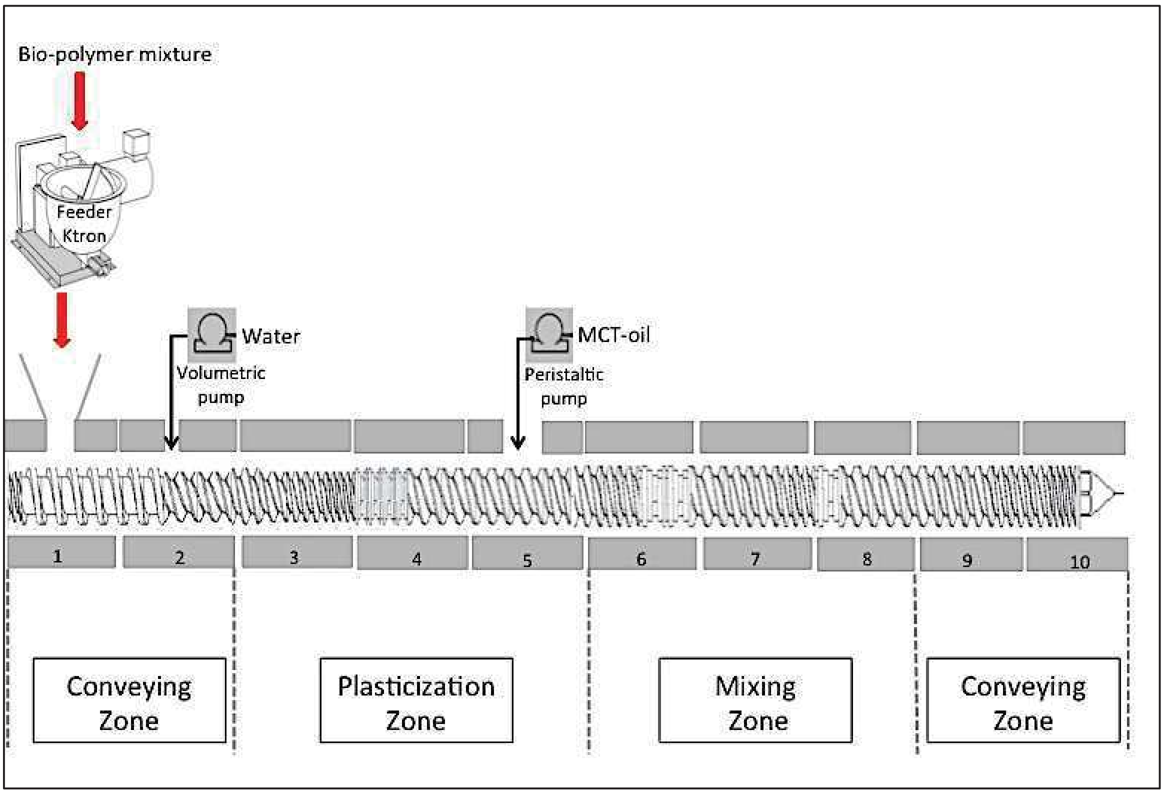


Figure 1. Twin-screw extruder Evolum HT25 configuration and screw profile for the elaboration of the bio-based matrix.

2.3. Physicochemical characterization

2.3.1. Moisture content

Moisture content of the samples was determined by gravimetric method (NF-V-ISO03-921). One gram of each sample was weighted and leave to dry in an oven at 103±2°C for 24 hours until there were no mass variation of the sample. Measurements were run in triplicates for each sample.

2.3.2. Hygroscopicity: Sorption isotherms

Water sorption isotherms were performed on a Dynamic Vapor Sorption (DVS) Advantage System from Surface Measurement Systems (Alperton, UK). This machine is equipped with a very accurate recording microbalance, capable of measuring changes in the sample mass as low as 0.1µg. Samples were exposed to a constant temperature (25°C) and programmed relative humidities varying from 0 to 90% divided into 15% increments (14 steps). A mixture of dry and moisture-saturated nitrogen flowing over the samples assured the changes in the relative humidity of the DVS-chamber. Ten milligrams of the sample were placed inside the chamber and before starting the data acquisition, all the samples were dried for 300 minutes under a stream of dry nitrogen (0% RH) at 103°C in order to obtain the dry weight. Equilibrium was achieved when the changes in the mass of the sample were lower than 5.10^{-3} % min^{-1}.

2.3.3. Glass transition temperature

Glass transition temperature (T_g) of the extrudates were measured on a Triton Technology Dynamic Mechanical Analysis (DMA) apparatus (Triton Technology, UK), by a thermal scan in the simple geometry of the single cantilever-bending mode. The amplitude (25µm) and the multi-frequency (1 and 10 Hz) mode were kept constants during the analysis. Samples were placed in an aluminum pocket and the temperature range of analysis was set from -100°C to 200°C at a scanning rate of 2°C/minute.

2.3.4. Colorimetry

The colors of the extrudates and three raw materials were measured by a Chroma-Meter CR-410 colorimeter, Minolta, (Carrière-sur-Seine, France). Extrudate samples were placed on a glass support of 50 mm of diameter directly on the top of the apparatus for measurement. The CIELAB color scale was used to measure three colors parameters: L*=0 (black) to L*=100 (white), -a* (greenness) to +a* (redness), and –b* (blueness) to +b* (yellowness).

The total color difference ΔE* with respect to the control were calculated using the following equation:

$$\Delta E *= \sqrt{(\Delta L *)^2 + (\Delta a *)^2 + (\Delta b *)^2}$$

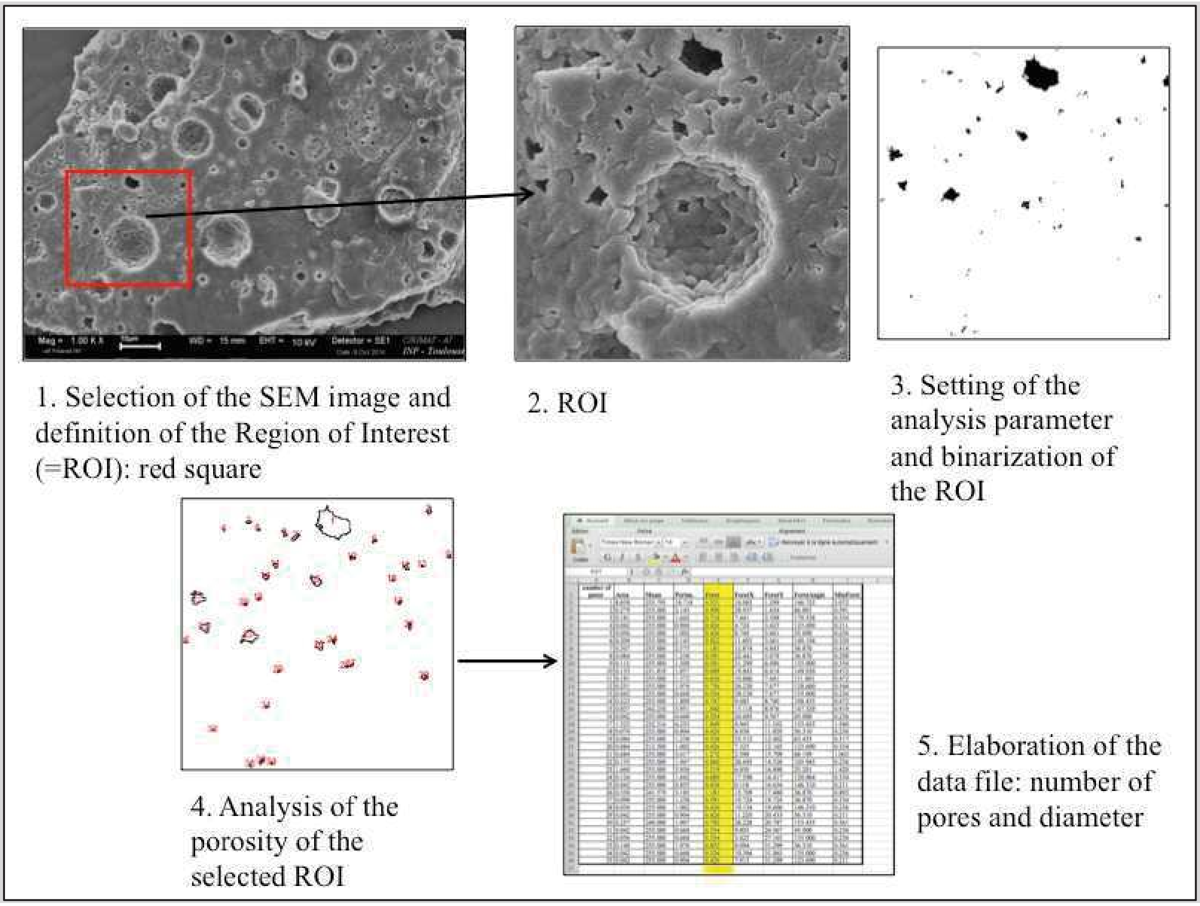
Each formulation has its own control sample, which corresponds to the extrudate without any MCT-oil.

2.3.5. Morphological analysis: SEM and X-ray tomography

Morphology of the extrudates was examined by scanning electron microscopy (SEM). SEM observations were performed with a LEO435VP scanning electron microscope LEO Electron microscopy Ltd., (Cambridge, UK) operated at 8kV. All the samples were metallized with silver under vacuum before observation.

The porosity of the samples was analyzed by ImageJ. ImageJ is an open source Java-based software developed by the National Institute of Health (Bethesda, USA). This image treatment allowed characterizing the extrudate in terms of morphological aspects (i.e. porosity) and other structural heterogeneities (e.g. surface and texture).

For each sample, distinct images at different magnifications were analyzed, in order to have an exhaustive morphological analysis of the sample. The imageJ parameters were the same for all the analyzed samples. First, the image was selected and the region of interest was chosen. The ROI (region of interest), defined as a square having the following dimensions W=270 and H=270, was the same for all the samples. A binary image was generated and the software made an automatic calculation of the porosity, after the calculation parameters were fixed. The image treatment process by ImageJ is presented on Figure 2.



1. Selection of the SEM image and definition of the Region of Interest (=ROI): red square

2. ROI

3. Setting of the analysis parameter and binarization of the ROI

4. Analysis of the porosity of the selected ROI

5. Elaboration of the data file: number of pores and diameter

Figure 2. Procedure of the morphological analysis.

X-ray tomography was carried out on a Skycan 1174 from Brucker (Kontich, Belgium), equipped with a 50kV and 800µA X-ray source. The scan was performed with a 10µm voxel size. Images were reconstructed by NRecon program from the same supplier.

The advantage of the SEM compared to the Microtomography X-ray is that it is possible to observe porosity going below 10 µm. Therefore these two techniques are considered to be complementary for morphological studies.

2.4. Model compound content: ASE extraction

Extraction of the MCT-oil from the extrudates was performed on a pressurized liquid extractor ASE 350 form Thermo Scientific Dionex (Villebon sur Yvette, France). Seven grams of extrudate, grinded at 2 mm particle size, were mixed with three grams of

Fontainebleau sand from VWR (Fontenay-sous-Bois, France) and put into a 10 mL stainless steel cell extraction. The cells were equipped with a stainless steel frit and cotton glass to avoid the solid particles to block up the collection pipe. Cyclohexane, from Sigma-Aldrich (St Quentin Fallavier, France) was used as solvent extraction. The oven temperature was set to 60°C and three cycles, at 150 bars, were required to extract all the MCT-oil contained in the matrix. Solvent was evaporated thanks to a rotary evaporation from BUCHI (Flawil, Switzerland) and the extracted MCT-oil was gravimetrically weighted. Each sample was run in triplicates.

The encapsulation efficiency (EE), the encapsulation rate (ER) and the incorporation rate (IR) were defined by equations (1), (2) and (3) showed below:

$$EE\ (\%) = \frac{mMCToil\ extracted}{mMCToil\ initial} * 100 \tag{1}$$

$$ER\ (\%) = \frac{mMCToil\ extracted}{ME} * 100 \tag{2}$$

$$IR\ (\%) = \frac{mMCToil\ initial}{ME} * 100 \tag{3}$$

$mMCToil_{extracted}$: amount of MCT-oil extracted by ASE extraction

$mMCToil_{initial}$: amount of MCT-oil initial incorporated in the mixture

ME: mass of the extrudate (dry matter) used for the extraction

Remark: all the experiments that were carried out in this chapter were performed on samples coming from one extrusion production.

3. RESULTS

The following part is focused on the procedures that were undertaken in order to establish the extrusion processing conditions for the encapsulation of a model hydrophobic compound.

3.1. Mild temperature extrusion

3.1.1. Introduction

Melt-extrusion can be considered as complex multivariable technology, where thermal, chemical and mechanical stresses are generated by the screws and as a result transform the raw materials. The object final properties and its morphology strongly depend on the extrusion parameters [5,13,29]. However due to the complexity of the mechanisms involved during extrusion, it is difficult to establish a correlation between the product properties and the process parameters [3,4]. To get a better understanding of all the mechanisms taking place inside the extruder and how the active compounds are dispersed within the molten matrix, modeling of extrusion has recently appeared as an accurate tool to better adjust extrusion parameters according to the desired product final properties [30].

In our case, we first optimized the process parameters (i.e. screw configuration and temperature profile) in order to obtain sufficiently flexible conditions to extrude different formulations and matrix compositions with various amounts of MCT-oil. Secondly, in order to investigate the influence of the composition on the process parameters, and thereafter try to understand the impact of these process conditions on the final properties of the delivery system, extrusion conditions determined in the first part of this study were kept constant for the different formulations tested. Therefore, the effects of the addition of PPI, Hi-cap100, and the different incorporation rates of the MCT-oil were evaluated.

3.1.2. Twin-screw extrusion

Extrusion results using a conical twin-screw extruder with a backflow channel (see Chapter 2) allowed understanding the behavior of the solid state of maltodextrins and confirmed our choice on the maltodextrin DE-12 as main ingredient of the matrix.

However, all the setting conditions determined in the mini extruder were not directly transferable to a twin-screw extruder [31]. The main reasons were on one hand the low torque of the mini compounder compared to that of Evolum 25. And on the other hand, the screw geometry of the mini compounder did not exactly reproduce the shear strength imparted by the real extruder. Moreover, the micro-extruder operates in a recirculating mode with a mixture prepared in advance which is not the case on a regular twin-screw extrusion process.

For all experiments, the mixtures were at the beginning extruded with maximum water content and then the solid content was increased up to the setting value. Then, the water content was decreased to a minimum value still allowing a processable melt extrusion and obtaining an extrudate at the exit of the extruder.

The screw speed was regulated so as to prevent high torque (100% torque corresponds to 200 Nm).

For this study, extruding the mixture through an exit die at the end of the barrel was not possible, because the mixture solidified really quickly and provoked an emergency shutdown of the extruder (mixture solidified inside the extruder's barrel or either was to sticky to put into shape). [32] reported the same difficulty to shape the extrudate at the exit of the extruders.

3.1.3. Optimization of the process parameters

3.1.3.1. Screw profile

Four different profiles (Figure 3) were tested in order to obtain the adequate shear mixing forces without degrading the biopolymer blends. The screw profile was set up based on the screw profile used to plasticize starch [31,33–35] but without putting high pressure/shearing zones in order to avoid protein or active compound degradation.

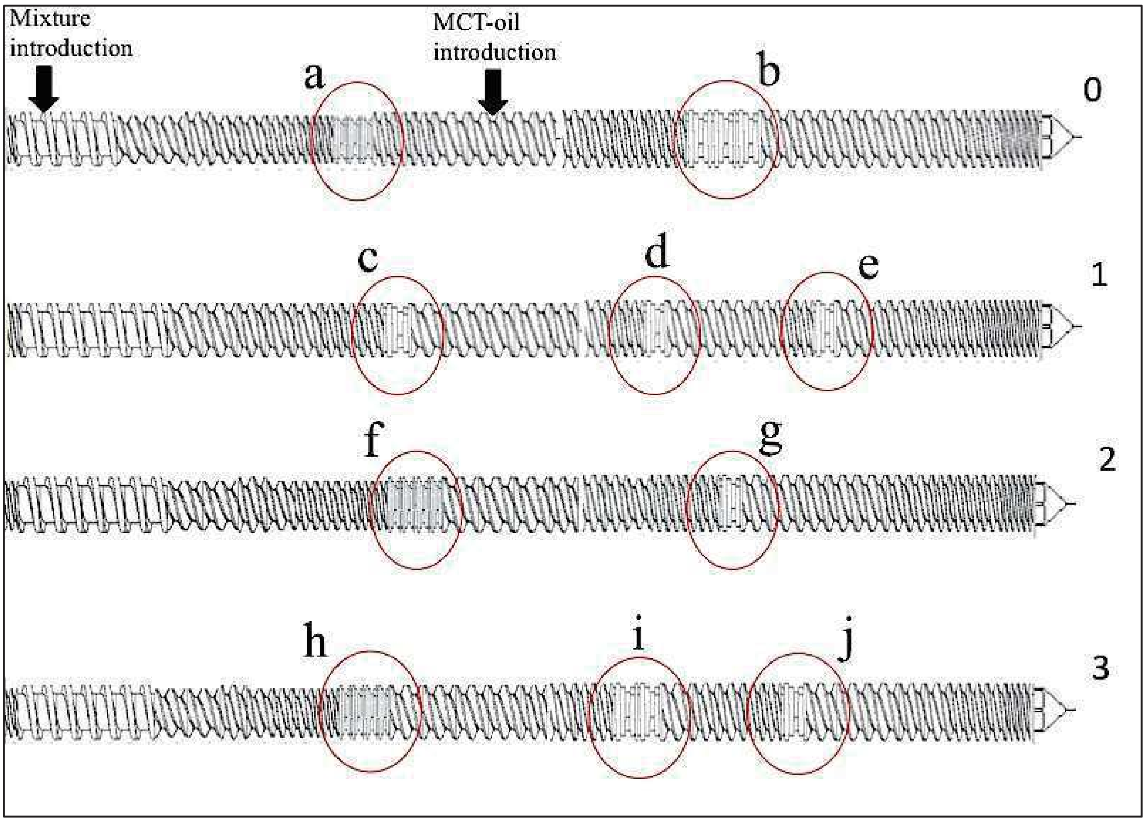


Figure 3. Different screw profile configurations.

The screw profile 0, very similar to the above cited profile, was composed of a plasticizing zone (a) and mixing zone (b) using kneading elements of different lengths (BL22/90° of 1 and 0.5mm). Unfortunately, it was too restrictive to allow the conveying of the mixture. For this reason the profile 1 was composed of three smaller plasticizing and mixing (c, d, e) zones (only kneading elements of 1mm of length). However there was neither plasticization of the matrix nor dispersion of the MCT-oil. Therefore, for the configuration of profile 2, kneading elements BL22/+45° of 0.5mm of length (f) were employed to enhance the conveying and plasticizing of the bio-polymer and water mixture, but the mixing zone (g) was still not important enough to guarantee the mixing. Finally profile 3 was defined with an important plasticizing zone composed of kneading elements BL22/+45° of 0.5mm of length (h) and two mixing zones constituted of kneading elements BL22/90° (i, j). The separation of the mixing zones allowed inducing compression and expansion of the matter, in order to facilitate the dispersion of the active ingredient and to obtain a homogeneous extrudate.

Table 4 gives a detailed description of the main characteristics of each screw element composing profile 3, used for our study.

Profile 3 allowed plastifying, dispersing and mixing all the different ingredients in order to obtain at the exit of the extruder, the bio-based delivery system. The challenge was to reduce at a maximum the exudation of the MCT-oil. For this reason, different incorporation rates were tested to establish the optimal incorporation of the oil that the biopolymer mixture could retain. The objective was also to set up soft extrusion conditions in terms of mechanical shear and temperature so as to avoid denaturation/fragmentation and degradation of the raw materials while dispersing the active compound.

Table 4. Screw elements used in the final screw profile and their mechanical effects.

Screw description	Scheme	Mixing power	Shearing power	Conveying power	Remarks
T1F (trapezoid, conjugated section with simple thread)		-	-	+	Feeding and transport
C2F (conjugated section with double thread)		+	+	+++	Feeding and transport
BL22/90° (bloc element)		+++++	++	-	Mixing, radial compression, high shear, increase of the residence time

BL22/+45° (bloc element)		+++++	++	+	Mixing, conveying, radial compression, high shear, increase of the residence time

3.1.3.2. *Temperature profile*

The mild temperature conditions used was one of the originality of this study as compared to other studies that employed hot melt extrusion temperature above 100°C presented Table 5.

Table 5. Examples of some extrusion encapsulation profile temperatures.

References	Extrusion profile temperature (°C)
[32] (Tackenberg, Krauss, Schuchmann, et al., 2015)	113-140
[4] (Emin & Schuchmann, 2013b)	140
[3] (Emin & Schuchmann, 2013a)	130-170
[28] (Emin et al., 2012)	150
[36] (Chang et al., 2010)	80-105
[29] (Zasypkin & Porzio, 2004)	104-113
[37] (Carvalho & Mitchell, 2000)	40-170
[38] (Kollengode & Hanna, 1997)	60-120
[39] (Ilo, Tomschik, Berghofer, & Mundigler, 1996)	150-160
[40] (Carr, Wing, & Doane, 1991)	70-125

This temperature range is very attractive to work with thermolabile and highly flammable active compounds without fearing their thermal degradation and reducing the risk of Maillard reactions to take place.

The temperature profile (Table 6) was adjusted in order to avoid thermal degradation of the active ingredient and reduce PPI degradation/fragmentation [41] and was also modulated in order to enhance flowability of the mixture inside the extruder barrel. The maximum temperature value was set as 50°C. As mentioned above, the water content was first increased to reach the adequate viscosity of the blend.

Table 6. Extrusion profile temperature.

Module of the barrel	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**
Temperature **(T°C)**	20	20	40	50	50	40	40	40	40	40

3.1.3.3. Formulations: sample preparation

The elaboration by extrusion of a bio-based material composed only of maltodextrin DE-12 was impossible, since the window of processability was very narrow. Indeed at high water content (around 30% (w/w)) the mixture was not extrudable since no shear was applied and the mixture was too diluted. On the contrary, at low water content (below 15% (w/w)) the maltodextrin concentration in the solution was so high, that in the mixing elements the shear was very strong and as a consequence, maltodextrins were immediately dehydrated and thus caramelized. For these reasons, extrusion of maltodextrins alone was not possible.

Therefore additives were required to enhance the extrudability of maltodextrins. Classic compatibilizer agent, Hi-cap100 and an innovative one, pea protein isolate were used for the bio-based mixture.

Four matrices formulations were then tested, with the following compositions:

- Maltodextrin I-12/pea protein (90/10): **A-10, containing 10% (w/w) of PPI;**
- Maltodextrin I-12/pea protein (85/15): **B-15, containing 15 % (w/w) of PPI;**
- Maltodextrin I-12/pea protein/ modified starch (89/10/1): **C-1, containing 10% (w/w) of PPI and 1% (w/w) of Hi-cap100;**
- Maltodextrin I-12/pea protein/modified starch (85/10/5): **D-5, containing 10%of PPI and 5% (w/w) of Hi-cap100.**

PPI has been selected to increase the viscosity of the blend, but also to improve the affinity between the hydrophilic matrix and the hydrophobic active compound thanks to their amphiphilic character, which confers good emulsifying properties (due to the chemical structure of various PPI amino acids (Table 1)). Hi-cap100 was added only as compatibilizing agent as it is classically used in encapsulation field.

3.1.4. Effect of MCT-oil and Hicap100 content on SME

Incorporation of MCT-oil has been carried out with the four different matrices, with a content varying from 8 to 15% (w/w). Table 7 summarizes the optimized extrusion processing conditions for the different incorporation rates of MCT-oil tested.

Table 7. Summary of the final processing conditions.

Extrusion parameters	
Screw speed (rpm)	225
Feed rate solid mixture (kg.h^{-1})	4.40
Feed rate water (kg.h^{-1})	1.80
Feed rate MCT-oil 8% (kg.h^{-1})	0.53
Feed rate MCT-oil 10% (kg.h^{-1})	0.66
Feed rate MCT-oil 12% (kg.h^{-1})	0.83
Feed rate MCT-oil 15% (kg.h^{-1})	1.13

The specific mechanical energy (SME) corresponds to the work input from the drive motor into the material being extruded, from which a part is dissipated as heat. SME characterizes the extrusion process by indicating the amount of mechanical energy transferred from the extruder to the material, involving its physical and chemical transformation. In fact, SME not only gives an indication about the performance of extrusion, but also about the final product characteristics such as density, solubility, and expansion index (Guerrero, 2012).

For example, a high SME could be associated to molecular breakdown or degradation of the material being extruded.

The screw speed, the extruder temperature, the moisture content, the screw configuration, and the feed composition directly influence SME. Viscosity of the extruded material is the most evident response variable that can be adjusted in order to modify the SME. In general, high SME corresponds to high viscosity.

The SME corresponding to the extruder used in this study is given by the following equation:

$$SME = \frac{Power}{feed\ rate} = \frac{Pm * \frac{T}{Tmax} * \frac{N}{Nmax}}{feed\ rate} = \frac{U * I * cos\varphi * \sqrt{3} * \frac{T}{Tmax} * \frac{N}{Nmax}}{feed\ rate}$$

P_m: power supplied by the drive motor (Watts)

Feed rate: (kg/h)

T: torque (%)

T_{max}: maximum torque of the extruder (T_{max}=100%)

N: screw speed (rpm).

N_{max}: maximum screw speed of the extruder (N_{max} = 1200 rpm)

U: electric potential (U = 400 V)

I: electric current (I = 57A)

Cos φ: theoretical efficiency of the extruders engine (cos φ = 0.9)

Without any addition of MCT-oil, extrusion of matrices showed a decrease of SME values, with the increase of the content of both PPI and HiCap100 (Table 8). Adding 5 additional parts of PPI significantly decreases the SME value, which seemed to indicate that long chain polymers incorporation facilitate maltodextrin extrusion. With the same amount of pea protein (10% w/w), the increase of the Hi-cap 100 ratio provoked also a marked decrease of the SME. Adding only 1% of Hi-Cap 100 gave better processability than 5 additional parts of PPI (C-1 compared to B-15).

When MCT-oil was added, two behaviors were observed depending on the matrix compositions. With protein only, SME tended to decrease with the increasing amount of incorporated MCT-oil, this effect was really remarkable for formulation A-10. This was expected since MCT-oil besides being the encapsulated compound may act as a lubricating agent. This behavior was also observed in other studies [28,42] where the MCT-oil was premixed with the active compound, and the SME was immediately lowered when it was employed.

Table 8. Variations of the SME at different MCT-oil incorporation rates.

Formulation	Incorporation rate of MCT-oil (%)	Torque (%)	Total feed rate (kg.h^{-1})	SME (Wh.kg^{-1})	Moisture content (%)
A-10	0	32	5.75	370.87	9.4±1.9
	9	35	6.37	366.16	-
	10	30	6.40	312.38	9.3±0.1
	11	29	6.45	298.70	9.3±1.0
	12	25	6.50	255.13	8.6±0.1
B-15	0	18	6.20	193.47	10.2±0.3
	8	16	6.73	158.43	9.4±0.3
	10	16	6.86	155.43	9.9±0.0
	12	18	7.03	170.63	11.4±0.1
C-1	0	16	6.20	171.98	10.2±0.3
	8	20	6.73	198.04	12.5±0.1
	10	20	6.86	194.29	13.0±0.2
	12	14	7.03	132.71	10.6±0.1
D-5	0	13	6.20	139.73	8.5±0.4
	8	24	6.73	237.65	12.0±0.1
	10	19	6.86	184.57	12.6±0.1
	12	20	7.03	189.59	10.3±0.2
	15	13	7.33	118.19	11.9±0.6

With the addition of Hi-cap 100, SME tended to first increase with MCT-oil content, indicating a specific interaction between both compounds, confirming the Hi-cap 100 emulsifying properties.

It is important to highlight that the obtained values of the SME were relatively lower compared to values reported on other studies. For example, values of the SME ranging from 569 to 2894 Wh.kg^{-1}were found in [42]. This can be explained by the harsher extrusion conditions employed: high temperature and screw speed, and lower water content.

It is worth noticing that the innovative part of these delivery systems is the use of PPI, because there are few studies reporting the use of proteins in extrusion for the elaboration of bio-based materials (composites or /and films) [43].

3.2. Physical properties of the delivery systems

The next section is addressed to the characterization of these new bio-based delivery systems, in terms of physicochemical properties, as well as the determination of their encapsulation performances.

3.2.1. Incorporation of MCT-oil

ASE extraction allowed evaluating the quantity of MCT-oil incorporated in the extrudate after stabilization (when the torque and the screw speed values are on stable regime). Comparing formulation A-10 to formulation B-15 (Figure 4), the incorporation of 5 additional parts of PPI allowed doubling the encapsulation efficiency. This demonstrated that PPI helped to increase the affinity between the matrix and the MCT-oil. Gharsallaoui et al. (2012) also noticed that thanks to PPI, the encapsulation rate of MCT-oil/maltodextrin spray-dried microparticles was improved. This result proved that PPI acts as a compatibilizer agent due to its amphiphilic structure.

The same results were observed for formulation C-1 when compared to formulation A-10. The addition of 1% (w/w) of modified starch improved by a factor 2 the encapsulation efficiency (and that, for all the formulations at different incorporation rates of MCT-oil).

Both Hi-cap100 and PPI, due to their chemical structures, behaved as surfactant by increasing the affinity between the hydrophilic matrix and the hydrophobic MCT-oil and by improving the dispersion of the MCT-oil droplets (as demonstrated by the SEM later in this chapter).

However, it should be noted that the encapsulation efficiencies for formulation B-15, at 8 and 10% (w/w) of incorporation rate, were higher (92 and 93 % respectively) than formulation C-1 (83% and 86% respectively).

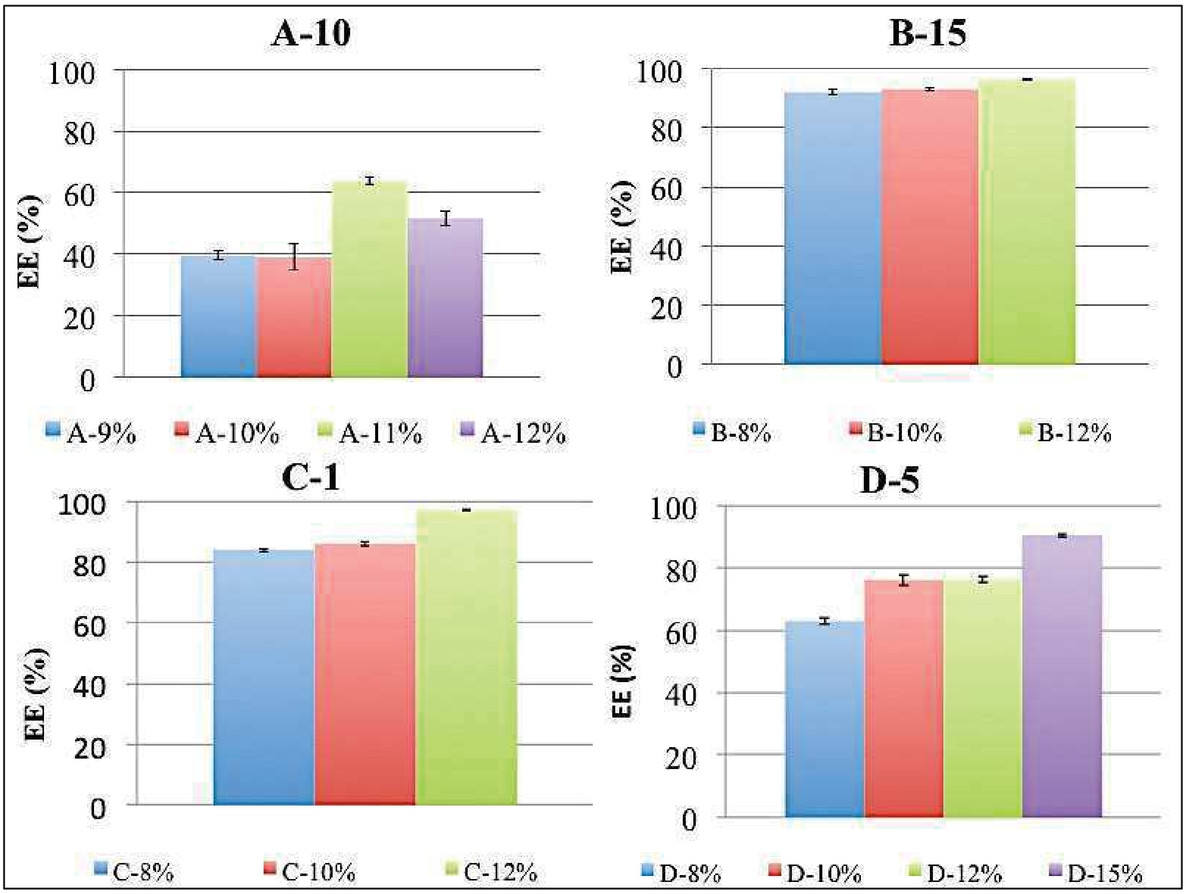


Figure 4. MCT-oil encapsulation efficiency.

For both formulations it was not possible to increase the incorporation rate of MCT-oil above 12% (w/w) as we observed that the active was not incorporated into the matrix and even splashed out at the exit of the extruder's barrel.

Formulation D-5, with 5 additional parts of modified starch, allowed a higher MCT-oil incorporation rate up to 15% (w/w), without exudation and with a valuable encapsulation efficiency (90%) corresponding to a final encapsulation rate of 15.41%. However, for lower MCT-oil incorporation rates, the encapsulation efficiencies were lower than the corresponding ones for formulations B-15 and C-1. This was particularly remarkable for 8% of incorporation rate, suggesting that a too high Hi-cap100 content was not suitable to encapsulate low amount of MCT-oil.

Table 9. Incorporation rate, encapsulation efficiency, encapsulation rate of the extruded formulations.

Formulation	**Incorporation rate (%)**	**Average Encapasulation rate (%)**	**Average Encapsulation Efficiency (%)**
A-9% (w/w) MCT-oil	9.00	3.56	39.60 ±1.52
A-10% (w/w) MCT-oil	10.00	3.90	39.05 ±4.26
A-11% (w/w) MCT-oil	11.00	7.00	62.63 ±1.02
A-12% (w/w) MCT-oil	12.06	6.21	51.75 ±2.52
B-8% (w/w) MCT-oil	8.69	8.00	92.06 ±0.92
B-10% (w/w) MCT-oil	11.06	9.52	93.07 ±0.44
B-12% (w/w) MCT-oil	13.21	12.83	96.28 ±0.13
C-8% (w/w) MCT-oil	9.00	7.55	83.88 ±0.48
C-10% (w/w) MCT-oil	11.06	9.52	86.10 ±0.71
C-12% (w/w) MCT-oil	13.21	12.83	97.16 ±0.20
D-8% (w/w) MCT-oil	8.95	5.63	62.95 ±1.14
D-10% (w/w) MCT-oil	11.01	8.37	76.07 ±1.78
D-12% (w/w) MCT-oil	14.49	11.04	76.15 ±1.01
D-15% (w/w) MCT-oil	17.03	15.41	90.53 ±0.62

The encapsulation efficiencies obtained for our bio-based delivery systems (Table 9) were found very encouraging as compared to the literature. For example, Emin et al. (2012)

reported to have an encapsulation efficiency of β-carotene, in a starch-based matrix, of about 70% by twin-screw extrusion.

PPI showed to be a good compatibilizer agent almost as efficient as Hi-cap100. Indeed, PPI allowed a good dispersion and stabilization of the MCT-oil within the matrix and obtaining a homogeneous extrudate.

3.2.2. Color

The color of an object is one of the principal characteristics that industrials have to give particular attention to, in order to fulfill consumer's acceptance. For example, in the case of cosmetics, hygiene, pharmaceuticals and food products, there is a tendency for white products, since this color is often associated to clean and high quality (e.g. purity).

L, a* and b* color values, as well as the total color difference (ΔE*) of the extrudate are presented in Table 10.

Table 10. Color mean values (L*, a*, b*, and ΔE*) of the raw materials and the extrudates.

Formulation	MCT-oil content (%)	L*	a*	b*	ΔE*
Pea protein		80.38	4.71	18.20	-
Maltodextrin DE-12		94.30	-0.20	1.27	-
Hi-cap100		98.75	-0.15	0.94	-
MCT-oil		35.34	0.52	-0.23	-
A-10	0	87.54	0.90	9.57	control
	9	87.09	1.76	13.05	3.61
	10	80.45	3.72	18.50	11.75
	11	83.20	2.85	17.21	9.00
	12	80.90	3.48	18.60	11.50
B-15	0	88.80	1.57	11.69	control
	8	85.07	2.91	15.84	5.74
	10	82.80	3.26	16.71	8.00

	13	79.49	3.45	18.72	11.82
C-1	0	87.65	0.95	9.88	control
	8	87.09	1.76	13.05	3.32
	10	85.67	1.45	12.94	3.68
	13	84.70	2.24	15.09	6.12
D-5	0	89.52	1.23	10.77	control
	8	84.72	1.89	14.10	5.88
	10	85.76	1.75	13.89	4.91
	13	81.38	3.31	18.63	11.51
	15	87.76	1.83	12.45	2.51

Looking only at the values of (ΔE^*), we were able to evaluate the impact of the MCT-oil incorporation on the color change of the extrudates. In this case, each formulation was compared to its control, which corresponds to the extrudate without any MCT-oil. Hence, important values of (ΔE^*) suggested that the difference in terms of color were considerable. The color change was visually noticeable for (ΔE^*) values above or equal to 5.

There are three interesting trends to discuss according to the extrudate color changes: (i) the impact of the addition of PPI and Hi-cap100 on the color of the extrudates. Herein, only the extrudates without any MCT-oil are compared. (ii) The impact of the incorporation of the MCT-oil on the color of the extrudates. (iii) And last but not the least, the impact of the processing conditions on the color (i.e. process temperature and screw speed).

Of course, all these three phenomena occurred at the same time, but to get a better understanding of what happened, we decided to analyze them separately.

The increase of PPI (from 10% to 15%) and the addition of Hi-cap 100 (1% and 5%) induced no significant change of the lightness (L^*). Since PPI has a yellowish color, we could expect the biopolymer mixture would turn "darker". This was confirmed by the slight increase of a^* and b^* values.

However, for all the formulations, the L* values decreased after extrusion compared to the L* value of DE-12 maltodextrin (L*=94,3), the main constituent of the bio-based matrices. The extrudates turned darker, probably due to the incorporation of PPI and eventually the formation of Maillard's products and change of conformations in the polysaccharides and protein chains.

Related to the extrudates containing MCT-oil, it can be seen that when the MCT-oil content was increased, the extrudates became visually more brownish (Figure 5). Indeed, a* and b* increased as the MCT-oil content and this was observed for all the extrudates. Specially the increase of the b* parameter implied a tendency to yellow.

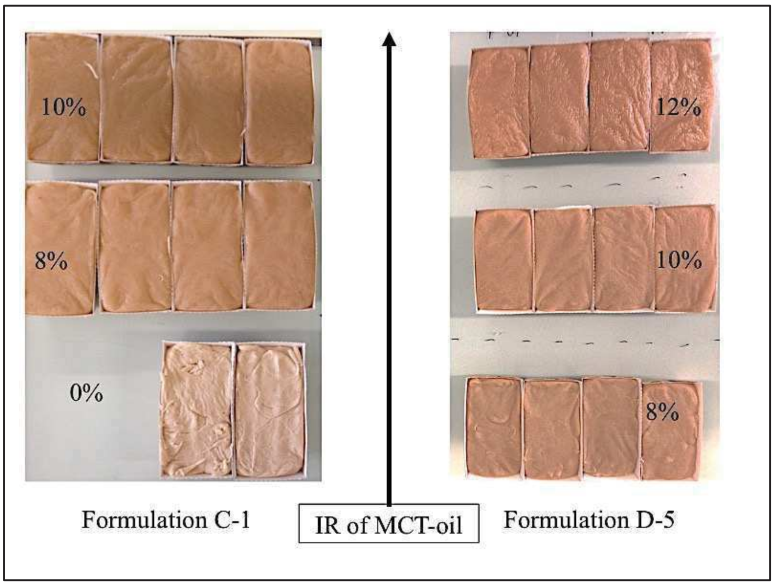


Figure 5. Observable color changes of the extrudates at different incorporation rates of MCT-oil. Herein presented formulation C-1 and D-5.

Ruiz-Gutierrez et al.^{44} (2014) demonstrated that increasing the screw speed (from 225 to 325 rpm) and/or the extrusion temperature (140°C), induced the increase of a* and b* parameters of their starch-based matrices, indicating a tendency towards yellow-reddish extrudates. These results allowed proving that harsh extrusion conditions enhance Maillard's reactions. And it is known that Maillard's products are responsible of the browning and darkening of the extrudates.

Guerrero[45] (2012) pointed out that the extrudate browning was due to Maillard's reactions between soy protein isolates and sugars. These reactions involve the formation of a protein-carbohydrate complex, where the amine group in the protein reacts with the carbonyl group of the reducing sugar to form a Schiff base, which further forms an Amadori compound (Figure 6) (also known as melanoidins) which is an insoluble polymer characterized by its brown color [46].

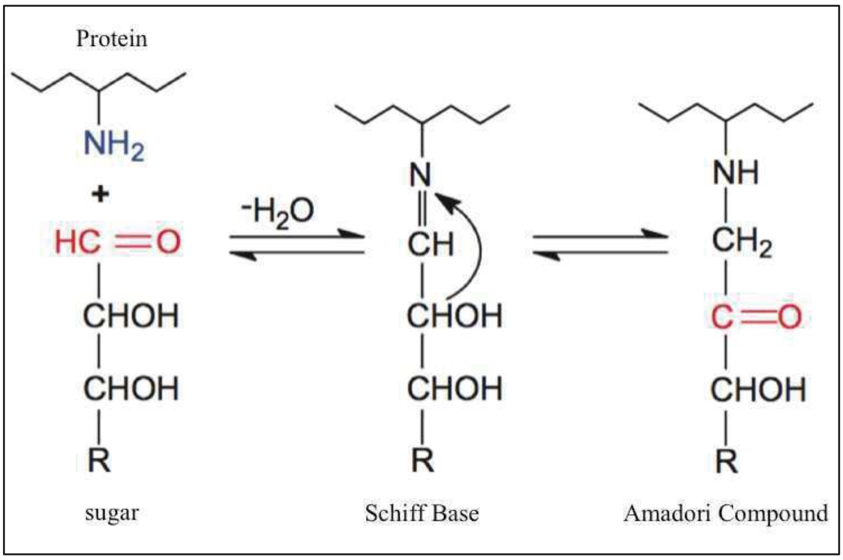


Figure 6. Maillard's reaction between amine protein and sugar take from [45].

In our case, the extrusion conditions were considered to be mild (screw speed of 225 rpm and extrusion temperature of 50°C) compared to other studies [4,42,44,47], but the observed color changes suggested that Maillard reactions could occur between PPI and maltodextrin DE-12.

What is a fact is that the darkening of the extrudates involved by the MCT-oil content increase might be linked to the SME variations (Table 8).

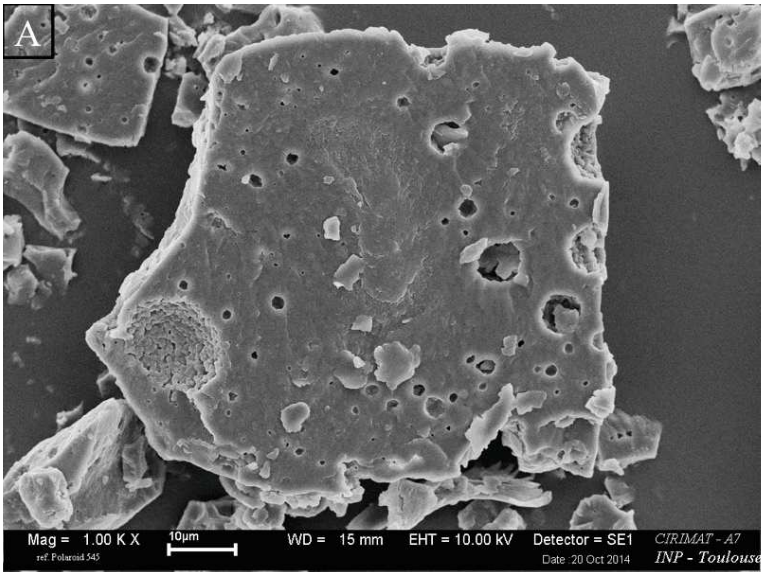
3.2.3. Morphology analysis

The aim of the SEM and microtomography was to establish a gross description of the morphological structure (i.e. porosity) and surface heterogeneities of the extrudate, related to the incorporation of MCT-oil.

The surface morphology of the extrudates was similar from one formulation to another.

All the extrudates presented a multi-varied porosity, with a high proportion of macropores (pores diameter ≥ 50 nm), according to the **IUPAC** classification on pores.

SEM of the four different extrudates corresponding to formulation A, B, C and D (with 8-9% MCT-oil) are presented on Figure 7.



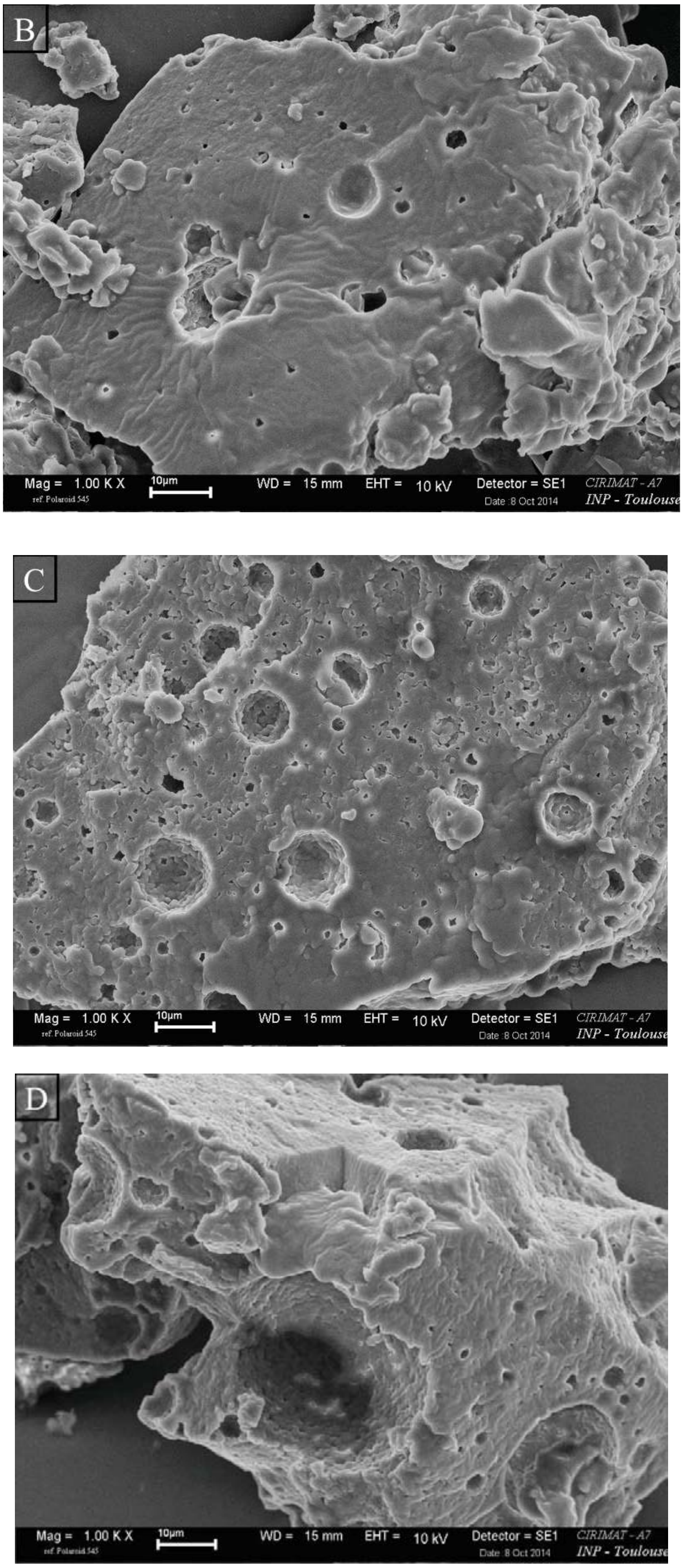


Figure 7. A) A-8 (%, w/w) MCT-oil 10μm scale x1.00K; B) B-8 (%, w/w) MCT-oil 10μm x1.00K; C) C-8 (%, w/w) MCT-oil 10μm x1.00K; D) D-8 (%, w/w) 10μm x1.00K.

The disappearance of the granular structure and the homogeneity of the surface evidenced that plasticization of the materials inside the extruder occurred.

Looking at the average diameter for all formulations, increase of the MCT-oil content in the extrudate provoked a pore widening resulting from the heterogeneous mixture, the oil tending then to coalesce (Table 11). The increase of hydrophobic compound rate may result in a less efficient mixing in the extruder.

A large variety of pores of different sizes were observable on the surface, therefore the extrudate presented a multipore structure with a broad porosity distribution (Figure 7).

Thanks to the imageJ morphological analysis, a statistical distribution of the number and type of pores could be measured for all the different extrudates at different MCT-oil incorporation rates.

Table 11. Porosity distributions of the different formulations.

Formulation	Incorporation rate of MCT-oil (%)	Total number of pores	Porosity ranged (µm)	Average pore diameter (µm)
A-10	0	24	0.58-1.69	0.95
	9	18	0.83-10.36	1.87
	10	8	0.65-2.03	1.87
	11	8	0.92-1.64	1.55
	12	12	1.86-6.78	2.56
B-15	0	79	0.55-9.77	1.37

	8	66	0.72-7	2.47
	10	nd	nd	nd
	12	nd	nd	nd
C-1	0	39	0.64-1.70	0.91
	8	20	0.55-1.64	0.71
	10	10	0.68-7.85	4.51
	12	6	3.19-7.88	5.53
D-5	0	nd	nd	nd
	8	33	0.55-8.08	2.49
	10	9	0.33-6.12	3.53
	12	nd	nd	nd
	15	9	4.5-37.1	6.96

The average pore diameter was used as an indicator of the major pore population that represents the most the type of porosity of the sample. For example, formulation A-10 at 10% (w/w) of MCT-oil content had an average pore diameter of 1.87µm. In other words, the most representative population of pores of this sample had an average pore diameter of 1.87µm.

Preliminary analysis obtained by X-ray tomography (Figure 8) corroborated SEM results, meaning that the extrudates presented multiple porosity morphology and a more or less disrupted surface. However, it is worth noticing that at 10µm voxel size, smaller porosity was not determined, thus the existing micro porosity of the extrudate at the interior of the extrudate was not estimated.

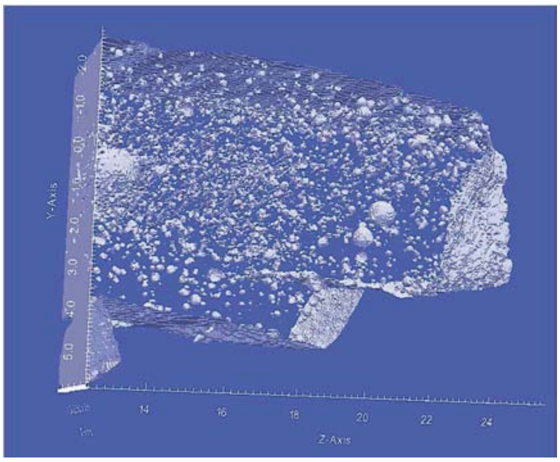


Figure 8. X-ray tomography image of formulation B-8% (w/w) MCT-oil. Grey color corresponds to the border between two different phases.

It is important to understand the process parameters in order to achieve the control of the morphology of the final product. The droplet dispersion and size can vary depending on screw speed and configuration and on feeder rate. These parameters can also affect the viscosity and therefore the droplet breakup and coalescence.

Emin and Schuchmann (2013) have evaluated the dispersive mixing efficiency of a hydrophobic compound in a starch-based matrix by twin-screw extrusion. They demonstrated that increasing the oil content led to bigger droplet size. This result was expected because of the higher collision frequency involved by a higher droplet number, resulting in more coalescence during extrusion.

Herein, the droplet size increased with the oil content for all tested formulations, which is consistent with previous studies.

When comparing the effect of the “stabilizing” agent (so the role played by PPI and Hi-cap100), for low incorporation rates of MCT-oil (<10%), the use of Hi-cap100 led to structures containing more pores, with an improved distribution size, while for higher amounts, PPI allowed to get more smaller pores.

Incorporation of 10% of MCT-oil revealed itself as an optimal incorporation rate since for all tested formulations the pore distribution seemed to be more homogeneous, the dispersion of the pores was improved and the pore diameter reduced.

The addition of 1% (w/w) of Hi-cap100 allowed having a more narrow pore distribution: the number of pores of the same diameter was increased, and also the pores were of smaller size. Whereas the addition of either 5% of PPI or 5% of Hi-cap100 (for formulations B and D the same behavior was observed) led to broader pore distribution, with a pore size significantly increased.

In conclusion Hi-cap100 had a better performance as a compatibilizing agent than PPI, based on the size of droplet formation. Hi-cap100 decreases the surface tension of the diverse ingredients and facilitates dispersion of the MCT-oil within the matrix. Smaller droplet size would indeed prevent probability for two droplets to coalesce.

3.2.4. Storage stability: evolution of the moisture content

Water is the crucial parameter to control to have a successful extrusion and thus, to obtain adequate properties of the final product [29,32,36]. Water not only plays an important role as a plasticizer by decreasing the glass transition temperature and/or the viscosity of the polymer mixture, but also because it ensures transferring the heat from the extruder to the polymer mixture [48].

Increasing the amount of water can decrease the viscosity of the polymer mixture, and as a consequence decrease the torque and the SME. In our case, the water content during the extrusion was maintained around 20 and 30% (w/w) so as to ensure mild extrusion conditions in terms of screw speed and barrel temperature. In that manner, the mixture had the adequate viscosity to be conveyed without fearing degradation of the polymer mixture due to high mechanical stress [28,49,50]. The use of large amount of water for extrusion was already reported in other studies as “wet-extrusion”, where the feed moisture content was greater than 40% (w/w) [51].

The high amount of water added during extrusion is also the reason why we measured lower SME values compared to other works. Most of them employed harsh extrusion conditions such as low water content below 10% (w/w) [32], screw speed above 250 rpm [28,40] and high extrusion temperatures around 140°C [3,28,32,37,39,44]. Even though all these reported studies had different extrusion conditions, at the end, all the extrudates had more or less the same range of equilibrium moisture content. The recurrent moisture content found in the literature for bio-based materials made either for food applications (encapsulation of pigments, flavors or just extruded food products) or for encapsulation of nutraceutical, can vary from 8 up to 15% (w/w) [29,36,44,47,52].

An advantage for working at low water content is to ensure longer shelf-life stability of the extrudates. [53,54]

In our study, all the formulations presented desorption moisture content varying from 8 to 13% (w/w) after drying and stabilization at 60% RH (Figure 9).

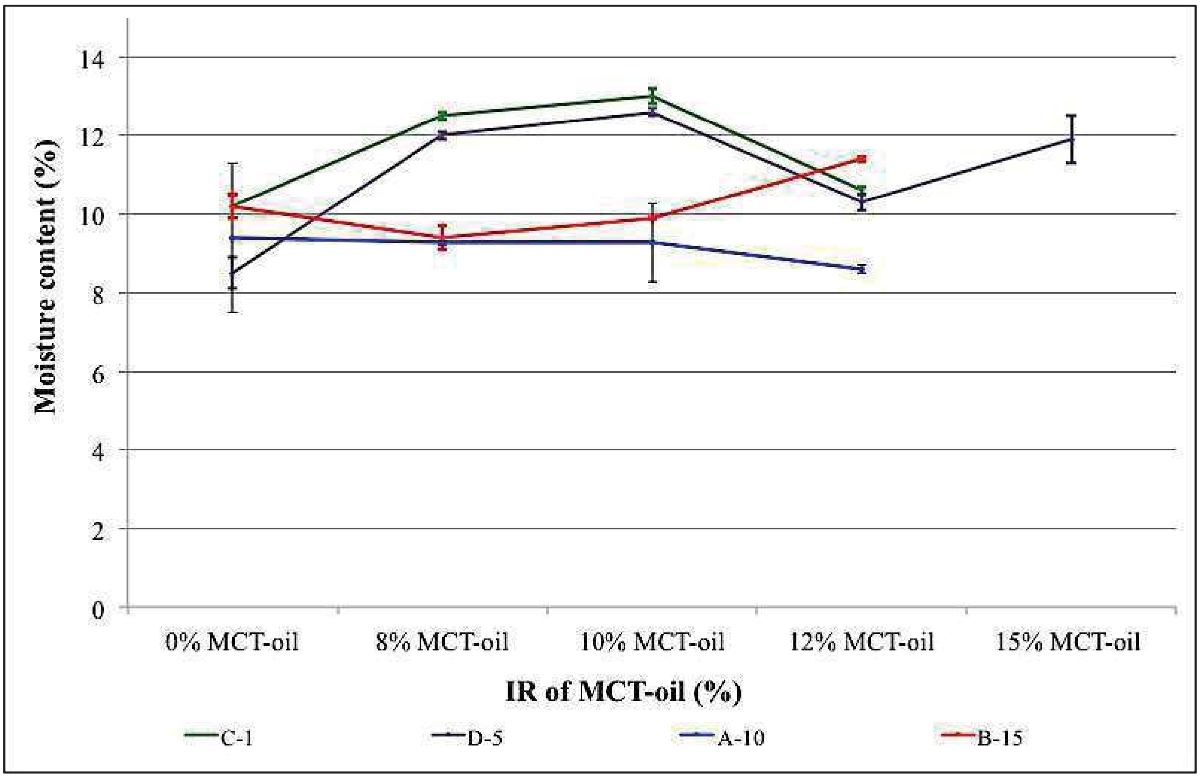


Figure 9. Influence of the incorporation rate of MCT-oil on the moisture content of extrudates (n=3, corresponds to the repetition of this experiment).

For the extrudates without MCT-oil, formulation D-5 was the one with the lowest moisture content and interestingly formulations B-15 and C-1 had the same moisture content. Comparing formulation A-10 to formulation B-15, increasing the protein content of 5 parts increased the moisture content. Thus, increasing the pea protein content slightly enhanced the water sorption. For both formulations (A-10 and B-15) the moisture content did not present any significant variation with the amount of MCT-oil, at least until 10% of MCT-oil, indicating that MCT-oil incorporation did not involve changes in the intermolecular interactions between macromolecular chains. Conversely, for the maximum MCT-oil incorporation rate (13%), the significant moisture increase for formulation B-15 could be caused by a modification of protein chain interactions, inducing the release of hydrophilic sites. It is difficult to comment any further because of the difference of the encapsulation efficiency between both formulations (Figure 4).

Moisture content of formulations C-1 and D-5 had the same behavior when the MCT-oil content was increased. Increasing the MCT-oil content up to 8% (w/w) provoked an increase of the moisture content. This could be explained by the fact that Hi-cap100 hydrophobic moieties were mobilized by MCT-oil, freeing hydrophilic parts in the matrices and thus enhancing water uptake.

Above 10% of MCT-oil, moisture content decreased for both formulations, once the MCT-oil content continued to increase.

3.2.5. Hygroscopicity

Looking to adsorption isotherms obtained from dried samples, the behavior towards water was sensibly different (Figures 10 and 11).

In general, the isotherm of the extrudate without any hydrophobic compound presents an S shape curved, which corresponds to a type II isotherm. The adsorption of water is done step-by-step, first by the constitution of the monolayer, and then once all the available sites are occupied, the multilayer system is formed.

On the contrary for the extrudates with MCT-oil, isotherms are more associated to a type III, where water is immediately absorbed as a multilayer system, and then a strong absorption of the free water due to the morphology of the material.

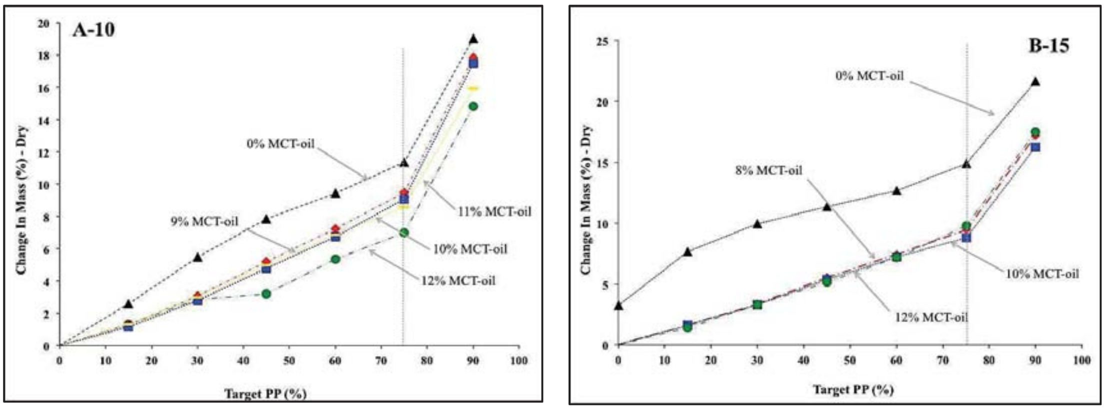


Figure 10. Sorption isotherm of formulations A and B: Impact of the MCT-oil content.

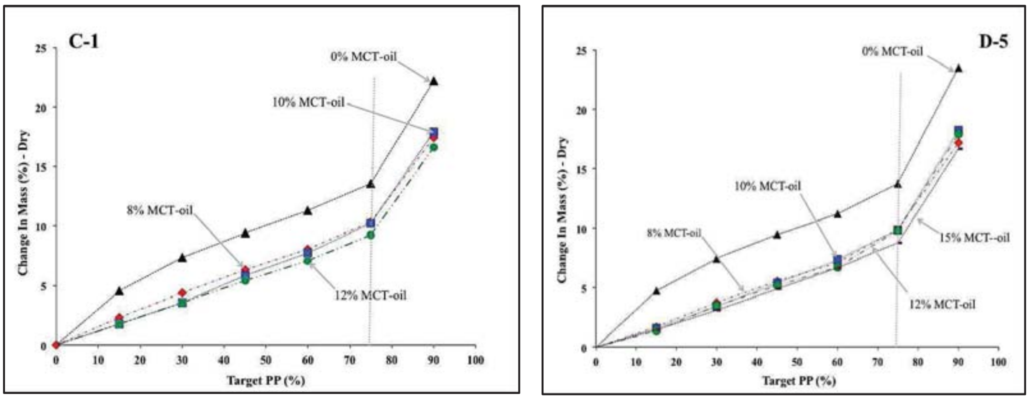


Figure 11. Sorption isotherm of formulations C and D: impact of the MCT-oil content.

In general, hygroscopicity decreased with the increase of the incorporation rate of MCT-oil, for all formulations. This was expected since the part of hydrophobic compound in the mixture was increased and consequently the matrice loses its hygroscopic character.

For all the samples, the drastic sorption change remained around 75% RH., which corresponds to the occurrence of unbound water on the matrice.

Therefore there would be a limit of the load of the hydrophobic compound to be encapsulated if the main characteristic of the delivery system is to be highly hygroscopic.

Therefore, an incorporation rate around 8 and 10% (w/w) of MCT-oil will be ideal because extrudates presented a homogeneous pore size distribution and no exudation was observed.

3.2.6. *Thermal properties*

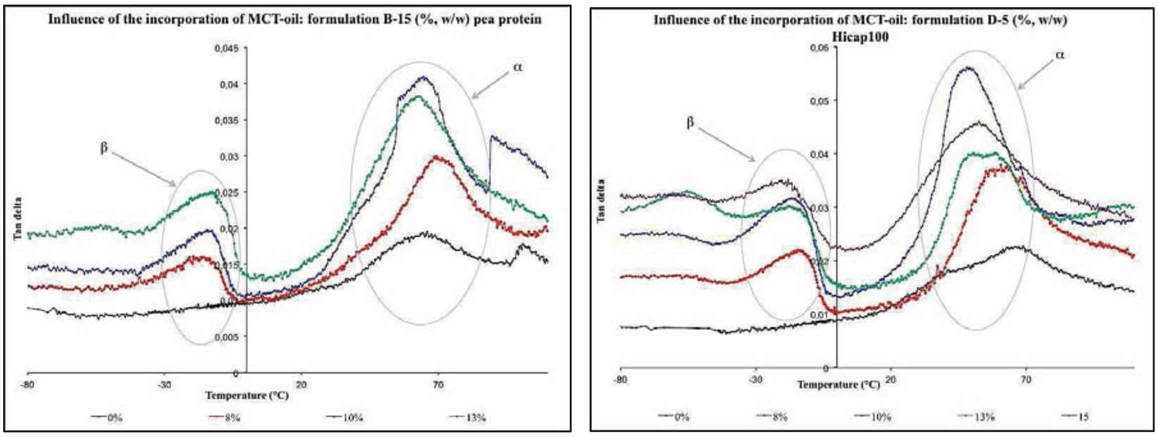


Figure 12. DMA graphs for formulations B-15 and D-5 at different incorporation rates of MCT-oil.

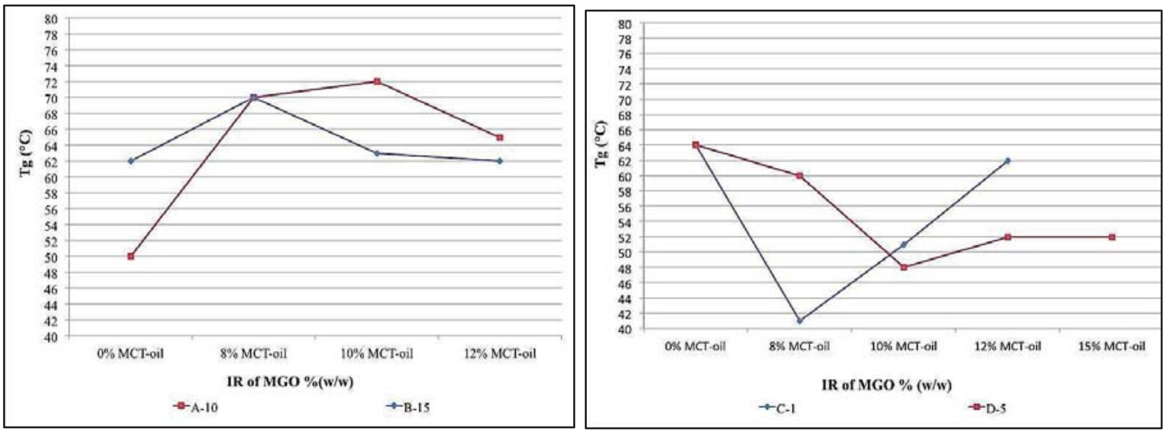


Figure 13. Influence of the MCT-oil incorporation rate on the glass transition temperature for formulations A-10, B-15, C-1 and D-5.

For all the formulations, the bio-based delivery systems obtained were in a glassy state at room temperature (Figures 12 and 13) since their α relaxation was above 40°C. The β relaxation that corresponds to a zone richer in plasticizer did not varied for formulations A-10 and B-15 (-14°C), neither for formulations C-1 and D-5 (around -10 to -20°C).

For formulations A-10 and B-15, and MCT-oil incorporation rate below 8% (w/w), MCT-oil acted as an antiplasticizing agent. Inversely, above 8%, it acted as a plasticizer. On the other hand, for formulations C-1 and D-5, the tendency was reversed, as observed in Figure 12.

The β relaxation observed on the left side of the graphs, seemed to be associated to the MCT-oil since it appeared at the same temperature whatever the formulation or the IR of MCT-oil. This relaxation was not observed for the formulation without MCT-oil. The temperatures for that β relaxation varied form -14°C to -20°C for all the formulations.

The reason why the encapsulation efficiency of our delivery system was so high compared to the values mentioned on the literature could be because the glass transition temperature of the matrices were, at least, 20°C above the room temperature. As a result our bio-based systems were at a glassy state at room temperature and so the active is protected and the diffusion of the active molecules was slowed down.

4. DISCUSSION

Encapsulation of a hydrophobic model compound (MCT-oil) in a maltodextrin-based matrix by twin-screw extrusion was only possible thanks to the use of some compatibilizing biopolymers (i.e. pea protein isolate and Hi-cap 100). Extrusion operating conditions were kept mild (a maximum temperature of 50°C and a specific mechanical energy between 120 and 370 Wh.kg^{-1}) but allowing, in the best conditions, to reach encapsulation rate and efficiency of respectively 12.8% and 96.3%.

PPI was selected because proteins are composed of amino acids among which some bear hydrophobic side chains (such as leucine, alanine, valine or isoleucine for the most abundant in PPI (Table1)). Therefore, PPI can act as a compatibilizer agent, facilitating the incorporation of the hydrophobic MCT-oil.

During the twin-screw extrusion process, PPI could also be considered as a texturing agent: without any PPI it was impossible to process maltodextrins alone and the addition of 5 more parts (from A-10 to B-15) provoked a drop of the SME by a factor 2 from 370.9 to 193.5 Wh.kg^{-1}.

The use of only 10 % of PPI was not sufficient to ensure the encapsulation of the MCT-oil. SME was still high; encapsulation rate did not exceed 7% (for an incorporation rate of 11%), its moisture content was stable whatever the amount of MCT-oil incorporated and MCT-oil had a significant anti-plasticizing effect on the matrix glass transition.

Thanks to an additional incorporation of 5parts of PPI or of a classical compatibilizing agent (i.e. Hi-cap 100), encapsulation was clearly improved. But each component did not seem to act with the same mechanism. Formulation B-15, with 15 % of PPI, proved, whatever the incorporation rate (below 13%), high encapsulation efficiency, a relatively high number of pores and a stable and high glass transition temperature (around 62-63°C).

While with the use of Hicap100, SME first increased with addition of MCT-oil before decreasing, encapsulation rate reached 15.4% but with some lower efficiencies than for B-15. Pores were smaller for low incorporation rates and started to grow largely with the increasing amount of MCT-oil, equilibrium moisture content increased with the increasing of the incorporation rate and inversely glass transition tended to decrease (especially for D-5). All these observations would let us think that, with the use of PPI, the MCT-oil is trapped during the extrusion in the hydrophobic region of the proteic structure, in smaller domains and that these domains do not take part in the glass transition of the matrix, which is more governed by hydrophilic interactions. While with the use of Hicap100, its smaller chains (Hi-cap100 is produced by chemical modification of a hydrolyzed starch) play the role of surfactant getting all around the MCT-oil droplets and permitting its incorporation in bigger hydrophilic domains of the matrix and thus acting slightly on the material glass transition (Figure 14). But taking into account the complexity of the whole system, more experiments would be mandatory to further confirm these assumptions, such as FTIR or affinity chromatography.

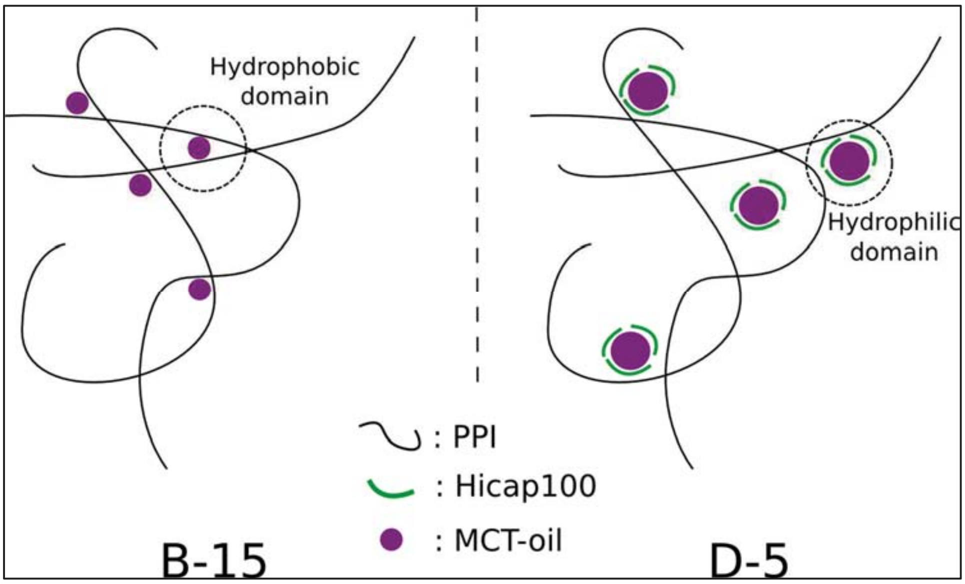


Figure 14. Influence of the Hicap100 addition on the entrapment of MCT-oil droplets in maltodextrin/PPI matrices.

5. CONCLUSIONS

Melt extrusion used as an encapsulation technology for the protection of a model hydrophobic compound, employing biopolymer mixtures of maltodextrins, pea protein isolates and modified starch was possible and optimized in order to obtain bio-based delivery systems. Extrusion allowed obtaining homogeneous mixture with multiple porosity morphology. The surface texture is, in general, smooth with several irregularities (pores, cracks).

The innovative part of these delivery systems is the use of pea protein isolate as the compatibilizer agent, which not only allowed to have better encapsulation rates of the MCT-oil, by increasing the affinity between the two immiscible phases but also improved the extrudability of the mixture. Among all the formulations tested, formulation B (containing 15% (w/w) of PPI) had the highest encapsulation efficiency (96%) for an encapsulation rate of 13 %. Based on these results, pea protein isolate performed similarly than 1-5% of Hi-cap100, but without influencing the material glass transition temperature.

In chapter four, the validation of the extrusion encapsulation conditions that were found, are tested with a real active compound mixture, which will correspond to the incorporation of a fragrance in our bio-based matrices.

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Transition chapter 3 to chapter 4

To summarize, in chapter 3 the extrusion encapsulation conditions were fixed in terms of:

- Screw and temperature profile: (ranging for 20 to 50°C),
- Screw speed (225 rpm),
- Formulations amongst which four were retained: **A-10**, containing 10% (w/w) of PPI, **B-15**, containing 15 % (w/w) of PPI, **C-1**, containing 10% (w/w) of PPI and 1% (w/w) of Hi-cap100, **D-5**, containing 10%of PPI and 5% (w/w) of Hi-cap100)
- Moisture content (20% (w/w)).

These conditions allowed having blends with acceptable texture and viscosity so as to enhance the dispersion of the active liquid compound.

MCT-oil was selected as a model hydrophobic compound to be encapsulated in our agro-materials. The satisfactory incorporation rates of MCT-oil were found to vary between 8 to 12% (w/w). The obtained delivery systems were at a glassy state at room temperature guaranteeing the protection of the active compound. They remained stable at 60% RH for a large period of time. And they presented very promising encapsulation efficiencies up to 90%.

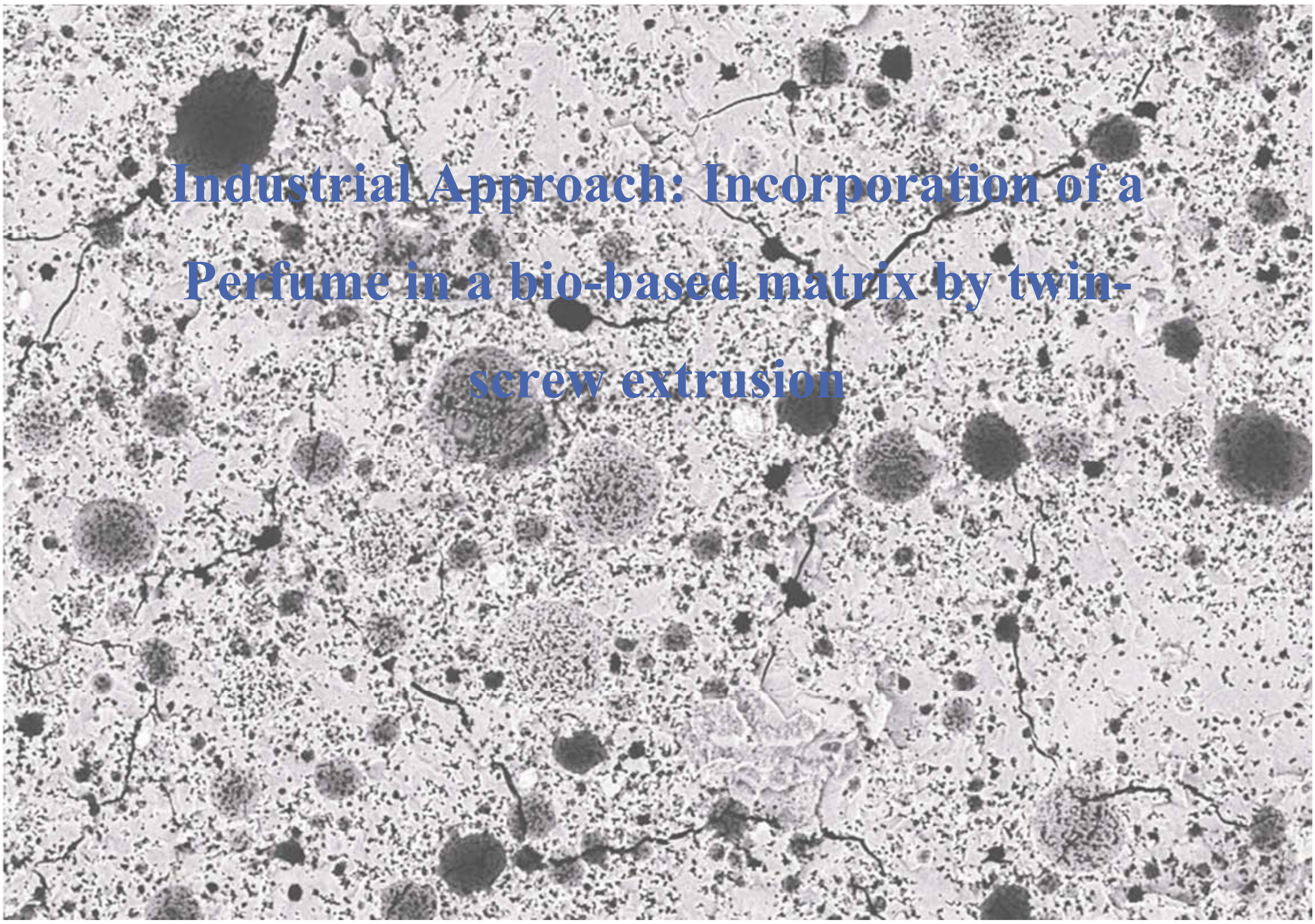
In chapter four these extrusion encapsulation conditions were tested for the incorporation of a specific perfume composed by Givaudan. The perfume was specially designed to study the interactions between the odor active compounds and the matrix. For that reason it is composed of molecules representing different chemical groups as well as all range of volatilities.

This perfume was mixed with MCT-oil at two different contents before being incorporated in the maltodextrin-based matrices. The delivery systems were characterized for thermal and morphological properties. Additionally, a kinetic release study of the fragrance under different environments was investigated.

The olfactive performances of the obtained agro-materials were studied in order to evaluate their possible application in the fragrance industry.

CHAPTER 4

Industrial Approach: Incorporation of a Perfume in a bio-based matrix by twin-screw extrusion



Industrial Approach: Incorporation of a perfume in a bio-based matrix by twin-screw extrusion

Natalia Castro[a, b], Vanessa Durrieu[a, b], Antoine Rouilly[a, b], Christine Raynaud[a, b]

[a] *Université de Toulouse, INP-ENSIACET, LCA (Laboratoire de Chimie Agro-industrielle), F-31030 Toulouse, France*

[b] *INRA, UMR-1010-CAI, F-31030 Toulouse, France*

Email: Antoine.rouilly@ensiacet.fr

Phone number: (+33) 5 34 32 35 09

Fax: (+33) 5 34 32 35 97

Abstract

Twin-screw extrusion encapsulation is a flourishing technology not only in the pharmaceutical and food fields, but also in the world of fragrances. This environmentally friendly and cost effective process could become an interesting counter part of spray drying. However, there is still a lot of fundamental studies to be done, that require more investigations in order to get a better comprehension of the physical processes involved during extrusion and thus, control the final properties of the product. Based on the statements mentioned above and with the aim of elucidate the plausible correlations between the process parameters and final characteristics of the product, the encapsulation of a pre-defined fragrance, in a bio-based matrix composed of maltodextrins and a compatibilizing agent, using a twin-screw extruder was investigated. Therefore the purposes of this chapter were the thermal and morphological characterization of the delivery systems obtained. Additionally, the study of the release of the perfume under different environments was also carried out so as to determine the impact of water. Encouraging encapsulation rates above 60% were measured, and interesting interactions between the aldehydes composing the perfume and the matrix ingredients were also observed. Olfactory tests were

carried out in the framework of a future application of these bio-based matrices in the fragrance industry.

Key words: *Twin-screw extrusion, carbohydrate mixture, encapsulation perfume, perfume*

1. INTRODUCTION

Perfume is a very complex and sensitive mixture that can be easily affected by external agents, such as temperature, moisture content, light and oxygen exposure. Perfume actually consists of mixtures of different volatile ingredients having their own physicochemical properties (volatility, solubility, viscosity) and reactivity. Depending on applications, the number of perfumery ingredients in a composition could range from 20 (for instance, in surface care applications) up to 500 (in fine fragrances and luxury products).

Perfume composition is usually described as what is known in the art as olfactive pyramid (Figure 1): (i) top notes, which correspond to low molecular weight compounds and thus, the most volatiles. Top notes are responsible of the first scent impression, (ii) middle or heart notes, corresponding to medium molecular weight molecules that are more or less volatile, which represent the core of the fragrance and which scent last for a couple of hours. (iii) And bottom notes that are the heaviest molecular weight compounds, and are responsible of the long lasting scent of the perfume.

One of the key responsibilities of perfumery houses is first to create hedonically pleasant composition but also to design fragrances stable in the different applications. One way to protect fragrances is to encapsulate the composition so that the perfumery ingredients do not react/degrade with base components therefore creating coloration, viscosity or even solubility issues. Although several techniques of encapsulation are known in the art (i.e. spray-drying, interfacial polymerization, polymerization), little is known regarding extrusion as a process to encapsulate fragrances. As mentioned in the other chapters, extrusion has demonstrated its high interest for encapsulation of food and pharmaceutical domains and therefore could be one of the most suitable and innovative technology to be employed for the encapsulation of perfumes [1,2].

Results obtained in Chapter 3, allowed establishing mild extrusion conditions for an hydrophobic model compound:

- Low extrusion temperature (≤50°C)
- Medium screw speed (225 rpm)
- Low mechanical stress applied (adequate screw profile)

In terms of encapsulation efficiency, using MCT-oil as model hydrophobic compound, the results were very promising (up to 90%). Besides, the delivery systems showed good long-term stability when exposed to 60% of relative humidity at 25°C.

At this state of the project, several challenges have emerged. The main issues are on one hand, to have high encapsulation efficiency of all the molecules composing the perfume, and on the other hand, to be able to release them in a homogeneous way. Controlling release profile thus raises high challenges and consequently highlights the lack of control and knowledge on the interactions occurring between the active compounds and the matrix components, as there has been poor investigation in the literature about the type of interactions that can take place when a complex mixture, as a perfume, is encapsulated in a carbohydrate matrices by twin-screw extrusion.

The purpose of this chapter was to encapsulate a model perfume into the four different matrices studied in chapter 3, and thereafter to evaluate their release profile at distinct environments. In this perspective, the influence of the moisture content as well as other thermal analysis of the matrices were analyzed in order to better understand the kinetic and olfactive release of the perfume.

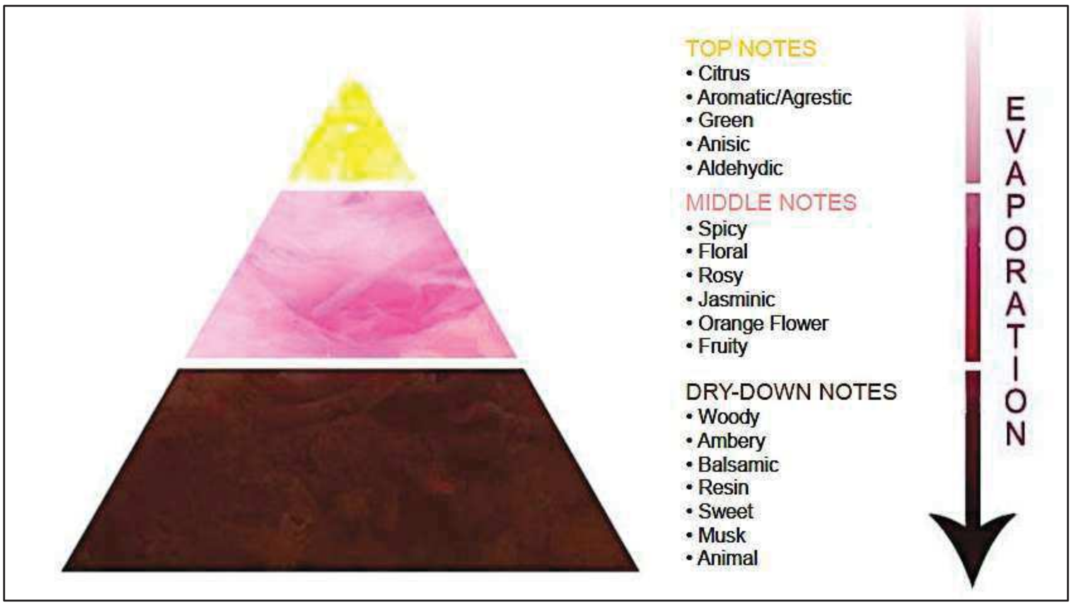


Figure 1. Structure of a perfume, courtesy of Givaudan France SAS.

All experiments presented in this chapter were issued from a single extrusion campaign. These results correspond to preliminary assays to establish more accurate conditions for future up-scale process.

The obtained results allowed having a first insight into the mechanical and analytical behavior of these new encapsulants materials.

2. MATERIALS AND METHODS

2.1. Raw materials

2.1.1. Matrix components

Maltodextrin DE-12 (Glucidex IT-12) and Pea Protein Isolate (PPI), SFP-87, were supplied by Roquettes Frères (Lestrem, France). Hi-cap100 was provided by Ingredion GmbH (Hamburg, Germany). And Miglyol 812N Neutraloel (CAS=65381-09-1), was supplied by CREMER Oleo, GmbH & Co. KG (Hamburg, Germany).

The four formulations used for the encapsulation of the perfume were the same formulations employed for the encapsulation of the MCT-oil (see Chapter 3).

The formulations were the following:

- Maltodextrin I-12/pea protein (90/10): **A-10, containing 10% (w/w) of PPI;**
- Maltodextrin I-12/pea protein (85/15): **B-15, containing 15 % (w/w) of PPI;**
- Maltodextrin I-12/pea protein/ modified starch (89/10/1): **C-1, containing 10% (w/w) of PPI and 1% (w/w) of Hi-cap100;**
- Maltodextrin I-12/pea protein/modified starch (85/10/5): **D-5, containing 10% of PPI and 5% (w/w) of Hi-cap100.**

2.1.2. Citrus: a model perfume

Citrus was a model perfume, exclusively designed by **Givaudan's** perfumers and scientists.

This Citrus perfume was created in order to be hedonically pleasant but also to represent with only 25 molecules a wide variety of fragrance molecules in terms of different physicochemical properties:

- Only twenty molecules composed the perfume
- Volatility
- Solubility in water

- Chemical reactivity thanks to the presence of distinct chemical groups: aldehydes, ketones, alcohols, …

The design of the perfume was also conceived in order to facilitate GC/MS analyses by reducing the number of isomers and co-elutions.

Our objectives with this model perfume was first to encapsulate it in the bio-based matrix, then to establish the possible interactions between the perfume's molecules and the matrix, and last but not the least, to determine the effect of the incorporation of the perfume on the physicochemical properties of the delivery system. The olfactive footprint of Citrus had citrus and fresh notes, with light floral facets. Another key point was that one of the molecules composing the perfume, Isopropyl Myristate, also called IPM, could be employed as a solvent, which acted as a compatibilizer agent too, enhancing the incorporation of the perfume into matrices. Its composition is detailed in Table 1 and the olfactive structure is presented below (Figure 2).

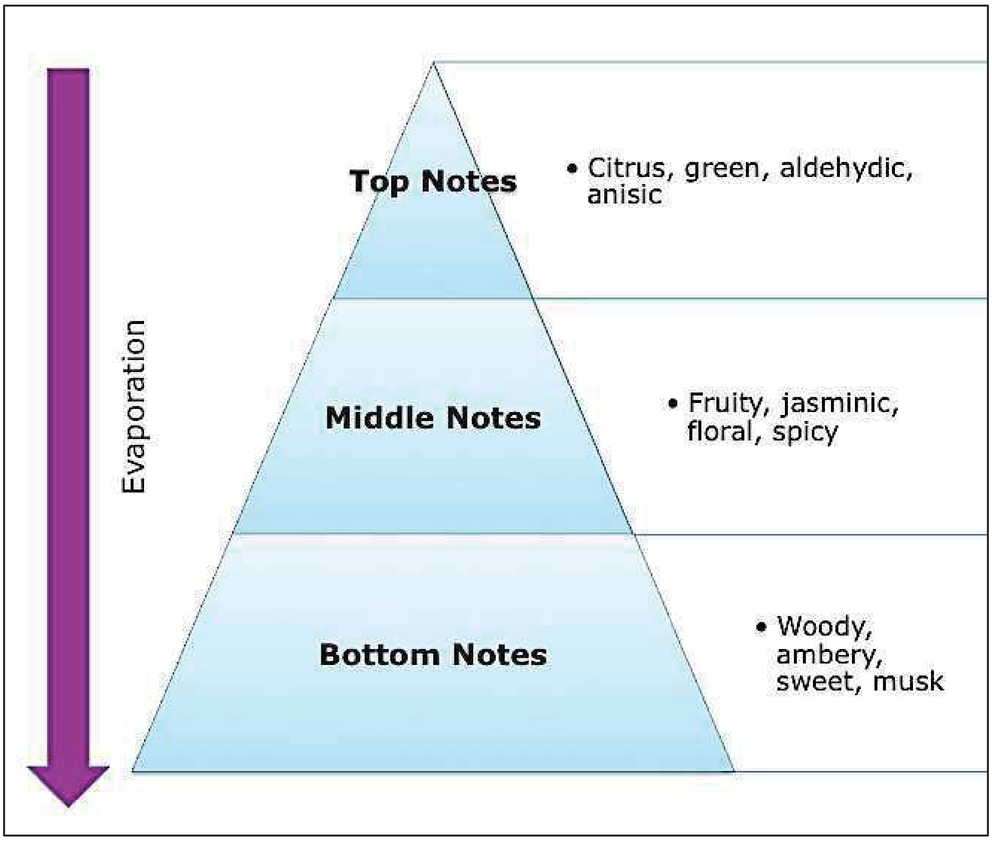
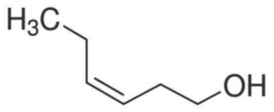
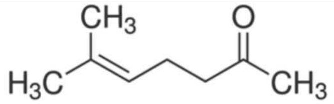
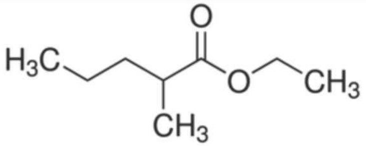
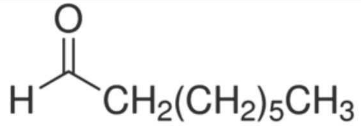
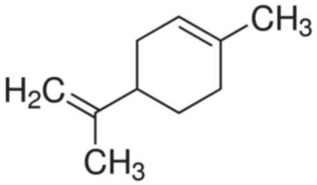
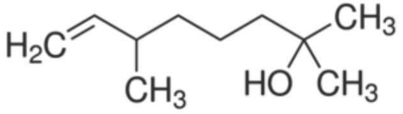
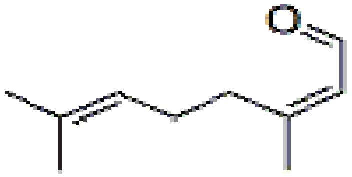
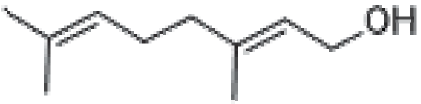
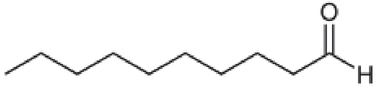
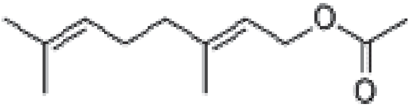
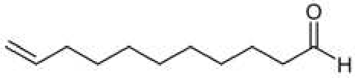
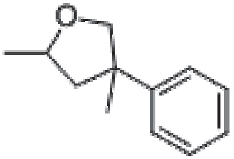
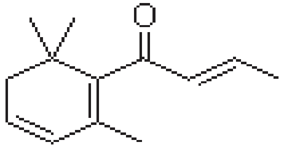
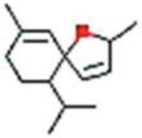
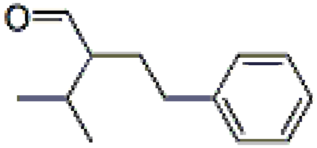
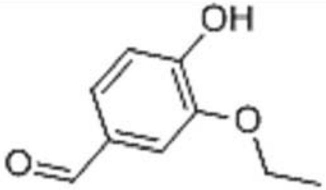
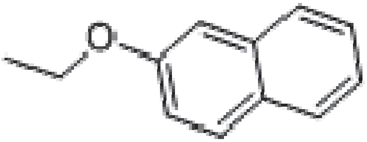


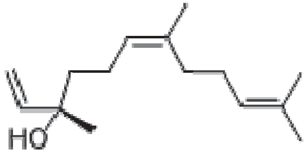
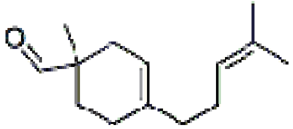
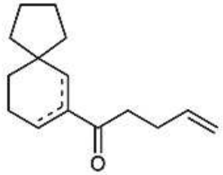
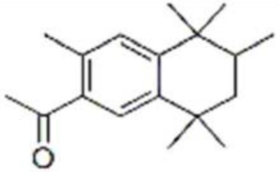
Figure 2. Structure of Citrus.

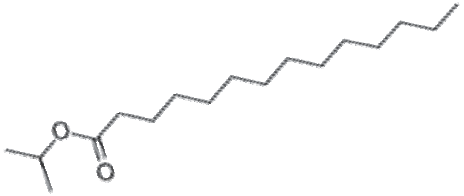
Table 1. Composition of Citrus: physicochemical properties of each volatile compound. The compounds underlying are the ones followed in this study

Commercial name	IUPAC name	CAS number	Structure	Kovats index (DB5-ms)	MW (g.mol^{-1})	Proportions (%/1000)
HEXENOL-3-CIS	3-hexene-1-ol	928-96-1		853	100	10
METHYL HEPTENONE PURE	6-methyl-5-heptene-2-one	110-93-0		934	126.2	15
MANZANATE	ehtyl-2-methylpentanoate	39255-32-8		889	144	5
ALDEHYDE C 8 OCTYLIQUE FCC	n-octanal	124-13-0		950	128.21	15
DIPENTENE	limonene	138-86-3		974	136.2	50
DIHYDRO MYRCENOL	2-methyl-6-methyleneoctan-2-ol	18479-59-9		1036	156.3	150

ALDEHYDE C 9 NONYLIC	n-nonanal	124-19-6		1108	142.2	30
CITRAL TECH	3,7-dimethyl-2,6-octadienal	5392-40-5		1265	152.20	80
GERANIOL 980	(2)-3,7-dimethylocta-2,6-diene-1-ol	106-24-1		1247	154.30	100
ALDEHYDE C 10	n-decanal	112-31-2		1228	156.30	50
ACET GERANYL SYNTH	(2E)-(3,7-dimethylocta-2,6-dienyl)acetate	105-87-3		1299	196.3	60
ALDEHYDE C 11 UNDECYLENIQUE	10-undecenal	112-45-8		1270	168.30	10

RHUBAFURAN	furan-tetrahydro-2,4-dimethyl-4-phenyl	82461-14-1		1358	176.20	0.7
DAMASCONE DELTA	2-beten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl), €	23726-93-4		1408	190.30	10
NEOCASPIRENE	2,9-dimethyl-6-propane-2yl-1-oxaspiro (4,5-)-deca-3,9-diene	89079-92-5		1444	206.32	5
FLORHYDRAL	3-(3-isopropylphenyl) butanal	125109-85-5		1463	190.28	10
ETHYL VANILLINE	3-ethoxy-4-hydroxybenzaldehyde	121-32-4		1480	166.17	0.2
NEROLINE	2-ethoxynaphthalene	93-18-5		1511	177.20	30

LILIAL	3-(4-tert-butylphenyl)-2-methylpropanal	80-54-6		1554	204.30	100
NEROLIDOL	(3-S-6-Z)-3, 7, 11-trimethyldodeca-1,6,10-triene-3-ol	142-50-7		1557	222.40	90
PRECYCLEMONE B	1-methyl-4-(4-methylpent-3-enyl)-cyclohex-3-ene-1-carbaldehyde	52475-86-2		1585	206.30	30
TRIDECENE NITRILE	tridec-2-enenitrile	22629-49-8		1592	193.30	0.1
SPIROGALBANONE PURE	1-spiro-(4,5)-dec-9-ene-9-ylpent-4-ene-1-one	224031-71-4 / 224031-70-3		1700	218.30	2
FIXOLIDE	Ethanone, 1-(5, 6, 7, 8-tetrahydro-3,5, 5, 6, 8, 8-hexamethyl-2-naphthalenyl)-	21145-77-7		1789	258.40	100
ISOPROPYL	propane-2-	110-27-0		1860	270.50	47

MYRISTATE	yltetradecanoate	

2.2. Twin-screw extrusion encapsulation

Different blends were tested using a co-rotating and co-penetrating twin-screw extruder Evolum HT25 Clextral (Firminy, France). The extruder is composed of ten thermo-regulated modules of 10 cm length each. The screws have a 25 mm diameter and are distance of 21 mm. As it was described in Chapter 3, the extruder screw profile was divided in four different zones: conveying, plasticizing and two mixing zones, and optimized to allow extrusion for all the four formulations.

The solid mixture (4.4kg.h^{-1}) was fed into the first part of the extruder with gravimetric feeder K-Tron KML KT20, Coperion K-Tron Pitman, Inc. (Sewell, USA) and, it was immediately mixed with water 20-22% (w/w), to ensure thanks to the thermo-mechanical stress, the plasticization of the blend before the active compound addition at barrel 5.

To verify the stability of the extrusion conditions, two temperature sensors were placed in barrel 4 (during the plasticization zone) and in barrel 10 (before exiting the extruder) to monitor the temperature of the blend. The experimental set-up of the extruder is presented in Figure 3.

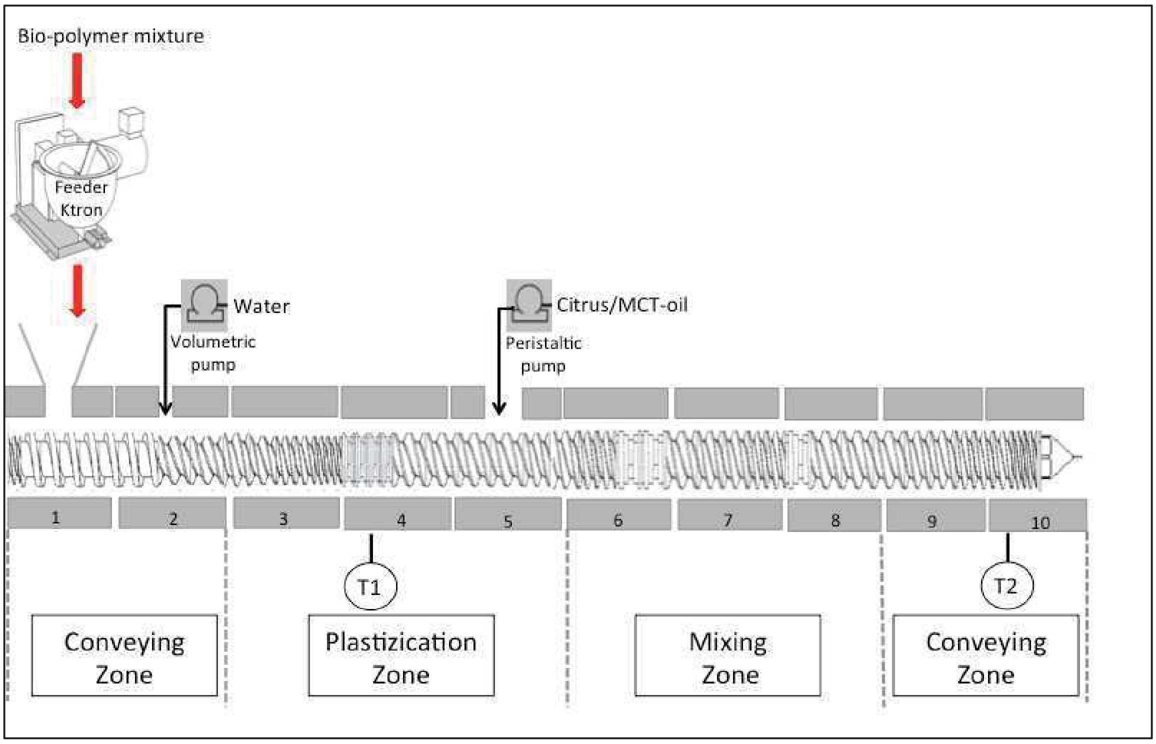


Figure 3. Twin-screw extruder Evolum HT25 configuration and screw profile for the elaboration of the bio-based matrix

The extrusion operating conditions are summarized in Table 2. Taking into account the advantages of using MCT-oil as a solvent vector to favor the incorporation rate of the active in the matrix [3–5], we decided to test two different mixtures of Citrus/MCT-oil e.g. 50/50 and 80/20. For both mixtures, the perfume loading was fixed at 10% (w/w) with respect to the solid polymer mixture. Citrus/MCT-oil mixture was introduced into the plasticized mixture at barrel 5 thanks to a peristaltic pump PM600 JOUAN S.A. (Saint-Herblain, France).

Table 2. Extrusion process conditions

Extrusion parameters	Specifications
Screw speed (rpm)	225
Feed rate solid mixture (kg.h^{-1})	4.40
Feed rate water (kg.h^{-1})	1.40
Feed rate Citrus/MCT-oil(80/20) (kg.h^{-1})	0.63
Feed rate Citrus/MCT-oil (50/50) (kg.h^{-1})	1.10

The temperature profile was optimal because all the barrel temperatures were fixed at a much lower temperature than the flash point of the molecules of the perfume. In our case, extruding at low temperature, was clearly an advantage because it reduced the risk to have thermal degradation of the active molecules, minimized their evaporation and prevented flash-off the molecules. The temperature profile corresponds to the profile temperature used for the encapsulation of MCT-oil (Table 3).

Table 3. Extrusion profile temperature

Module of the barrel	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Temperature (T°C)	20	20	40	50	50	40	40	40	40	40

The eight formulations are detailed on Table 4. The composition was calculated in relation to the initial feeder rates and the solid mixture dry matter.

Table 4. Formulations tested: initial composition introduced in the extruder. The load of Citrus was fixed to 10% (w/w) related to the solid mixture, for all the formulations

Formulation	Maltodextrin DE-12 (%)	PPI (%)	Hi-Cap100 (%)	Citrus (%)	MCT-oil (%)	Water (%)
A-10 + Citrus/MCT-oil (50/50)	57.6	6.4	0	8	8	20
A-10 + Citrus/MCT-oil (80/20)	61.2	6.8	0	8	2	22
B-15 + Citrus/MCT-oil (50/50)	54.4	9.6	0	8	8	20
B-15 + Citrus/MCT-oil (80/20)	57.8	10.2	0	8	2	22
C-1 + Citrus/MCT-oil (50/50)	56.96	6.4	0.64	8	8	20
C-1 + Citrus/MCT-oil (80/20)	60.52	6.8	0.68	8	2	22
D-5 + Citrus/MCT-oil (50/50)	54.4	6.4	3.2	8	8	20
D-5 + Citrus/MCT-oil (80/20)	57.8	6.8	3.4	8	2	22

The samples were collected in an aluminum pan and dried at 50°C during two minutes. After drying, the samples were stored inside a blister and hermetically stored in a climatic chamber at 0%RH at 25°C during three weeks before the kinetic study.

2.3. Physicochemical characterization of the delivery system

2.3.1. Moisture content: Karl Fisher

Moisture content of all samples was determined thanks to the Karl-Fisher-Titration method. The measurement of the moisture content in the samples was conducted with a volumetric titrator V-20 METTLER-TOLEDO (Giessen, Germany). A commercial mixture of methanol and formamide (1:1), from Riedel-deHaen (Seelze, Germany) was employed as solvent, and Hydranal Composit-5 was used as a titrant.

Extrudates were stabilized in four controlled humidity chambers setup at 33%, 60%, 75% and 90% of relative humidity and 25°C for eight weeks before analysis.

All the titrations were performed in triplicates for each sample.

2.3.2. Glass transition temperature (DMA)

Glass transition temperature (T_g) of the extrudates was measured on a Triton Technology Dynamic Mechanical Analysis apparatus (Triton Technology, UK), by a thermal scan in the simple geometry of the single cantilever-bending mode. The amplitude (25µm) and the multi-frequency (1 and 10 Hz) mode were kept constants during the analysis. Samples were placed in an aluminum pocket and the temperature range of analysis was set from -100°C to 200°C at a scanning rate of 2°C/minute.

Extrudates were stabilized at four controlled humidity chambers set up at 33%, 60%, 75% and 90% of relative humidity and 25°C for eight weeks before analysis.

2.4. Morphological analysis: SEM

Morphology of the extrudate was examined by scanning electron microscopy (SEM). The particles were deposited on conductive double-faced adhesive tape and sputter-coated with silver. SEM observations were performed with a LEO435VP scanning electron microscope LEO Electron microscopy Ltd., (Cambridge, UK) operated at 8kV. All the samples were metallized with silver under vacuum before observation.

The porosity of the samples was analyzed by ImageJ. ImageJ is an open source Java-based software developed by the National Institute of Health (Bethesda, USA) and employed as an image-processing tool in the scientific domain. This image treatment allowed to characterized the extrudate in terms of morphological aspects (i.e. porosity) and other structural heterogeneities (e.g. surface and texture).

These analyses allowed having estimation about the morphological aspect of these extrudates.

2.5. Model perfume characterization

2.5.1. GC-MS-FID

The volatiles of the perfume in the different bio-based delivery systems were identified and quantified by combined Gas Chromatography (GC)/ Flame ionization detection (FID) / Mass spectrometry (Clarus® 500GC /TurboMass MS detector, Perkin Elmer, Villebon-sur-Yvette, France). The injector and the detector (FID) were heated respectively to 250°C and 280°C. A column (50m x 0.25 mm x0.25 µm) with a (5%-phenyl)-methylpolysiloxane phase Agilent J&W DB-5ms, from Agilent Technologies (Les Ulis, France) was employed. The temperature started at 40°C, held for one minute and ended at 70°C with a temperature ramp of 20°C/min. Followed by a temperature ramp of 2°C/min, starting at 70°C and ending at 200°C. Then, to ensure that nothing remained in the column, a temperature ramp of 20°C/min was applied, from 200°C to 280°C, and the program ended by equilibration for 10 minutes at 280°C. Helium was used in a splitless mode as a gas carrier and the column flow rate was fixed at 1.52 mL/min. The injections of 0.5µL of each of these solutions were performed in quadruplicate. Mass spectra were recorded in electron impact mode (70eV) between 35 to 400 *m/z* mass range at a scan speed of 3.63 scan s^{-1}.

First, the perfume was injected so as to identify and quantify all its twenty molecules. Their identification was made based on their retention index (Table 1) and mass spectra. Results were checked with the NIST2011 Mass Spectral Library.

The same analytical method was employed to identify the perfume's composition, to prepare the calibration curves, as well as for the quantification analysis of the volatiles in the extrudates. Results were expressed as mean values with the corresponding standard deviations.

2.5.2. GC Calibration of Citrus

An internal calibration, using methyl decanoate 99% (CAS: 110-42-9, MW=186.29 $g.mol^{-1}$) as an internal standard from Sigma Aldrich (St Quentin Fallavier, France), was made in order to establish a calibration curve for six molecules among the twenty molecules composing the perfume. The concentration of the internal standard was fixed at 1.10^{-4} $g.mL^{-1}$. The six selected molecules were considered to be representative of the whole range of volatility of the perfume: limonene, dihydromyrcenol, n-decanal, lilial, IPM and fixolide. For instance, the perfume was used as the standard mixture solution for quantification and determination of the calibration curves. Figure 4 illustrates the chromatogram of Citrus and the six selected molecules.

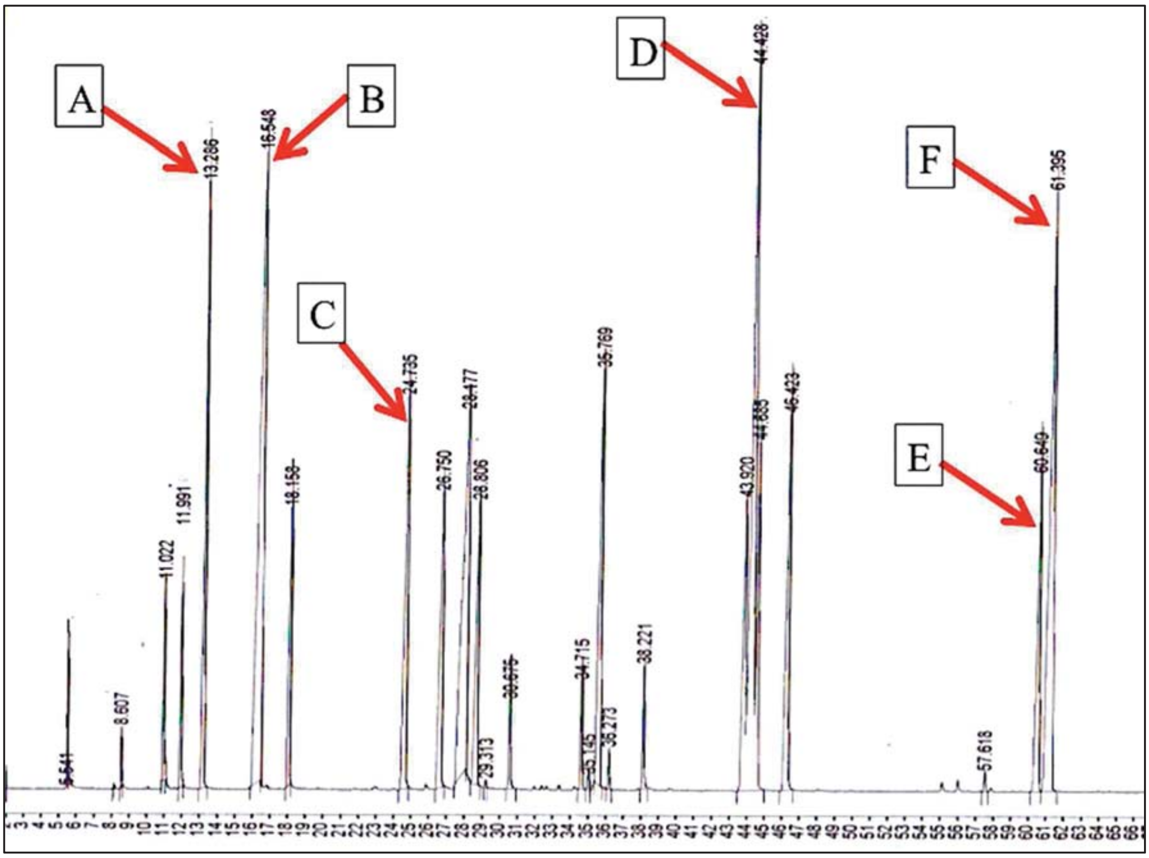


Figure 4. Chromatogram of Citrus (Area=f (retention time (min)). A=limonene; B=dihydromyrcenol; C=n-decanal; D=lilial; E=IPM and F=fixolide

Thus, five geometric dilutions of the standard solution with different concentrations ranging from 0.39 $mg.mL^{-1}$ to 6.25 $mg.mL^{-1}$ were prepared in methanol HPLC (≥99.9%) grade (CAS: 67-56-1, MW=32.04 $g.mol^{-1}$) supplied by Sigma Aldrich (St Quentin Fallavier, France), and analyzed in a gas chromatograph Clarus®500, coupled to a mass spectrometer Turbo Mass Perkin Elmer® in the same conditions as described in 2.5.1.

Knowing the concentration of the internal standard and its peak area, the value of the concentration of each molecule in each solution could be determined by plotting the ratio of the areas as a function of the concentration ratios. The linear relationship of the calibration curves are expressed as follow:

$$\frac{A\ xVOC}{A\ MD} = f\left(\frac{Q\ xVOC}{Q\ MD}\right)$$

Where, $A\ xVOC$ is the area of the corresponding volatile organic compound of the perfume, $A\ MD$ is the area of the corresponding internal standard, $Q\ xVOC$ is the concentration of the corresponding volatile organic compound and, $Q\ MD$ is the known concentration of the internal standard. In this case Q MD corresponded to 1. 10^{-4} g.mL$^{-1.}$

Linear calibrations plots were obtained with a coefficient of determination of 0.9976 (limonene), 0.9991 (dihydromyrcenol), 0.9985 (n-decanal), 0.9718 (lilial), 0.9960 (IPM), and 0.9997 (fixolide). An illustration of the calibration curve of fixolide is presented below (Figure 5).

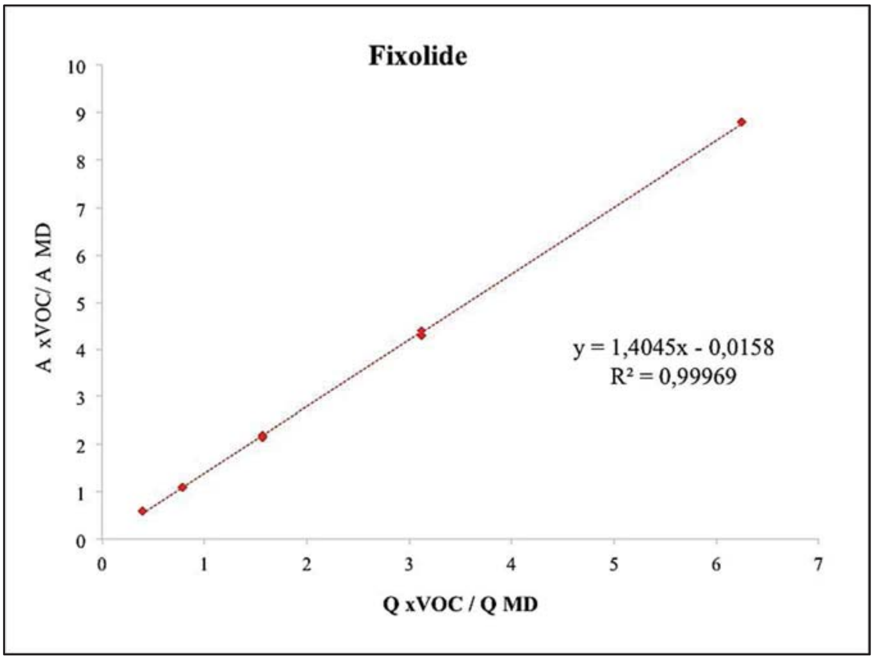


Figure 5. Calibration curve of fixolide

2.5.3. Solid/liquid extraction

Approximately one gram of the extrudate containing the perfume was grinded and added to a 50 mL volumetric flask. Then, 3 mL of water was added in order to enhance the dissolution of the extrudate. To the volumetric flask 50 μL of a standard solution of Methyl decanoate at 1.10^{-4} $g.mL^{-1}$ was introduced. The volumetric flask was completed to the mark with methanol. The solutions were ultrasonicated (>20kHz) for 20 minutes. Before the solutions were placed into a GC-vial, they were filtered thanks to a PTFE filter (13 mm of diameter and 0.45μm pore size) from VMR International S.A.S (Fontenay-Sous-Bois, France).

Extractions were conducted in duplicates for each extrudate. The molecules were identified based on their retention time and specific mass spectrum.

As a result the amount of perfume remaining on the matrix was determined by interpolation using the linear relationship form the calibration curves.

2.6. Kinetic release study

The aim of this kinetic study was to investigate the effect of humidity on the release profile of the perfume. As reminder the principal characteristics of these delivery systems were that they must be highly hygroscopic, and so release of the active could be triggered by humidity. Four different climatic chambers were used in order to analyze the behavior of the bio-based delivery systems.

Two climatic chambers were fixed at 60% and 75% RH at 25°C. The other two climatic chambers were home made thanks to hermetical boxes of 30 L and saturated salt solutions: $MgCl_2$ (CAS: 7757-79-1, MW=101.10 $g.mol^{-1}$) for the chamber at 33% RH at 25°C. And, KNO_3 (CAS: 7791-18-6, MW=203.31 $g.mol^{-1}$) for the chamber at 90% RH at 25°C. Both salts were provided by Sigma Aldrich (St Quentin Fallavier, France).

The kinetic study started after the extrudates were stabilized for three weeks, so all could samples reach equilibrium, at hermetically closed chambers at 0% RH at 25°C after they were produced and was conducted over 28 days. The different stages of the process are illustrated in Figure 6, and the climatic chambers employed are shown in Figure 7.

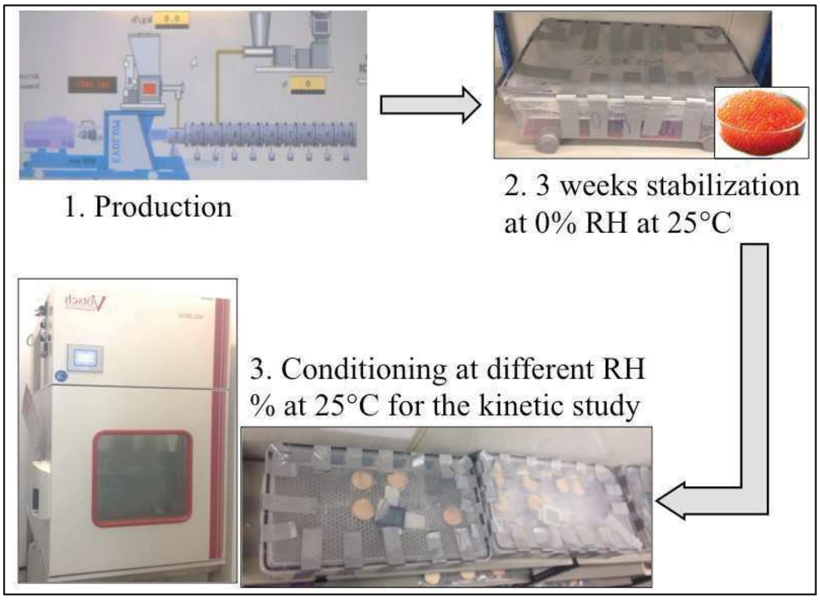


Figure 6. Scheme of the 3 stages of the process: The production, then stabilization at 0% RH and the conditioning for the kinetic study

Figure 7. Pictures of the climatic chambers: A=33% RH at 25°C; B=60% RH at 25°C; C=75% RH at 25°C; D=90% RH at 25°C

The kinetic study for the samples that were conditioned at 33% RH at 25°C started a month later as the climatic chamber needs to go back to equilibrium.

2.7. Sensory tests

In this section, the olfactive performance of the bio-based delivery systems produced by twin screw-extrusion were tested against market products for 2 application fields: powder detergent and air care applications.

It is noteworthy to remind, that based on the specifications of the new encapsulated systems (highly hygroscopic and glassy at ambient temperature) the evident targeted applications for the extrudates were air fresheners and perfume for detergent or WC block products.

The first olfactive evaluation was to analyze the extrudate as an air freshener product. The second olfactive test was to investigate the fragrance bloom of the extrudate when added in a detergent-base powder.

An intern expert panel, consisting of Givaudan employees with strong knowledge and understanding of the market products, evaluated the samples for their performance and their olfactive character.

To evaluate the olfactive performance of the extrudates, a 5-points hedonic scale test (Figure 8) was carried out to measure the odor intensity. This type of sensory experiment judges if an odor is perceived (detection of the odor threshold of the perfume) and determines how strong is the perceived odor [8].

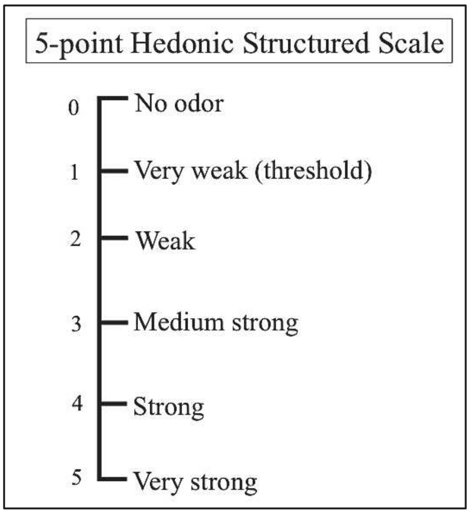


Figure 8. 5-points hedonic scale used for olfactive evaluation

Prior to the olfactive assessment, all samples were analyzed in order to determine the quantity of perfume in the matrix.

The olfactive evaluation was conducted at iso-oil loading versus benchmarks containing the same citrus perfume model.

Remark: all the olfactive tests were carried out with extrudate that were stocked for more than nine months on a hermetic blister at 4°C. Only extrudates of a Citrus/MCT-oil content of (80/20) were analyzed.

2.7.1. Air-fresheners

Air fresheners are products used for eliminating or masking the malodors, or just simply to perfume indoor spaces. Usually, market products have a lifetime between 30 and 50 days. They exist in wide range of products in the market, from sprays, gels, plug-ins to candles and incense burners.

The sensory booths are humidity- and temperature-controlled hermetic rooms. The booths are in aluminum because it's a neutral material relating to absorption or emission of volatile organic compounds.

The testing was carried out in a large booth (25m^3 booths, 21°C, 50% RH) specifically designed with a constant airflow. The airflow was turned off during the testing. The testing was conceived so that each sample contains the same amount of fragrance and has approximately the same surface area. The samples were placed on the booths 40 minutes before the olfactive tests begin to ensure diffusion of the perfume in the room. The fans were turned on for 5 seconds to dispersed the franfrance around the booth and then the assessment began.

The olfactive performance and character of the system was first assessed through a porthole in the door of the booth, then Givaudan expert panel enter the booth to complete the assessment. The overall perceived by the Givaudan expert panel suing à 0-5-intensity scale judged intensity.

Tests were performed over a month and evaluations were realized at three-time points to understand the evolution of hedonic intensity and profile: on day-1, day-19 and day-28.

2.7.2. Powder detergent

Although fragrance is used in relatively low dosage (<2%) and does not intervene in the cleaning performance of the detergent, special attention is always given to perfume because it is the first aspect entering in contact with consumers [9]. Indeed, an inadequate fragrance can impact on the cleaning efficiency perceived by the consumer and ultimately deteriorate the image of a good detergent.

For the detergent evaluations, an actual “in use” test of a powder detergent product in “hand-washing” conditions was performed. This type of ‘in use’ tests allow to give direct information about the bloom of the fragrance [10].

The extrudates were first finely grinded to powder and then mixed to an unperfumed powder detergent base. From now on it will be reefer as the mixture. The bio-based delivery systems were compared to two reference products. The first one was the so-called “free-oil”, which corresponds to the perfume directly mixed with the perfume-free detergent base powder. The free-oil was considered as the control sample, since the “bloom” of the perfume for this sample was the strongest. The second product used is Givaudan proprietary technology, Bloomtech®, a water-soluble spray-dried starch-based matrix containing up to 50% of perfume.

All samples were tested at iso-oil loading, 15 grams of the mixture were added to three liters of water in a vessel. The solution was immediately stirred thanks to a glass rod and trained panelists scored the bloom of the perfume

<u>Remark</u>: all the experiments were performed on samples that were issued from one extrusion production.

3. RESULTS AND DISCUSSION

3.1. Characterization of the delivery system

In this first section, the analysis of the physicochemical properties of the delivery systems and the influence of the process parameter are addressed. Correlations between results obtained in Chapter 3 and in this Chapter are also discussed.

3.1.1. Study of the process parameters: influence of the SME and the temperature profile

The specific mechanical energy as well as the temperature profile of the extruder were two of the process parameters monitored in order to evaluate the impact of the formulations on the final properties of the delivery system. One of the key advantages for this study is that all the independent process variables were kept constant. Besides, the incorporation rate of the fragrance, for all the eight formulations, was maintained constant at 10% (w/w), but two perfume/MCT-oil ratios were tested (50/50 and 80/20, (w/w)). So, a direct correlation between formulations, SME and temperature profile could be established.

SME corresponding to the extruder herein employed was given by the following equation, as defined on Chapter 3:

$$SME = \frac{Power}{feed\ rate} = \frac{Pm * \frac{T}{Tmax} * \frac{N}{Nmax}}{feed\ rate} = \frac{U * I * cos\varphi * \sqrt{3} * \frac{T}{Tmax} * \frac{N}{Nmax}}{feed\ rate}$$

P_m: power supplied by the drive motor (Watts)

Feed rate: (kg/h)

T: torque (%)

T_{max}: maximum torque of the extruder (T_{max}=100%)

N: screw speed (rpm).

N_{max}*: maximum screw speed of the extruder (*$N_{max} = 1200$ *rpm)*

U: electric potential (U = 400 V)

I: electric current (I = 57A)

Cos φ: theoretical efficiency of the extruder's engine (cos φ = 0.9)

The recorded process parameters are summarized in Table 5.

The temperature profile of the extruder was stable during the extrusion experiments and for all the formulations tested. There was neither auto-heated zones nor drastic increase of the temperature when the Citrus/MCT-oil mixture was incorporated. The stability of the torque and profile temperature during extrusion was remarkable as no increase was observed for the different MCT/citrus oil ratio tested at different loading/incorporation rates.

It is important to stress out that, for both thermic sensors, the measured temperatures were 10 to 20°C below the set temperature, respectively 50 and 40°C. This may be explained because the thermic sensors only measure the temperature at the surface of the mixture extruded and not the barrel's temperature or the temperature of the whole mixture inside the barrel. This was also noticed by Emin & Schuchmann[11], (2013a) and Godavarti & Karwe[12], (1997). As a result, the temperature measured by the thermic sensors only served, in our case, as an alert signal that helped to indicate if dehydration of the mixture was occurring on the zones were the mechanical shear was stronger (generally, in the zones where the kneading and mixing screw elements were set). Due to high mechanical shear, the mixture could indeed dehydrate and its temperature could rapidly increase through viscous friction.

Table 5. Extrusion process parameters values

Formulation	Torque (%)	Total feed rate ($kg.h^{-1}$)	SME ($Wh.kg^{-1}$)	T(°C) module 4	T(°) module 10
A-10	10	5.80	114.90	35	31
A-10 + Citrus/MCT-oil (50/50)	10	6.90	96.58	33	31

A-10 + Citrus/MCT-oil (80/20)	13	6.43	134.73	34	30
B-15	10	5.80	114.90	34	29
B-15 + Citrus/MCT-oil (50/50)	9	6.90	86.92	33	30
B-15 + Citrus/MCT-oil (80/20)	10	6.43	103.64	33	30
C-1	13	5.80	149.37	32	34
C-1 + Citrus/MCT-oil (50/50)	12	6.90	115.90	30	34
C-1 + Citrus/MCT-oil (80/20)	11	6.43	114.00	28	33
D-5	11	5.80	126.39	35	34
D-5 + Citrus/MCT-oil (50/50)	10	6.90	96.58	34	30
D-5 + Citrus/MCT-oil (80/20)	11	6.43	114.00	33	30

All the measured SME values were below 200 $Wh.kg^{-1}$ implying that the mixtures were plasticized [13].

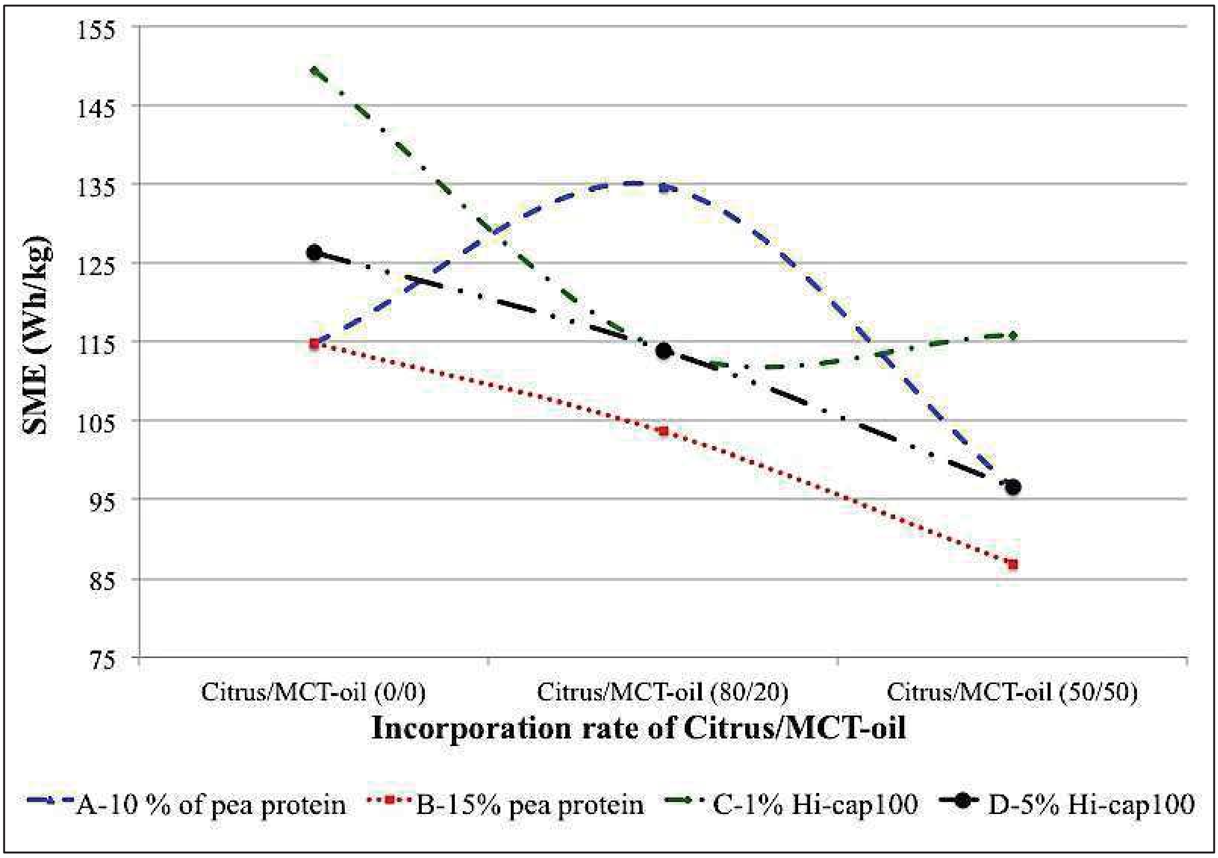


Figure 9. Influence of the IR of Citrus/MCT-oil on the SME

SME expresses how much energy is required to transform and mix all the ingredients inside the extruder in order to obtain a homogeneous mixture. In Chapter 3, MCT-oil was considered to act as a lubricating agent because it decreased the SME as its content was increased.

At first sight (Figure 9), in the case of the active mixture Citrus/MCT-oil, the SME of formulations B, C and D had the same tendency; SME decreased as MCT-oil content increased. However, for these formulations, the active mixture was splashed out at the exit of the extruder, suggesting that either there was no sufficient mixing to incorporate the entire active compound mixture inside the matrix, or that the amount of the active mixture was too high and therefore not everything could be incorporated.

As a matter of fact, for formulations containing the active mixture at (50/50) % (w/w) of content, there was less solid available for the same load of Citrus (10 % (w/w)). When the active mixture is added at (80/20) % (w/w) of content, the total load of the hydrophobic actives was fixed at 10 % (w/w), whereas for an active mixture at (50/50) of content, the load of the hydrophobic active mixture was at 16% (w/w). So, not only there was less solid available but also there was an excess of active to be incorporated, which explained its splashing out at the exit of the extruder and the strong exudation of the delivery systems after extrusion. So the SME continue ti decrease from loading of Citrus/MCT-oil of 80/20 to 50/50, not because the process requires less energy but because there was an exces of active mixture to be incorporated.

These results confirmed what it was demonstrated on Chapter 3, good incorporation rates were obtained for an active load ranging below 12 % (w/w).

So, the SME decreased because there was an excess of active compound, and therefore there was no shearing stress that could be applied into the mixture, which was more liquid than solid. As a consequence the torque and the SME were lower because of an excess of lubricating agent.

Formulation C-1% (w/w) presented the highest SME when no active mixture was incorporated but showed the most important decrease in SME value when incorporating active mixture. This result might be related to the fact that to low amount of Hi-cap100 was required to allow a better incorporation of the active compound mixtures at distinct contents.

Formulation B-15% (w/w) had the lowest SME values for the different incorporation rates, suggesting that this formulation was the best in terms of mixing efficiency and rheology compared to the other three formulations.

Formulation A-10% (w/w) presented the highest SME value for an active mixture content of Citrus/MCT-oil at (80/20). This was expected, since the same pattern was found on chapter 3, where formulation A, with an incorporation rate of 11% of MCT-oil, had the highest SME value (298 $Wh.kg^{-1}$), indicating that only 10% of PPI was not sufficient to increase the affinity between these two immiscible phases (e.g. the hydrophilic matrix and the hydrophobic active mixture), and thus more energy was required to ensure a good encapsulation and an efficient mixing. Even with an incorporation rate of MCT-oil of 12%, the SME value remained higher (255 $Wh.kg^{-1}$) than the other formulations. So, in this case the SME value suggested that affinity between the hydrophilic matrix and the hydrophobic active mixture were poor, and thus more energy was needed to ensure an efficient mixing of all the ingredients.

3.1.2. Thermal properties

The bio-based matrix was elaborated with three different raw materials (e.g. maltodextrins, PPI and Hicap100) and water, which of course, is one of the key parameters in this study since it controls the shelf-life stability of the final product [14]. The thermal properties of the matrices were very difficult to analyze, due to their complex nature; not only because they were elaborated with more than two ingredients, but also because of the complexity of the active compound mixture that was incorporated. In fact, Citrus/MCT-oil revealed itself as a mixture of a large variety of molecules with different chemical properties. The chemical properties of the molecules composing the active mixture modified indeed the thermal behavior of the wall material as it can be seen on the thermograms of the Figure 10.

Determining the glass transition temperature of delivery systems is crucial since it serves as an indicator to know in which conditions the product must be stored but also used. Besides, the glass transition temperatures can help to get a better understanding of how the active compound could be released [15,16].

Herein, we considered that maltodextrins, PPI and Hi-cap100 were miscible and, as a result the matrix mixture obtained was homogeneous. Therefore, the α relaxation temperature was associated to the glass transition temperature of the whole matrix. This statement allowed focusing our attention on the impact of the incorporation of Citrus/MCT-oil compound on the thermal behavior of these four matrices. In addition, the evolution of the glass transition temperature of each matrix that was exposed to different relative humidities was also investigated.

For all the matrices that were analyzed at different humidities, the thermal behavior was similar. For all the samples, three or four thermic relaxation temperatures were observed testifying the complexity of the system. The thermograms presented below (Figure 11) showed the DMA of extrudates C-1 exposed to different relative humidities.

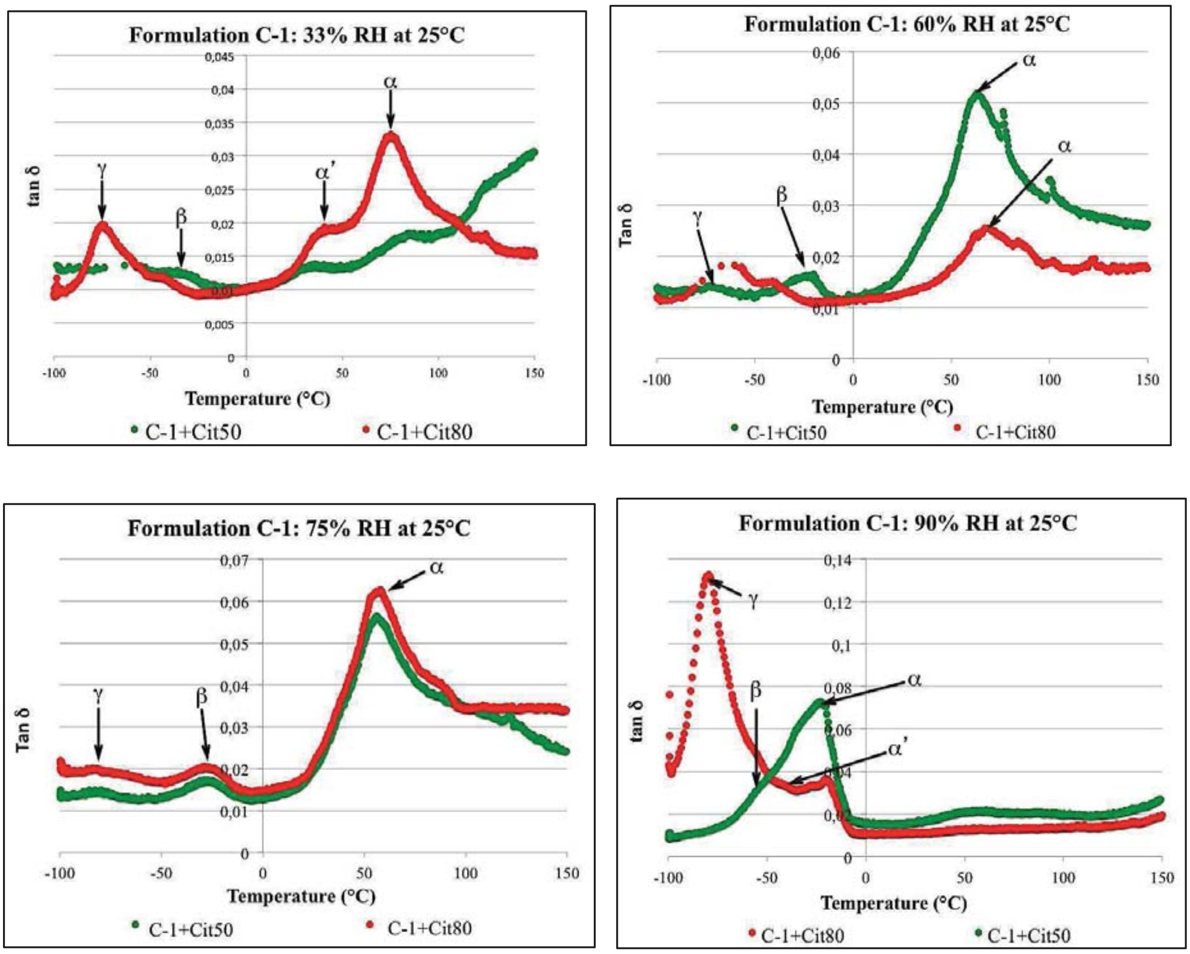


Figure 10. DMA thermograms of formulation C-1 exposed to different relative humidities

The thermogram (Figure 11) of the matrices exposed to 33% RH at Citrus/MCT-oil content of (80/20) put in evidence all the possible existing thermal relaxations, since under this environment the effect of water was limited and consequently, liberation of the active compound was expected to be very low. Three to four thermal relaxations were detected: α, α', β and γ.

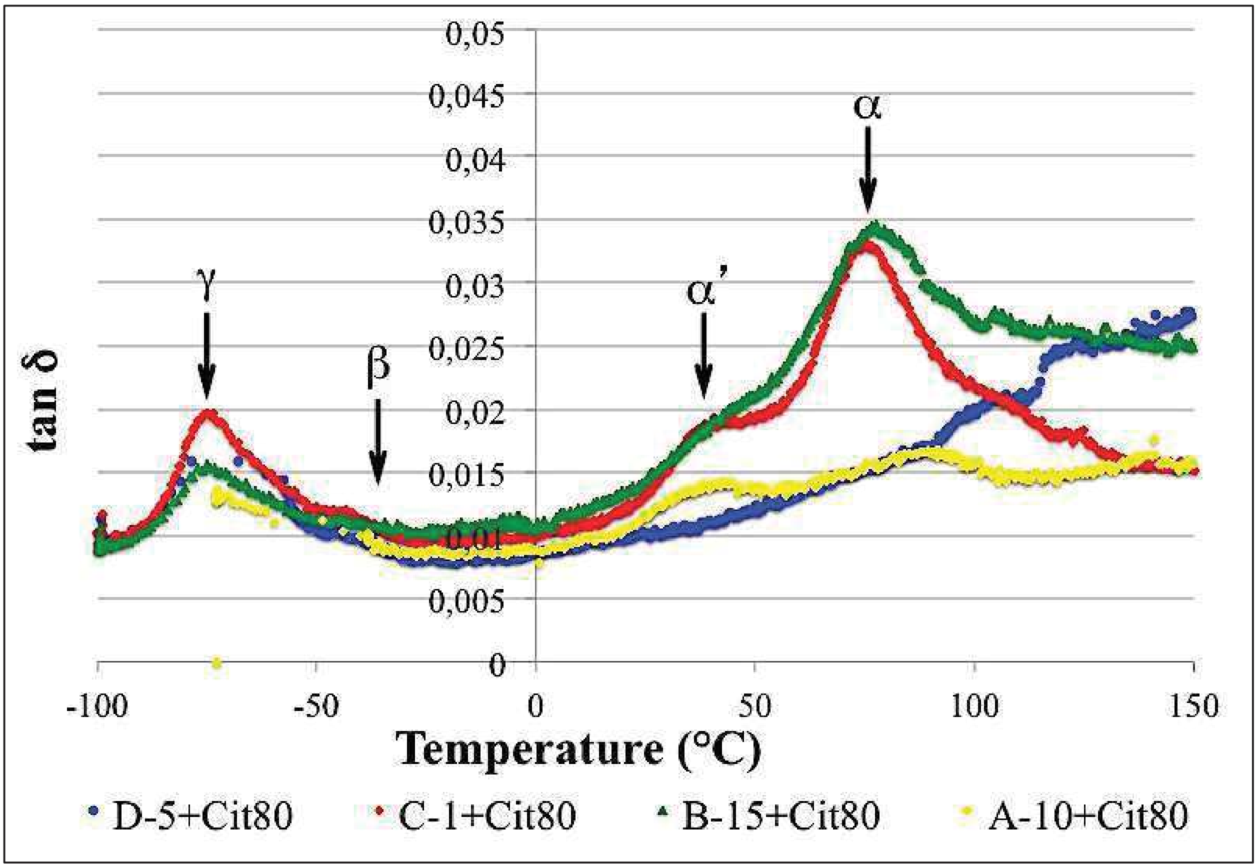


Figure 11. DMA of the 4 formulations exposed to 33% RH at 25°C

Based on the results obtained in Chapter 3, the thermal relaxation due to the plasticizing effect of the MCT-oil was identified and associated to a β relaxation temperature around -30°C to -10°C (for the formulations containing Citrus/MCT-oil at (50/50) the relaxation β was observable). Indeed, the β relaxation that often appeared as a well-defined peak for the compositions containing Citrus/MCT-oil at 50/50% (w/w) and as a small and large peak for compositions containing Citrus/MCT-oil at 80/20% (w/w) was attributed to the effect of MCT-oil. Respectively, the γ thermal relaxation was associated to the effect of a second plasticizer. In general the lowest thermal relaxations are related to small, polar plasticizer, as polyols. This second plasticizer effect was attributed to the polar molecules of the perfume like hexenol-3-cis, geraniol, and dihydromyrcenol all presenting a hydroxyl group and low molecular weight.

The thermograms showed the possible competition existing between the plasticizer effects of each active principle. The different thermal relaxations pointed out the presence of distinct regions. On one hand, a region rich in hydrophobic plasticizer corresponding to the MCT oil, and on the other hand a zone rich in hydrophilic plasticizer associated to the polar molecules of the perfume. The more the thermal relaxations tend to very low temperatures, the more they are assigned to polar and small plasticizers such as water, which glass transition temperature is modeled around -137°C /-139°C [17–20].

In the following section, the analysis of the evolution of the glass transition temperature as well as the influence of the active compound compositions of the delivery systems were discussed. The values of the β are not presented in here.

Thermal relaxations for the formulation containing Citrus/MCT-oil at (50/50):

Related to the evolution of the glass transition temperature of the bio-based matrices we observed that when the relative humidity increased, the glass transition temperature for all the four formulations decreased (Figure 12). This behavior was expected since the water absorption by the extrudate provoked a depression of the glass transition temperature [21–23]. Moreover, the glass transition temperatures of all formulations at 33%, 60% and 75% RH were above the room temperature (between 60°C to 80°C), confirming that the delivery systems were at a glassy state. These delivery systems presented very low exudation onto the surface after stabilization and, were very rigid and brittle materials. Moreover, they were stable, in terms of texture and appearance, under these conditions over a period of eight months, fulfilling partly the industrial specifications. On the contrary, samples kept at 90% RH developed mold after 3 months of stabilization.

As expected, the formulations stabilized at 33% of relative humidity presented the highest glass transition temperature whereas the extrudates stored at 90% RH had the lowest glass transition temperature (Tg). Their Tg, herein noted as the α relaxation temperature was below the ambient temperature, meaning that extrudates were at a rubbery state. The measured values of the Tg were in agreement with the texture of the extrudates. In fact, extrudates at 90% RH were malleable molten dough, with visual noticeable exudation onto the surface. Considering these Tg values, it was expected that volatile retention for these bio-based matrices must be very low.

Figure 12 showed the evolution of the glass transition temperature for each formulation (with 50/50 perfume/MCT-oil mixture) depending on the relative humidity that they were exposed to. Formulation A-10 presented the highest glass transition temperature for all tested relative humidities. Meaning that thanks to the addition of 5% (w/w) of PPI or of 1 to 5% (w/w) of Hi-cap100 the glass transition temperature of the mixture could be decreased without changing the experimental conditions in terms of extrusion screw, temperature profile and water.

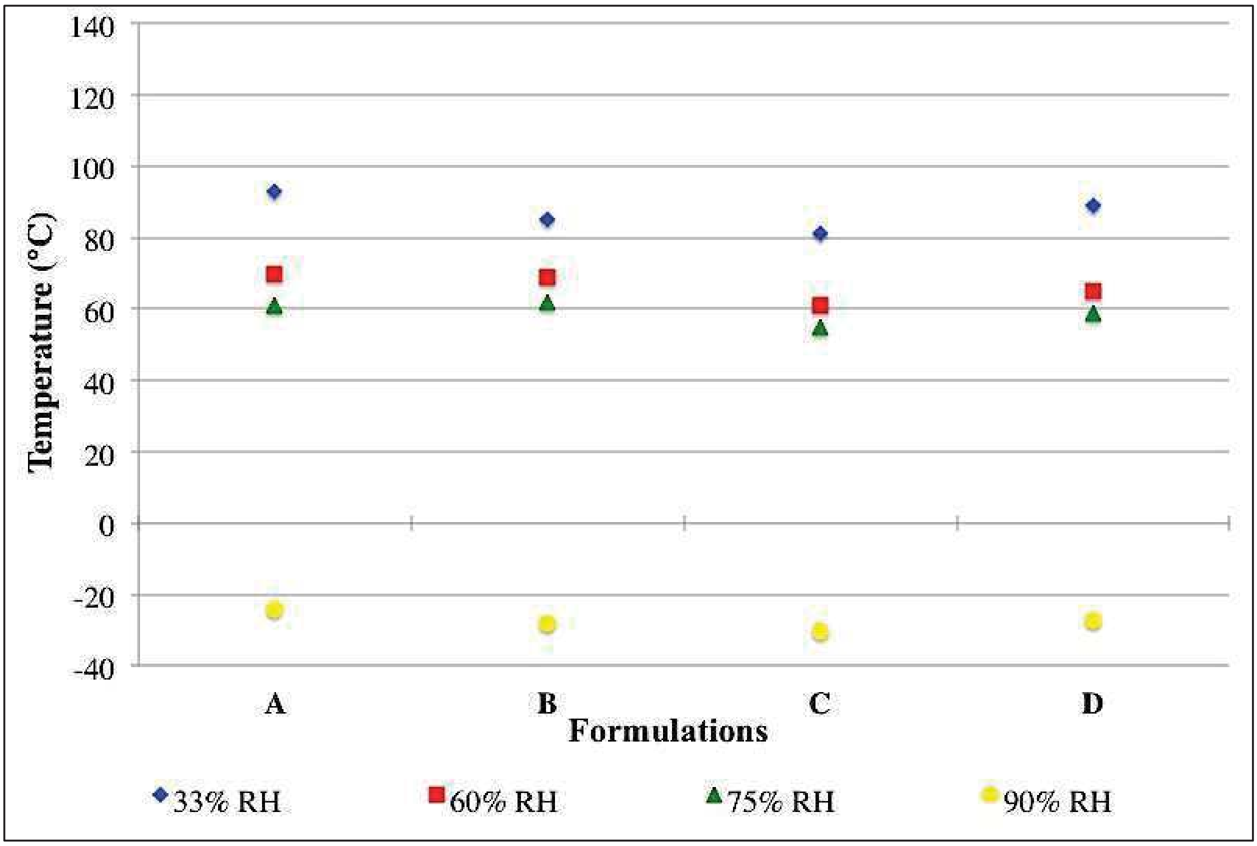


Figure 12. Impact of the relative humidity on the glass transition temperature of extrudates containing Citrus/MCT-oil (50/50) mixture. Comparison between formulations A, B, C and D.

<u>Thermal relaxations for the formulation containing Citrus/MCT-oil at (80/20):</u>

In this case, the same tendency for the evolution of the glass transition temperature of the extrudates was observed. The glass transition temperature decreased as the relative humidity increased (Figure 13). Again, the formulations stabilized at 90%RH presented a glass transition temperature below the room temperature (from -11 to -34°C).

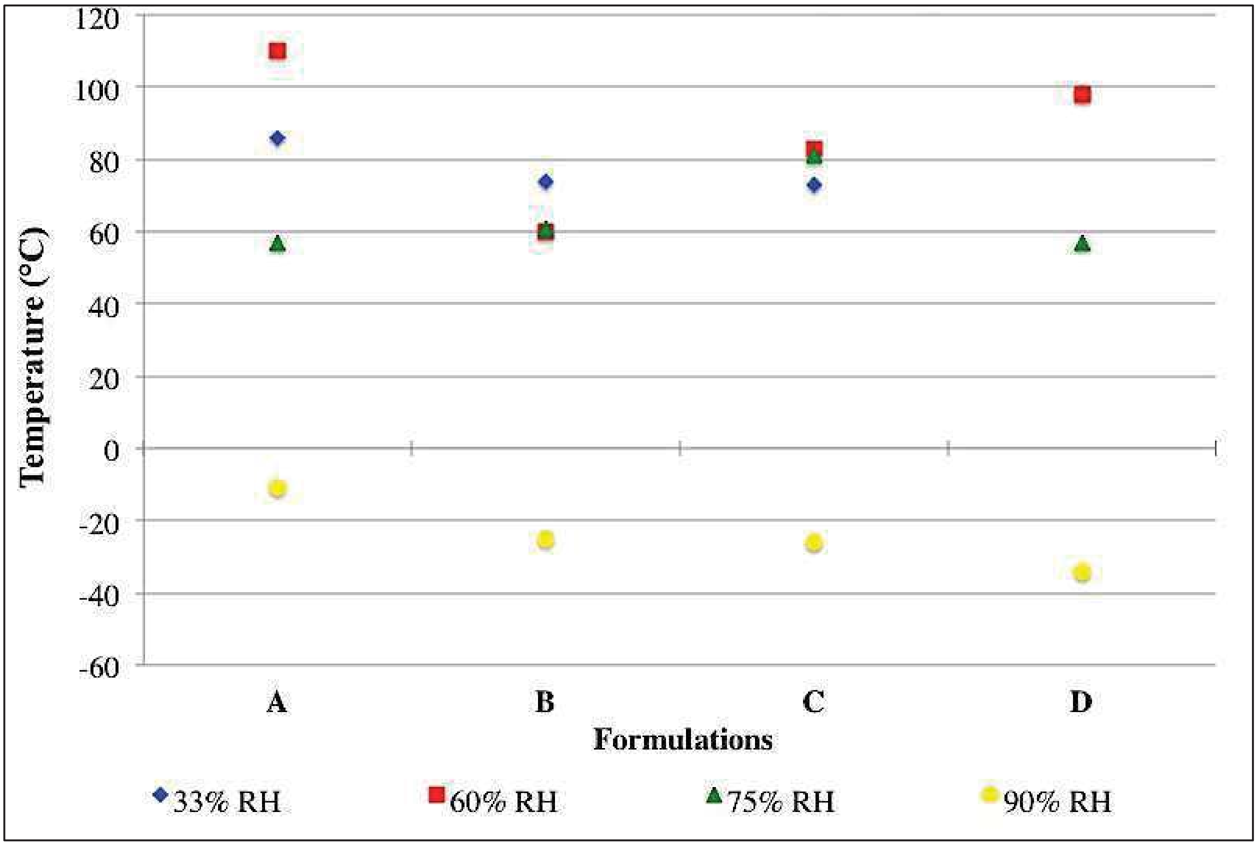


Figure 13. Impact of the relative humidity on the glass transition temperature of extrudates containing Citrus/MCT-oil at (80/20) of content. Comparison between formulations A, B, C and D.

Herein the impact of the incorporation of Citrus/MCT-oil at (80/20)% (w/w) could be appreciated for formulations A and C, whose glass transition temperature was higher when exposed to 60%RH than at 33%RH. Perhaps, this could be explained by a higher loss of volatiles during stabilization at these humidities, since water uptake by the samples enhanced the diffusion of the volatiles molecules through the matrix. As a consequence, the small plasticization effect of the perfume molecules was lost and so the glass transition temperature increased. It is important to take into account that when the glass transition temperature was decreased two phenomena could occur. On one hand if the glass transition temperature of the matrix is decreased, mobility of the active compound is favored, and thus its release is boosted. On the other hand the release of the active compound could induce a slight increase of the glass transition temperature of the system, if the active compound is considered to have a plasticizing effect. In our case, variations of the glass transition temperatures of the matrix were clearly provoked by the effect of water.

These types of phenomenon are related to the homogeneity of the wall material, in terms of moisture content and distribution of the active compound. Indeed, the absorption of water, and thus the glass transition temperature of the different layers of the materials are slightly different [15,24]. That could explain why formulation A and C, respectively at 60% and 75%RH presented a higher glass transition temperature than at 33%RH.

Again, extrudates exposed at 90% RH were not considered to be interesting since their glass transition temperature was too low and as a consequence samples were not stable under these circumstances.

3.1.3. Moisture content

Moisture contents of the extrudates stabilized in controlled climatic chambers at different humidities are presented in Table 6. As expected, when the extrudates were stabilized at higher relative humidities, the moisture content in the extrudates increased. These results pointed out that the matrices are good adsorbent materials. For extrudates stocked at 33% RH, formulations A and B were more hygroscopic than formulations C and D. On the contrary, at 90%RH the formulations C and D were more hygroscopic than formulations A and B. These different behaviors can be explained to the fact that at high humidities pea proteins are more susceptible to be degraded and so, the hygroscopic character was reduced [25,26]. Extrudates stocked at 60% RH presented almost the same moisture content.

It is noteworthy to mention that extrudates were very stable for more than eight months under stocking conditions at 60 and 75% RH, since no sights of mold or fungi were observed. Besides, the physical structure of the extrudates remained unchanged, and very solid. On the contrary, for the extrudates at 90%RH after two months of stabilization, mold and fungi were developed.

This is a positive sign because it confirms that the delivery systems were biodegradable.

The issue is that at humidity levels above 75% RH the extruded agro-materials were not stable.

Table 6. Moisture content of the extrudates containing Citrus/MCT-oil at (80/20)% (w/w) of content

Relative Humidity (%)	Formulation	Moisture content % (w/w)
33	A-10	8.26 ±1.0
	B-15	8.81 ± 0.6
	C-1	7.42 ± 1.0
	D-5	8.07 ± 1.4
60	A-10	10.72 ± 1.0
	B-15	10.47 ± 1.3
	C-1	10.58 ± 2.3
	D-5	10.21 ± 3.5
75	A-10	11.13 ± 0.6
	B-15	12.02 ± 1.0
	C-1	7.98 ± 2.2
	D-5	11.65 ± 0.3
90	A-10	13.49 ± 1.8
	B-15	12.73 ± 1.3
	C-1	24.83 ± 2.1
	D-5	14.31 ± 1.6

The moisture contents measured at 90% RH seemed surprisingly low when compared to those measured at 75% RH. Especially, when looking to their corresponding glass transition temperatures. It is then possible that the analysis had been compromised, maybe because of the trigger of their biodegradation.

3.1.4. Morphology

The same morphological analysis by SEM and ImageJ treatment that was made on Chapter 3 was also run for the extrudates containing Citrus/MCT-oil as the active compound. Formulation B-15+Citrus/MCT-oil (80/20) and formulation C-1+ Citrus/MCT-oil (80/20 were investigated.

Results were expressed as the average pore diameter, which corresponds to the ratio between the pore diameter (for each population of pores determined) and the total number of pores of the sample (Table 7).

Table 7. Morphological structures of Extrudates B and C at Citrus/MCT-oil at (80/20): pore distribution

Formulation	Citrus theoretical load (% (w/w))	Pore population	Ferret Diameter (µm)	Average Ferret Diameter (µm)
B-15+ Citrus/MCT-oil (80/20)	10	83	0.61	3.60
		156	2.37	
		73	9.61	
C-1+ Citrus/MCT-oil (80/20)	10	21	0.64	1.24
		113	1.7	
		56	0.55	

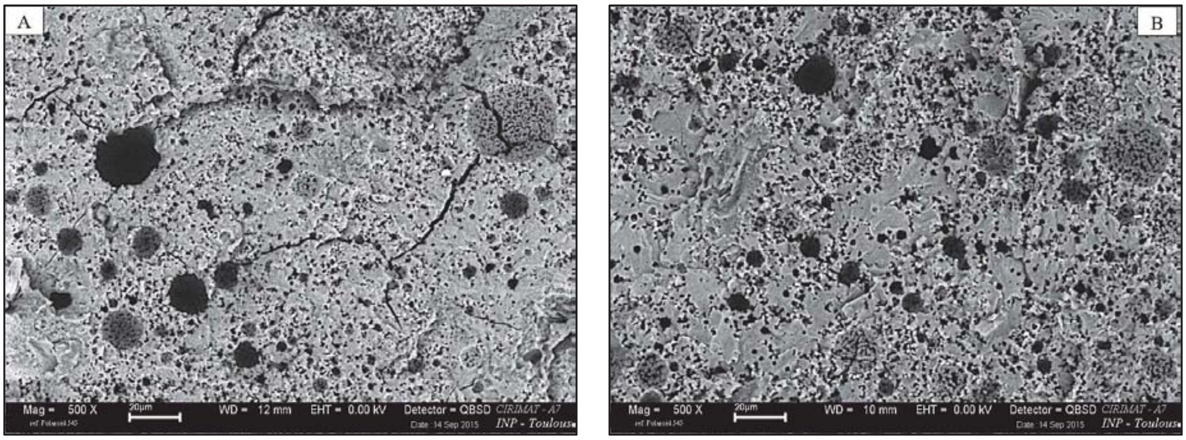


Figure 14. SEM of Citrus extrudates at Citrus/MCT-oil (80/20)% (w/w) content; A: formulation B-15 at 20µm x500 and B: formulation C-1 at 20µm x500

According to the results exposed in Table 15 and the SEM images of Figure 14, both of the delivery systems presented a macroporosity structure and a homogeneous pore distribution. Indeed, the surface of the extrudates was less fractured and smoother, compared to the extrudates with 100% of MCT-oil obtained in chapter 3. As illustrated on Figure 15, formulation B had bigger pores than formulation C. The smaller size of the pores of formulation C corresponded to a better stabilization of the active compound droplets within the material. Comparison of these two formulations lead to the conclusion that the addition of 1% of Hi-cap enhanced the formation of a narrow population of small pore diameter, whereas PPI favored the organization of a broad population of larger pore diameter, what was already observed in Chapter 3.

This demonstrated that Hi-cap100, in terms of droplet formation, reduced more the interfacial tension between the active mixture compound and the matrix and thus generated smaller droplet size with a more homogeneous pore distribution than PPI.

3.2. Kinetic release study of Citrus

The aim of this section was to evaluate the efficiency of the extrusion encapsulation process on volatile retention, especially the impact of the process itself on the preservation of the perfume. Furthermore, the release performances of these delivery systems, when exposed to different relative humidities, were also discussed. The liberation of the perfume was followed for a month.

3.2.1. Evaluation of extrusion process on volatile retention

To judge about the encapsulation performance of the twin-screw extrusion process, the encapsulation rate of the four formulations at Citrus/MCT-oil content of (80/20) was measured by applying the following equation:

$$Encapsulation\ Rate\ (ER) = \frac{MC_{\text{ extracted}}}{ME} * 100$$

$MC_{extracted}$: mass of all the molecules of Citrus extracted (mg)

ME: mass of the dry extrudate (g)

The extrudates that were evaluated were kept under stocking conditions before analysis (4°C on a hermetic blister for nine months). The encapsulation rates are presented on Table 8.

Table 8 Encapsulation rates of the four formulations after extrusion. RSD*=relative standard deviation

Formulation	**Encapsulation Rate (%)**	**RSD* (%)**
A-10 + Citrus/MCT-oil (80/20)	6.7 ± 0.4	6.5
B-15 + Citrus/MCT-oil (80/20)	6.2 ± 0.1	1.2
C-1 + Citrus/MCT-oil (80/20)	7.4 ± 0.2	2.9
D-5 + Citrus/MCT-oil (80/20)	7.9 ± 0.1	0.9

The encapsulation rate values obtained for all the four formulations were acceptable. Indeed, encapsulation efficiencies of about 67 to 79% were obtained for all the formulations, where formulations C and D showed the best results. The performances obtained for our delivery systems were comparable to those mentioned in the literature for twin-screw extrusion encapsulation and summarized on Table 9.

The encapsulation efficiency is defined as:

$$Encapsulation\ Efficiency\ (EE) = \frac{MC_{\text{ extracted}}}{MC_{\text{ initial}}} * 100$$

MC

$MC_{extracted}$: *mass of all the molecules of Citrus extracted (mg)*

$MC_{initial}$: *mass of all the molecules of Citrus incorporated initially (mg)*

Table 9. Encapsulation efficiencies reported in the literature for twin-screw extrusion encapsulation

References	Active compounds	Type of matrix	EE (%)
27	orange terpenes and tocopherols	maltodextrins DE-12+ sucrose	60-95
28	antrazine	starch	68-89
29	orange oil	maltodextrin DE-2+ saccharose + lecithin	85
30	butter and lemon flavor	OSAN starches+dextrose, lactose	80
4,31	ascorbic acid	starch	<97
32	natural pigments	starch	36-77

However, due to the differences of the matrix material and the active compound employed, as well as the differences related to the analytical quantification methods and process parameters it was difficult to judge about the efficiency and performance of our system compare to others.

For instance, it is important to remind that, for twin-screw extrusion encapsulation, the load of the active compound has been settled to be between around 10 to 12% (w/w), and that the most common quantification method employed is GC-FID. Although each sampling method has always to be adapted to the encapsulated compound, the type of matrix and the process employed, it is difficult to compare these results and to have a constructive feedback about it. Taking into account these statements, we will try to highlight the advantages and limitations of our delivery systems, as well as to discuss the points that are considered to be as future improvements for the extrusion encapsulation process.

Figure 15 and 16 roughly corresponds to a plot representing the amount of molecules of Citrus that had been lost during the extrusion process as the analysis was conducted on different matrices which had been stocked on a blister at 4°C just after being extruded. The secondary axis corresponds respectively to the volatility and polarity of the molecules and, it helps to have a mapping about how molecules were released based on this characteristic. Hence, we were able to get a better understanding of the possible interactions that could take place between the perfume molecules and the matrix ingredients, according to their physicochemical properties

Thanks to these figures, there are two important points that need to be highlighted. The first point corresponds to the low impact of the extrusion process on the perfume composition and the second point is the evidence of existing interactions between the molecules of Citrus and the matrix.

To begin, for all four formulations, it was remarkable to notice that most of the highly volatile compounds such as manzanate and methyl heptenone for example, were preserved. Therefore, the process could be considered as efficient regarding the conservation of volatiles. Indeed an encapsulation process can be classified as performant based on the preservation of the most sensitive active compounds. Herein, it proves that our processing conditions were mild in terms of temperature and mechanical shear since these molecules still retained in the matrices after extrusion (almost 30% of the molecules remained on the matrix).

This clearly might be contemplated as an advantage compared to other extrusion encapsulation processes, in view that no pre-encapsulation of the active ingredient, usually by spray drying or freeze-drying, was required prior to extrusion. This was the case of Ruiz-Gutiérrez et al.[32] (2015), Yuliani et al.[33] (2006) and Kollengode & Hanna[34] (1997) which encapsulated the active ingredient before extrusion to ensure its protection. Sure their procedures guaranteed the conservation of the active ingredient against harsh extrusion conditions, but their encapsulation efficiencies were not higher than the ones obtained in this study. On top of that, we could also assume that pre-encapsulating the active ingredient generates extra cost and time production, therefore being less competitive to our process.

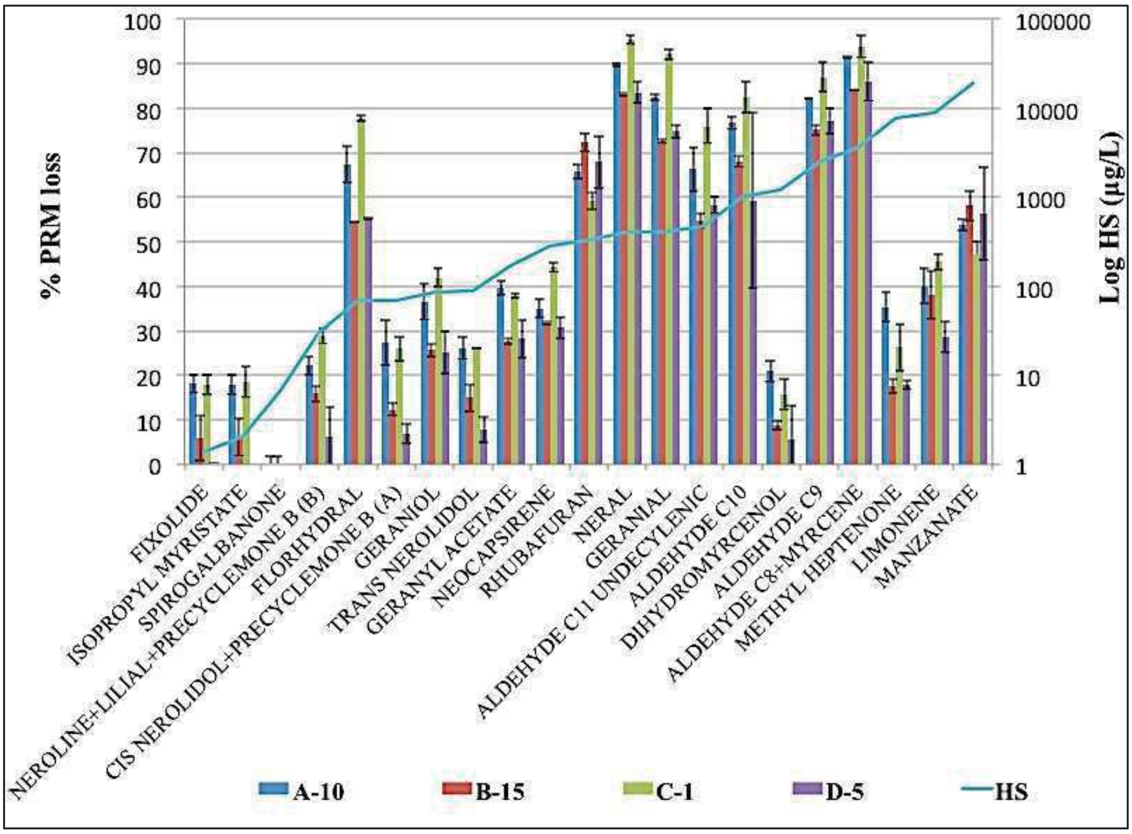


Figure 15. Influence of the matrix ingredients on the volatility (HS= head space (µg/L) of the molecules of the perfume

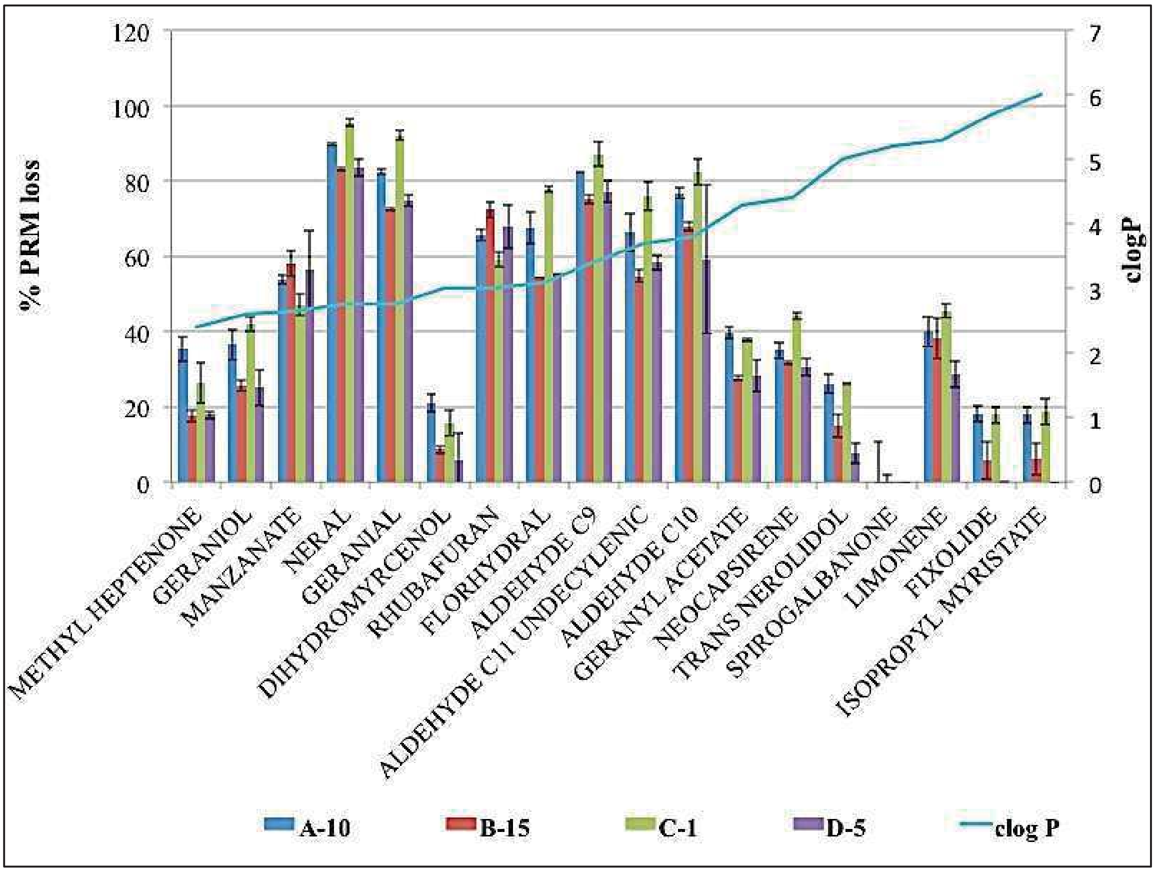


Figure 16. Influence of the matrix ingredients on the polarity of the molecules of the perfume

Turning to the active/wall interactions, a trend that stands out was that for all the four delivery systems, molecules having an aldehyde function, such as aldehyde C8, C9, C10, C11, florhydral, neral, geranial, and rhubafuran presented very high PRM loss values (around 70% and 80%) compared to higher volatile molecules as manzanate, suggesting that interactions between aldehydes and the matrix ingredients took place. One can suspect that these types of interactions were covalent interactions, occurring between these free aldehydes compounds and the amino acid of the PPI. The fact that volatile compounds like methyl heptenone had smaller PRM loss value than neral, for example; lead us to believe that only covalent interactions between this compound and the matrix could be responsible of this behavior.

In Chapter 3, Maillard's reaction occurred between the carbonyl group of the reducing sugar of maltodextrins and the amino acid of PPI so as to obtain the so-called Amadori compounds [35,36,36]. As for the Maillard reaction, the reactivity comprises aldehyde and amine functions that may form covalent binding. We could envision a similar reactivity between the aldehyde compounds present in the perfume and the amino acid of PPI.

Besides, all the aldehyde compounds presented in the Citrus formulation were relatively hydrophobic (clogP value between 2 and 4), which also might explain the high interaction between the matrices.

Several studies on the flavor and matrices interactions have indeed demonstrated that proteins used as wall materials interacted with aldehyde compounds either by covalent irreversible binding or by hydrophobic interactions, or both [38–42]. Gremli[37] (1974) studied the interactions between soy protein isolate and some flavors compounds in solutions with the aim of understanding the consequences of flavoring soy products. He determined that aldehydes showed higher retention percentages (between 37 and 100%) than ketones (between 5 and 68%) in a 5% (w/w) solution of soy protein. Alcohols did not interact with soy protein. The problem is that extrusion modifies the structure of proteins and as a consequence, the interactions between an extruded protein and an aldehyde would not be the same as they could be when proteins and the aldehydes are in solution. In extrusion cooking for example, is believed that proteins are opened and rearranged in a fibrous way favoring the formation of reversible interactions with volatile compounds.

Our delivery systems permitted to put in evidence two facts:

- Interactions between the matrix and the aldehydes compounds were noticed by the high PRM loss values compared to the other perfume ingredients.
- Adding 5% of PPI or Hi-cap100, allowed reducing the retention of all the aldehydes except for rhubafuran. The first case could be explained by the fact that when the amount of PPI was increased perhaps the interactions between maltodextrins and PPI were favored, and thus less amino groups of PPI were available to react with free aldehydes compounds. In the second case, we could make the assumption that the excess amount of Hi-cap100 could surround the aldehyde compounds, and thus preventing them from react with the amino group of PPI.

This behavior was found to be very interesting because for a specific amount of compatibilizing agent, either Hi-cap100 or PPI seemed to prevent or favor the aldehyde/matrix interactions.

A more accurate study would be required in order to better elucidate the type of interactions herein involved. After all, not only the chemical properties of the active and the wall materials are responsible of the interactions, but also the physical properties of the delivery systems [38,43–45].

For this reason, surface energy analysis (IGC-SEA) could be of strong interest, because it would allow to determine the enthalpy of adsorption of the injected compound, (i. e. one of the molecules composing the perfume) on the surface of the stationary phase (e.g. the stationary phase is composed of the wall material without any active ingredient, at a precise moisture content) at different physical states by only varying the temperature profile of the column [46,47]. In that way the influence of the physical state of the delivery system on the interactions with the active ingredients can be studied.

In general, the extrusion conditions developed turned out to be performant. However the interactions between the aldehydes and the matrix ingredient could be seen as a major drawback, because the main objective of encapsulation is to protect the active without interfering with it.

Regarding the polarity of the different perfumery ingredients, no relationship was noticed related to their weight loss during extrusion.

3.2.2. Study of the release behavior under different relative humidities

In this complex system, it is difficult to establish if the core/matrix is a single phase or a biphasic system. Therefore it is quite difficult to propose a release mechanism to describe the release profile of the six molecules studied. Under these circumstances, we assumed that perfume release from our delivery systems was mostly done by diffusion.

Release profile of all the molecules followed in this study had the same pattern, meaning that the major perfume lost occurred during the first day of the kinetic study. The major release was observed for the extrudates that were stocked at 90%RH and 25°C (Figure 17), which was expected since the extrudates were at a rubbery state under these conditions. This was confirmed by the low values of their glass transition temperature (A=-11°C, B=-25°C, C=-26°C and D=-34°C) for all four formulations at a Citrus/MCT content of (80/20). Therefore, diffusion of the active compounds inside the matrix was favored. Besides, the high moisture content values of the extrudates (around 12-14% (w/w)) also contributed to the release of the encapsulated molecules.

Kinetic release study for the formulations containing Citrus/MCT-oil (80/20) at 90%RH:

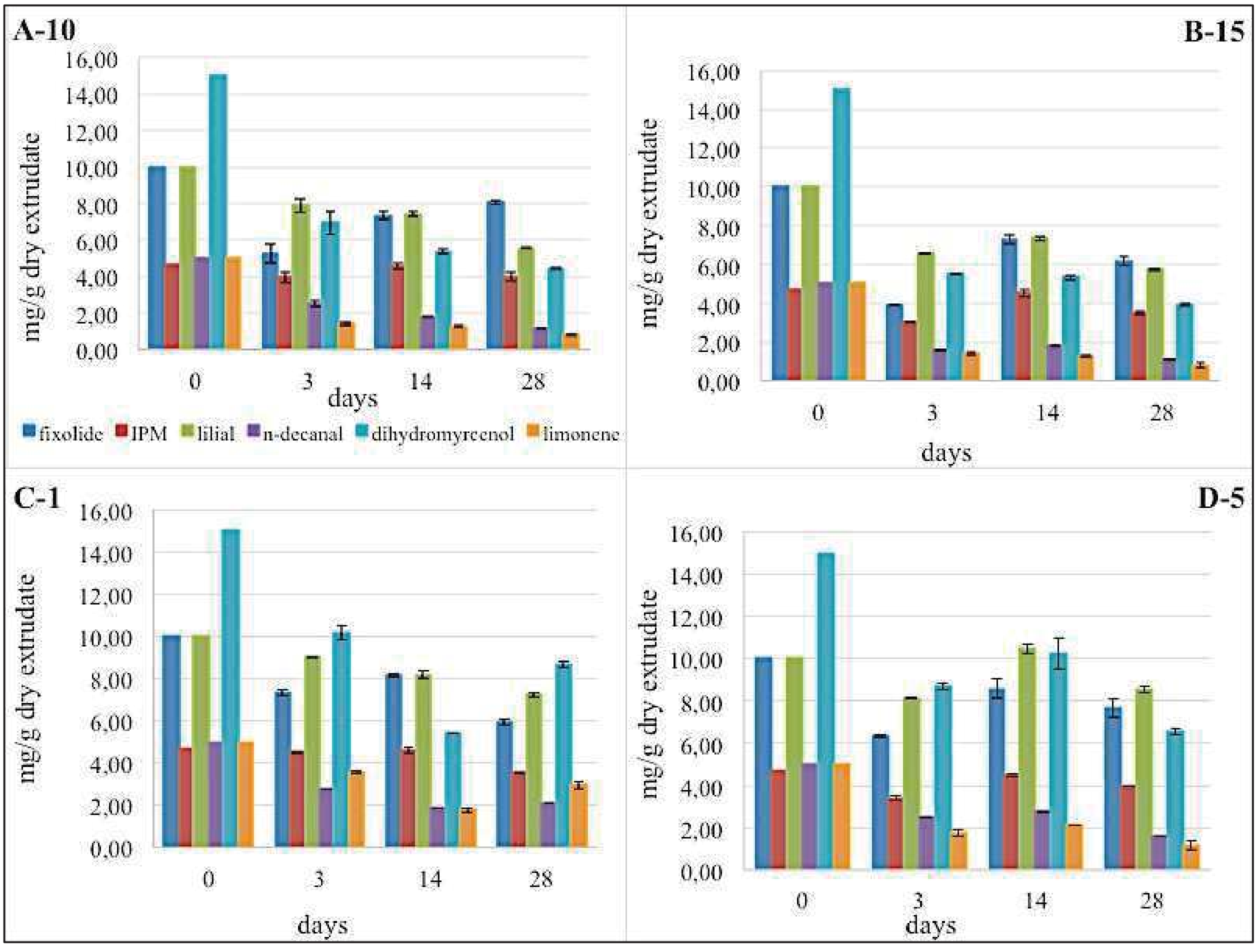


Figure 17. Kinetic release study of the four extruded formulations with Citrus/MCT-oil (80/20) stabilized at 90%RH and 25°C. 0 days corresponds to the theoretical composition of the perfume on 1g of extrudate loaded at 10% (w/w).

From the graphics showed on Figure 18, illustrating the amount of molecule remaining on the extrudate over time, there was not too much information that could be analyzed since for the first days the major part of the perfume was already released. And in the following days, we noticed that the amount of molecules remaining on the extrudate increased. This phenomenon was more marked for low volatile molecules such as fixolide and IPM. Actually it seemed like if the extrudates re-adsorbed the molecules that were released. The fact was that two of our climatic chambers (33% and 90% RH) were hermetically closed and no renewable air was ensured. This could be the reason why the amounts of molecules remaining on the extrudates through time were increased. Besides, these climatic chambers were the smallest in volume compared to the other two. Indeed, our climatic chambers were more as an equilibration room, where the released molecules and the environment reached equilibrium.

Kinetic release study for the formulations containing Citrus/MCT-oil (80/20) at 33%RH:

The same technical issue was observed on the climatic chamber at 33% and 25°C, because they were hermetically closed chambers where no renewable air was ensured. From day 3 to day 14 the amount of molecules remaining on the extrudate was slightly increased, almost for all molecules (Figure 18).

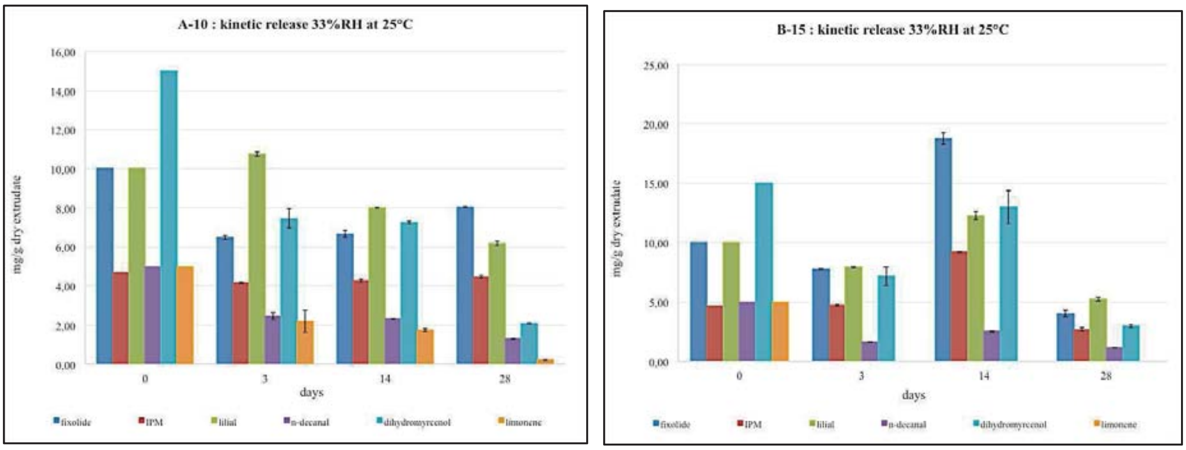


Figure 18. Kinetic release histograms of formulations A and B at 33% RH at 25°C. 0 days correspond to the theoretical composition of the perfume on 1g of extrudate loaded at 10% (w/w).

For this reason a global analysis of the kinetic release behavior was made at 33%RH without going into details. For the general release behavior of our delivery systems, under this condition, it was observed that the amount of perfume remaining in the extrudates after three days was higher compared to the other relative humidities. This trend was valid for all the four formulations.

Despite this drawback in our experimental setting, we could however estimate high retention values of the extrudates knowing the measured values of the glass transition temperature. As it was highlighted on §3.2.1, the glass transition temperatures of the extrudates were the highest at this relative humidity. Thus, the delivery systems were in a glassy state, and so the retention of the active compounds should be guaranteed.

Kinetic release study for the formulations containing Citrus/MCT-oil (80/20) at 60% and 75%RH:

The formulations that were stocked at 60%RH and 75R%RH (Figures 19 and 20) showed the same behavior in terms of release. The most volatiles molecules (i.e. limonene, n-decanal) were rapidly released, but the high molecular weight molecules, like IPM, lilial and fixolide, were more retained, since they showed little variation during the kinetic analysis.

No significant differences were observed for all the extrudates exposed to 60 and 75% RH at 25°C.

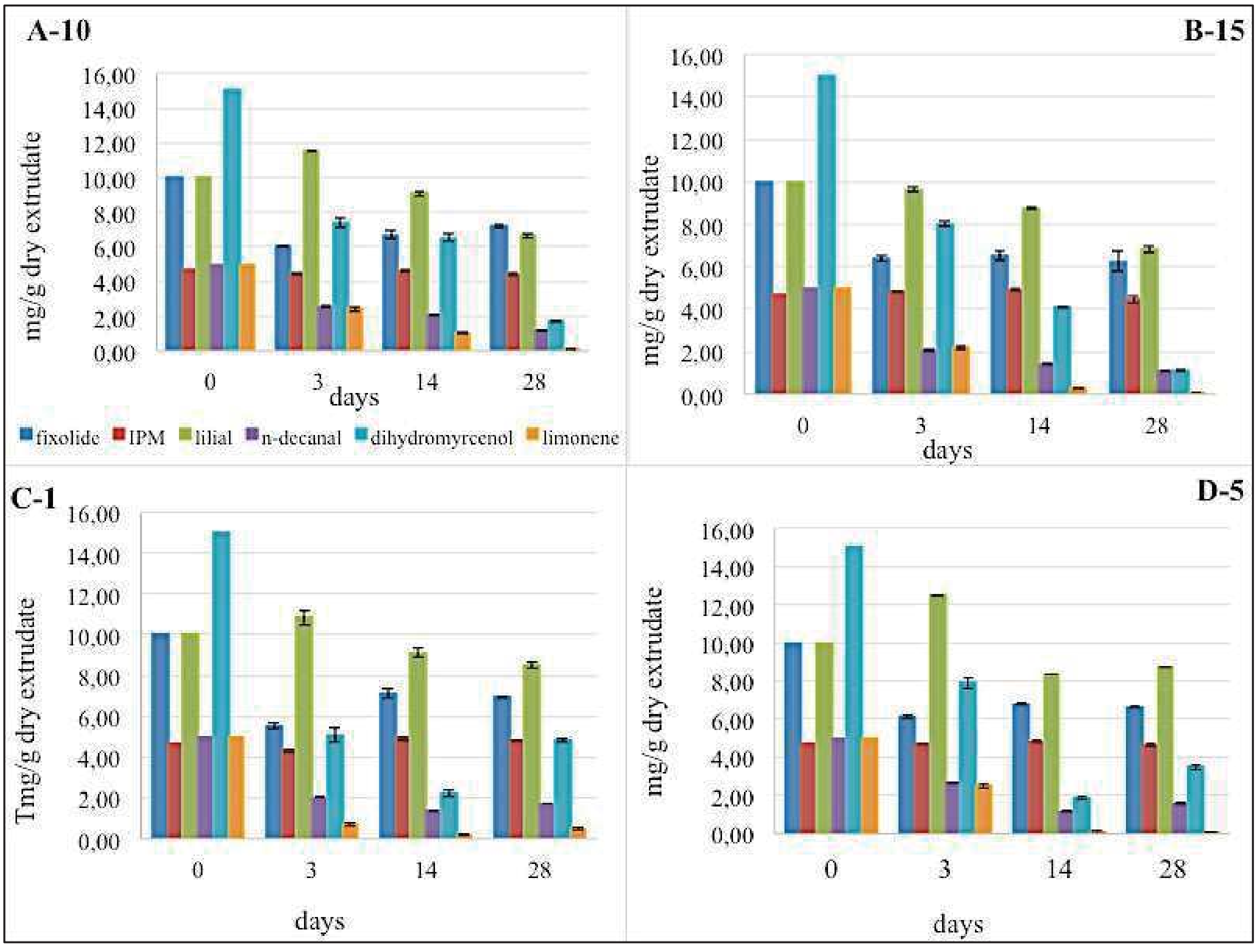


Figure 19. Kinetic release histograms of four extruded formulations with Citrus/MCT-oil (80/20) at 60% RH and 25°C. 0 days correspond to the theoretical composition of the perfume on 1 g of extrudate loaded at 10% (w/w).

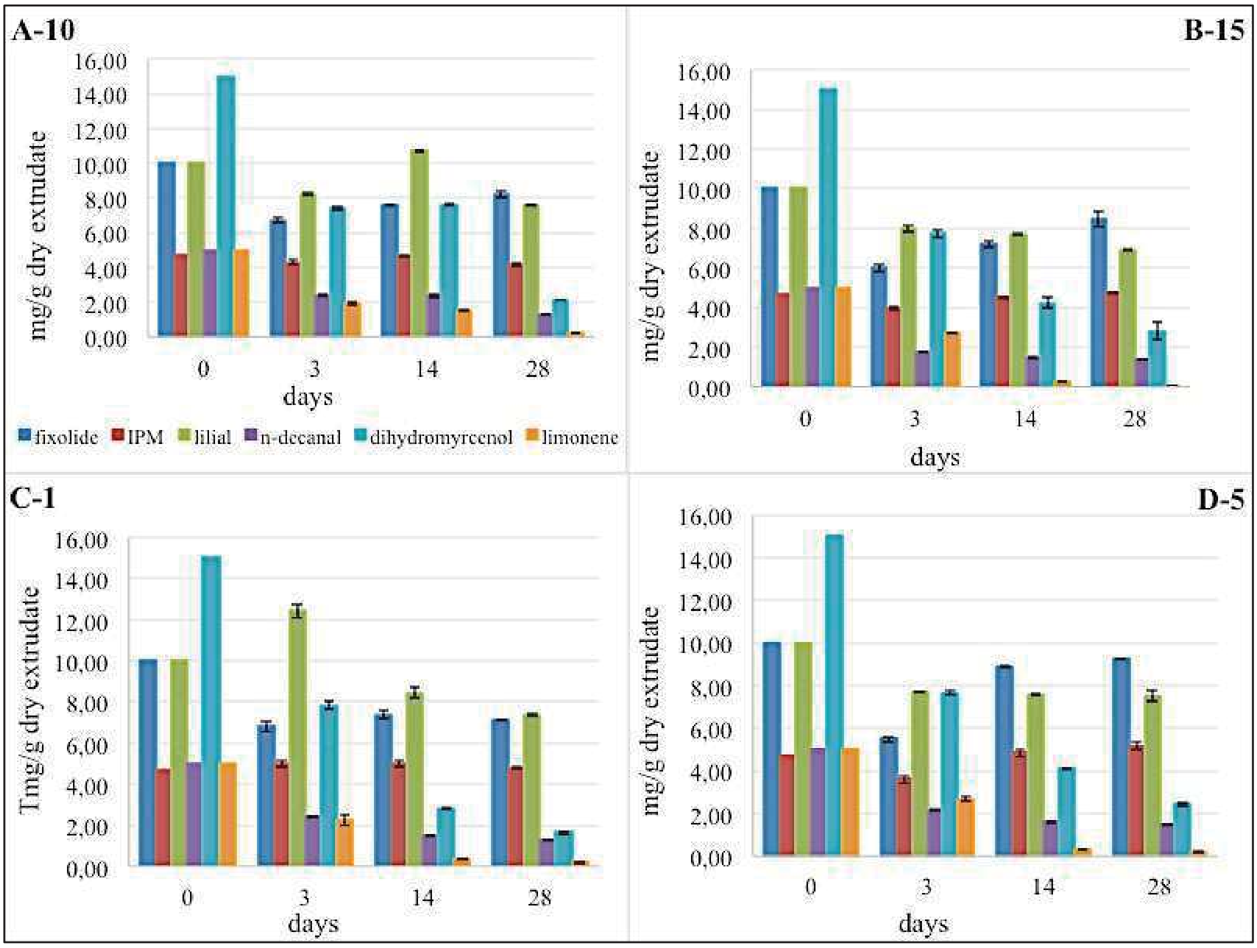


Figure 20. Kinetic release histograms of four extruded formulations with Citrus/MCT-oil (80/20) at 75% RH and 25°C. 0 days correspond to the theoretical composition of the perfume on 1 g of extrudate loaded at 10% (w/w).

To summarize, the major part of the perfume was lost in the first days of the kinetic study, showing that the four matrices had a rapid release profile when exposed to different humidities. Indeed, we observed that the perfume release appeared to stabilize over time as no variations were observed after 14 days.

We also observed that less aldehyde were lost when the PPI content was increase.

3.2.3. Study of the release behavior: influence of the Citrus/MCT-oil content

Formulations B-15 and C-1 were the ones chosen to compare the effect of the Citrus/MCT-oil content on the release of the perfume. This choice was established on the results obtained in Chapter 3, where addition of 5% (w/w) of PPI or of 1% (w/w) of Hi-cap100 led to the same behavior in terms of compatibilizing agents. Therefore in this part, we have attempted to understand the behavior of these two compatibilizing agents when the amount of active principle added is modified.

Retention of the active mixture was found to be better for formulation at (80/20) of content than for formulations at (50/50) for both of the formulations B-15 and C-1 at 60% and 75%RH (Table 10 and 11). For example in the case of fixolide, the remaining amount in the extrudate for formulation B-15+Citrus80 was of 6.38 mg.g^{-1} of dry extrudate and on the contrary for formulation B-15+Citrus50 it was around 2.38 mg.g^{-1} of dry extrudate, at 60%RH in the case of formulation C-1 at 75%RH, the amount of n-decanal retained in the matrix for the composition of the active mixture at (80/20) was of 2.07 mg.g^{-1} of dry extrudate and for the active at (50/50) it was of 1.13 mg.g^{-1} dry extrudate.

Low retention values were obtained for the formulations containing Citrus/MCT-oil at 50/50. These results were expected considering that during extrusion, splashing out of the extruder was observed and the extrudates presented high exudation levels after stabilization at 0%RH at 25°C.

In our case, we believed that this poor retention of the active within the matrix was provoked to an excess of the MCT-oil, which acted as a solvent in which the perfume was dissolved ant thus increased the dissimilarities between the hydrophilic matrix and the hydrophobic active mixture. Both ingredients could also have been on competition when incorporated in the matrix. To confirm these statements, further experiments will be required.

Table 10. Amount of n-decanal remaining on the extrudate after 3 days under 60 and 75%RH at 25°C

Relative Humitity %RH at 25°C	**Citrus/MCT-oil content**	**B-15 (mg/ g of dry extrudate)**	**C-1 (mg/ g of dry extrudate)**
60	(50/50)	1.16 ± 0.05	1.35 ± 0.06
	80/20)	1.78 ± 0.01	2.03 ± 0.01
75	(50/50)	1.13 ± 0.04	1.28 ± 0.02
	(80/20)	2.07 ± 0.02	2.41 ± 0.02

Table 11. Amount of fixolide remaining on the extrudate after 3 days under 60 and 75%RH at 25°

Relative Humitity %RH at 25°C	Citrus/MCT-oil content	B-15 (mg/ g of dry extrudate)	C-1 (mg/ g of dry extrudate)
60	(50/50)	2.57 ± 0.23	2.43 ± 0.08
	80/20)	6.38 ± 0.11	5.52 ± 0.02
75	(50/50)	2.27 ± 0.04	2.40 ± 0.11
	(80/20)	5.98 ± 0.18	6.81 ± 0.26

There were no significant differences between the two compatibilizing agents tested in terms of retention of the perfume (Figure 21 and 22).

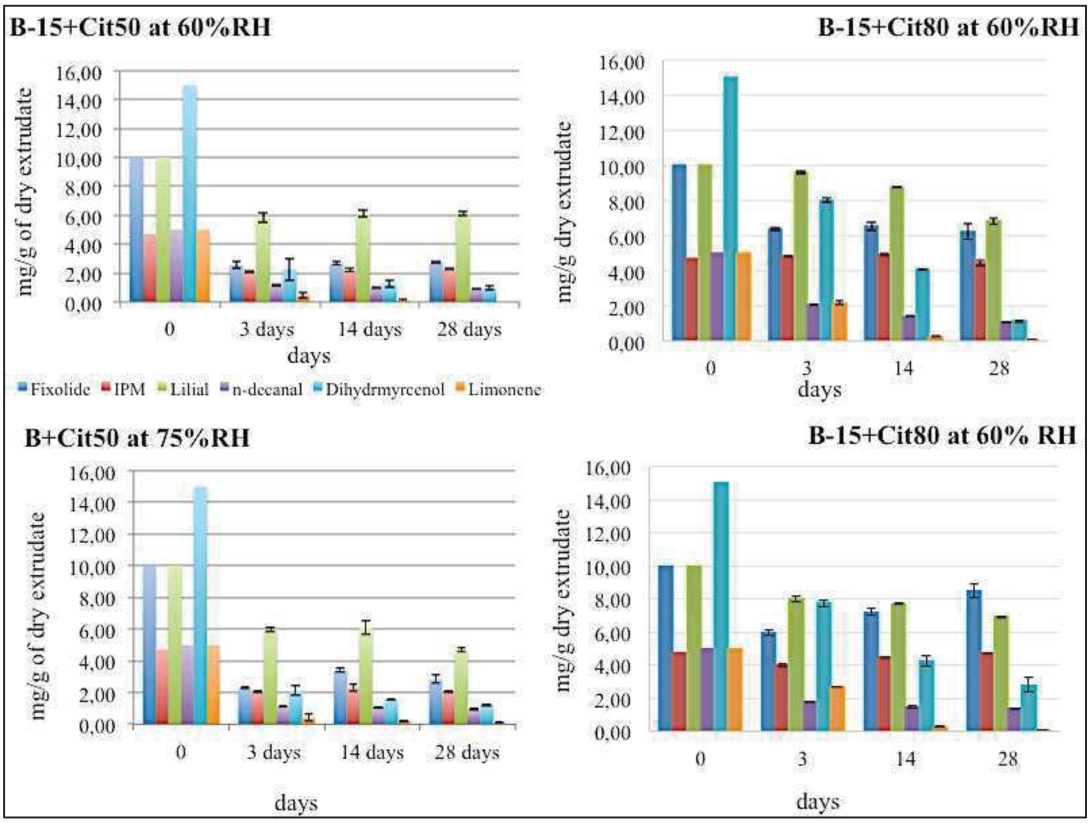


Figure 21. Kinetic release histograms of formulation B at different Citrus/MCT content at 60% RH and 75%RH at 25°C. 0 day corresponds to the theoretical composition of the perfume on 1 g of extrudate loaded at 10% (w/w).

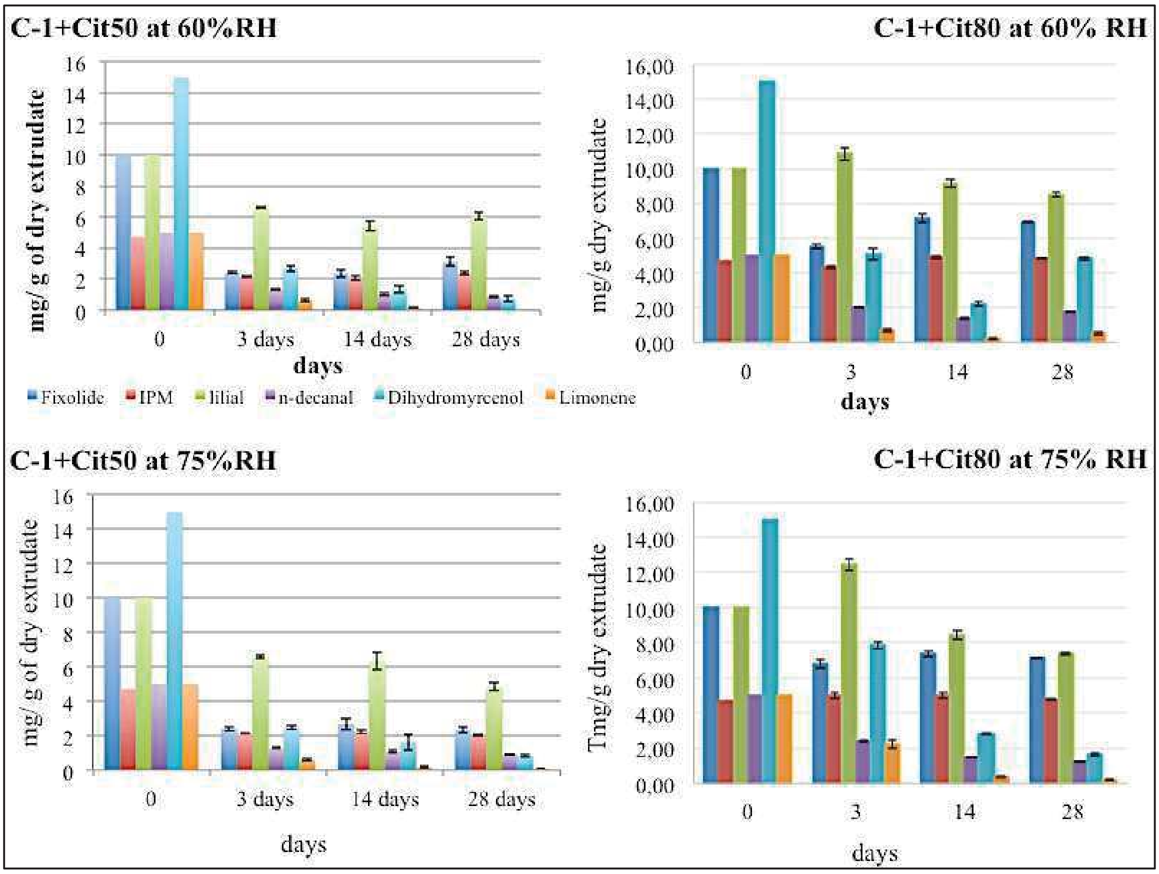


Figure 22. Kinetic release histograms of formulation C at different Citrus/MCT content at 60% RH and 75%RH at 25°C. 0 day corresponds to the theoretical composition of the perfume on 1 g of extrudate loaded at 10% (w/w).

3.3. Outlooks: industrial application of the bio-based delivery systems

The objectives of these olfactive tests were to identify the possible application of our bio-based delivery system in the fragrance industry. Results will provide a first glimpse on how the new extruded bio-based delivery systems behaved when encapsulating a complex mixture of fragrances. These experiments were an attempt to initiate a real use of these delivery systems.

3.3.1. Air Fresheners

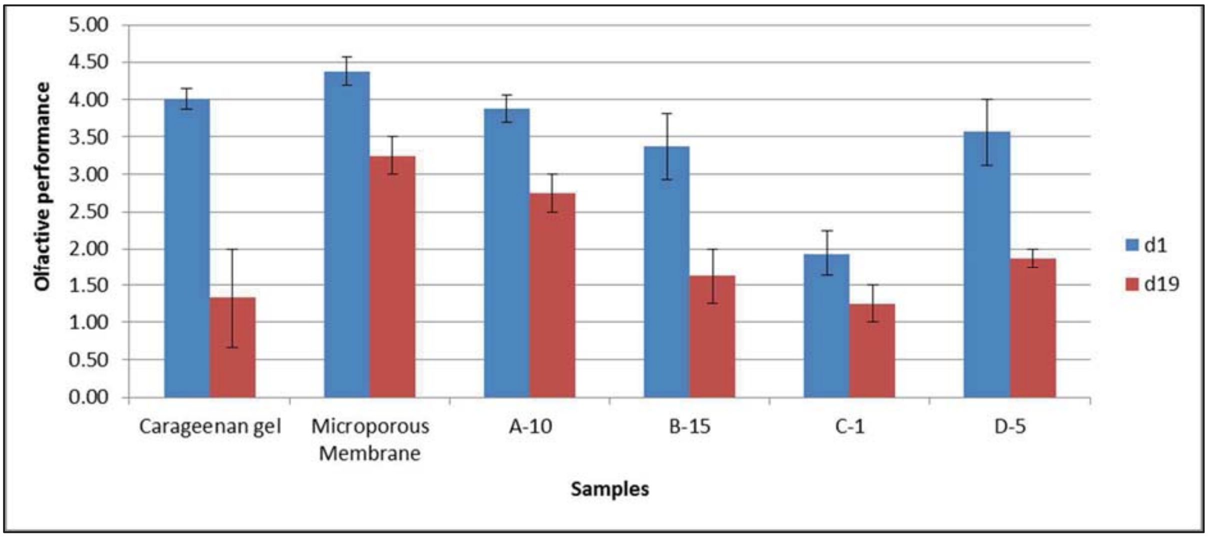


Figure 23. 5-point hedonic scale test of the olfactive performances of the extrudates matrices vs benchmarks over time, tested for air freshener application; d1= day 1 and d19= day 19.

The data presented in Figure 23 indicate the olfactive performance (i.e. intensity) of the different samples over time. As expected, the 2 benchmarks demonstrated very good performance at initial time, with olfactive character very close to the pure fragrance. The performance of the carrageenan gel significantly drops at day 19 when compared to microporous membrane.

The performance of the extruded delivery systems were overall in line with benchmarks at d1 for all formulations, except for formulation C-1 which was really underperforming. Formulation C-1 had the poorest olfactive performance and hedonics, due to the strong smell of the matrices itself that perhaps masked the olfactive character of the Citrus. However, for the other formulations, the olfactive footprint of Citrus was still perceivable and recognizable even though the character was perceived to be less room filling and with more bottom notes than the microporous membrane. To evaluate the performance overtime, a second olfactive analysis was conducted on day 19. All matrices were more underperforming to the microporous membrane benchmark, once again confirming the rapid release of the perfume in our bio-based matrices.

In parallel, a weight loss study on the different samples was carried out under controlled environments in order to better understand how the perfume was released from the extrudate when tested as air fresheners. Around twenty to twenty-five grams of the extrudates were weighted and put on controlled climatic chambers; one setup at 22°C at 45%RH and the second one was fixed at 37°C at 75%RH. The evolution of the mass of the sample was followed over a period of two to five weeks. The amount of residual water of the extrudates was taken into account to correct the curves of weight loss of the sample (Figure 24). The weight loss curves, confirmed the fact that most of the perfume was released within the first 3 days of stabilization. This corroborates the results obtained for all the formulations regarding the kinetic study. Our delivery systems display rapid release profile of the active when exposed to high levels of humidities.

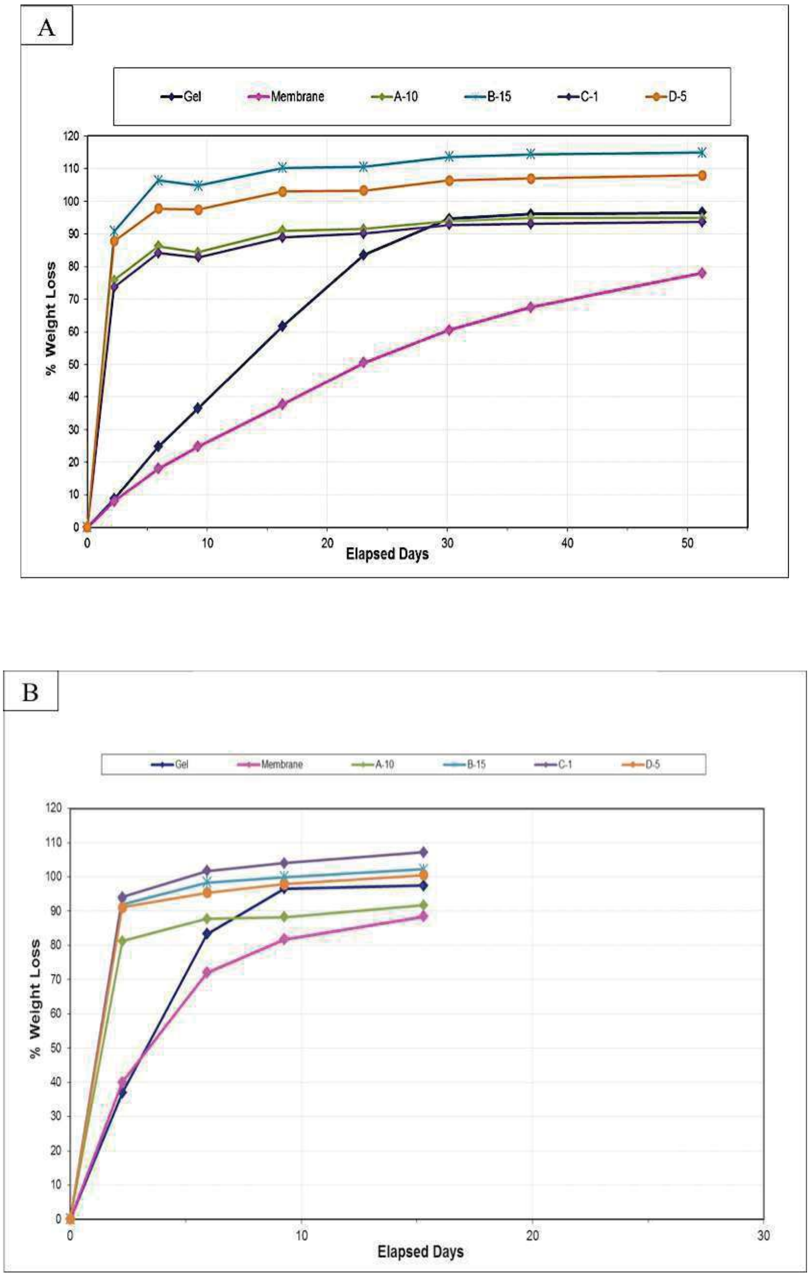


Figure 24. Evaporation profiles of different samples at iso-oil loading and comparable surface at different environments. A: corresponds to 22°C at 45%RH and B: corresponds to 37°C at 75%RH

All extruded delivery systems presented a very rapid release profile of volatiles compared to the gel and the membrane for both conditions.

At 22°C and 45% RH, all extruded samples reached a plateau after 5 days, showing that there is no more water or perfumery ingredient loss whereas the slopes for both gel and membrane are more linear, with less than 30% of weight loss over 5 days.

The samples exposed at 37°C at 75%RH showed more similitude, although the release profile of all extruded samples are still much more rapid than for membrane or gel. In this case, the impact of both temperature and humidity could be visualized.

These curves are very important because they help us to understand why the olfactive performances of the extrudates were so low for day 19 compared to the olfactive performance of the membrane. However, the granulometry of our delivery systems was not homogenous, and thus this could induce some difference on how the perfume was released when exposed to different conditions, because the surfaces of the samples were not exactly the same. This might also explain the differences on weight loss between the benchmarks and our samples. Further studies detailing the weight loss per surface would be quite interesting to really compare and understand the release profile of the extruded matrices versus known benchmarks.

Nevertheless, it is important to emphasize that for day 1, the olfactive performance of formulations A-10, B-15 and D-5 were in line with standard benchmarks and that no strong olfactory shift was noticed.

3.3.2. *Detergence*

In the detergence field, new encapsulating materials have to respect certain aspects in terms of olfactive performance, solubility, visual aspect and texture, anticaking power, and process production.

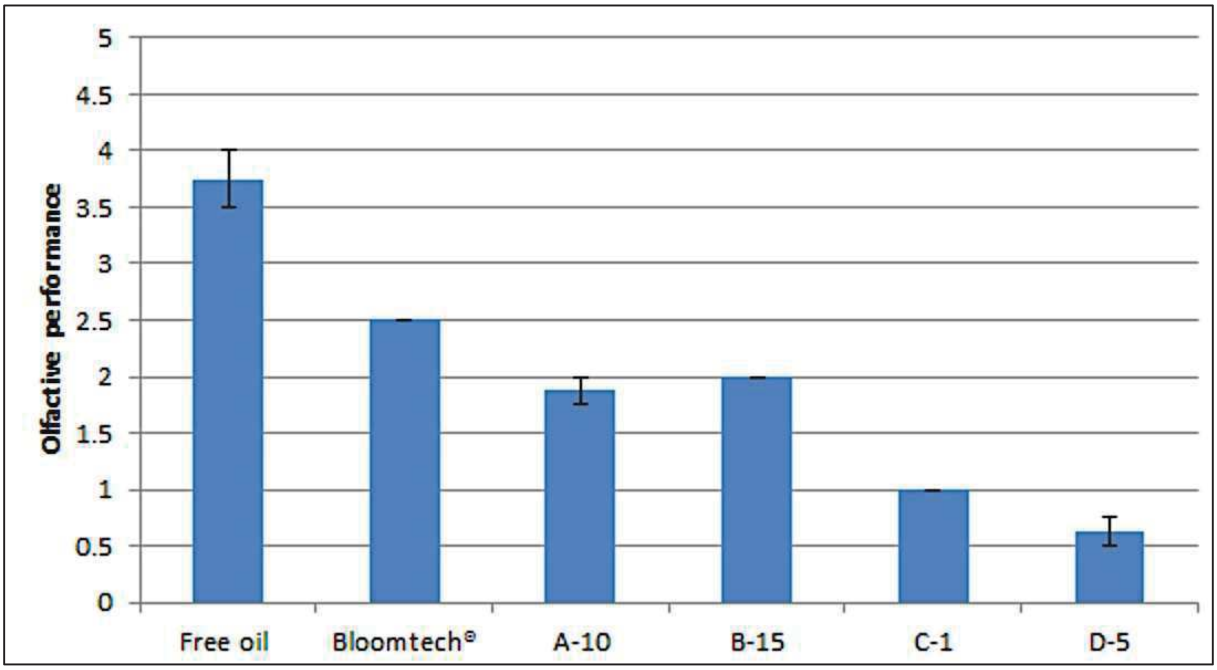


Figure 25. Evaluation of olfactive performance of bloom stage at iso-oil for free oil, spray-dried starch particles and extruded matrices: Detergence application

Preliminary dissolution tests, for rapid implementation of extrudate, were run in order to have a global idea of their solubility when compared to the benchmark product, i.e. Bloomtech® herein employed. As well as for the air freshener's olfactive tests, the samples were manually grinded, and as a consequence the particle size was not homogeneous. Indeed, our delivery systems were very hard solid matrices and so, their grinding was very difficult.

Performances of all four formulations A, B, C and D (Figure 25) were significantly lower when compared to the free-oil sample. However, it is surprising that among the four formulations tested, the performance of A and B were quite similar to the performance of Bloomtech®. However, due to the difficulty of the sample preparation, and their low solubility in water (their dissolution took twice as long as the Bloomtech®) it was decided that the test must not be continued.

Indeed, on top of the dissolution aspects, our extrudates, used as such, could not fit to other specifications required for powder detergent applications as they presented agglomeration and particle size homogeneity issues.

Despite the results related to the olfactive performance of the delivery system for detergence applications, the formulation but also the processing of the extrudates could be improved in order to fulfill the requirements needed in this domain. For example to avoid the agglomeration and particle size issues an anticaking agents as microcrystalline cellulose or lecithin may be added.

4. DISCUSSION

It is important to stress out that our extrusion encapsulation process is a very attractive alternative for the encapsulation of active compounds, because of the mild conditions that were established. This mild extrusion process, which reported SME values below 200 Wh.kg^{-1} (Table 9), allowed preserving volatile compounds such as manzanate (Figure 15). This is a very positive result since twin-screw extrusion is considered to be a harsh technology for encapsulation of sensitive ingredients (i.e. thermolabile and volatile compounds) due to the high mechanical shear and elevated temperature required for some matrices (Table 9 of Chapter 1). Therefore, in other studies, they have reported a pre-encapsulation step prior to extrusion in order to avoid degradation of the active compounds.

Even though, our matrices slightly modified the olfactive profile of the perfume, the storage stability and the encapsulation rates obtained (6.2 to 7.8%) were encouraging and are comparable to those divulged in the literature (Table 13), not only for the same type of encapsulation process, but also comparable to other encapsulation process, as for example spray-drying.

These results confirmed Uhlemann & Reiss's[48] (2010) statement related to the impact of extrusion on flavor or fragrance encapsulation. Indeed, extrusion encapsulation allowed having a longer shelf-life stability of the delivery system during the stocking conditions (above months stability) at expenses of a modified flavor profile. It is inevitable not to have any interactions between the active compounds and the wall material ingredients and as a consequence of those interactions; the olfactive profile of the perfume might be changed.

As a matter of fact, the matrix ingredients seemed to have an important affinity towards aldehyde compounds and this statement was valid for all four formulations. Indeed, we suspect that these interactions occurred between the amino groups of PPI and the aldehyde compounds of the perfume and thus, they are of the covalent type binding [39,41]. These reactions are known as part of Maillard's reaction and are often found in flavor encapsulation. Not to mention that hydrophobic interactions could also take place between fragrances and modified starch as mention by Guichard's[39] work (2002).

Nevertheless, there were some differences observed between the four formulations with respect to their behavior towards aldehydes. As mentioned on § 3.2.1, interactions towards aldehydes were not the same if the PPI content was increased or not. For example, it appeared that the addition of 5% of PPI (comparing formulation A to formulation B) helped to reduce the interactions between aldehydes and proteins. The same trend was observed when 5% of Hi-cap100 was added (comparing formulation A to formulation D) interactions between aldehydes/PPI decreased. Conversely, the addition of 1% of Hi-cap100 increased the aldehyde retention. These results suggested that by the addition of these compatibilizer agents, we could modified the microenvironment around the active compounds, and thus modulate their retention and their release [49].

About the retention of volatile compounds, in spray drying for example, low molecular weight molecules with low clogP values, always presented poor retention rates as they are highly volatile and have less affinity with hydrophobic encapsulating agents. For example, Charve and Reineccius[50] (2009) spray-dried (E)-2-hexenal in different matrices material and all presented high losses of this compound. So, it is expected to see that very small and high volatility compounds such as methyl-heptenone, manzanate, geraniol had poor retention values in extrusion encapsulation. In contrast, molecules with high molecular weight and clogP, like fixolide, IPM, trans-nerolidol and neocaspirene, showed higher retention. The analytical results as well as the sensory evaluations confirmed these slight changes perceived on the olfactive footprint of Citrus, even though it was overall pretty in line to the original Citrus.

With respect to the thermal behavior, the most important part was to confirm that for all the four matrices, the glass transition temperatures were above the room temperature. Again, having the delivery systems at a glassy state was ideal in order to reduce molecular diffusion of the perfume at stocking conditions. This is one of the industrial specification that was achieved: we were able to produce matrices, that for a large range of relative humidities (33 to 75% RH at 25°C) could be at a glassy state and thus, protect the active components. Although this may be true for all the extrudates except for the extrudates that were stock at 90%RH which had a glass transition temperature below 0°C.

Regarding the kinetic results and the determined glass transition temperature of the extrudates, the release profile of the perfume could be anticipated. The retention of volatiles decreased as the relative humidity of the surrounding environment was increased, what was expected and, in agreement with the reported literature.

What is important to note, regarding the glass transition temperature of our delivery systems, is that for such different extrusion conditions (in terms of extrusion temperature, matrix ingredients, initial water content, and screw speed) we were able to obtained similar or even higher glass transition temperature than the other studies [4,18,28,30,31,33,48,51,52]. This is very attractive since mild extrusion temperatures could be beneficial to encapsulate thermolabile components, like perfumes.

The encapsulation of Citrus/MCT-oil at (80/20) was considered to give better results than extrudates of Citrus/MCT-oil at (50/50), not only because during extrusion there were fewer active mixture losses and the extrudates showed little exudation, but also because best retention values and longer stability were obtained. As a matter of fact, the formulations containing Citrus/MCT-oil at (50/50) were already above the maximum amount of active that could be incorporated if we take into account both ingredients (e.g. Citrus and MCT-oil) as active compounds. This excess of active compounds was also confirmed by the lowest values of SME measured (around 96 $Wh.kg^{-1}$) and, by the splashing out of the active mixture at the exit of the extruder. The excess amount of MCT-oil was also observed thanks to appearance of segregation zones on the thermograms (Figure 10) suggesting that there might be competition between these two active ingredients.

Last but not least, concerning the sensory evaluation, the olfactive performances of our delivery bio-based systems were indeed relatively lower than the benchmark product they were compared to. The olfactive footprint of Citrus was recognizable even though a slight smell of base was perceived.

Furthermore, formulation B presented higher olfactive performance than formulation C, for the detergence and air freshener applications. This could be explained by the fact that formulation B had more important pore population and bigger average pore diameter than formulation C (Table 11). This implies that morphology might enhance release of the perfume, resulting in a more important bloom.

It is noteworthy to mention that the olfactive test of the extrudates were carried out after nine months of being produced and kept at stocking conditions (hermetic blister at 4°C). The fact that the analyzed samples were old could be impairing to their olfactive performances. Hence these preliminary results are encouraging for a plausible application, perhaps in other industrial domain.

5. CONCLUSIONS

The extrusion conditions that were established for the encapsulation of the perfume were considered to be efficient since for a 10% (w/w) of Citrus load the encapsulation rates obtained (60-80%) were comparable to those reported in the literature for twin-screw extrusion.

Besides, the process conditions allowed to preserve high volatile compounds such as manzanate and methyl-heptenone, which is an asset for this kind of technology that are considered to be harsh. This proved that the process parameters established were mild, and it was also confirmed by the low SME values measured.

Sure, extrusion encapsulation of flavors and fragrances provides slight modifications of active compound profile conversely to other encapsulation techniques, but nevertheless it offers longer shelf-life stability thanks to the higher glass transition temperature and low moisture content.

However, in terms of release and thus, olfactive profile of the perfume, there are still some parameters that could be improved. For example, the formulation of the matrix can be improved in order to decrease core/wall interactions, in particular for aldehydes. In this case, the formulation of the perfume or the matrix could be modulated to reduce the affinity between these compounds and as a result boost their release. However, investigations related to active compound and extruded delivery systems interactions are few, due to the complexity of the systems, therefore more research are required. Solubility of the delivery systems could also be improved if the targeted application will be detergence. In that case the amount of PPI in the matrix could be reduced or replaced for example by Hi-cap100, which has a better solubility in water.

Conversely, the process parameters could be adjusted to modify the properties of the matrices. Either by increasing the solid feed rate to increase the encapsulation rate or, increasing the screw speed to reduce the residence time of the mixture inside the extruder and as a result also increase the encapsulation rates [11].

The biggest challenge related to flavor and/or fragrance encapsulation is to produce a matrix material where the flavor or fragrance will keep exactly the same sensory characteristics at the different stages of the industrial process (during encapsulation, stocking and when employed). Obviously, for us it will be very interesting to be ale to put into shape the delivery system directly after extrusion, because in that way all the delivery system will have the exactly same shape. Consequently, it will be easier to study the release behavior and avoid the problems related to agglomeration and particle size homogeneity.

In conclusion, the obtained results are very encouraging, not only in terms of encapsulation but also in terms of scientific discovery related to the interactions occurring between an extruded matrix and the core material. Off course, all these results came from a single extrusion campaign. Therefore, a critical analysis is recommended to put into perspective the observations and hypostesis herein exposed. So, more experiments (meaning more extrusion campaigns) are mandatory to corroborate the tendencies and to verify the hypothesis mentioned in this thesis but also to have a more accurate understanding of the complexes phenomenons involved. This only shows that there are still many phenomena to be explored in order to master twin-screw extrusion encapsulation of perfumes.

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GENERAL CONCLUSIONS

One of the areas of expertise of the Laboratory of Industrial-Chemistry (LCA) is the encapsulation of active principles, using natural polymers, by spray drying. Willing to extend this topic and combining it with the long lasting experience of the lab in twin-screw extrusion, the frame of this PhD was then to develop an innovative delivery system, by incorporating a hydrophobic compound in a bio-based hydrophilic matrix in a one-step extrusion process. Therefore, the driving force behind this PhD project was related to the understanding of core/wall interactions in such thermo-mechanical process and their impact on the final properties of the delivery system.

Simultaneously, the industrial purpose of this work was to elaborate by twin-screw extrusion a perfume diffuser from natural and biodegradable materials that was sensitive to the environmental conditions. For example, the moisture content of the surrounding could trigger the release of the perfume. Therefore, the specifications of the industrial partner were to implement an innovative encapsulation process for fragrance's protection. The delivery system had to be made from biopolymers, be water-soluble and at a glassy state at room temperature. And the most important requirement was, that the delivery system had to respect a perfume load of 10% (w/w).

After an exhaustive bibliographic study on all melt extrusion encapsulation processes (experimental conditions, wall materials, active ingredients, physicochemical properties of the encapsulating materials), we decided to elaborate maltodextrin-based matrices for the encapsulation of the perfume as it is commonly employed in the flavor industry and as it is highly water sensitive. However as these polymers are never used alone in twin-screw extrusion (short chain length and too hydrophilic to retain hydrophobic compounds), the use of a compatibilizing agent seemed mandatory.

To differentiate our bio-based delivery systems from current techniques used in flavor encapsulation, we have chosen to use a non-common compatibilizing agent, in this case pea protein isolate (PPI). Vegetal proteins are known (and our lab has a specific experience in that domain) to demonstrate some interesting encapsulation efficiency as they bear hydrophobic moieties allowing the proteins to blend correctly with hydrophobic compounds during thermo-mechanical process. The performance of PPI was then compared to a classical compatibilizer, a modified starch (i.e. Hi-cap 100). Another challenge that we decided to impose was to develop our process within the frame of the green chemistry principles, by trying to reduce the number of steps during the encapsulation process, the production of by-products and by avoiding the use of toxic solvents.

These objectives were achieved through the use of twin-screw extrusion technology as the encapsulation process. This technique was at the heart of our research project.

Before exploring the extrusion experimental conditions, a series of elementary experiments were performed on a lab scale on five different DE-value maltodextrins in order to select the most appropriate for this application. Indeed, formulation of new bio-based products always demands for knowledge on the physicochemical properties of the raw materials to be employed, so as to better orientate the type of process and the conditions to be applied.

According to the rheological and thermal results, we were able to conclude that maltodextrins are very peculiar materials, because their window of processability is very narrow. As we tested different plasticizers, we noticed that if more than 20% (w/w) of plasticizer is used, materials become so ductile that they cannot be handled, whereas with less than 10% (w/w) of plasticizer, materials were too brittle. Taking this into account, maltodextrin DE-12 was the most convenient one for the wall material, after all, it had the right viscosity at low moisture content (i.e. 12% w/w) at 80°C (its flow consistency was about 33838 Pa.s) and presented an adequate hygroscopic character for environments having a relative humidity below 75% RH at 25°C. During this work, the correlation between the physicochemical properties of maltodextrins with their DE was also discussed, as DE alone cannot describe accurately the molecular weight distribution of maltodextrins.

Up-scaling the process to industrial twin-screw equipment, it was observed that when only maltodextrin and water mixtures were extruded without any compatibilizing agent (i.e. PPI or Hi-cap100), it was no possible to obtain a product. Actually, the addition of a compatibilizer like PPI and/or Hi-cap100 was necessary to render the mixture extrudable and to facilitate the incorporation of the active ingredient. Due to this, four formulations were optimized for the elaboration of biopolymer mixture matrices:

- **A-10**, containing 10% (w/w) of PPI,
- **B-15**, containing 15 % (w/w) of PPI,
- **C-1**, containing 10% (w/w) of PPI and 1% (w/w) of Hi-cap100,

- **D-5**, containing 10% (w/w) of PPI and 5% (w/w) of Hi-cap100

The addition of the compatibilizing agents permitted to put in evidence the following facts related to their mechanism of action:

- The addition of PPI not only allowed to process maltodextrin, but also allowed to increase the encapsulation rates of MCT-oil, a model hydrophobic compound. Indeed, the highest encapsulation efficiencies were obtained for formulation B-15, that were around 96% for an encapsulation rate of MCT-oil of 13%. Besides, the obtained delivery systems presented stable glass transition temperatures and very low exudation, as long as the incorporation rate of MCT-oil was below 13%. This may be explained by the fact that MCT-oil is trapped in the hydrophobic regions of the proteic structure, and these domains do not take part in the glass transition temperature of the matrix (62-72°C).

- The addition of 1 to5% of Hi-cap100 clearly improved the encapsulation rates (up to 15.4%). However, the encapsulation efficiencies were lower than for formulation B-15. Interestingly, the smaller size of the pores indicated that Hi-cap100 acted as a surfactant by getting all around the MCT-oil and thus facilitating its dispersion into the hydrophilic domains of the matrix. For this reason the glass transition temperature of these formulations (40-60°C) were lower than for formulations B-15.

Noteworthy, the color of the extrudates changed with the addition of PPI; they became slightly brownish, due to Maillard's reactions between the carbonyl group of reducing sugars of maltodextrins and the amino group of PPI. The specific mechanical energy (SME) values measured for the bio-based delivery system varied between 120 to 370 Wh.kg^{-1}, which indicated very mild conditions for the process

Regarding the results obtained for the delivery systems containing the perfume, we noticed that even though the MCT-oil was employed as a vector to enhance the incorporation of the perfume, there was a limit amount for which the MCT-oil did not play such a role, and acted more as another core material. Indeed, when the perfume was incorporated in a mixture of Citrus/MCT-oil at (50/50) % of content, a part of this mixture was splashed out of the extruder, suggesting that no all the mixture was incorporated. This was confirmed by the amount of perfume remaining in these extrudates, which were much lower than for the formulations containing Citrus/MCT-oil at (80/20) % of content. In this particular case the SME value was not taken as an indicator of mild extrusion conditions but more as an indicator of an excess of lubricant agent.

On the contrary, results obtained for the delivery system with Citrus/MCT-oil at (80/20) were interesting. Encapsulation rates of 6.7 to 7.9% for initial load of the perfume of 10% (w/w) were considered acceptable. The SME values were much lower than those obtained for the systems containing only MCT-oil as the active.

Results of the kinetic study were not compelling at this stage for a perfumery application, given the fact that the major part of the perfume was released during the first days, and this was observed for all the formulations in all the different climatic chambers they were exposed to. Thereafter, the release over time did not show significant variation after 14 days of study.

Our delivery system showed a stronger affinity for aldehyde compounds during processing. These compounds were more retained than the others perhaps due to covalent binding with the amino acids of PPI. Due to these interactions, the olfactive profile of Citrus was obviously modified. Yet, we observed that the addition of 5% of Hi-cap100 or PPI, helped to decrease aldehyde retention. This suggested that by modulating the formulation of the matrix or the perfume these interactions could be avoided.

The olfactive performances, for air freshener and detergence application, of our bio-based delivery system were underperforming compared to the benchmarks employed (e.g. gel, microporous membrane and starch-based capsule, all tested at iso-oil loading. For detergent application, we suspected that the matrix was not soluble enough to instantly release the perfume upon addition in water.). For air freshener application, this was explained by the fact that our extrudates had a very rapid release within the first days. Samples reached a plateau after 5 days, suggesting that there was no more water or perfume released. Nevertheless, it is important to highlight that at day 1 of the air freshener tests, the olfactive performance of formulation A, B and C were acceptable since no strong olfactory shift was noticed and their performance were in line with benchmarks.

Scanning electron microscopy permitted to determine the morphology of the bio-based delivery systems. In general, our extrusion conditions induced the formation of homogeneous extrudates, with multiple macroporosity morphology (pore diameter was superior to 50 nm) and with several irregularities onto the surface.

The advantages of the twin-screw extrusion encapsulation process developed herein can be outlined as follow:

- One-step process allowing the incorporation of an active compound in a polymeric matrix without any pre- or post-treatments. This results in a cost-effective technology.
- No production of by-products during the processing. No waste of materials.
- Low water consumption. The amount of water needed to plasticize the mixture was less than 25 % (w/w).
- The ingredients employed are from natural origin (except for the modified starch that had been chemically treated) and are biodegradables.
- Mild extrusion conditions: In one hand the extrusion temperatures fixed were all below 50°C and on the other hand SME values measured were considered to be low (< 370Wh.kg^{-1}), for both the active ingredients tested. These mild extrusion conditions are highly attractive for the encapsulation of very sensitive and thermolabile compounds such as manzanate and methyl heptenone in our case but that should be extended in the future to other sensitive molecules (i.e. pigments or polyphenols).
- Even though the extrusion conditions were mild, the mixing efficiency of the designed screw profile was still performing. This was demonstrated by the encouraging encapsulation efficiencies of MCT-oil obtained. They could reach encapsulation efficiencies above 80% for encapsulation rates varying between 8-13%. And in the case of Citrus/MCT-oil at (80/20) of content, the encapsulation efficiencies attempted were acceptable (between 67 to 79%) for an incorporation rate of 10% (w/w).
- Adaptability of the extrusion conditions. No adjustments of the established extrusion conditions (e.g. screw profile, screw speed, feeders rates and temperature profile) were required even though we varied the formulations and the active compounds.
- No use of an external organic plasticizer was necessary. Obtained delivery systems were at glassy state at room temperature ensuring the protection of the active compounds.
- The delivery systems were stable for a long period of time at 25°C at relative humidities ranging from 33 to 75% RH, which is an important factor to consider for industrial applications.
- The use of PPI is considered innovative since it not only acted as a compatibilizer agent enhancing the incorporation of hydrophobic compounds into hydrophilic matrices, but

also acted as a texturing agent by improving the viscosity of the blend and thus facilitating its extrusion.

However this process presented several inconvenients:

- The major drawback of this process was the impossibility of putting into shape the material mixture at the exit of the extruder's die. This technological issue may be responsible of the sampling problems for the kinetic release study, since the samples did not present the same exposed surface. This issue could be explained by the fact that the mixture was very complex and as it was already mentioned for pure maltodextrins, the workability window was very narrow. The use of a die provoked an important increase of the shear stress applied onto the mixture when exiting the extruder's barrel followed consequently by the flash-off of the water remaining in the sample. Specific die design or post-treatment of the extrudate would have to be studied in order to produce better-defined encapsulating objects.
- In the case of the incorporation of the perfume, suspected interactions between the aldehydes composing the perfume and the amino acids of the PPI were observed. These interactions are problematic because they modified the olfactive profile of the perfume. In encapsulation, the wall materials must be inert to the active compound so to avoid their modification or degradation.
- Extrusion is a multivariable input output process that most of the time is seen as a black box. Therefore several process parameters can influence the same final property and are sometimes difficult to study.
- The distribution/dispersion of the active compound inside the extruder barrels was scantily investigated. So, for the same formulation, depending on the time of the sampling, it is plausible that one extrudate has more core material than the second extrudate.
- The thermal and the sorption isotherms proved that the core/wall systems created in this project were very complex systems in different aspects: mechanical, thermal and analytical speaking (because of the interaction with volatile compounds with specific chemical groups). Therefore, their analysis and interpretation were very challenging

Even though during this project, several studies were carried out in order to elucidate the phenomena involved during the extrusion encapsulation process, trying to correlate the processing conditions and the product final properties, there are still some scientific aspects that required further investigations:

- Interactions between the matrix ingredient and the aldehydes of the active compound are very interesting because it proved that extruded protein also interact by covalent binding to the aldehydes. Therefore, perhaps modification of the proteins would be necessary in order to reduce their affinity for aldehydes.
- Working on the formulation of the perfume or the matrix materials in order to reduce or to avoid these types of interactions. The perfume composition can be designed in a manner that all the components could be released in the same way (modulate their vapor pressure), of course if they do not interact with the matrix.
- The pre- or post-treatment of the matrix or the active ingredient could be implemented so as to improve the encapsulation efficiency of the delivery system and improve their release properties.
- Increasing the encapsulation rates by tuning the extrusion processing parameters like the screw speed or the solid feed rates. For example in the case of the incorporation of a perfume, it is better either to increase the mechanical shear of the extruder so as to improve the mixing efficiency and thus increase the incorporation and retention of the active compound, and/or to adjust the formulation in order to decrease the possible interactions that reduce the incorporation and retention of the active compounds. As the formulations and the properties of the delivery systems are better characterized, it would be extremely valuable to further modulate the extrusion process parameters.

We can conclude that thanks to the mild extrusion processing parameters that we were able to establish that a hydrophobic model compound, such as MCT-oil, and a technical perfume, such as Citrus, could be incorporated into bio-based matrices by twin-screw extrusion. It is also important to mention that all this work has been conducted in respect of the industrial specifications: a natural, biodegradable and hygroscopic matrix.

However, lots of areas remained unexplored and required further investigations. Especially to improve the encapsulation rates, to better understand the core/matrix interactions and the behavior of the mixture during extrusion.

APPENDIX

Recovery rate

The recovery rate helps to evaluate the general performance of an analytical procedure. The recovery rate allows verifying if there is a systematic or random error throughout the different stages of the analytical procedure (either at the extraction step or the analytical response). In the food chain the recovery rate is often used as a mandatory control quality method [6].

The aim of the recovery rate in this particular case was to determine the possible matrix effect on the analysis of the analytes. Meaning that, the recovery rate may assess the matrix effect on the perfume at the extraction stage. The protocol was based on the extraction protocol described in section 2.5.4. A known quantity of the analyte was added to the "blank extrudate". The blank extrudate corresponds to an extrudate without any perfume. And then the blank extrudate and the analyte were subjected to the same extraction procedure.

The perfume load for extrusion experiments was fixed at 10% (w/w) with respect to the solid polymeric mixture. For this reason, the perfume was also added at 10% (w/w) with respect to the "blank extrudate".

Recovery rate for formulations containing Citrus/MCT-oil (80/20):

In a 50 mL volumetric flask approximately 0.87 g of blank extrudate, 0.1 g of Citrus and 0.026 g of MCT-oil were added. Then 3mL of water and 50µL of the Methyl decanoate standard at $0.1 g.mL^{-1}$ were introduced. The sample was mixed to favor the dissolution of the solid and then completed to the mark with methanol. The volumetric flask was ultrasonicated (>20kHz) for twenty minutes. The solution was filtered with a PTFE filter and was put in a GC-vial and analyzed with the same analytical method described before. For the formulations containing Citrus/MCT-oil (50/50) the recovery rate procedure was identical, except that the amount of blank extrudate, Citrus and MCT-oil were adapted.

Results of the recovery rate were the following:

Recovery rate results for Citrus/MCT-oil (80/20):

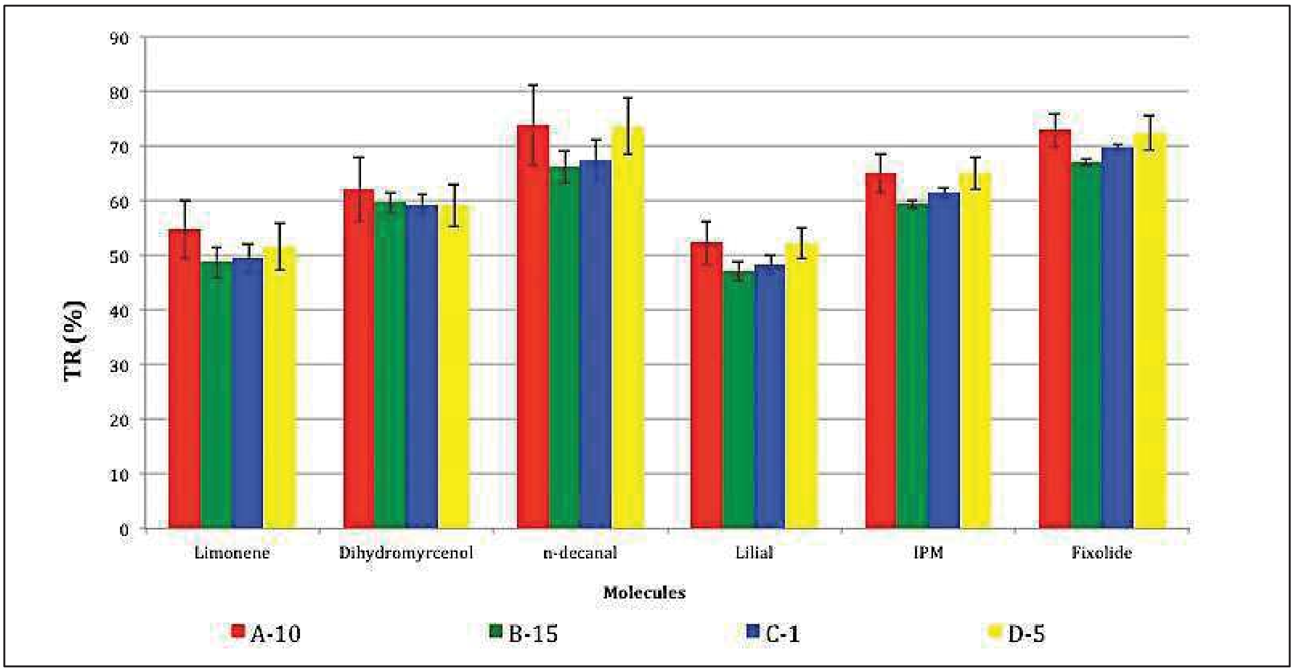

Figure 5. Summarizing histogram of the recovery rate values for all the four formulations at Citrus/MCT-oil (80/20) (n=4).

For all the four formulations and all the molecules except limonene and lilial, the mean recovery values (Tables 5 and 6) were within the range of acceptability, i-e 70 and 120% [7]. Besides, results presented a repeatability RSD ≤ 10%, which means that the matrix effect on the perfume can be considered to be low and as a consequence the extraction procedure was approved. So, the recovery rate value was not required for the quantification of the amount of perfume encapsulated.

However, this experiment put into evidence the possible interactions between the matrix components and the perfume (Figure 5). Even though there were not significant differences between the four formulations, it seemed that formulations B and C retained more all the six molecules than formulations A and D.

Based on this recovery rates, the extraction protocol selected could be improved in manner to find the right balance between the extraction solvent(s) and the molecules composing the perfume. And at the same time, that extraction solvent(s) have the fewer interaction with the constituents of the matrix.

Then again, this experiment does not take into account the influence of the process on the mixing of all the ingredients, since here they were just put into solution.

Note that the results obtained by measuring the recovery rate did not reproduce identically what happened inside the extruder barrel in terms of encapsulation. In fact, in extrusion all the ingredients were intimately mixed and transformed (e.g. plasticized). For the recovery rate, the ingredients were only added to a solution. The active compound was not physically encapsulated but more precisely emulsified. Besides, there was no plasticization at all. Therefore, the interactions between the matrix and the perfume could have been underestimated.

Recovery rate results for Citrus/MCT-oil (50/50):

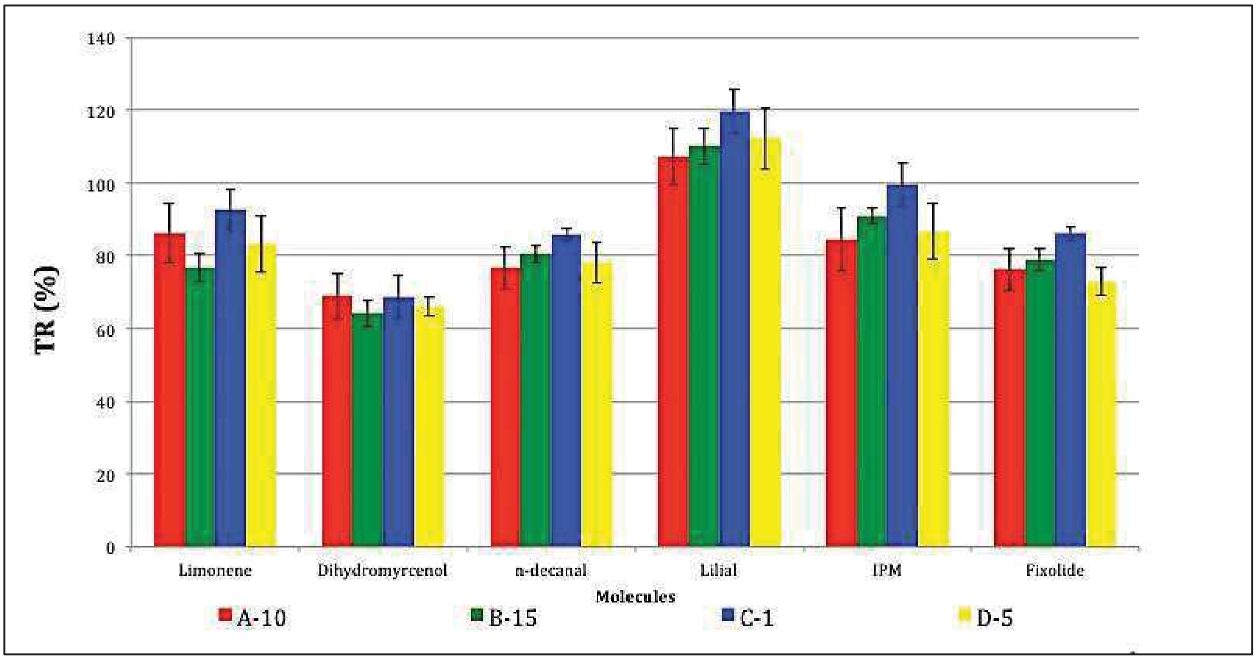

Figure 6. Summarizing histogram of the recovery rate values for all the four formulations at Citrus/MCT-oil (50/50) (n=4).

The same trend was observed for formulations containing Citrus/MCT-oil at (50/50). All the recovery rates measured were within the range of acceptability (Tables 7 and 8), except for lilial that presented recovery values equal or superior to 120%. Thus, the recovery rate was not taken into account either for the calculation of the amount of perfume encapsulated in the matrices.

In this case, formulation A had a more important retention for medium and low volatility compounds compared to formulation C (Figure. 6). These results corroborated the fact that interactions between the matrix and the perfume do exist, but also that, comparing formulations with different proportions of Citrus/MCT-oil; MCT-oil seemed to play a role on the matrix retention behavior. Here, as all the ingredients were put into solution, it appeared that the increase of the MCT-oil facilitated the extraction of the perfume.

Dans le contexte actuel, les communautés scientifiques et politiques sont centrées sur les différentes manières de mieux préserver et utiliser les ressources naturelles de notre planète. Dans le but de réduire la consommation des matières issues du pétrole, et de développer de nouveaux produits et procédés industriels plus propres, l'industrie des fragrances et des arômes cherche aujourd'hui à développer de nouveaux matériaux bio-sourcés pour protéger leurs molécules volatiles odorantes. Dans ce travail de thèse, les maltodextrines ont été choisies comme composé majoritaire, les protéines de pois et un amidon modifié ont été sélectionnés comme additifs compatibilisants pour la composition des matrices d'agro-matériaux. L'incorporation des molécules volatiles odorantes, ainsi que l'élaboration des matrices encapsulantes ont été réalisées en une seule étape, grâce à la technologie d'extrusion bi-vis à basse température. Les caractéristiques physicochimiques, thermiques et morphologiques de ces nouvelles matrices enrobantes ont été analysées, de même que la détermination de leur efficacité d'encapsulation et du profil de libération du principe actif. Les différentes investigations menées ont permis de mieux comprendre l'impact des formulations et de l'incorporation des molécules volatiles odorantes sur les paramètres opératoires. Les interactions entre la matrice enrobante et le principe actif ont également été étudiées. Les conditions d'extrusion établies, ainsi que les caractéristiques de ces nouvelles matrices encapsulantes, s'avèrent être pertinentes pour le domaine de la parfumerie.

Mots clés : maltodextrines, molécule volatile odorante, agro-matériaux, principe actif, matrice enrobante, extrusion bi-vis, encapsulation, protéines de pois, amidon modifié, propriétés thermiques, efficacité d'encapsulation, hygroscopicité.

Nowadays, scientific and political communities are focused on ways to better preserve and manage the natural resources of our planet. In order to reduce consumption of fossil resources, and to develop more environmentally friendly industrial processes, the industry of flavors and fragrances became interested in developing new bio-based encapsulating materials. In the present work, maltodextrins have been chosen as main component of the matrix, and pea protein isolate and a modified starch were selected as compatibilizing additives. The incorporation of volatile odorant compounds and the elaboration of the new bio-based delivery systems were performed, all in one single step, by low temperature twin-screw extrusion. The physicochemical, thermal and morphological properties of these matrices were studied, as well as the encapsulation efficiency and the release profile of the active compounds. These investigations have led to a better understanding of the impact of the formulations and of the incorporation of the active compound on the process parameters. The interactions between the wall and the encapsulated materials were also analyzed. The characteristics of the new bio-based delivery systems and the established extrusion process conditions were found to be very promising to be employed in the field of perfumery.

Key words: maltodextrins, volatile odorant compound, agro-material, active principle, wall material, twin-screw extrusion, encapsulation, pea protein, modified starch, thermal properties, encapsulation efficiency, hygroscopicity