Characterization of Starch Using Flow Field-Flow Fractionation Techniques

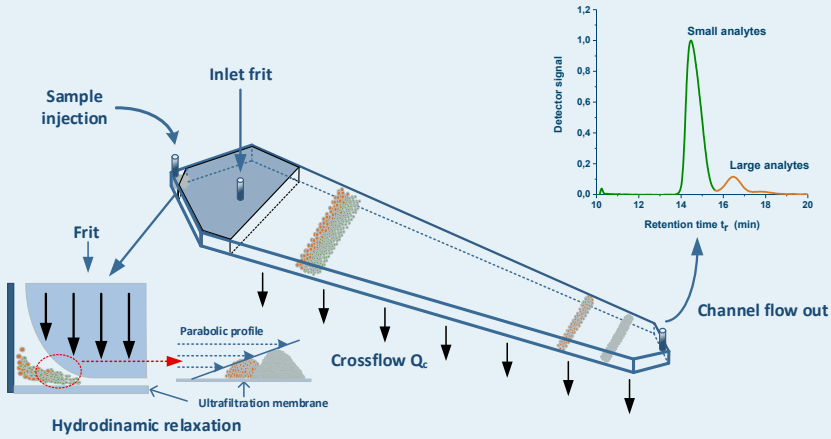
Fuentes, Catalina

2019

Document Version:
Publisher's PDF, also known as Version of record

Characterization of Starch Using Flow Field-Flow Fractionation Techniques

CATALINA SANDRA FUENTES ZENTENO
DEPARTMENT OF FOOD TECHNOLOGY, ENGINEERING AND NUTRITION | LUND UNIVERSITY



Characterization of Starch Using Flow Field-Flow Fractionation Techniques

Catalina Sandra Fuentes Zenteno

LUND
UNIVERSITY

DOCTORAL DISSERTATION
by permission of the Faculty of Engineering, Lund University, Sweden.
To be defended in Hall C at the Center for Chemistry and Chemical Engineering.
Friday, 12th of April 2019 at 10:15 am.

Faculty opponent
Dr. Frédéric Violleau

Department of Agricultural and Food Sciences,
Ecole d'Ingénieurs de Purpan, Toulouse, France

Organization
LUND UNIVERSITY
Department of Food Technology, Engineering and Nutrition
Faculty of Engineering
P.O.Box 124, SE-22100 Lund, Sweden
Author(s) Catalina Sandra Fuentes Zenteno

Document name DOCTORAL DISSERTATION

Date of issue

2019 - 04 - 12

Sponsoring organization Swedish International Development Cooperation Agency (SIDA)

Title and subtitle: Characterization of Starch Using Flow Field-Flow Fractionation Techniques

To improve the properties and functionality of starch, it is important to understand the physicochemical and structural characteristics as raw material, after being modified to increase its characteristics and/or during changes due to the processing conditions. For this reason, the objective of the present thesis is to improve the understanding of characteristics and molecular properties like molar mass (M), root-mean-square radius (r_{rms}) and their relation to other physicochemical properties of starch extracted from different sources. Due to its complex structure, however, the analysis of starch can be challenging and few techniques are suitable. Nevertheless, some sub-techniques of the field-flow fractionation (FFF) family, especially asymmetric flow field-flow fractionation (AF4) have been shown to be appropriate for this task.

Regarding the different FFF techniques, large-scale full-feed depletion mode of split flow thin cell fractionation (FFD-SF) was used as a preparative fractionation of corn and potato starch granules showing to be a useful technique for this task. In addition, the fractions obtained by large-scale FFD-SF were analyzed using optical microscopy (OM) and gravitational field-flow fractionation (GrFFF) which have been shown to be also suitable methods for determining the average size diameter (d_{avg}), with good agreement between these two techniques.

Moreover, some slight improvements related to the analysis of starch as a polymer in solution using AF4 were made. First, with the use of a modified channel known as frit inlet (FI-AF4). It has been shown that almost double the amount of mass can be injected into the FI-AF4 channel in comparison with the conventional channel, without observing overloading effects. Second, the downturn phenomena that could be observed in the analysis of starch and/or other polymers, which is usually considered as an artifact that occurs during the analysis, was shown to be a result of co-elution of species with similar M but different conformation.

Otherwise, physicochemical properties such as granule size, crystallinity, pasting properties among others as well as structural properties such as r_{rms}, weight-average molar mass (M_w) and apparent density (ρ_{app}) were analyzed in five different starches from Andean crops (three grains and two roots). To evaluate the relation between all these properties a statistical analysis was performed and a model was proposed that relates pasting properties i.e. peak viscosity and final viscosity with ρ_{app}, gelatinization enthalpy, granule size and amylose content. Additionally, thermal properties were examined in relation to the granule size. For this, potato starch and its fractions (obtained using large-scale FFD-SF), as well as Andean roots, chosen for their similarity in botanical origin, were analyzed showing a correlation between enthalpy and granule size. Another factor affecting the thermal properties seems to be the amylose content.

In the analysis of starch nanocomponents, it seems that the procedure used for obtaining non-solvent precipitated starch (non-SPS) resulted in new materials with amorphous structures that do not show particle characteristics when in solution, which suggests that designating these as nanoparticles might not be appropriate. Finally, in the analysis of starch extracted from different types of breads, the results show that M and size are affected by the baking process. In the case of breads, α-amylase enzyme was added. M and size decreased while the degree of branching (DB) and the number of reducing ends H-1(β-r) and H-1(α-r) increased. Furthermore, in starches extracted from Bolivian breads, it seems that the content of water during the preparation of the dough could affect the production of resistant starch.

Key words Asymmetric Flow Field-Flow Fractionation, Starch, Molar Mass, Conformation

Classification system and/or index terms (if any)

Supplementary bibliographical information — Language English

ISSN and key title — ISBN (print) 978-91-7422-643-0
ISBN (digital) 978-91-7422-644-7

Recipient's notes — Number of pages — Price

Security classification

Signature  Date 2019 – 03 - 07

Characterization of Starch Using Flow Field-Flow Fractionation Techniques

Catalina Sandra Fuentes Zenteno

LUND
UNIVERSITY

Coverphoto by Catalina Sandra Fuentes Zenteno

Faculty of Engineering
Department of Food Technology, Engineering and Nutrition

ISBN 978-91-7422-643-0 (printed version)
ISBN 978-91-7422-644-7 (digital version)

Printed in Sweden by Media-Tryck, Lund University
Lund 2019

To my family (A mi familia)

Table of Contents

List of Figures and Tables

Popular Scientific Summary

The food industry is constantly trying to improve the quality of food products by attempting different approaches. Food today should not only be fresh-looking, with good stability and produced under high sanitary conditions, it also must be nutritious, have a longer shelf life until being consumed and must provide functional characteristics such as higher content of fiber and preservation of its nutritional properties after processing like high vitamins content to name some.

Starch is one of the principal components in daily food like roots, cereals and bread, or is used as a functional ingredient in other food. Starch is a polysaccharide that constitutes the major source of energy in the human diet. To improve the properties and functionality of starch, it is important to understand the physicochemical and structural characteristics at molecular level as raw material, as well as after being modified to improve its characteristics and/or during changes due to the processing conditions.

For instance, bread is preferably consumed while fresh, but unfortunately, bread freshness is maintained only for a few days. Bread loses its freshness during storage, which causes alteration in the physical, chemical and sensorial characteristics such as taste, smell and texture. This is a result of moisture migration from the crumb to the crust. In recent studies at molecular level, it is suggested that changes in amylopectin - an important component of starch - are responsible for the increase of bread firmness.

In general, physicochemical and structural properties of polysaccharides (e.g. amylose content, gelatinization temperatures, molar mass (M), etc.) play an important role when applied in complex mixtures such as food. Especially M, size and their distributions are important because physical and mechanical properties can depend on them (such as viscosity). Furthermore, the separation and analysis of polysaccharides can be very challenging and few analytical techniques are suitable for this task. The difficulties encountered are typically related to the high molar mass and dispersity in polysaccharide population, as well as the presence of aggregated structures and degradation due to shear that occurs during the analysis of big polysaccharides like starch because of its branched structure.

Therefore, academic research and the food industry are in need of reliable and straightforward analytical methods for the analysis of branched polysaccharides. One of these methods is asymmetric flow field-flow fractionation (AF4), which is a separation technique similar to the well-known size exclusion chromatography (SEC), but instead of using a separation column, species are separated in an open channel. AF4 system is able to separate mixtures of molecules with different sizes and masses based on their size. The separation is based on the amount of time

molecules need to leave the AF4 channel, in which smaller molecules migrate faster than larger ones.

In recent years, AF4 was successfully used in the analysis of different branched polysaccharides such as starch, obtaining less degradation in structure during the analysis compared to other separation techniques. Additionally, AF4 connected to appropriate detectors is useful to determine the structure and conformation of the molecules. Therefore, in the present study mainly AF4 was used to fractionate and characterize starch samples such as Andean roots as raw material, waxy maize with some treatments/modifications to obtain nanocomponents, and starches extracted from breads as a final product.

Principal results show that some particular characteristics can be highlighted in the starches from the Andean crops (three grains and two roots) studied. For instance, amaranth starch seems to be virtually free of amylose and had very high molar mass and size, which means that very few molecules formed the amaranth granule. In contrast, achira starch contained high amylose content. Additionally, quinoa and canihua starches seem to be stable after the heating and cooling process, which could provide good properties for the production of food products that need to have good stability at high temperatures.

For the three different types of non-solvent precipitated starch that were analyzed (i.e. starch nanoparticles prepared from waxy maize starch granules), the results demonstrate that they display different conformational properties while being dispersed in aqueous solution. In addition, the molar masses were different indicating that the preparation procedure had a strong influence on the size and conformation.

Furthermore, the analysis of starch extracted from breads showed that starch has a different molar mass and size depending on some processing conditions such as the amount of water used in the elaboration of the dough or the addition of enzyme to increase the life-time of the bread. Moreover, a greater branching structure could be reflected when the molar mass and size decreased due to the baking process, i.e. starch in the raw material is less branched than in the final product. Additionally, the changes in the molecular properties could be related to the content of amylose and the formation of resistant starch (a type of dietary fiber).

Resumen Científico Popular

La industria alimentaria está constantemente tratando de mejorar la calidad de los productos alimenticios desde diferentes enfoques. Los alimentos de hoy no solo deben tener un aspecto fresco, una buena estabilidad y ser producidos en condiciones sanitarias elevadas, también deben ser nutritivos, tener una vida útil más larga y deben proporcionar características funcionales tales como un mayor contenido de fibra y la conservación de sus nutrientes después de ser procesados por ejemplo tener un alto contenido de vitaminas.

El almidón es uno de los componentes principales de los alimentos diarios, se puede encontrar en raíces, cereales, pan y se utiliza también como un ingrediente en otros alimentos. El almidón es un polisacárido que constituye la principal fuente de energía en la dieta humana. Para mejorar las propiedades y la funcionalidad del almidón, es importante comprender las características fisicoquímicas y estructurales a nivel molecular como: materia prima, después de haber sido modificado (para mejorar sus características) y/o durante los cambios producidos debido a las condiciones de procesamiento.

Por ejemplo, el pan se consume preferiblemente mientras está fresco, pero desafortunadamente, la frescura del pan se mantiene pocos días. El pan pierde su frescura durante el almacenamiento, lo que provoca alteraciones en las características físicas, químicas y sensoriales como ser sabor, olor y textura. Esto es el resultado del traslado de la humedad de la miga a la corteza. En estudios recientes a nivel molecular, se sugiere que los cambios en la amilopectina (un componente importante del almidón) son responsables del aumento de la firmeza del pan.

En general, las propiedades fisicoquímicas y estructurales de los polisacáridos (por ejemplo, contenido de amilosa, temperatura de gelatinización, masa molar, etc.) desempeñan un papel importante cuando se aplican en mezclas complejas como los alimentos. Especialmente la masa molar y el tamaño de la molécula son importantes porque otras propiedades físicas y mecánicas pueden depender de ellas (como por ejemplo la viscosidad). Por otra parte, la separación y el análisis de los polisacáridos pueden ser muy complicada y pocas técnicas analíticas son adecuadas para esta tarea. Las dificultades encontradas en su análisis suelen estar relacionadas con la alta masa molar que tiene y la dispersión dentro de la población de polisacáridos, así como con la formación de estructuras agregadas y la degradación de su estructura debida a la ruptura de sus ramificaciones que se produce durante el análisis de grandes polisacáridos como el almidón.

Por lo tanto, la investigación en la parte académica y la industria alimentaria necesitan métodos analíticos confiables y directos para el análisis de polisacáridos ramificados. Uno de estos métodos es el fraccionamiento en flujo de campo asimétrico AF4 por sus siglas en inglés, que es una técnica de separación similar a la conocida cromatografía de exclusión por tamaño SEC por sus siglas en inglés,

pero en lugar de usar una columna de separación, los componentes de la muestra se separan en un canal sin empaque. El sistema AF4 es capaz de separar mezclas de moléculas de diferentes tamaños y masas según su tamaño. La separación se basa en la cantidad de tiempo que las moléculas tardan en recorrer el canal AF4, donde las moléculas más pequeñas recorren más rápido que las más grandes.

En los últimos años, la técnica AF4 se utilizó con éxito en el análisis de diferentes polisacáridos ramificados como el almidón, obteniendo una menor degradación de su estructura durante el análisis en comparación con otras técnicas de separación (como ser SEC). Además, AF4 conectado a apropiados detectores útil para determinar la estructura y la conformación de las moléculas. Por lo tanto, en el presente estudio se utilizó principalmente la técnica AF4 para fraccionar y caracterizar muestras de almidón como ser cultivos Andinos (tres granos y dos raíces) como materia prima, almidón de maíz waxy (que significa libre de amilosa) que fue sometido a diferentes tratamientos y/o modificaciones para obtener nano componentes de su almidón, y finalmente se analizó almidones extraídos de panes.

Los principales resultados muestran que algunas características particulares pueden ser resaltar en los almidones estudiados de cultivos Andinos. Por ejemplo, el almidón de amaranto parece estar virtualmente libre de amilosa, tuvo una masa molar y un tamaño muy elevados, lo que significa que muy pocas moléculas formaron el gránulo de amaranto. En contraste, el almidón de achira contenía un alto valor de amilosa. Además, los almidones de quinua y cañahua parecen indicar que son estables después de ser calentados y enfriados, lo que podría proporcionar buenas propiedades para la producción de alimentos que necesitan tener una buena estabilidad a altas temperaturas.

Por otro lado, se obtuvieron y analizaron tres diferentes tipos de nano-componentes del almidón de maíz mediante un proceso de nano-precipitación utilizando un no solvente (alcohol etílico). Es decir, se obtuvo nano partículas de almidón preparadas a partir de gránulos de almidón de maíz. Los resultados demuestran que las nano partículas (o nano-componentes) tienen diferente conformación molecular cuando se encuentran dispersas en solución acuosa. Además, las masas moleculares fueron diferentes en las tres muestras, lo que indica que el procedimiento de preparación tuvo una gran influencia en el tamaño y la conformación.

El análisis de los almidones extraídos de panes mostró que el almidón tiene una masa molecular y un tamaño diferente dependiendo de algunas condiciones durante el procesamiento, como ser la cantidad de agua utilizada en la elaboración de la masa o la adición de enzimas para aumentar la vida útil del pan. Además, una estructura más ramificada se reflejaría cuando la masa molar y el tamaño de la molécula disminuyen debido al proceso de horneado. Es decir, el almidón en la materia prima es menos ramificado que en el producto final. Además, los cambios en las propiedades moleculares podrían estar relacionados con el contenido de amilosa y la formación de almidón resistente (que es un tipo de fibra dietética).

Abstract

To improve the properties and functionality of starch, it is important to understand the physicochemical and structural characteristics as raw material, after being modified to increase its characteristics and/or during changes due to the processing conditions. For this reason, the objective of the present thesis is to improve the understanding of characteristics and molecular properties like molar mass (M), root-mean-square radius (r_{rms}) and their relation to other physicochemical properties of starch extracted from different sources. Due to its complex structure, however, the analysis of starch can be challenging and few techniques are suitable. Nevertheless, some sub-techniques of the field-flow fractionation (FFF) family, especially asymmetric flow field-flow fractionation (AF4) have been shown to be appropriate for this task.

Regarding the different FFF techniques, large-scale full-feed depletion mode of split flow thin cell fractionation (FFD-SF) was used as a preparative fractionation of corn and potato starch granules showing to be a useful technique for this task. In addition, the fractions obtained by large-scale FFD-SF were analyzed using optical microscopy (OM) and gravitational field-flow fractionation (GrFFF) which have been shown to be also suitable methods for determining the average size diameter (d_{avg}), with good agreement between these two techniques.

Moreover, some slight improvements related to the analysis of starch as a polymer in solution using AF4 were made. First, with the use of a modified channel known as frit inlet (FI-AF4). It has been shown that almost double the amount of mass can be injected into the FI-AF4 channel in comparison with the conventional channel, without observing overloading effects. Second, the downturn phenomena that could be observed in the analysis of starch and/or other polymers, which is usually considered as an artifact that occurs during the analysis, was shown to be a result of co-elution of species with similar M but different conformation.

Otherwise, physicochemical properties such as granule size, crystallinity, pasting properties among others as well as structural properties such as r_{rms}, weight-average molar mass (M_w) and apparent density (ρ_{app}) were analyzed in five different starches from Andean crops (three grains and two roots). To evaluate the relation between all these properties a statistical analysis was performed and a model was proposed that relates pasting properties i.e. peak viscosity and final viscosity with ρ_{app}, gelatinization enthalpy, granule size and amylose content. Additionally, thermal properties were examined in relation to the granule size. For this, potato starch and its fractions (obtained using large-scale FFD-SF), as well as Andean roots, chosen for their similarity in botanical origin, were analyzed showing a correlation between the enthalpy of gelatinization and granule size. Another factor affecting the thermal properties seems to be the amylose content.

In the analysis of starch nanocomponents, it seems that the procedure used for obtaining non-solvent precipitated starch (non-SPS) resulted in new materials with amorphous structures that do not show particle characteristics when in solution, which suggests that designating these as nanoparticles might not be appropriate. Finally, in the analysis of starch extracted from different types of breads, the results show that M and size are affected by the baking process. In the case of breads, α-amylase enzyme was added. M and size decreased while the degree of branching (DB) and the number of reducing ends H-1(β-r) and H-1(α-r) increased. Furthermore, in starches extracted from Bolivian breads, it seems that the content of water during the preparation of the dough could affect the production of resistant starch.

List of Papers

The thesis is based on the following Papers, referred in the text by their roman numerals.

I. **Fractionation and characterization of starch granules using field-flow fractionation (FFF) and differential scanning calorimetry (DSC)**
Fuentes, C., Kang, I., Lee, J., Song, D., Sjöö, M., Choi, J., Lee, S., and Nilsson, L.
Manuscript submitted to Analytical and Bioanalytical Chemistry.

II. **Comparison between conventional and frit-inlet channel in separation of biopolymers by asymmetric field flow-field fractionation**
Fuentes, C., Choi, J., Zielke, C., Peñarrieta, J.-M., Lee, S., and Nilsson, L.
Manuscript submitted to Analyst

III. **Co-elution phenomena in polymer mixtures studied by asymmetric flow field-flow fractionation**
Zielke, C., Fuentes, C., Piculell, L., and Nilsson, L.
Journal of Chromatography A, 1532 (2018) 251–256.

IV. **Physicochemical and structural properties of starch from five Andean crops grown in Bolivia**
Fuentes, C., Perez-Rea, D., Bergenståhl, B., Carballo, S., Sjöö, M., and Nilsson, L.
International Journal of Biological Macromolecules 125 (2019) 829–838.

V. **Characterization of non-solvent precipitated starch using asymmetrical flow field-flow fractionation coupled with multiple detectors**
Fuentes, C., Saari, H., Choi, J., Lee, S., Sjöö, M., Wahlgren, M., and Nilsson, L.
Carbohydrate Polymers 206 (2019) 21–28.

VI. The effect of baking and enzymatic treatment on the structural properties of wheat starch
Fuentes C., Zielke, C., Prakash, M., Kumar, P., Peñarrieta, J.-M., Eliasson, A.-C., and Nilsson, L.
Food Chemistry 213 (2016) 768–774.

VII. Characterization of molecular properties of wheat starch from three different types of breads using asymmetric flow field-flow fractionation (AF4)
Fuentes C., Castañeda, R., Rengel, F., Peñarrieta, J.-M and Nilsson, L.
Manuscript submitted to Food Chemistry Journal

The author's contribution to the Papers:

Paper I The author performed part of the experimental work, participated in the planning of the study and wrote the manuscript in collaboration with the other co-authors.

Paper II The author was involved in the design of the study, performed all the experiments, analyzed the results and was responsible for writing the manuscript.

Paper III The author participated in performing part of the AF4 runs and analyzed the results.

Paper IV The author performed the analysis of total starch, total protein content and AF4 runs, took active part in the data evaluation and wrote the paper in collaboration with the other co-authors.

Paper V The author participated in the planning of the study, performed all experimental work except obtaining the samples, took active part in the data evaluation and wrote the major part of the paper.

Paper VI The author performed most experimental work except obtaining the samples, took active part in the data evaluation and was responsible for writing the manuscript.

Paper VII The author designed the study, performed the experimental work, took active part in the data analysis and was responsible for writing the manuscript.

Other papers (not included in the thesis):

Production of starch nanoparticles by dissolution and non-solvent precipitation for use in food-grade Pickering emulsions
Saari, H., Fuentes, C., Sjöö, M., Rayner, M., and Wahlgren, M.
Carbohydrate Polymers 157 (2017) 558–566 (open access)

Application of asymmetric flow field-flow fractionation (AF4) and multiangle light scattering (MALS) for the evaluation of changes in the product molar mass during PVP-b-PAMPS synthesis
Fuentes C., Castillo, J., Vila, J., and Nilsson, L.
Analytical and Bioanalytical Chemistry (2018) 410:3757–3767 (open access)

Assessment of pre-requirements of HACCP and analysis of critical control points for safety during production of artisanal and industrial bread
Castañeda R., Fuentes, C., Peñarrieta, J.-M.
Revista Boliviana de Química (Rev.Bol.Quim. 2016) Vol. 33, No.5, pp. 196-208 (open access)

Determination of charge distribution in octenyl succinic (OSA) starch and cereal beta-glucan using electrical AF4
Choi J., Fuentes C., Zielke, C., and Nilsson, L.
Manuscript

Abbreviations and Symbols

AF4	Asymmetric flow field-flow fractionation
cryo-TEM	Cryogenic transmission electron microscopy
d_{avg}	Average size diameter
d_c	Cut-off diameter
ρ	Density (g/mL)
ρ_{app}	Apparent density (Kg/m^3)
D	Diffusion coefficient (m^2/s)
DB	Degree of branching
DMSO	Dimethyl sulfoxide
dRI	Differential refractive index
DSC	Differential scanning calorimetry
FE	Fractionation efficiency
FFD-SF	Full-feed depletion mode of split flow thin cell fractionation
FFF	Field-flow fractionation
FI	Frit inlet
FV	Final viscosity (mPa·s)
GrFFF	Gravitational field-flow fractionation
ΔH	Enthalpy of gelatinization (mJ/mg)
^1H NMR	Nuclear magnetic resonance spectroscopy of proton
M	Molar mass (g/mol)
MALS	Multiangle light scattering
M_v	Weight average molar mass determined by viscosity
M_w	Weight average molar mass
Non-SPS	Non-solvent precipitated starch
OM	Optical microscopy
OSA	n-octenyl succinic anhydride
PEO	Poly(ethylene oxide)

PT	Pasting temperature (°C)
PV	Peak viscosity (mPa·s)
Q_c	Crossflow (mL/min)
r_h	Hydrodynamic radius (nm)
r_{rms}	Root-mean-square radius or radius of gyration (nm)
SdFFF	Sedimentation field-flow fractionation
SEC	Size exclusion chromatography
SEM	scanning electron microscope
SPLITT	Split-flow thin-cell
t_r	Retention time (s)
T_c	Conclusion temperature (°C)
T_o	Onset temperature (°C)
T_p	Peak temperature (°C)
TP	Throughput
ThFFF	Thermal field-flow fractionation
WM	Waxy Maize starch
XRD	X-ray diffraction

1 Introduction

Starch is one of the main carbohydrates utilized in the food and other industries. It can be used as a raw material, modified to improve desired functional characteristics, or added as an ingredient to other foods as a thickener, gelling agent, stabilizer, emulsifier, fat replacer or encapsulating agent, among other applications (Ai & Jane, 2016; Bai, Shi, Herrera, & Prakash, 2011; Chung, Lee, Han, & Lim, 2010). The diversity of applications for starch is mainly due to the changes occurring inside the semi-crystalline structure of the starch granules. These changes take place during the heating process (gelatinization, pasting and gel formation) and cooling process (retrogradation) of the starch granules in the presence of excess water.

A specific model for the structure of the starch granule explaining the precise changes that occur during the heating and cooling process is still under discussion. Nevertheless, there is a general acceptance that amylopectin is responsible for the semi-crystalline structure. It is well known that the proportion of amylose and amylopectin can produce different physicochemical properties in food products and affect the formation of resistant starch - a type of dietary fiber - that the human body cannot digest, which means that it is not absorbed in the small intestine and its fermentation process feeds beneficial bacteria present in the gastrointestinal tract (Lan, Hoover, Jayakody, Liu, Donner, Baga, et al., 2008; Leeman, Karlsson, Eliasson, & Björck, 2006; Lu, Wang, Li, & Huang, 2018; Tao, Li, Yu, Gilbert, & Li, 2019; Yoshimoto, Tashiro, Takenouchi, & Takeda, 2000). However, several details in the structure are still uncertain.

For these reasons, the analysis and characterization of the starch granule and its principal components, i.e. amylose and amylopectin, is still important. This analysis can not only serve as a quality control of the products; it can also help to understand the relationships between the structures and functional properties, allowing for the development of new types of starch and/or new processing conditions to obtain desirable functionality for specific applications in food and other industries.

Among the properties of polymers, molar mass (M), and size are of fundamental importance because they can be closely related to other properties in the final product, such as viscosity, adsorption kinetics and film properties to name a few (Duthen, Rochat, Kleiber, Violleau, Daydé, Raynaud, et al., 2018; Garin, Tighzert, Vroman, Marinkovic, & Estrine, 2014). Moreover, it is essential to consider that the analysis of polymers, especially those with branching structures such as starch, can

be very challenging due to their complex structures. The structure is often very large, could be sensitive to shear degradation and have the tendency to form aggregates. For these reasons, it is necessary to apply a reliable and straightforward analytical method.

In recent years, field-flow fractionation (FFF) techniques with emphasis on the sub-technique Asymmetric flow field-flow fractionation (AF4) were successfully used for the analysis of different branched polysaccharides such as starch (You, Stevenson, Izydorczyk, & Preston, 2002). It has been shown, for branched polymers, that similar techniques such as Size Exclusion Chromatography (SEC) can generate degradation in the structure due to shear scission (Cave, Seabrook, Gidley, & Gilbert, 2009; Žigon, The, Cheng, & Grubišić-Gallot, 1997). In addition, it has been reported that the use of AF4 can produce less degradation in polymers with branching structures like starch during their analysis (Makan, Otte, & Pasch, 2012; Otte, Pasch, Macko, Brüll, Stadler, Kaschta, et al., 2011). Moreover, AF4 connected to suitable detectors such as multiangle light scattering (MALS) and differential refractive index (dRI) allows the determination of additional information like size, apparent density and conformation.

2 Objectives

The overall aim of the present thesis is to improve the understanding of characteristics and molecular properties like molar mass and size and their relation to other physicochemical properties of starch extracted from different sources: first, as raw material; second, when modified to obtain starch nanocomponents; and third, during the changes due to processing conditions like breadmaking. The specific objectives of each paper, which is part of the present thesis, are listed in Table 1.

The study of these starch samples at different stages i.e. raw material and/or after processing conditions could allow the improvement or modification of procedures in the production of food to obtain desirable characteristics in the final product. For example, as raw material, the characterization of starch - where M can be related with pasting or thermal properties - allows the establishment of mathematical models that can be used during the formulation of a product. In another example, as a final product, the determination of M after cooking could show the formation of resistant starch, or the changes produced due to the addition of an enzyme. To do so, FFF techniques, mainly the sub-technique AF4, were used to fractionate and characterize starch samples as granules and as polymers in solution. In addition, to look for possible improvements during its analysis a modified channel known as frit inlet (FI-AF4) was used.

The work included in this thesis was mostly conducted at the Department of Food Technology, Engineering and Nutrition, Lund University, Sweden. Some experimental work was also performed at the School of Chemistry, Faculty of Pure and Natural Science, Universidad Mayor de San Andres (UMSA), La Paz, Bolivia as part of the collaboration between Sweden and Bolivia.

Table 1. Specific objectives of each paper
Overview of type of samples, FFF techniques used, and research objective

Paper	Sample Type	FFF techniques used and its purpose	Complementary techniques	Research Objective
Paper I	Corn and potato starch granules.	Large-scale FFD-SF was used as preparative separation method. In addition, GrFFF connected to UV detector was used for the determination of the size distribution of starch granule fractions.	Optical Microscopy (OM) to determine the size distribution of the fractions. Differential scanning calorimetry (DSC) to evaluate the thermal properties of starch granule fractions.	To separate the corn and potato starch granules into fractions of different sizes. Additionally, GrFFF and OM were used to determine the size distributions of the fractions collected from the large-scale FFD-SF channel and DSC was used to evaluate the thermal properties of the starch granules fractions.
Paper II	Pullulan, glycogen and waxy maize starch.	AF4 connected to MALS and dRI detectors was used to obtain the elution profiles of the polymers in solution. Additionally, two different types of AF4 channels were used i.e. the conventional channel and a modified channel known as frit inlet (FI).	No additional techniques were used in this study.	To compare two channels (conventional and FI-AF4 channels) in terms of the plate height (H), resolution (R_s) and the mass recovery. In addition, waxy maize starch was analyzed to compare the overloading effect between AF4 and FI-AF4 channel.
Paper III	Pullulan, glycogen and Poly (ethylene oxide) (PEO).	AF4 connected to MALS and dRI detectors was used to determine molar mass (M) and root-mean-square radius (r_{rms}) distributions during the separation of the samples.	No additional techniques were used in this study.	To study the downturn phenomenon.
Paper IV	Starch granules extracted from amaranth, quinoa, canihua, achira and maca.	AF4 connected to MALS and dRI detectors was used to determine the weight average values of M_w, r_{rms} and apparent density (ρ_{app}) of the starch polymers in solution.	Scanning electron microscope (SEM) to determine the morphology and size of the starch granules. Moisture, total starch, protein and amylose content to determine the chemical composition. X-ray diffraction (XRD) pattern to analyze the crystallinity. DSC to evaluate the thermal properties. ^{1}H NMR to determine the degree of branching (DB). Rheology to determine the pasting properties of starch granules.	To evaluate the relation between fundamental properties (such as granule size, amylose content, ρ_{app}, etc.) and pasting properties.

Paper V	Waxy Maize starch (WM) and Non-Solvent Precipitated Starch (non-SPS) obtained from WM i.e. untreated (SP) pre-treated via acid-hydrolysis (AHSP) and modified using OSA (OSASP).	AF4 connected to MALS, dRI and dynamic light scattering (DLS) detectors was used to determine the weight average values of M_w, r_{rms}, r_h, ρ_{app} and the conformation of the starch non-SPS in solution.	SEM to determine the morphology and size of the dry non-SPS. Cryogenic transmission electron microscopy (Cryo-TEM) to determine the morphology in solution of the non-SPS.	To investigate the influence of preparation methods for non-SPS, e.g., acid hydrolysis and OSA-modification, on the size, M, ρ_{app} and conformation when non-SPS is subjected to different reconstitution procedures. Additionally, it was studied if they remain as particles after re-dispersion under different conditions.
Paper VI	Starch extracted from breads baked with and without the addition of α-amylase enzyme.	AF4 connected to MALS, and dRI detectors was used to determine the averages values of M_w, r_{rms} and ρ_{app} of the starch polymers extracted from bread.	^{1}H NMR to determine the DB and the number of reducing ends.	To investigate the influence of the baking process on size, M, ρ_{app}, conformation and DB of wheat starch with and without the addition of α-amylase enzyme (i.e. Novamyl 10000 BG).
Paper VII	Starch extracted from three different type of breads made in artisanal manner and industrial production.	AF4 connected to MALS, and dRI detectors was used to determine the weight average values of M_w, r_{rms}, ρ_{app} and the conformation of the starch polymers extracted from bread.	Total starch, resistant starch, protein, moisture and amylose content to determine the approximate chemical composition.	To investigate the changes in the molecular properties such as M_w, r_{rms}, ρ_{app} and conformation of starch extracted from three different types of breads, commonly the most consumed in Bolivia.

3 Structure of Starch and Its Characteristics

Starch is synthesized in a granular form in special organelles. The biosynthesis of the granule is initiated at the hilum and the starch granule grows by apposition (Pérez & Bertoft, 2010; Zobel, 1988). The starch granules vary greatly in size from about 1 µm, for e.g. quinoa or amaranth, to more than 100 µm e.g. potato (Jane, Kasemsuwan, Leas, Zobel, & Robyt, 1994; Lindeboom, Chang, & Tyler, 2004). Granules can adopt different shapes such as spheres, ellipsoids, polygons and irregular tubules, depending on their botanical origin. Starch granules are insoluble in water at room temperature and densely packed with semi-crystalline structures, with a density of about 1.5 g/cm^3.

The starch granule is composed of two polymers, amylose and amylopectin. Amylose consists mainly of linear chains of α-(1-4)-D-glucose units with some branches in large molecules, while amylopectin has a highly branched structure with α-(1-4) linked D-glucose backbones with α-(1-6) linked branches (Pérez & Bertoft, 2010). The proportion of amylose/amylopectin varies considerably between the different types of plants, as well as within species of the same plant. Commonly, the amylose content is between 15 – 35 % and the amylopectin between 65 – 85 % of the total starch granule. However, it is possible to find some types of starches almost free of amylose, which are called waxy starches (Šárka & Dvořáček, 2017).

Most starch granules are arranged as concentric rings of alternating amorphous and semi-crystalline shells that are between 100 and 400 nm in thickness depending on botanical origin (Ali Razavi & Amini, 2016). These structures are called “growth rings” (Pérez & Bertoft, 2010) and grow by apposition from the hilum of the granule during biosynthesis. The dense layer in a growth ring consists of alternating crystalline and amorphous lamellae (see Figure 1A and 1B).

The “blocket” concept was supported and discussed by Gallant et al. (Gallant, Bouchet, & Baldwin, 1997), who suggest that semi-crystalline and amorphous growth rings are subdivided into respectively spherical blocklets. These blocklets are proposed to contain crystalline and amorphous lamellae. Today, it is the general opinion that crystalline region is therefore mainly attributed to double helices

formed by amylopectin branches, and that the amorphous region corresponds to branching points.

It is important to mention that this approach (amylopectin forming double helices) easily integrates into the currently accepted cluster model (Figure 1C), where the short linear chains of the branches are intertwined into double helices. This double-helix formation can occur between adjacent branches in the same amylopectin cluster or between adjacent clusters in three dimensions (Oates, 1997), while the α-(1-6) branch points are located in the more amorphous regions between the clusters of double helices (Pérez & Bertoft, 2010).The model of the double helices packing configuration supported by results from XRD pattern, explain the difference between different types starches.

Starch granules can therefore be classified as A-type for cereal starches, B-type for tuber- and amylose-rich starch and C-type for legume starches (Buléon, Colonna, Planchot, & Ball, 1998; Pérez & Bertoft, 2010). A-type starch granules consist of a monoclinic unit cell (Figure 1D) with 12 glucose residues located in two left-handed chains that contain eight water molecules between the helices (Buléon, Colonna, Planchot, & Ball, 1998).

In the B-type pattern, chains are packed in a hexagonal array (Figure 1D) and the hexagonal unit is formed by two left-handed, parallel-stranded double helices. This cell contains 12 glucose residues and 36 water molecules located in the central cavity (Oates, 1997). The C-type is an intermediate form – a mixture of A- and B-type patterns. Additionally, the crystalline V-form characteristics of amylose complexed with fatty acids is rarely detected in native starch; this crystalline form usually appears after gelatinization (Buléon, Colonna, Planchot, & Ball, 1998).

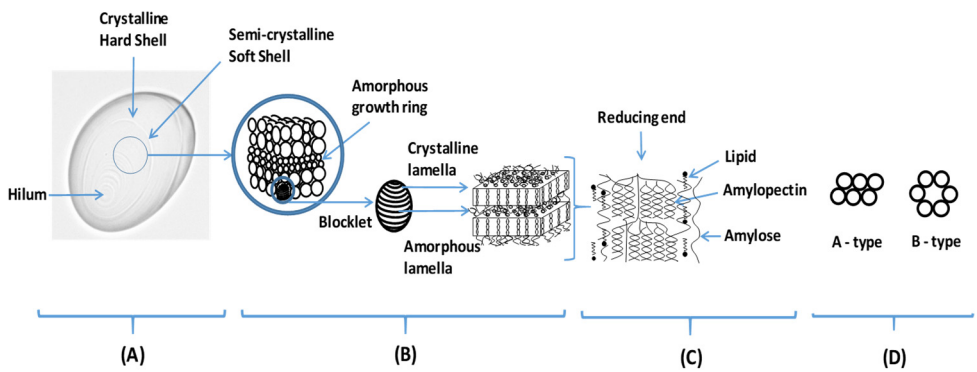


Figure 1. Schematic diagram of the structure of a starch granule
This figure illustrates the structure of a starch granule. A) a single potato granule formed by amorphous and crystalline regions, B) an extension of the breaking down of the amorphous and semi-crystalline rings showing a blocket formed by amorphous and crystalline lamellae, C) the cluster structure of amylopectin within the semi-crystalline layer of the growth ring surrounded by amylose and lipid and D) double-helix packing configuration according to crystalline type. Adapted from Gallant, Bouchet et al. 1997.

Other components in minor amounts that are also present in starch granules include, for instance, lipids and phosphate groups which in cereals originate from phospholipids (Morrison, Milligan, & Azudin, 1984). In tubers, especially potato starch, phosphate can be covalently linked to amylopectin (Hizukuri, Tabata, Kagoshima, & Nikuni, 1970). Additionally, small amounts of proteins are also found in starch granules and some minerals such as potassium, magnesium, calcium and iron are present in trace amounts (Blennow, Sjöland, Andersson, & Kristiansson, 2005).

Food properties can be affected by starch characteristics. For instance, bread loses its freshness during storage, which causes alteration in the physical, chemical and organoleptic properties. In particular, bread staling is of concern because it is a complex process that may be difficult to control and causes a number of interactions and changes inside the bread (Fadda, Sanguinetti, Del Caro, Collar, & Piga, 2014; Purhagen, Sjöö, & Eliasson, 2011).

Starch retrogradation and moisture redistribution inside the bread are recognized as dominant factors for staling (Gray & Bemiller, 2003). The crumb loses its softness and shows an increase in firmness whereas the crust loses its crispiness and becomes soft and loosely attached to the crumb as a result of moisture migration from the crumb to the crust (S. Cauvain, 2015). These changes in amylopectin, in particular are thought to be responsible for the increase in the bread firmness (Gray & Bemiller, 2003; Schoch & French, 1947).

The analysis and characterization of starch at the molecular level is important. Nevertheless, the analysis of starch can be challenging, as it is a complex polymer. Furthermore, it can be part of more complex matrix such as food. For these reasons, it is commonly necessary to extract the starch before its analysis when its molecular characteristics, such as M, are going to be analyzed. It is also necessary to have an appropriate dissolution method to ensure that the starch is present as individual and non-degraded polymer, and it has to be fractionated before its analysis because starch has high M and wide distribution of species.

Moreover, it is often necessary to determine the proportions of amylose and amylopectin in food as their ratio can produce different physicochemical properties. For instance, starch can be used as a thickener during the manufacture of products like soups, sauces or puddings. Depending on the product, the starch must have desirable characteristics such as high or low viscosity and it is known that higher amylose content will produce higher viscosity (Ji, Yu, Liu, Bao, Wang, & Chen, 2017; Tao, Li, Yu, Gilbert, & Li, 2019; Xie, Yu, Su, Liu, Wang, Liu, et al., 2009).

In addition, other qualitative analyses for the characterization of starch are also important to determine its physical properties. For example, the thickening capacity of the starch could be due to the swelling of the granules or due to the dissolution

of amylopectin. When higher viscosity occurs due to the swelling of the granules, the determination of granule shape, pasting and gelatinization properties became important. However, if the viscosity is due to the dissolution of amylopectin, the molar mass and size becomes important parameters.

Furthermore, some quantitative analyses can be conducted, such as determination of total starch content due to the formation of iodine complex. This is determined gravimetrically by collecting, drying and weighing the precipitates formed. In addition, other methods such as enzymatic or hydrolysis degradation with later determination of the glucose content are also used. There are also other techniques such as low angle light scattering, which is used to determine the size and M of starch. Some of the methods are briefly explained below.

4 Methods for Fractionation and Characterization of Starch

As described above the analysis of starch can be challenging and few techniques can be suitable for this task. One of the techniques that can be used in the analysis of starch is field-flow fractionation (FFF), which contains a family of separation devices with a large number of sub-techniques used mainly for the separation and characterization of macromolecular, colloidal and particulate species in a wide size range from a few nanometer to more than 400 μm (Schimpf, Caldwell, & Giddings, 2000). The separation occurs in a thin channel through which a channel flow is pumped resulting in a laminar flow (see Figure 2) with a parabolic velocity profile. The sample is injected onto the channel and eluted, an external field is applied across the channel (with perpendicular direction to the channel flow), which moves the sample close to the bottom of the channel (accumulation wall). Due to the interaction between the external field and the liquid flow, the separation of the analytes is achieved.

In this study, FFF techniques such as full-feed depletion mode of split-flow thin cell fractionation (FFD-SF), gravitational field-flow fractionation (GrFFF), and in particular the sub-technique AF4 were applied for the fractionation and characterization of starch samples. In some cases, the samples were fractionated and analyzed as granule and in other cases as polymers in solution after being dissolved for instance, in DMSO applying high temperature. The techniques used in this study and some others that can be used for the analysis of starch are briefly described below.

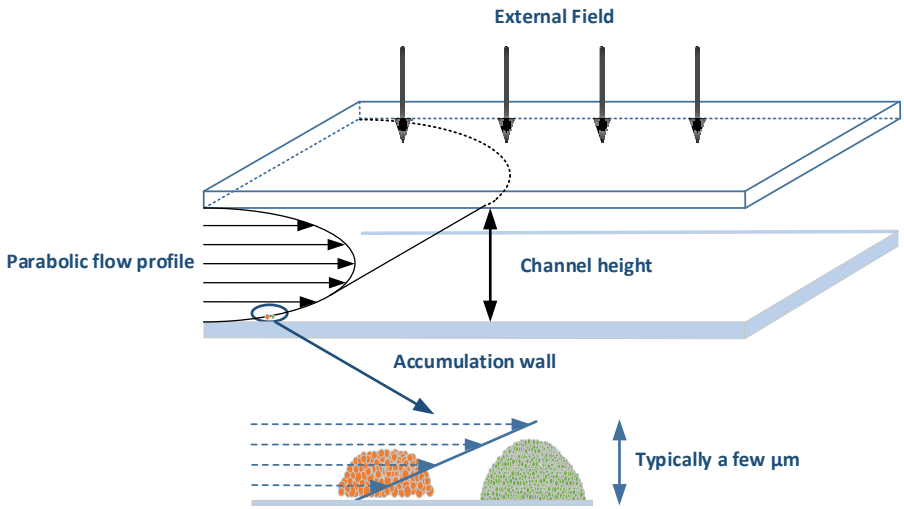


Figure 2. Illustration of the separation mechanism in FFF techniques
Separation of two analytes of different size where smaller species elutes earlier than larger ones.

4.1 Fractionation and Characterization of Starch Granules

Large-scale FFD-SF is a technique based on FFF principles used as a preparative separation method. This technique uses a relatively large amount of samples (i.e. mg or g), which can be fractionated in a relatively short period of time. In large-scale FFD-SF, the sample can be introduced continuously into the channel through inlet a' (i.e. in this technique the carrier and the sample are introduced via an inlet that is on the top part of the channel). This means that the sample does not have to be diluted and there is no need for the application of stream splitters. Additionally, the size of the channel can be larger than in other FFF techniques (C. Contado, Dondi, Beckett, & Giddings, 1997; Lee, Lee, Cho, Kim, Kang, Kwen, et al., 2010).

The large-scale FFD-SF technique was chosen to fractionate the samples in Paper I (i.e. corn and potato starch) into different fractions of various granule sizes. The fractionation is based on the size and the density of the sample, which are combined in the definition of the cut-off diameter (d_c) (C. Contado, Dondi, Beckett, & Giddings, 1997; Lee, et al., 2010) given by the following equation:

$$d_c = \sqrt{\frac{18\eta}{bLg\Delta\rho}\left(V(a') - V(b)\right)} \qquad (1)$$

where η is the viscosity of the carrier liquid, b is the breadth and L is the length of channel, g is the value of Earth's gravity, $\Delta\rho$ is the difference between the density of the sample and the carrier liquid, $V(a')$ and $V(b)$ are the volumetric flow rates entering the inlet-*a'* and exiting the outlet-*b*, respectively. Usually the d_c and the sample-feeding flow rate $V(a')$ are chosen first, and then $V(b)$ is determined using Eq. 1. Once $V(b)$ is determined, flowrate exiting the outlet-a, $V(a)$ becomes $V(a')$ - $V(b)$. Higher $V(a')$ allows higher sample throughput (TP), which is defined as the mass of the sample that can be processed in a unit time period (g/min or g/hours) (Sanz, Galceran, & Puignou, 2004). This technique allows the separation of considerable amount of samples in short period of time.

The results of Paper I show that large-scale FFD-SF was a useful technique for the fractionation of starch granules with d_c lower than 50 µm, showing high separation efficiencies in the fractions in short period of time. It is important to mention that granules larger than 50 µm that correspond to fraction-c in potato starch, were removed using static sedimentation as was not possible to performed the separate. In addition, the fractionation efficiency (*FE*) of fraction-c was 92%. Moreover, the d_c set in large-scale FFD-SF for the separation of corn and potato starch granules were 15 and 30 µm, respectively. For corn starch, fraction-a had granules < 15 µm and fraction-bbb had granules with diameter > 30 µm. In the case of potato starch, fraction-a had granules with diameter < 30 µm, fraction-bbb granules with diameter > 30 µm and < 50 µm. The *FEs* of total fraction-a were over 95% for both samples. For total fraction-b, the *FEs* for corn starch was 93% and for potato starch was 84%.

Another sub-technique is GrFFF, which can be used for the fractionation of samples in the range of micrometers. In this technique, Earth's gravitational force is used as an external field. A carrier liquid is continuously pumped through a channel and then a suspension of the sample is injected. Larger particles elute earlier than the smaller ones and the elution mechanism involved is often called the "steric elution mode". At relatively low flow rates, particles remain near to the bottom of the channel while the carrier liquid is driving them along the channel. As the flow rate increases, the analytes are subject to hydrodynamic lift forces, which raise them some distance above the wall to form a thin equilibrium layer where gravitational and lift forces are balanced (Giddings, Myers, Caldwell, & Pav, 1979; Pazourek, Wahlund, & Chmelík, 1996).

GrFFF was prevously shown to be a useful tool for the separation of various types of samples such as starch granules, blood cells, yeast, bacteria and environmental particles (Cardot, Gerota, & Martin, 1991; Chmelík, Krumlová, Budinská, Kruml, Psota, Bohačenko, et al., 2001; C. Contado & Dondi, 2001; C. Contado, Dondi, Beckett, & Giddings, 1997; Catia Contado, Reschiglian, Faccini, Zattoni, & Dondi, 2000; Janoušková, Budinská, Plocková, & Chmelik, 2001; Merino-Dugay, Cardot, Czok, Guernet, & Andreux, 1992; Sanz, Galceran, & Puignou, 2004; Sanz, Puignou,

Reschiglian, & Galceran, 2001; Shin, Choi, Kim, Lee, Kim, Lee, et al., 2018). In Paper I, GrFFF was used in the analysis of corn and potato starches. From the results, the average size diameter (d_{avg}) was shown to be in good agreement with the d_{avg} determined by optical microscopy (OM), especially for corn starch. In addition, OM and GrFFF are shown to be suitable methods for the determination of d_{avg} of the granules. Moreover, the results from OM and GrFFF for the corn starch granules were comparable, while those for the potato starch granules were slightly different. An explanation for that could probably be the non-spherical (ellipsoidal) shape of the potato starch granules.

4.2 Fractionation and Characterization of Starch Polymers

Physicochemical and mechanical properties of polymers depend on their molecular architecture, molar mass, size and their distributions (Auriemma, De Rosa, Di Girolamo, Malafronte, Scoti, Mitchell, et al., 2016). The two fundamental properties, molar mass (M) and size, can be experimentally determined in different ways using different types of techniques, all having advantages and disadvantages. For instance, batch-mode determinations can be performed with techniques such as viscometry, light scattering and osmometry. Some of these techniques are briefly described below. Among them, more attention will be paid in AF4, since it is the most used technique for determining the M and r_{rms} in the experiments described in the following sections.

Viscometry was one of the first methods used to determine M in polymers. Hermann Staudinger was the first person to establish an empirical relationship between the increase in viscosity and the M (Staudinger, 1930). This relationship is based on the dissolution of the polymer in solvents, a process that increases the viscosity. Usually, the viscosity can be measured using an Ubbelohde capillary viscometer. The molar mass average determined by viscosity (M_v) sometimes can be very close to weight average molar mass (M_w). However, M_v value can often be far from M_w each makes the determination quite uncertain.

Static light scattering is a technique that can determine the intensity of the scattered light at a certain scattering angle. The method is based on, as its name suggests, the physical phenomenon of light scattering caused by the interaction between light and the analyte. When the light, strikes a particle in solution, it causes the electrons around the particle to oscillate in synchronism with the energy of the incident light. The incident light generates an oscillating dipole in the particle. The strength of this dipole moment is proportional to the energy of the incident light. The oscillating dipolo is a source of energy-scattering light in all directions at the same wavelength

as the incident light. It is possible with a light scattering measurement to determine the M_w and the r_{rms} of the analyte in solution (Wyatt, 1993).

Osmometry is based on the determination of osmotic pressure at several different sample concentrations, which are measured directly using a semi-permeable membrane and then the M is determined (Ward, 2009). Some drawbacks of this technique are related to the fact that the osmotic pressure is inversely proportional to M and molecules with very high M can contribute very little. Thus, the sample must be almost free of low molecular species to get accurate results. In addition, the membrane used in the analysis can be damaged by the use of organic solvents that generally are applied in the analysis of polymers (Josua Timotheus, Thomas, & Antje, 2015). In the case of starch, DMSO is used frequently for its dissolution.

These techniques provide differently size or M averages, but little or no information is obtained about size and M distribution. In order to generate an accurate characterization, separation methods connected to various detectors are typically used. The most commonly used separation method for polymers is size exclusion chromatography (SEC). In this technique, the size and M can be estimated either with a standard calibration curve or by absolute determination of both the M and average size by utilizing online MALS in combination with concentration detector, for instance, dRI.

Nevertheless, SEC has some limitations, which include the adsorption of sample components to the column, the degradation of large species due to shear forces in the column and the co-elution effects that can arise, for instance, from the presence of branches in the polymers (Barth, 1982; Otte, et al., 2011; Žigon, The, Cheng, & Grubišić-Gallot, 1997). This can produce abnormal elution effects, making the determination of the M distribution difficult or impossible (Cave, Seabrook, Gidley, & Gilbert, 2009; Otte, et al., 2011).

Some of the drawbacks of SEC can be avoided by instead utilizing AF4 (Litzén & Wahlund, 1991; K.-G. Wahlund & Nilsson, 2012; K. G. Wahlund & Giddings, 1987). AF4 has been shown to be suitable for the analytical separation of macromolecules and aggregated structures and interest in the method has increased in recent years (Malik & Pasch, 2016; Nilsson, 2013). The separation mechanism is illustrated in Figure 3. It is based on the longitudinal laminar flow of a carrier liquid through a separation channel in combination with a crossflow (Q_c) in a perpendicular direction over the channel.

The Q_c forces the sample components toward the ultrafiltration membrane, which acts as an accumulation wall. At the accumulation wall, the components are confined to a thin concentrated layer. In Brownian separation mode, the Q_c induced transport is counteracted by the diffusion of the sample components and at a steady state, a concentration profile is established in the sample zone. The result is that

sample components with a higher diffusion coefficient (D) (i.e. components with small size), on average, will be distributed farther away from the accumulation wall than components with a lower D. As the flow profile along the separation channel is parabolic, the components distributed farther away from the accumulation wall will travel faster downstream and, thus, size separation is achieved.

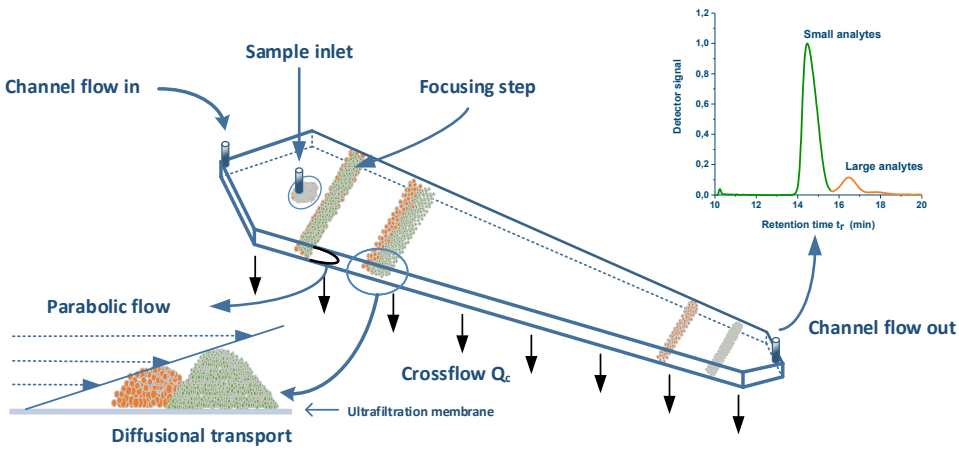


Figure 3. Illustration of the separation mechanism in AF4
Separation of two analytes of different size. First, the homogeneous mixture is injected into the channel. This is followed by a focusing/relaxation step. After that, the elution starts. Modified after Litzén and Wahlund 1991.

Additionally, one important parameter of the ultrafiltration membrane, which makes up the accumulation wall, is the cut-off, which must be sufficiently low to keep the sample in the channel because sample components smaller than the cut-off may permeate through the membrane. It is also important to consider the material of the membrane as some samples can be adsorbed during the analysis due to interactions between the sample and the membrane. Finally, for the determination of size and M distributions, an AF4 system is most suitably connected to a MALS detector and a concentration detector (such as dRI) in a similar way as for a SEC system.

The analysis in MALS is based on the angular dependence of the intensity of light scattered by an analyte. The light scattering data obtained from the detectors are fitted to different models such as Debye (Debye, 1944), Zimm (Zimm, 1948a, 1948b), Berry (Berry, 1966). This allows the determination of r_{rms}, by performing a fit to the angular dependence of the scattered light intensity. The most robust method utilized for an unknown sample analyzed by AF4 connected to MALS detector is Berry model (Andersson, Wittgren, & Wahlund, 2003). Furthermore, the specific refractive index increment (dn/dc) for the sample has to be known (or determined) and the concentration has to be measured using a dRI detector or any other detector that determines concentration (e.g. UV detector). In addition, the average of M, or

more specifically, the average M_w of each fraction, can then be determined from the intersection of the fitted curve (from the model that was chosen).

Moreover, the use of dRI as detector during the analysis of starch is suitable because, as starch does not have UV-absorbing analytes, the UV detector cannot be used. Nevertheless, it should be noted that some challenges could also be present. For instance, in the analysis of starch, a low injected amount is usually used to avoid overloading, which can result in a very weak dRI signal (Perez-Rea, Bergenståhl, & Nilsson, 2015; Van Bruijnsvoort, Wahlund, Nilsson, & Kok, 2001). This could complicate the determination of M for very large species because there will be a strong signal in the MALS detector but only a low signal in the dRI detector.

5 Methodological Aspects in the Fractionation and Characterization of Starch Polymers

5.1 Possible Improvements in the Fractionation and Characterization of Starch Polymers

The by far most utilized and versatile of all sub-techniques in the family FFF is AF4. It has been used widely in the analysis of starch molecules (Dou, Zhou, Jang, & Lee, 2014; Perez-Rea, Bergenståhl, & Nilsson, 2015, 2016; Rojas, Wahlund, Bergenståhl, & Nilsson, 2008; K.-G. Wahlund, Leeman, & Santacruz, 2011). This technique uses a secondary mobile phase, known as Q_c, as an external field to drive sample components towards the accumulation wall to perform the separation.

A brief summary of the separation mechanism was described in the previous section, but more detailed technical aspects focusing on the separation device are described below. In AF4, the separation takes place in a channel that has a solid wall on the top and a permeable wall on the bottom. After injecting the sample into the channel, there is an important step for the separation efficiency – the focusing/relaxation step (see Figure 3).

The sample is focused by two counter-directed flows (one from the channel inlet and the other from the channel outlet). This step allows the sample components to establish a diffusion-dependent equilibrium concentration profile. Nevertheless, this step might, in some cases, cause a loss of sample as a higher concentration of the sample is close to the accumulation wall (i.e. to the membrane) (K. G. Wahlund & Giddings, 1987) and adsorption could take place. Another reason could be the formation of aggregates, resulting in low recoveries (Moon, Kwon, & Park, 1997b).

A way to avoid the focusing step is the utilization of a modified channel frit inlet (FI) illustrated in Figure 4. The separation is based on a hydrodynamic relaxation of the sample (Liu, Williams, Myers, & Giddings, 1991; Moon, Kwon, & Park, 1997a). One advantage of this channel is that the injected amount of sample could probably be higher than for the conventional channel. That could be beneficial in the analysis of branched polymers such as starch, where overloading (in MALS-signal) occurs

easily in the AF4 channel (Perez-Rea, Bergenståhl, & Nilsson, 2015; Rojas, Wahlund, Bergenståhl, & Nilsson, 2008).

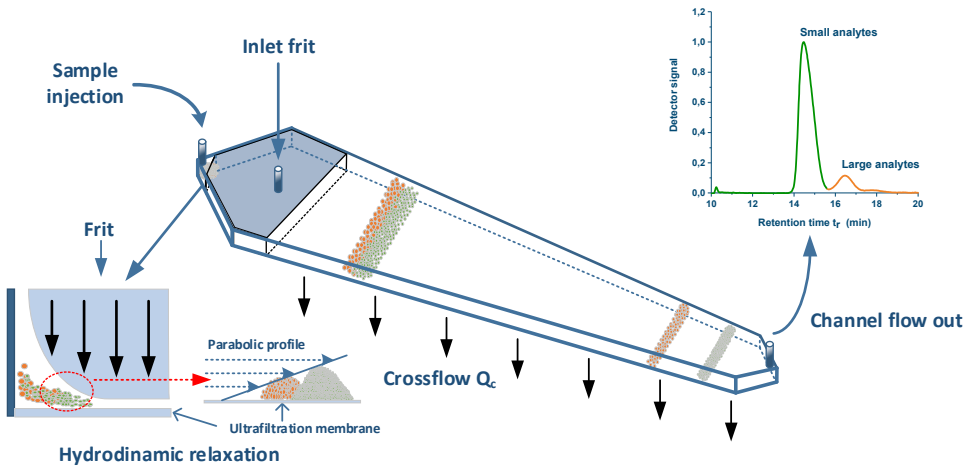


Figure 4. Illustration of the separation mechanism in FI-AF4
Separation of two analytes of different sizes. First, the homogeneous mixture is injected into the FI-AF4 channel and then the hydrodynamic relaxation takes place. After that, the elution starts. Modified after Litzén and Wahlund 1991 and Moon, Kwon et al. 1997.

In Paper II, one of the objectives was the comparison of conventional and FI-AF4 channels to examine the overloading effect during the analysis of WM starch. The same volume of sample (10 µL) was injected in a series of different concentrations from 0.125 mg/mL to 1.000 mg/mL. The results from MALS signal at 90° scattering angle are shown in Figure 5. It is possible to observe that in the conventional AF4 channel (see Figure 5A), the WM sample has some overload effect at a sample concentration of 0.330 mg/mL and above this concentration, the effect of the overload is significant. Similar results for WM were reported previously (Perez-Rea, Bergenståhl, & Nilsson, 2015; Van Bruijnsvoort, Wahlund, Nilsson, & Kok, 2001) where overloading occurs at similar injected amount of sample (≥ 2 µg).

The overload effect is more obvious at a sample concentration of 0.500 mg/mL where it can be noticed that there is a change in the trend of r_{rms} vs. t_r which indicates that the fractionation has been affected. In the case of FI-AF4 channel (Figure 5B), the t_r of the peak maxima remains constant until a concentration of 0.500 mg/mL (i.e. 5 µg) which means that overloading does not occur at lower concentrations and it is possible to notice that r_{rms} vs. t_r remains also without change suggesting no overloading. In short, the sample injection mass was twice higher using FI-AF4 channel than the mass injected in the AF4 channel without observed overloading.

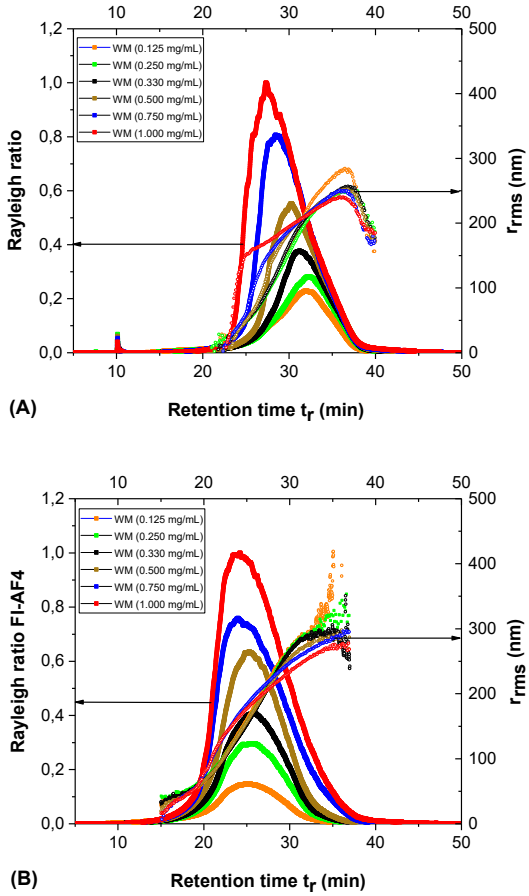


Figure 5. Effect of sample concentration on waxy maize starch (WM)
Fractograms from AF4 channel (A) and FI-AF4 channel (B) showing Rayleigh ratio (MALS signal at 90° scattering angle) vs retention time t_r (min) from Paper II.

5.2 Co-elution Phenomena – Understanding Downturn

A downturn in the M distribution vs. t_r can sometimes be observed in the analysis of broadly distributed polymer samples containing heterogeneous structures such as starch. The downturn that is shown in Figure 6 was observed during the analysis of starch extracted from bread (Paper VI). In Paper IV, a similar behavior was noticed in the analysis of some starch from Andean crops. Several equivalent observations have been previously made by other authors (Otte, et al., 2011; Pitkänen & Striegel, 2014).

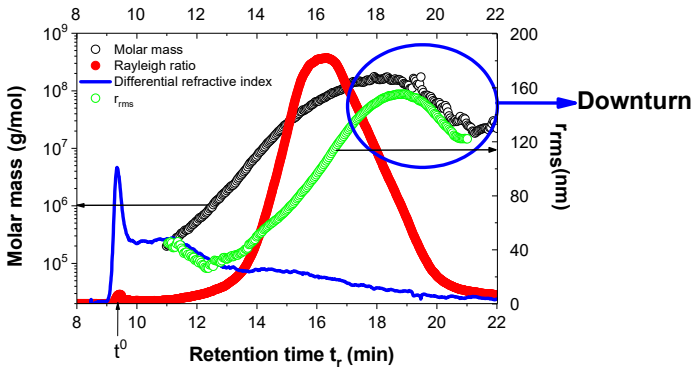


Figure 6. Downturn phenomenon in AF4
Fractograms from AF4 channel of starch from bread without added enzyme (SBR) from Paper VI. Showing Rayleigh ratio (MALS signal at 90° scattering angle red line), dRI signal (blue line), Molar mass M (open black dots) and root-mean-square radius r_{rms} (green open dots) vs retention time t_r (min).

This downturn is often explained as an artifact of the fractionation and could be attributed to various errors during detection or data processing. Nevertheless, this phenomenon could be due to co-elution of species with different conformations. For this reason, the downturn phenomenon was studied in more detail in Paper III using a mixture of pullulan, glycogen and PEO, which have different conformations and hydrodynamic sizes, but with overlapping M distribution. Pullulan and PEO are linear macromolecules and glycogen is a branched polymer. It is important to mention that branched and linear polymers with the same M will have different hydrodynamic sizes, i.e. linear polymers will display a random coil conformation and branched polymers will display a branched conformation.

In Figure 7A, we can see that glycogen elutes at shorter t_r (about 14 min) than pullulan (about 17 min) and there is a range in the M distribution where both M signals, showing different M, are eluting at the same t_r. In addition, a downturn in M distribution of the mixture can be seen at about 14 min (see Figure 7A) followed by a subsequent upturn at about 17 min. Furthermore, for the mixture of the two linear polymers, i.e. pullulan and PEO (see Figure 7B) (PEO elutes at shorter t_r than pullulan), no downturn was observed in the M distribution in the region where both have an overlapping signal in MALS at 90° scattering angle. It is important to mention that there is an overlapping by small species from pullulan in the whole region where PEO elutes (t_r between 10 to 15 min). In addition, a small upturn in M distribution of the mixture curve can be observed when the contribution from PEO starts to decrease (t_r about 14 min).

It should be mentioned that the r_{rms} distribution (data can be found in Paper III) for the separation of both mixtures (linear-branched and linear-linear polymers) is showing a steady monotonic increase in size over the complete fractograms without

observing a downturn, indicating that the size separation was performed successfully. From these results, it can be concluded that there is the possibility of a significant loss of M selectivity in mixtures of complex polymers with different size distributions, especially when the individual polymers have different conformations, i.e. different hydrodynamic radii (r_h) and M. A downturn or upturn can take place in the region where co-elution of components with different conformation occurs during the separation of the mixture. This could mean that the separation of a single sample consisting of a single type of polymer could include species with different conformations, which causes the up- or downturn. Thus, the sample itself could comprise a mixture of species with some species having a different conformation but the same M over the size range.

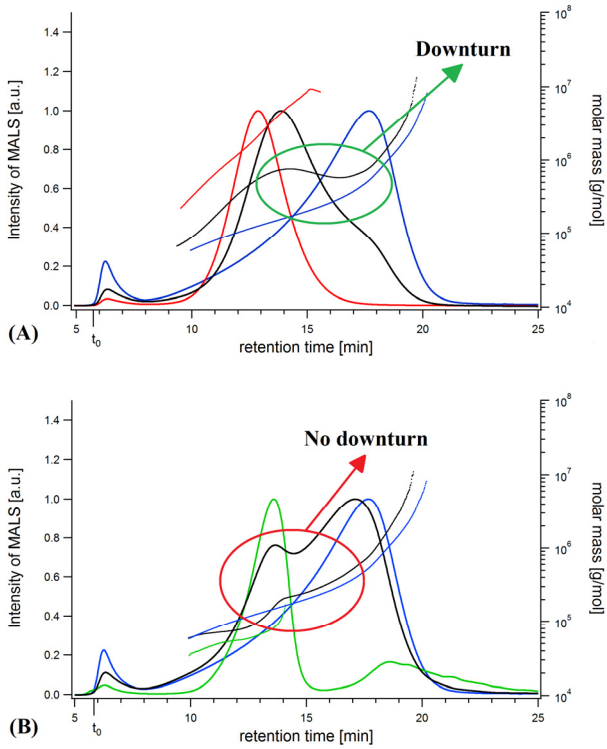


Figure 7. Downturn phenomenon studied in AF4 using a mixture of polymers
Fractograms of A) pullulan (blue), glycogen (red) and a mixture of both (black) and B) pullulan (blue), PEO (green) and the mixture of both (black) of Rayleigh ratio from MALS (solid lines, left y-axis, a.u.) and M distribution (right y-axis, g/mol) adapted from Paper III (Zielke, Fuentes, Piculell, & Nilsson, 2018).

6 Characterization of Starch Granules and Starch Polymers from Andean Crops

The Andean region extends through seven countries, and unique plant species are endemic to this are. In Bolivia, some endemic plants have been used for centuries for popular consumption in traditional cooking such as quinoa, amaranth and maca. Nevertheless, there is a lack of information about them. For this reason, five of the most common crops (three grains and two roots) were chosen to investigate their starch properties due to the importance of knowing the relationship between the granule sizes, thermal properties as well as fundamental properties. In paper IV, these crops were investigated, the physicochemical properties determined were granule size and morphology, crystallinity, amylose, protein and phosphorus content, degree of branching and gelatinization and pasting properties. In addition, structural properties such as M, r_{rms} and ρ_{app} for the starch polymers of these crops were determined using AF4 connected to MALS and dRI detectors.

It is well known that the diameters of starch granules vary depending on their botanical source and have a wide range from less than one to more than 100 μm (Jane, Kasemsuwan, Leas, Zobel, & Robyt, 1994; Nienke, Peter, & Robert, 2004). The starch granules do not have a size classification. However, some literature mentions a certain order that goes from very small to large granules. For instance, very small starch granules (i.e. 0.3-2 μm) are present in quinoa, amaranth and cow cockle. Small starch granules (2-10 μm) are found in oats, rice, and buckwheat (Nienke, Peter, & Robert, 2004). Medium-sized starch granules (5-30 μm) exist in tapioca, barley, maize and sorghum (Hall & Sayre, 1970; Jane, Kasemsuwan, Leas, Zobel, & Robyt, 1994) and large starch granules are found in tubers such as potato or canna, which have the largest granules known with sizes about 100 μm (Cisneros, Zevillanos, & Cisneros-Zevallos, 2009). Furthermore, some types of starch, such as potato, can have a broad range of granule sizes (1 to 110 μm) (Hoover, 2001).

It has been reported that some physicochemical and functional properties of starches, such as viscosity, pasting and thermal properties, can change according to the granule size (Dhital, Shrestha, Hasjim, & Gidley, 2011; Nienke, Peter, & Robert, 2004). In addition, larger granules usually show high amylose contents and lower

lipid, protein and mineral contents. Large granules also have a higher degree of crystallinity, which might be related to the differences in pasting and in thermal properties (Dhital, Shrestha, Hasjim, & Gidley, 2011). Additionally, the peak temperature (T_p) and conclusion temperature (T_c) of gelatinization increase slightly with decreasing granule size (Dhital, Shrestha, Hasjim, & Gidley, 2011; A.-C. Eliasson & Karlsson, 1983; Singh & Kaur, 2004). Furthermore, the endothermic enthalpy of gelatinization (ΔH) decreases when the granule size decreases in the starches (Dhital, Shrestha, Hasjim, & Gidley, 2011; Singh & Kaur, 2004).

Otherwise, in Paper I that was described above, two types of starches (corn and potato) were fractionated and the thermal properties according to the starch granule's size were determined. The results for the whole corn starch and its fractions showed no differences in thermal properties and granule size. Nevertheless, it was possible to find differences in the thermal properties of whole potato starch granule and its fractions. Taking this data and the results of the two Andean roots from Paper IV into consideration (due to the similarity of their botanical origin), was made table 2. It shows the summary of the results of thermal properties according to the granule size and the amylose content from the two Andean roots.

It is possible to see from Table 2, that ΔH increases with increasing granule size. These results are in agreement with previous results (Dhital, Shrestha et al. 2011). Additionally, no trend was found for T_o, T_p and T_c when maca and achira starch are considered in the analysis. Nevertheless, it seems that the amylose content can affect the thermal properties of the starch greatly according to the granule size. There is an increase of amylose content with increasing granule size for maca and achira (see Table 2), which is in agreement with previous reports showing that higher amylose content is present in larger starch granules (Diego, Walter, Henry Alexander, & José, 2017; Kaur, Singh, McCarthy, & Singh, 2007; Singh & Kaur, 2004; Utrilla-Coello, Agama-Acevedo, Barba de la Rosa, Rodríguez-Ambriz, & Bello-Pérez, 2010). In addition, high amylose starches have somewhat higher gelatinization temperatures (Hoover & Manuel, 1996; Yoshimoto, Tashiro, Takenouchi, & Takeda, 2000), which is in agreement with the results obtained from maca and achira starch.

Table 2.
Thermal properties of Andean root starches and fractions from potato starch prepared in a 1:3 m/v (starch/water) ratio according to the granule size

Andean roots	Granule size (μm)	T_o (°C)	T_p (°C)	T_c (°C)	ΔT	ΔH (mJ/mg)	Amylose (%)
Maca	10 ± 3[a]	46.0 ± 0.1	48.7 ± 0.2	53.5 ± 0.4	7.5	13.6 ± 0.1	29
Achira	50 ± 14[a]	61.5 ± 0.2	65.6 ± 0.2	70.0 ± 0.6	8.5	15.4 ± 0.2	48
Whole potato	37.7 ± 4[b]	60.7 ± 0.2	65.1 ± 0.2	70.2 ± 0.6	9.6	14.9 ± 0.7	
Fraction – a (potato)	15.3 ± 6[b]	61.5 ± 0.6	66.7 ± 0.2	72.5 ± 0.2	10.9	13.0 ± 0.4	
Fraction-bbb (potato)	49.0 ± 6[b]	61.9 ± 0.2	65.9 ± 0.2	70.8 ± 0.4	8.9	16.8 ± 0.2	
Fraction-c (potato)	68.1 ± 16[b]	61.6 ± 0.1	65.6 ± 0.1	70.4 ± 0.1	8.8	17.1 ± 0.2	

[a] Mean granule size diameter by measuring the diameter of 400 granules
[b] Mean granule size diameter by measuring the diameter of 500 granules
T_o: Onset temperature, T_p : Peak temperature, T_c: Conclusion temperature, $\Delta T = T_c - T_o$: Gelatinization range, ΔH: Enthalpy of gelatinization, Mean values ± standard deviation (n = 3).

Furthermore, with all parameters determined in the analysis of the five Andean crops in Paper IV, a multiple linear regression analysis was performed to find a correlation between their physicochemical properties such as granule size, crystallinity, pasting properties and structural properties such as r_{rms}, M and ρ_{app}. It was possible to find some correlations and some equations were proposed that describe the relationship between the parameters, which are shown as follows:

$$PV = -5.26 + 1.04(T_p) - 0.23(\rho_{app}) \quad (2)$$

The first equation relates the peak viscosity (PV) with T_p and apparent density (ρ_{app}), showing a correlation between these variables of $R^2 = 0.97$.

$$FV = 479.1 + 55.6(Amylose\ content\ \%) + 25.8(\Delta H) \quad (3)$$

The second equation relates the final viscosity (FV) to the amylose content and ΔH, showing a correlation between these variables of $R^2 = 0.98$.

Additionally, considering the possible correlations between all properties that were studied in Paper IV, it is possible to propose models to estimate pasting properties (i.e. PV and FV) from fundamental properties such as ρ_{app}, amylose content, ΔH and granule size. These equations are shown below:

$$PV = -1371.8 - 73.3(\rho_{app}) + 225.5(\Delta H) - 0.52(Granule\ size) + 109.4(Amylose\ content\ \%) \quad (4)$$

$$FV = -360.3 + 71.1(\rho_{app}) + 36.7(\Delta H) + 13.6(Granule\ size) + 43.8(Amylose\ content\ \%) \quad (5)$$

Finally, particular characteristics can be highlighted in some of the Andean starches. The amaranth starch appeared virtually free of amylose and had the highest M_w and r_{rms}, which means that very few molecules formed the amaranth granule. Quinoa and canihua starches displayed very low breakdown, indicating that these starches were stable after the heating and cooling processes. This might be related to the fact that those starches had small granule sizes, with canihua starch granules being the smallest.

7 Characterization of Starch Nanocomponents

In recent years, there has been great interest in producing nanostructures from starch granules, as described in some recent reviews (Ali Razavi & Amini, 2016; Le Corre, Bras, & Dufresne, 2010; Wang, Truong, & Wang, 2003). In particular, small-sized granules and modified granules have been shown to be used for stabilization of Pickering emulsions (Ge, Xiong, Li, Liu, Yang, Chang, et al., 2017; Liang, Jiang, Yokoyama, Yang, Cao, & Zhong, 2016; Rayner, Timgren, Sjöö, & Dejmek, 2012; Saari, Fuentes, Sjöö, Rayner, & Wahlgren, 2017; Yusoff & Murray, 2011). Nevertheless, to the best of our knowledge, there are no studies describing if the nanostructures obtained after being reconstituted behave like particles in dispersion or if they are like small structures that maintain their polymer characteristics. To study this, three different types of starch nanoparticles were obtained by non-solvent precipitation (non-SP) and were analyzed in Paper V.

The study focused on the analysis of reconstructed samples, i.e. after the non-SPS were obtained to investigate if they remain as particles when they are reconstituted. One of the samples was prepared with an acid hydrolysis pre-treatment (AHSP) another one was modified using n-octenyl succinic anhydride (OSA) obtaining OSASP. The third sample did not have any additional treatment (SP). Additionally, three different ways to re-disperse/dissolve the samples were tested a) dissolution at 100 °C in DMSO, b) dissolution at 100 °C in an aqueous solution (e.g., AF4 carrier liquid), and c) dispersion at room temperature in an aqueous solution (e.g., AF4 carrier liquid). The analysis was performed using AF4 connected to MALS and dRI detectors. The use of DMSO was to compare the behavior of the samples when they are dissolved or dispersed, as it is well known that starch is dissolved in DMSO. In addition, the ratio of r_{rms}/r_h gives us information about the conformation, where r_h was calculated from the AF4 theory in the samples prepared in aqueous solution at room temperature.

The results showed, that all the non-SPS had an amorphous structure, as the analysis using DSC did not show any transition in the endothermic curve (no peak was detected), which indicates that the granules were completely disrupted and the non-solvent precipitation did not induce any major formation of starch crystals previously reported (Das, Sanson, Fava, & Kumacheva, 2007; Kim, Lee, Kim, Lim,

& Lim, 2012). In addition, no significant differences were observed in the AF4 fractograms, in M and r_{rms} between the same sample dissolved in DMSO (blue) and boiling aqueous solution (green), respectively (see Figures 7A-C blue and green solid lines). This indicates that the three non-SPS samples were dissolved in both DMSO and boiling aqueous solution. In contrast, considering the samples prepared in aqueous solutions at room temperature (see red solid lines in Figures 7A-C), each non-SPS sample showed a different behavior.

AF4 fractograms of the AHSP sample prepared in an aqueous solution at room temperature (red solid line, Figure 8A) indicated the existence of two populations, which is not the case for the same sample prepared in DMSO (blue solid line) or boiling aqueous solution (green solid line). The first population is exhibiting an elution profile similar to those of the same sample prepared differently, with slight increases in M and r_{rms} distributions. Additionally, the second population at t_r between 37 and 45 min, showed higher M (about 10^9 g/mol) and r_{rms} (about 200 nm). The existence of the second population suggests the presence of some undissolved material in an aqueous solution at room temperature.

The conformation value for the AHSP sample (first eluting population) was 0.86, suggesting that the conformation lies in between a highly branched object ($r_{rms}/r_h \geq 1.0$) and/or a spherical object with a homogeneous mass distribution ($r_{rms}/r_h = 0.778$) (Burchard, 1999). To obtain more information about the conformation of this sample, a complementary analysis using SEM and cryo-TEM was performed (data shown in Paper V). The results from SEM show the presence of small particles (as the analysis was made with the dried sample) with a diameter of about 250 nm. However, upon dispersion in an aqueous environment, the cryo-TEM micrographs do not show such well-defined particles. Rather, the sample appears to consist of smaller and less well-defined objects like loose polymer aggregates.

Therefore, it can be concluded that upon dispersion, the AHSP sample loses its well-defined particle-like nature and more closely resembles an aggregate of polymers. Most likely, this is due to partial dissolution of the particles. It should also be noted that the second population in the AHSP sample is much larger than the particles observed in SEM and were not visible in the cryo-TEM results. This suggests that the second population might consist of either larger particles or aggregates of smaller particles, but conformational data could not be obtained for this population.

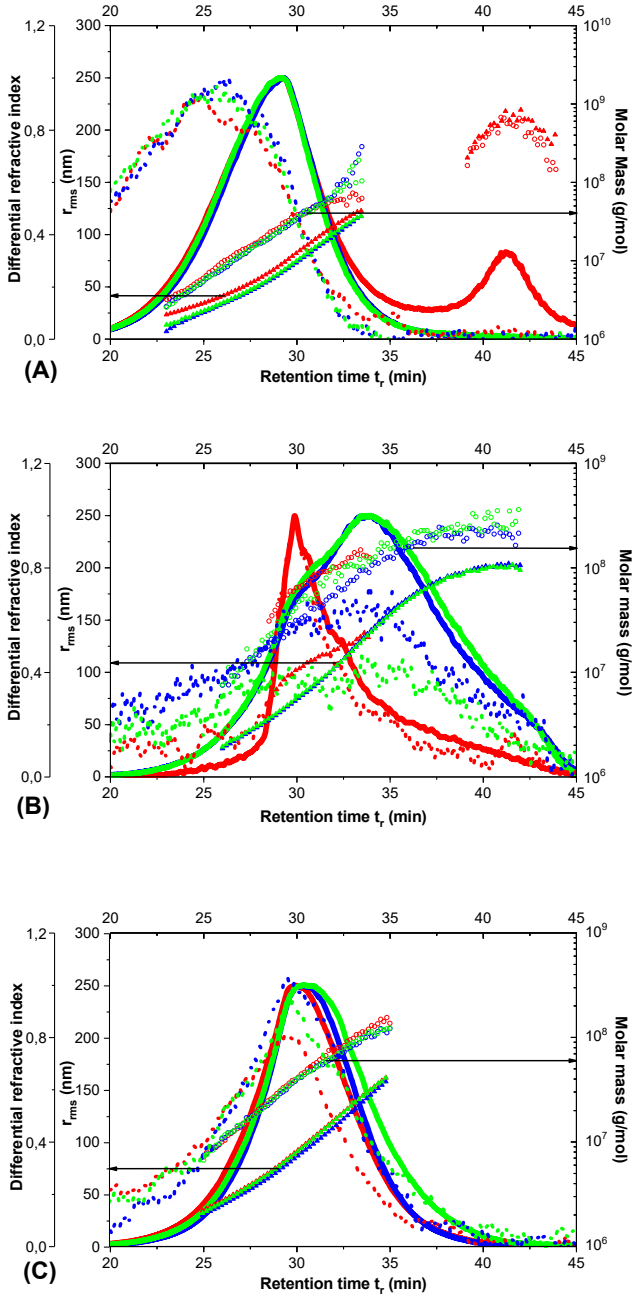


Figure 8. Fractograms of non-SPS samples (AHSP (A), SP (B) and OSASP (C))
Non-SPS dissolved in DMSO (blue), boiling aqueous solution (green), and aqueous solution at room temperature (red). Symbols denote the molar mass (g/mol) (○), root-mean-square radius r_{rms} (nm) (▲), MALS-signal at 90° scattering angle (—), and dRI signal (····) from Paper V.

The results of the SP sample prepared in aqueous solution at room temperature (see red solid line, Figure 8B) showed a much different elution profile than the one prepared in DMSO (blue solid line), thus indicating that dissolution was not complete. The elution profile was narrower than the one in DMSO, mainly because much of the later-eluting material is missing. For the same reason, the SP sample in an aqueous solution at room temperature exhibited lower M and r_{rms} distributions than the same sample prepared differently. The conformation value for this sample was 1.22, which corresponds to a highly branched macromolecule (Burchard, 1999) and is in good agreement for what has been reported for amylopectin (r_{rms}/r_h = 1.02 to 1.29) (Roger, Bello-Perez, & Colonna, 1999).

In the case of the OSASP sample, no significant differences were observed among the three AF4 fractograms in MALS signal at 90°of scattering angle (see red solid line, Figure 8C). Moreover, the M and r_{rms} distributions were similar, indicating that OSASP was dissolved well in all three preparation procedures and is found as a molecule in solution. The conformation value was 1.04, which also corresponds to a branched structure. In short, from the results, it is possible to conclude that none of the non-SPS samples shows a particle conformation (spherical) after being reconstituted, which suggests that they are not nanoparticles as the name implies, and that this might therefore not be the most appropriate designation for them.

8 Characterization of Starch after Breadmaking

One of the most popular foods with a high starch content that can be found in almost every home throughout the world is bread (S. P. Cauvain, 2005). Bread is an important part of human nutrition and breadmaking has been widespread. Although there is a great deal of information about starch extracted from bread during breadmaking, storage, etc. (Dewettinck, Van Bockstaele, Kühne, Van de Walle, Courtens, & Gellynck, 2008; A. C. Eliasson, Bergenstahl, Nilsson, & Sjoo, 2013; MacRitchie, 2016; Pareyt, Finnie, Putseys, & Delcour, 2011; Stamataki, Yanni, & Karathanos, 2017), it is still important to increase the knowledge about starch as raw material in bread at the molecular level, when modified and/or during the changes due to the processing conditions to improve the properties and functionality. This section thus examines molecular properties of starch extracted from different types of bread using AF4. This work is part of Paper VI and VII.

Various additives are currently applied in the baking industry to suppress staling and to some extent enhance the shelf life of bread. Enzymes and emulsifiers are the two most common types of additives used to reduce the crumb firmness of breads (León, Durán, & Benedito de Barber, 2002). The impact of enzymes on retarding the staling rate to preserve the freshness of bread for a longer period of time has been reported in several studies (Fiszman, Salvador, & Varela, 2005; Maningat, Seib, Bassi, Woo, & Lasater, 2009). Among the enzymes, α-amylase is used in breadmaking to reduce staling and was the enzyme used in Paper VI. Novamyl 10000 BG (α-amylase enzyme) is thermo-stable and well known for its anti-staling properties. Its commercial preparation is maltogenic α-amylase from Bacillus sp. TS-25, formerly B. stearothermophilus (Diderichsen & Christiansen, 1988).

The effect of the addition of 10000 BG (α - amylase enzyme) was studied. Breads were baked without (SBR) the addition of the enzyme and with the addition of he enzyme at two different concentrations low (SBL) and high (SBH). The starch was extracted from the crumb of the breads (Paper VI). Additionally, three different types of breads from Bolivia were analyzed (Paper VII). One type of bread was produced in an industrial way (PMOM) and the two others were made in an artisanal manner (PSAM and PMAM).

The formulation of the first bread was very similar to the reference bread (SBR) used in Paper VI. The two other breads have some differences in formulation. The water content in the dough for PSAM and PMAM was 600 and 700 g/Kg, respectively, while for PMOM the water content was about 500 g/Kg. Additionally, vegetable fat (90 g/Kg and 40 g/Kg of dough) was added to PSAM and PMOM, respectively. The starch from wheat flour was also analyzed for comparative proposes.

Table 3 shows a summary of the average results of molecular properties obtained from AF4 connected to MALS and dRI detectors and the content of resistant starch in starch extracted from Bolivian breads. The results show that all samples were high M species with a wide range of weight average $M_w = 6.6 \cdot 10^7$ - $1.6 \cdot 10^8$ g/mol. The M_w of starches extracted from the different types of wheat starches used during the elaboration of breads were similar. SBR and PMOM had similar average M_w. It should be noted that the preparation conditions in both types of breads were similar. In the case of breads baked in an artisanal manner, an increase in average M_w is observed, which suggests that the baking conditions (i.e. shorter baking time and apparent higher temperature) and recipes could have an influence on the change of M_w. It should be noted that because the real temperature in the air used in the baking process of artisanal breads was not possible to obtain, the apparent temperature is taken instead.

In the starches extracted from breads with added enzyme, a decrease in r_{rms} and M_w and increase in ρ_{app} as an effect of baking were observed. The effect is larger when the enzyme is added. Additionally, some complementary data collected using ^{1}H NMR show that there is an increase in average degree of branching (DB). Furthermore, it seems that the DB increases as the concentration of the α-amylase increases. It should be noted that the number of reducing ends H-1(β-r) and H-1(α-r) increases because of the baking process. In the case of starch breads from Bolivia M_w and r_{rms} increased in the order PMAM > PSAM > PMOM. The ρ_{app} had the same trend as M_w and r_{rms} for starch breads.

In Table 3, it can be noted that M, r_{rms} and ρ_{app} were higher in breads baked in an artisanal manner. Furthermore, in the PMAM sample, the r_{rms} value decreased rather than increased, which is because of the presence of the second population at $t_r = 35$ min (data presented in Paper VII). In addition, the changes in the molecular properties could be related to the moisture content of the breads due to the use of different water content in the preparation of the dough. PMAM had a higher moisture content (40.3%) than the other two breads (PMOM = 21.5% and PSAM = 36.2%), which could have contributed to the formation of resistant starch (see Table 3). It has been previously reported that a higher moisture content in the bread is consistent with a higher resistant starch content (Amaral, Guerreiro, Gomes, & Cravo, 2016; Siljeström & Asp, 1985).

Table 3.
Summary of average values of M, r_{rms}, ρ_{app}, mass recovery and resistant starch content.

Sample ID	Type of sample	$M_w \cdot 10^7$ (g/mol)	r_{rms} (nm)	ρ_{app} (Kg/m^3)	Recovery (%)[a]	Resistant starch (%)
S0	Starch from wheat flour	7.2	112	7.4	51	
SBR	Starch of bread whithout added enzyme	6.6	91	12.0	99	
SBL	Starch from bread (low concentration of enzyme, 65 mg/Kg)	3.3	63	16.6	101	
SBH	Starch from bread (high concentration of enzyme, 100 mg/Kg)	3.0	64	14.8	126	
M1	Starch from wheat flour used in PMOM bread	7.2	166	10.2	63	0.4
M2	Starch from wheat flour used in PSAM and PMAM bread	7.6	166	10.7	59	0.4
PMOM	Starch from pan molde	6.7	105	12.1	98	1.7
PSAM	Starch from pan sarnita	12.6	155	13.7	82	2.1
PMAM	Starch from pan marraqueta	15.7	179	17.4	88	7.4

[a] The mass recovery was determined from the ratio of the mass eluted from the separation channel (integration of the dRI signal) to the injected mass (based on the analyzed starch content of the sample)

9 Conclusions

The present study was performed to improve the understanding of the relation between physicochemical properties such as granule size, crystallinity, pasting properties among other and molecular properties like molar mass, ρ_{app} and r_{rms}. First, a model was proposed, where pasting properties i.e. peak viscosity and final viscosity with ρ_{app}, gelatinization enthalpy, granule size and amylose content are related to each other using five Andean crops that can be used as a raw material in food and other industries. Second, thermal properties were examined in relation to the granule size. For this, potato starch and its fractions (obtained using large-scale FFD-SF) as well as Andean roots were analyzed showing that the gelatinization enthalpy (ΔH) increases with increasing granule size and amylose appeared to greatly affect the thermal properties.

Moreover, waxy maize starch was modified to produce nano starch components. The non-solvent precipitated starch (non-SPS) obtained has amorphous structures. In addition, when the non-SPS were reconstituted in under different conditions, they did not show particle characteristics i.e. they displayed a branched conformation, which suggests that designating these as nanoparticles might not be appropriate. Otherwise, starch was analyzed as a final product after baking process where it was possible to observe that the addition of enzymes and different types of preparation methods and contents in the recipe produced changes in the molecular properties i.e. M, r_{rms} and ρ_{app}, which could have been modified due to the baking process and the applied conditions. Additionally, it seems that the resistant starch varies depending on the type of bread that was analyzed.

On the other hand, for the analysis of starch as a polymer, it was shown that using a modified AF4 channel i.e. frit inlet (FI) channel, almost double the amount of mass could be injected compared to the conventional AF4 channel, without observing overloading effects. Additionally, the downturn phenomenon usually considered as an artifact was shown to be a result of eluting species with similar M but different conformation.

Finally, starch from different sources was studied using FFF techniques, mainly AF4, first as a raw material, second when it was modified to obtain nano-starch components and third after breadmaking. Under different conditions, the results confirmed that the use of these techniques is suitable for the analysis of branched structures polysaccharides such as starch.

10 Future Outlook

There is extensive knowledge about the starch granule, its molecules and other characteristics. However, due to its complex structure, it is still necessary to investigate new aspects that complement the current knowledge. Further research is therefore suggested on the following topics:

1. In the present study, only five starches from Andean crops were analyzed. In order to obtain a greater number of correlations and better correlation values between fundamental and structural properties, it is necessary to add a larger number of samples, especially from roots and grains, to enable proper calculations. This could lead to the development of mathematical models to predict changes in fundamental and molecular properties. It could also help in the utilization of starches in technical and food applications. For instance, during the development of a new product using canihua starch, its behavior during cooking could be predicted.

2. Complementary analyzes should be performed with AF4 concerning nano components obtained from starch with a crystalline structure, as in the present work only non-SPS with amorphous structures were analyzed. Some studies mention that the crystalline part of starch can lead to the formation of starch nanoparticles, which could confirm e.g. that nanocomponents obtained from starch with crystalline structures remain as particles after being reconstituted.

3. The studies about starch extracted from bread were focused on the crumb of the bread and some work on the crust was done applying AF4. It could be necessary to complement the knowledge about the changes that occur during the baking process. This could be especially useful in the industry to predict the changes of some characteristics, for example, the formation of resistant starch according to the recipe or other parameters such as cooking temperature, in order to obtain functional products.

4. There is little knowledge about the molecular characteristics of resistant starch in food after cooking and its changes in the gastrointestinal tract. More studies in this area are thus need. These experiments can be performed using AF4 to analyze starch extracted from different foods after cooking. Additionally, it could be possible to carry out in vitro experiments where resistant starch is analyzed in different conditions simulating the gastrointestinal tract in order to determine the changes in M and conformation to ensure stability, i.e. if it remains resistant starch or if some changes occur.

Acknowledgements

Time passes very quickly and, silently and sometimes it is hard to notice that it has been flying. This PhD has been in progress for five years and the time for completion is near. So many things have happened during these five years and there are many people I want to thank.

First, I would like to express my sincere gratitude to my supervisor *Lars Nilsson* for his continuous support throughout this time. Thank you for choosing me as a student during the interview in Bolivia and giving me the opportunity to be part of this project. I know that in the beginning, it was hard for me to adapt to the work style and my English was very poor (maybe it is still not so good). Nevertheless, as the time passed we became a good working team (or that is what I think). Thank you for all the opportunities during the PhD. The exchange in Korea and the trips to the US and Germany were great experiences for me. I will carry those memories for the rest of my life.

I would like to thank the coordinator of my project *Mauricio Peñarrieta* for his collaboration in the administrative issues that I know are a pain in the neck and are time-demanding. Thank you for being part of the group that chose me for the PhD and gave me the opportunity to be part of this project.

I want to express my gratitude to Professor *Seungho Lee* for all his support and valuable advice not only in the academic sense, but also for the advice for the development of my personal life. Thank you for your valuable contribution to the papers.

I also would like to thank my co-authors:

Björn Bergenståhl, thank you for the nice discussions, especially from the statistical point of view, and all the support during the PhD.

Marie Wahlgren, thank you for the nice discussions about the differences between particles and molecules.

Malin Sjöö, thank you for the contribution and nice discussions about starch.

Claudia Zielke, thank you for being a nice co-worker. We spent so much time with the AF4 machines that we gave them names. We were not only co-workers, though – we also became great friends. We have many memories during our travels together to the US, Germany, Korea and Bolivia. You will always be in my heart.

Jaeyoung Choi, thank you for discussing with me the theory of AF4. Although theory is just theory, I guess it is possible to learn more from those who think differently from us and I learned a lot during our discussions. We became not only co-workers but also friends and you are my Korean brother.

Hisfazilah Saari, thank you for working with me and becoming my friend, spending time with me and cooking for me. I very much enjoyed working together.

Daysi Perez-Rea, thank you for your feedback. Your PhD work helped me as a starting point.

Rossio Castañeda and *Fanny Rengel,* my two master's students who became my friends and made my working days in Bolivia memorable together with *Jorge* and *Susy*.

This thesis and my stay in Lund would not have been possible without the funding sources from the Swedish International Development Agency (SIDA) in a collaborative project between the Higher University of San Andres UMSA and Lund University.

It was not all work during the PhD. There were good and bad times in my daily life in which I met new people and I made new friends that always will be in my heart. I want to sincerely thank *Alejandra Castro* for all your help and your friendship throughout my PhD. Muchas gracias por todo Ale, te quiero mucho. *Juan Salinas,* thank you for all our nice talks and walks when we needed to relax from work. *Carsten Lüdtke,* thank you so much for all your help and company, especially for keeping me writing the thesis and checking what I wrote. *Kataneh Aalaei*, thank you for great talks during our after-works and parties together. *Alejandro Romero,* thank you for always listening to me when I was complaining, *Shuai Bai*, thank you for becoming my friend so fast and for all your help. *Kajsa Nilsson*, thank you for nice talks after the courses and for your help. *Hans Bolinsson,* thank you for all your help during my PhD, especially when I had problems with my computer (which was quite often). I want to thank all my Korean and Chinese friends that made my days happy: *Meiyu Zhang, Yi Lu*, *In Kang, Jangjae Lee, Dongsup Song and Yeo Woon.*

I cannot forget all the Bolivians that I met during the PhD and remember that it is possible to learn more from those who think differently from us. I want to thank *Daniel Osorio*, *Atma-Sol Bustos, Pamela Canaviri, Cecilia Curi, Vanessa Castro, Sander Perez, Claudia Lazarte, Wendy Soria*, *Roxana Quiroga, Ivan Limachi, Oscar Rollano, Fabricio Montano, Paola Terrazas, Daniel Salas, Teresa Mercado and Etzar Gomez*. I learned a lot from all of you. Thank you for the good and the bad times together. I will always remember our first trip to Malmö when some of us lived together on Möllevångsvägen and we had to change trains many times to get back to Lund – a day of mass hysteria.

Finalmente quiero agradecer a mis amados padres *Norah Zenteno* y *Juan Fuentes* por todo su apoyo no solo durante el doctorado, toda mi vida. Siempre han sido mi pilar, mi inspiración, la luz que me guía y la fuerza para seguir adelante. Muchas gracias también a mis queridos hermanos *Alejandro* y *Boris*, mis cuñadas *Grisel* y *Sonia* y mis sobrinos *Juan Jose, Sahely, Raquel, Marcos* y *Matías* a quien quiero

con toda mi alma. Gracias a toda mi adorada familia mis tíos, tías, primos, primas y sobrinos que siempre se preocupan por mí.

También quiero dar las gracias a mis queridos amigos *Benjamín Montevilla* por apoyarme y ayudarme en todo lo que has podido, gracias por revisar mis manuscritos y corregir los errores en Ingles, *Jorge Velasco* gracias por los consejos tanto técnicos como personales*, Gonzalo Ibiett* gracias por escucharme, apoyarme, aconsejarme y darme ánimos para continuar, *Pamela Orellana* y *Lydia Callata* las amigas que siempre han esperado mi retorno y a las primeras que he visto después de cada viaje. Por ultimo un agradecimiento especial a la Dra*. Ana María Aguilar* quien me dio ánimos, consejos y apoyo durante todo el doctorado.

11 References

Ai, Y., & Jane, J.-l. (2016). Macronutrients in Corn and Human Nutrition. *Comprehensive Reviews in Food Science and Food Safety, 15*(3), 581-598.

Ali Razavi, S. M., & Amini, A. M. (2016). Starch nanomaterials: a state-of-the-art review and future trends. In *Novel Approaches of Nanotechnology in Food*, (pp. 237-269): Academic Press.

Andersson, M., Wittgren, B., & Wahlund, K.-G. (2003). Accuracy in Multiangle Light Scattering Measurements for Molar Mass and Radius Estimations. Model Calculations and Experiments. *Analytical Chemistry, 75*(16), 4279-4291.

Auriemma, F., De Rosa, C., Di Girolamo, R., Malafronte, A., Scoti, M., Mitchell, G. R., & Esposito, S. (2016). Relationship Between Molecular Configuration and Stress-Induced Phase Transitions. In G. R. Mitchell & A. Tojeira (Eds.), *Controlling the Morphology of Polymers: Multiple Scales of Structure and Processing*, (pp. 287-327). Cham: Springer International Publishing.

Bai, Y., Shi, Y.-C., Herrera, A., & Prakash, O. (2011). Study of octenyl succinic anhydride-modified waxy maize starch by nuclear magnetic resonance spectroscopy. *Carbohydrate Polymers, 83*(2), 407-413.

Barth, H. G. (1982). High-performance size-exclusion chromatography of hydrolyzed plant proteins. *Analytical Biochemistry, 124*(1), 191-200.

Berry, G. C. (1966). Thermodynamic and Conformational Properties of Polystyrene. I. Light-Scattering Studies on Dilute Solutions of Linear Polystyrenes. *The Journal of Chemical Physics, 44*(12), 4550-4564.

Blennow, A., Sjöland, A. K., Andersson, R., & Kristiansson, P. (2005). The distribution of elements in the native starch granule as studied by particle-induced X-ray emission and complementary methods. *Analytical Biochemistry, 347*(2), 327-329.

Buléon, A., Colonna, P., Planchot, V., & Ball, S. (1998). Starch granules: structure and biosynthesis. *International Journal of Biological Macromolecules, 23*(2), 85-112.

Burchard, W. (1999). Solution properties of branched macromolecules. In, vol. 143 (pp. 113-194).

Cardot, P. J. P., Gerota, J., & Martin, M. (1991). Separation of living red blood cells by gravitational field-flow fractionation. *Journal of Chromatography B: Biomedical Sciences and Applications, 568*(1), 93-103.

Cauvain, S. (2015). *Technology of Breadmaking (3rd ed.)*. Cham: Springer International

Cauvain, S. P. (2005). How much more bread research do we need? In S. P. Cauvain, S. S. Salmon & L. S. Young (Eds.), *Using Cereal Science and Technology for the Benefit of Consumers*, (pp. 127-131): Woodhead Publishing.

Cave, R. A., Seabrook, S. A., Gidley, M. J., & Gilbert, R. G. (2009). Characterization of Starch by Size-Exclusion Chromatography: The Limitations Imposed by Shear Scission. *Biomacromolecules, 10*(8), 2245-2253.

Chmelík, J., Krumlová, A., Budinská, M., Kruml, T., Psota, V., Bohačenko, I., Mazal, P., & Vydrová, H. (2001). Comparison of Size Characterization of Barley Starch Granules Determined by Electron and Optical Microscopy, Low Angle Laser Light Scattering and Gravitational Field-Flow Fractionation. *Journal of the Institute of Brewing, 107*(1), 11-17.

Chung, H.-J., Lee, S.-E., Han, J.-A., & Lim, S.-T. (2010). Physical properties of dry-heated octenyl succinylated waxy corn starches and its application in fat-reduced muffin. *Journal of Cereal Science, 52*(3), 496-501.

Cisneros, F. H., Zevillanos, R., & Cisneros-Zevallos, L. (2009). Characterization of starch from two ecotypes of andean achira roots (Canna edulis). *Journal Of Agricultural And Food Chemistry, 57*(16), 7363-7368.

Contado, C., & Dondi, F. (2001). Barley starch granules subject to SPLITT cell fractionation and Sd/StFFF size characterization. *Starch/Staerke, 53*(9), 414-423.

Contado, C., Dondi, F., Beckett, R., & Giddings, J. C. (1997). Separation of particulate environmental samples by SPLITT fractionation using different operating modes. *Analytica Chimica Acta, 345*(1-3), 99-110.

Contado, C., Reschiglian, P., Faccini, S., Zattoni, A., & Dondi, F. (2000). Continuous split-flow thin cell and gravitational field-flow fractionation of wheat starch particles. *Journal of Chromatography A, 871*(1), 449-460.

Das, M., Sanson, N., Fava, D., & Kumacheva, E. (2007). Microgels loaded with gold nanorods: photothermally triggered volume transitions under physiological conditions. *Langmuir: The ACS Journal Of Surfaces And Colloids, 23*(1), 196-201.

Debye, P. (1944). Light Scattering in Solutions. *Journal of Applied Physics, 15*(4), 338-342.

Dewettinck, K., Van Bockstaele, F., Kühne, B., Van de Walle, D., Courtens, T. M., & Gellynck, X. (2008). Nutritional value of bread: Influence of processing, food interaction and consumer perception. *Journal of Cereal Science, 48*(2), 243-257.

Dhital, S., Shrestha, A. K., Hasjim, J., & Gidley, M. J. (2011). Physicochemical and Structural Properties of Maize and Potato Starches as a Function of Granule Size. *Journal Of Agricultural And Food Chemistry, 59*(18), 10151-10161.

Diderichsen, B., & Christiansen, L. (1988). Cloning of a maltogenic alpha-amylase from Bacillus stearothermophilus. *FEMS Microbiology Letters, 56*(1), 53-60.

Diego, R.-T., Walter, M.-A., Henry Alexander, V.-H., & José, F. S.-D. (2017). Thermal and physicochemical properties of starches from three Colombian rice varieties. *Agronomía Colombiana, Vol 35, Iss 1, Pp 116-124 (2017)*(1), 116.

Dou, H., Zhou, B., Jang, H.-D., & Lee, S. (2014). Study on antidiabetic activity of wheat and barley starch using asymmetrical flow field-flow fractionation coupled with multiangle light scattering. *Journal of Chromatography A, 1340*, 115-120.

Duthen, S., Rochat, C., Kleiber, D., Violleau, F., Daydé, J., Raynaud, C., & Levasseur-Garcia, C. (2018). Physicochemical characterization and study of molar mass of

industrial gelatins by AsFlFFF-UV/MALS and chemometric approach. *PLoS ONE, 13*(10), 1.

Eliasson, A.-C., & Karlsson, R. (1983). Gelatinization Properties of Different Size Classes of Wheat Starch Granules Measured with Differential Scanning Calorimetry. *Starch - Stärke, 35*(4), 130-133.

Eliasson, A. C., Bergenstahl, B., Nilsson, L., & Sjoo, M. (2013). From molecules to products: some aspects of structure-function relationships in cereal starches. *Cereal Chemistry, 90*(4), 326-334.

Fadda, C., Sanguinetti, A. M., Del Caro, A., Collar, C., & Piga, A. (2014). Bread Staling: Updating the View. *Comprehensive Reviews in Food Science and Food Safety, 13*(4), 473-492.

Fiszman, S., Salvador, A., & Varela, P. (2005). Methodological developments in bread staling assessment: application to enzyme-supplemented brown pan bread. *European Food Research and Technology, 221*(5), 616-623.

Gallant, D. J., Bouchet, B., & Baldwin, P. M. (1997). Microscopy of starch: evidence of a new level of granule organization. *Carbohydrate Polymers, 32*(3), 177-191.

Garin, M., Tighzert, L., Vroman, I., Marinkovic, S., & Estrine, B. (2014). The influence of molar mass on rheological and dilute solution properties of poly(butylene succinate). *Journal of Applied Polymer Science, 131*(20), n/a.

Ge, S., Xiong, L., Li, M., Liu, J., Yang, J., Chang, R., Liang, C., & Sun, Q. (2017). Characterizations of Pickering emulsions stabilized by starch nanoparticles: Influence of starch variety and particle size. *Food Chemistry, 234*, 339-347.

Giddings, J. C., Myers, M. N., Caldwell, K. D., & Pav, J. W. (1979). Steric field-flow fractionation as a tool for the size characterization of chromatographic supports. *Journal of Chromatography A, 185*, 261-271.

Gray, J. A., & Bemiller, J. N. (2003). Bread Staling: Molecular Basis and Control. *Comprehensive Reviews in Food Science and Food Safety, 2*(1), 1-21.

Hall, D. M., & Sayre, J. G. (1970). A Scanning Electron-Microscope Study of Starches:Part II : Cereal Starches. *Textile Research Journal, 40*(3), 256-266.

Hizukuri, S., Tabata, S., Kagoshima, & Nikuni, Z. (1970). Studies on Starch Phosphate Part 1. Estimation of glucose-6-phosphate residues in starch and the presence of other bound phosphate(s). *Starch - Stärke, 22*(10), 338-343.

Hoover, R. (2001). Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate Polymers, 45*(3), 253-267.

Hoover, R., & Manuel, H. (1996). The Effect of Heat–Moisture Treatment on the Structure and Physicochemical Properties of Normal Maize, Waxy Maize, Dull Waxy Maize and Amylomaize V Starches. *Journal of Cereal Science, 23*(2), 153-162.

Jane, J.-L., Kasemsuwan, T., Leas, S., Zobel, H., & Robyt, J. F. (1994). Anthology of Starch Granule Morphology by Scanning Electron Microscopy. *Starch - Stärke, 46*(4), 121-129.

Janoušková, J., Budinská, M., Plocková, J., & Chmelik, J. (2001). Optimization of experimental conditions for the separation of small and large starch granules by

gravitational field-flow fractionation. *Journal of Chromatography A, 914*(1), 183-187.

Ji, Z., Yu, L., Liu, H., Bao, X., Wang, Y., & Chen, L. (2017). Effect of pressure with shear stress on gelatinization of starches with different amylose/amylopectin ratios. *Food Hydrocolloids, 72*, 331-337.

Josua Timotheus, O., Thomas, R., & Antje, P. (2015). Overview of Methods for the Direct Molar Mass Determination of Cellulose. *Molecules, Vol 20, Iss 6, Pp 10313-10341 (2015)*(6), 10313.

Kaur, L., Singh, J., McCarthy, O. J., & Singh, H. (2007). Physico-chemical, rheological and structural properties of fractionated potato starches. *Journal of Food Engineering, 82*(3), 383-394.

Kim, H. Y., Lee, J. H., Kim, J. Y., Lim, W. J., & Lim, S. T. (2012). Characterization of nanoparticles prepared by acid hydrolysis of various starches. *Starch / Stärke, 64*(5), 367.

Lan, H., Hoover, R., Jayakody, L., Liu, Q., Donner, E., Baga, M., Asare, E. K., Hucl, P., & Chibbar, R. N. (2008). Impact of annealing on the molecular structure and physicochemical properties of normal, waxy and high amylose bread wheat starches. *Food Chemistry, 111*(3), 663-675.

Le Corre, D., Bras, J., & Dufresne, A. (2010). Starch Nanoparticles: A Review. *Biomacromolecules, 11*(5), 1139-1153.

Lee, S., Lee, T. W., Cho, S. K., Kim, S. T., Kang, D. Y., Kwen, H., Lee, S. K., & Eum, C. H. (2010). Implementation of splitter-less SPLITT fractionation and its application to large scale-fractionation of sea sediment. *Microchemical Journal, 95*(1), 11-19.

Leeman, A. M., Karlsson, M. E., Eliasson, A.-C., & Björck, I. M. E. (2006). Resistant starch formation in temperature treated potato starches varying in amylose/amylopectin ratio. *Carbohydrate Polymers, 65*(3), 306-313.

León, A. E., Durán, E., & Benedito de Barber, C. (2002). Utilization of Enzyme Mixtures To Retard Bread Crumb Firming. *Journal Of Agricultural And Food Chemistry, 50*(6), 1416-1419.

Liang, R., Jiang, Y., Yokoyama, W., Yang, C., Cao, G., & Zhong, F. (2016). Preparation of Pickering emulsions with short, medium and long chain triacylglycerols stabilized by starch nanocrystals and their in vitro digestion properties. *RSC Advances, 6*(101), 99496-99508.

Lindeboom, N., Chang, P. R., & Tyler, R. T. (2004). Analytical, Biochemical and Physicochemical Aspects of Starch Granule Size, with Emphasis on Small Granule Starches: A Review. *Starch - Stärke, 56*(3-4), 89-99.

Litzén, A., & Wahlund, K. G. (1991). Zone broadening and dilution in rectangular and trapezoidal asymmetrical flow field-flow fractionation channels. *Analytical Chemistry, 63*(10), 1001-1007.

Liu, M. K., Williams, P. S., Myers, M. N., & Giddings, J. C. (1991). Hydrodynamic relaxation in flow field-flow fractionation using both split and frit inlets. *Analytical Chemistry, 63*(19), 2115-2122.

Lu, X., Wang, Y., Li, Y., & Huang, Q. (2018). Assembly of Pickering emulsions using milled starch particles with different amylose/amylopectin ratios. *Food Hydrocolloids, 84*, 47-57.

MacRitchie, F. (2016). Seventy years of research into breadmaking quality. *Journal of Cereal Science, 70*, 123-131.

Makan, A. C., Otte, T., & Pasch, H. (2012). Analysis of High MolarMass Branched Polybutadienesby SEC-MALLS and AF4-MALLS. *Macromolecules, 45*(12), 5247-5259.

Malik, M. I., & Pasch, H. (2016). Field-flow fractionation: New and exciting perspectives in polymer analysis. *Progress in Polymer Science, 63*, 42-85.

Maningat, C. C., Seib, P. A., Bassi, S. D., Woo, K. S., & Lasater, G. D. (2009). Wheat Starch: Production, Properties, Modification and Uses (3rd ed.). In J. BeMiller & R. Whistler (Eds.), *Starch - Chemistry and Technology*, (pp. 441-510). San Diego: Academic Press.

Merino-Dugay, A., Cardot, P. J. P., Czok, M., Guernet, M., & Andreux, J. P. (1992). Monitoring of an experimental red blood cell pathology with gravitational field-flow fractionation. *Journal of Chromatography B: Biomedical Sciences and Applications, 579*(1), 73-83.

Moon, M. H., Kwon, H., & Park, I. (1997a). Stopless Flow Injection in Asymmetrical Flow Field-Flow Fractionation Using a Frit Inlet. *Analytical Chemistry, 69*(7), 1436-1440.

Moon, M. H., Kwon, H., & Park, I. (1997b). Stopless Separation of Proteins by Frit-Inlet Asymmetrical Flow Field-Flow Fractionation. *Journal of Liquid Chromatography & Related Technologies, 20*(16), 2803.

Morrison, W. R., Milligan, T. P., & Azudin, M. N. (1984). A relationship between the amylose and lipid contents of starches from diploid cereals. *Journal of Cereal Science, 2*(4), 257-271.

Nienke, L., Peter, R. C., & Robert, T. T. (2004). Analytical, Biochemical and Physicochemical Aspects of Starch Granule Size, with Emphasis on Small Granule Starches: A Review. *Starch / Stärke, 56*(3-4), 89.

Nilsson, L. (2013). Separation and characterization of food macromolecules using field-flow fractionation: A review. *Food Hydrocolloids, 30*(1), 1-11.

Oates, C. G. (1997). Towards an understanding of starch granule structure and hydrolysis. *Trends in Food Science & Technology, 8*(11), 375-382.

Otte, T., Pasch, H., Macko, T., Brüll, R., Stadler, F. J., Kaschta, J., Becker, F., & Buback, M. (2011). Characterization of branched ultrahigh molar mass polymers by asymmetrical flow field-flow fractionation and size exclusion chromatography. *Journal of Chromatography A, 1218*(27), 4257-4267.

Pareyt, B., Finnie, S. M., Putseys, J. A., & Delcour, J. A. (2011). Lipids in bread making: Sources, interactions, and impact on bread quality. *Journal of Cereal Science, 54*(3), 266-279.

Pazourek, J., Wahlund, K.-G., & Chmelík, J. (1996). Effect of flowrate and ionic strength on retention of nonporous micron-sized silica gel particles in gravitational field-flow fractionation. *Journal of Microcolumn Separations, 8*(5), 331-338.

Perez-Rea, D., Bergenståhl, B., & Nilsson, L. (2015). Development and evaluation of methods for starch dissolution using asymmetrical flow field-flow fractionation. Part I: Dissolution of amylopectin. *Analytical & Bioanalytical Chemistry, 407*(15), 4315-4326.

Perez-Rea, D., Bergenståhl, B., & Nilsson, L. (2016). Development and evaluation of methods for starch dissolution using asymmetrical flow field-flow fractionation. Part II: Dissolution of amylose. *Analytical And Bioanalytical Chemistry, 408*(5), 1399-1412.

Pérez, S., & Bertoft, E. (2010). The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. *Starch - Stärke, 62*(8), 389-420.

Pitkänen, L., & Striegel, A. M. (2014). AF4/MALS/QELS/DRI characterization of regular star polymers and their "span analogs". *Analyst, 139*(22), 5843-5851.

Purhagen, J. K., Sjöö, M. E., & Eliasson, A.-C. (2011). Starch affecting anti-staling agents and their function in freestanding and pan-baked bread. *Food Hydrocolloids, 25*(7), 1656-1666.

Rayner, M., Timgren, A., Sjöö, M., & Dejmek, P. (2012). Quinoa starch granules: a candidate for stabilising food-grade Pickering emulsions. *Journal of the Science of Food and Agriculture, 92*(9), 1841-1847.

Roger, P., Bello-Perez, L. A., & Colonna, P. (1999). Contribution of amylose and amylopectin to the light scattering behaviour of starches in aqueous solution. *Polymer, 40*(25), 6897-6909.

Rojas, C. C., Wahlund, K.-G., Bergenståhl, B., & Nilsson, L. (2008). Macromolecular Geometries Determined with Field-Flow Fractionation and their Impact on the Overlap Concentration. *Biomacromolecules, 9*(6), 1684-1690.

Saari, H., Fuentes, C., Sjöö, M., Rayner, M., & Wahlgren, M. (2017). Production of starch nanoparticles by dissolution and non-solvent precipitation for use in food-grade Pickering emulsions. *Carbohydrate Polymers, 157*, 558-566.

Sanz, R., Galceran, M. T., & Puignou, L. (2004). Determination of Viable Yeast Cells by Gravitational Field-Flow Fractionation with Fluorescence Detection. *Biotechnology Progress, 20*(2), 613-618.

Sanz, R., Puignou, L., Reschiglian, P., & Galceran, M. T. (2001). Gravitational field-flow fractionation for the characterisation of active dry wine yeast. *Journal of Chromatography A, 919*(2), 339-347.

Šárka, E., & Dvořáček, V. (2017). Waxy starch as a perspective raw material (a review). *Food Hydrocolloids, 69*, 402-409.

Schimpf, M. E., Caldwell, K., & Giddings, J. C. (2000). *Field-Flow Fractionation Handbook*: Wiley.

Schoch, T. J., & French, D. (1947). Studies on bread staling I. The role of starch. *Cereal Chemistry, 24*, 231-249.

Shin, K., Choi, J., Kim, Y., Lee, Y., Kim, J., Lee, S., & Chung, H. (2018). Feasibility study for combination of field-flow fractionation (FFF)-based separation of size-coded particle probes with amplified surface enhanced Raman scattering (SERS) tagging

for simultaneous detection of multiple miRNAs. *Journal of Chromatography A, 1556*, 97-102.

Singh, N., & Kaur, L. (2004). Morphological, thermal, rheological and retrogradation properties of potato starch fractions varying in granule size. *Journal of the Science of Food and Agriculture, 84*(10), 1241-1252.

Stamataki, N. S., Yanni, A. E., & Karathanos, V. T. (2017). Bread making technology influences postprandial glucose response: a review of the clinical evidence. *British Journal of Nutrition, 117*(7), 1001-1012.

Staudinger, H. (1930). Über hochpolymere Verbindungen. 46. Mitteilung. Organische Chemie und Kolloidchemie. *Kolloid Zeitschrift, 53*(1), 19.

Tao, K., Li, C., Yu, W., Gilbert, R. G., & Li, E. (2019). How amylose molecular fine structure of rice starch affects functional properties. *Carbohydrate Polymers, 204*, 24-31.

Utrilla-Coello, R. G., Agama-Acevedo, E., Barba de la Rosa, A. P., Rodríguez-Ambriz, S. L., & Bello-Pérez, L. A. (2010). Physicochemical and Enzyme Characterization of Small and Large Starch Granules Isolated from Two Maize Cultivars. *Cereal Chemistry, 87*(1), 50.

Van Bruijnsvoort, M., Wahlund, K. G., Nilsson, G., & Kok, W. T. (2001). Retention behavior of amylopectins in asymmetrical flow field-flow fractionation studied by multi-angle light scattering detection. *Journal of Chromatography A, 925*, 171-182.

Wahlund, K.-G., Leeman, M., & Santacruz, S. (2011). Size separations of starch of different botanical origin studied by asymmetrical-flow field-flow fractionation and multiangle light scattering. *Analytical and Bioanalytical Chemistry, 399*(4), 1455-1465.

Wahlund, K.-G., & Nilsson, L. (2012). Flow FFF – Basics and Key Applications. In S. K. R. Williams & K. D. Caldwell (Eds.), *Field-Flow Fractionation in Biopolymer Analysis*, (pp. 1-21). Vienna: Springer Vienna.

Wahlund, K. G., & Giddings, J. C. (1987). Properties of an Asymmetrical Flow Field-Flow Fractionation Channel Having One Permeable Wall. *Analytical Chemistry, 59*(9), 1332-1339.

Wang, Y.-J., Truong, V.-D., & Wang, L. (2003). Structures and rheological properties of corn starch as affected by acid hydrolysis. *Carbohydrate Polymers, 52*(3), 327-333.

Ward, I. M. (2009). Polymers: Chemistry and Physics of Modern Materials, 3rd edition, by J.M.G. Cowie and V. Arrighi. *Contemporary Physics, 50*(6), 670-670.

Wyatt, P. J. (1993). Light scattering and the absolute characterization of macromolecules. *Analytica Chimica Acta, 272*(1), 1-40.

Xie, F., Yu, L., Su, B., Liu, P., Wang, J., Liu, H., & Chen, L. (2009). Rheological properties of starches with different amylose/amylopectin ratios. *Journal of Cereal Science, 49*(3), 371-377.

Yoshimoto, Y., Tashiro, J., Takenouchi, T., & Takeda, Y. (2000). Molecular Structure and Some Physicochemical Properties of High-Amylose Barley Starches. *Cereal Chemistry, 77*(3), 279.

You, S., Stevenson, S. G., Izydorczyk, M. S., & Preston, K. R. (2002). Separation and Characterization of Barley Starch Polymers by a Flow Field-Flow Fractionation Technique in Combination with Multiangle Light Scattering and Differential Refractive Index Detection. *Cereal Chemistry, 79*(5), 624.

Yusoff, A., & Murray, B. S. (2011). Modified starch granules as particle-stabilizers of oil-in-water emulsions. *Food Hydrocolloids, 25*(1), 42-55.

Zielke, C., Fuentes, C., Piculell, L., & Nilsson, L. (2018). Co-elution phenomena in polymer mixtures studied by asymmetric flow field-flow fractionation. *Journal of Chromatography A, 1532*, 251-256.

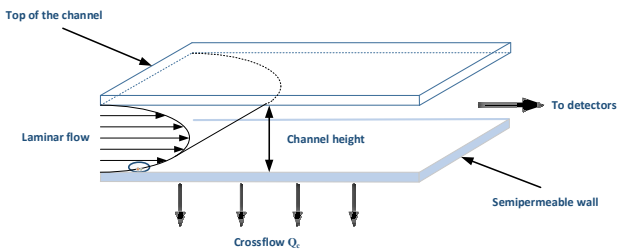
Žigon, M., The, N. K., Cheng, S., & Grubišić-Gallot, Z. (1997). Degradation of High Molecular Weight Polystyrenes During the SEC Separation Process, as Demonstrated by SEC Coupled with Lalls and by Static Light Scattering. *Journal of Liquid Chromatography & Related Technologies, 20*(14), 2155-2167.

Zimm, B. H. (1948a). Apparatus and Methods for Measurement and Interpretation of the Angular Variation of Light Scattering; Preliminary Results on Polystyrene Solutions. *The Journal of Chemical Physics, 16*(12), 1099-1116.

Zimm, B. H. (1948b). The Scattering of Light and the Radial Distribution Function of High Polymer Solutions. *The Journal of Chemical Physics, 16*(12), 1093-1099.

Zobel, H. F. (1988). Molecules to Granules: A Comprehensive Starch Review. *Starch - Stärke, 40*(2), 44-50.

Printed by Media-Tryck, Lund 2019 NORDIC SWAN ECOLABEL 3041 0903



LUND UNIVERSITY

Lund University
Faculty of Engineering
Department of Food Technology,
Engineering and Nutrition
ISBN 978-91-7422-643-0