PhD THESIS

# Biotechnological production of biobased xylo-oligosaccharides and fermentable sugars derived from wheat by-products as novel carbohydrates for potential food applications

PhD student **Gabriela PRECUP**

(SUMMARY OF THE DOCTORAL THESIS)

Scientific coordinator **Prof. dr. Dan Cristian VODNAR**

# SUMMARY

In the context of climate change mitigation and energy transition, the use of bioresources is essential for a shift towards a more sustainable society and economy. A popular research topic that tackles some of nowadays' challenges is circular economy. It focuses on creating a closed-loop system by promoting business models that encompass the maximal recycling and recovery of material and energy (Parliament, 2015) (European Commission, 2018). This topic is shaping the public policy around the world, the sustainable development goals being at the heart of the European Union's (EU) policymaking and action. Moreover, to help better decision-making, the framework of circular integration was recently proposed, that includes elements of circular economy, industrial ecology and process integration mixed in a multi-scale and holistic approach. Specifically, it provides an engineering toolbox to facilitate the design and implementation of sustainable and circular systems (Walmsley, Ong, Klemeš, Tan, & Varbanov, 2019).

The use of renewable sources of solar origin and biomass to produce energy, food, feed, chemicals and materials should be wisely integrated in the sustainable systems, in a way to avoid resource depletion and irreversible impacts on the environment, while meeting the human needs. Biomass comes from different sources including crops, trees, grass, algae and is generated in the form of residues or wastes from processing primary sources or as waste or discard from consumption (Andersen et al.., 2021). Lignocellulosic biomass is a promising raw material for biorefineries, since it is costless, renewable and abundant, and its valorization into food/feed ingredients, chemical compounds, or energy vectors, is of great interest for various stakeholders including researchers and entrepreneurs. The use of agro-residues for the production of value-added compounds is therefore an economically advantageous strategy, since it also helps in the management of billions of tons of residues generated annually (Amorim, Silvério, Prather, & Rodrigues, 2019). A plethora of valuable compounds can be obtained, such as monosaccharides (i.e., glucose, xylose, mannose, fructose, arabinose), oligosaccharides (i.e., fructo-oligosaccharides or FOS, xylo-oligosaccharides or XOS, galacto-oligosaccharides or GOS), bioactive compounds (i.e., flavonoids, phenolic acids, terpenes, carotenoids), biofuels (i.e., biodiesel, ethanol, hydrogen, butanol), cellulose, lignin (Cano et al.., 2020; Cho, Trinh, Song, Lee, & Bae, 2020). These compounds can be obtained from several agro-industrial wastes such as sugarcane bagasse (Jacobsen & Wyman, 2002), hardwoods (G Garrote & Parajó, 2002), corncobs (Gil Garrote, Dominguez, & Parajo, 2002), almond shells, wheat chaff and straw (Đorđević & Antov, 2018; Precup et al.., 2022; Sun et al.., 2005), vine shoots (Dávila, Gullón, Alonso, Labidi, & Gullón, 2019), sugar beet pulp (Elst et al.., 2018), banana peels (Pereira, Arruda, Molina, & Pastore, 2018) or others (Moure, Gullón, Domínguez, & Parajó, 2006).

For instance, wheat straw is an agricultural residue that presents many interesting characteristics that facilitate its biotechnological upgrade in a biorefinery framework. Wheat is the main cereal produced worldwide, 44 % of total cereal

production, being a herbaceous crop that can be transported in relatively high-density form and typically has a low water content that enables its easy storage. As a rough estimation, more than 170 million tons of wheat straw are produced yearly in Europe.

From a chemical point of view, lignocellulosic biomass is mainly composed by cellulose, hemicellulose and lignin. Specifically, cellulose is composed by a chain of glucose molecules joined by β-(1–4)-glycosidic bonds linked by hydrogen bonds between layers of polysaccharides, forming a crystalline conformation, while hemicellulose refer to various amorphous and heterogenous polymers found in the plant cell-wall matrix that have β-(1–4)-linked backbones, built up by pentoses (D-xylose, D-arabinose), hexoses (D-mannose, D-glucose, D-galactose) and sugar acids. They are commonly categorized into several groups such as xyloglucans, heteroxylans, (galacto) glucomannans, and arabinogalactans [1]. Lignin is a complex three dimensional biodegradable polymer composed by three major phenolic components, namely p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol [2].

The hemicellulose fraction could be used for production of xylo-oligosaccharides and manno-oligosaccharide, being rich in xylose, galactose, fructose, glucose and mannose. It accounts for 25-30% of lignocellulosic biomass, with a relatively low molecular weight (circa 15 kDa), consisting of mainly xylan, a polymer of xylose linked by -1,4-xylosidic bonds that can be depolymerized by several treatments to XOS (xylobiose, xylotriose, up to xylodecaose) [3, 4].

Xylo-oligosaccharides (XOS) are considered non-digestible fibres that attained commercial interest due to their prospective application in the food industry since they showed low caloric index and good stability at various temperatures and pH. They can be produced by autohydrolysis, chemical, and/or enzymatic methods. Associations in the scientific literature linked XOS with potential beneficial health outcomes related to the gut microbiota, thus being reported as emerging “prebiotics”.

The aim of this thesis was to present a holistic approach referring to the production of biobased XOS from wheat straw by means of alkali, enzymatic treatment, and the combinations thereof, that were further purified with ion-exchange resins. The first research direction was to compare different treatments for wheat straw fractionation in order to obtain the highest yield possible of XOS and further test different downstream processing methods to purify the fraction obtained. The second direction was to assess the XOS fractions in fermentation experiments with *Bifidobacterium bifido* subsp. *lactis* (BB-12) on wheat-flour doughs, to investigate the rheological and physicochemical properties for potential applications in bakery products. Additionally, considering that XOS were reported as potential “prebiotics”, the perception of Romanian consumers on this topic was investigated via an online survey.
The experiments on XOS production were performed at the Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB) in Potsdam, Germany (coordinated by Dr. Ing. Joachim Venus and Dr. Ing. Monika Heiermann) and the assessment of the XOS in fermentation experiments were performed in the Laboratory of Fermentative Biotechnologies within the Life Science Institute “King Michael I of Romania” of the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, under the supervision of Prof. Dr. Dan Cristian Vodnar and Dr. Bernadette Emőke Teleky .

## The main objectives of this PhD thesis were:

Lignocellulosic biomass such as wheat straw (WS) could represent a valuable source for biorefineries, since it is renewable and available in high amounts with very low value, with high potential to be valorized for the production of food/feed ingredients, chemical compounds or for energy purposes. A plethora of valuable compounds can be obtained, such as monosaccharides (i.e., glucose, xylose, mannose, fructose, arabinose), oligosaccharides (i.e., xylo-oligosaccharides or XOS), bioactive compounds (i.e., flavonoids, phenolic acids, terpenes, carotenoids), biofuels (i.e., biodiesel, ethanol, hydrogen, butanol), cellulose, lignin.

Xylo-oligosaccharides (XOS) are considered non-digestible fibres that attained commercial interest due to the associations showed by scientific literature with their potential beneficial effect in the gut microbiota, being considered novel "prebiotics".

In this regard, a first aim of the thesis was to investigate the current status of prebiotics research in Romania and explore the awareness and knowledge on this topic among Romanian consumers and how socio- demographic characteristics influence the behavior of consumers regarding dietary choices, through an online questionnaire.

A second aim was to identify an optimal strategy for the production of XOS from wheat straw by biotechnological means, such as autohydrolysis, alkali and enzymatic treatment, and the combinations thereof. In order to obtain a pure fraction of XOS for potential application in the food industry, a downstreaming strategy consisting of two steps was carried out: a first step of adsorption using the surface-active material activated charcoal or the polymer adsorber resin PUROLITE MN-502 to remove phenols and reduce the concentration of salt ions. A second step of an- and cation exchange chromatography was performed to further reduce the concentration of salts and minerals.

Finally, we were interested to test the effect of XOS supplementation in different percentages (1, 2, 5, or 10%) on the rheological and physicochemical characteristics of wheat flour doughs and comparing them with the glucose-enriched doughs in fermentation experiments with *Bifidobacterium bifidum* 12, under the same conditions. Sourdough fermentation was monitored and analysed for 48 hours, and samples were taken at 0, 18, 24, and 48 h for pH, cell viability, sugar and organic acids analysis, and rheological measurements.

In order to achieve the aim of the thesis, the following aspects were investigated and materialized into four objectives:

**O.1.** Investigation of the awareness and knowledge on the prebiotic concept among Romanian consumers and how socio- demographic characteristics influence the behavior of consumers regarding dietary choices

**O.2.** Development of an optimal strategy for the production of biobased XOS from wheat straw by biotechnological means, such as autohydrolysis, alkali and enzymatic treatment, and the combinations thereof

**O.3.** Development of a downstream process method consisting of filtration, decolorization, and an- and cation exchange resins to obtain a purified fraction of XOS and fermentable sugars for potential use in food applications

**O.4.** Assessment of the physicochemical and rheological properties of XOS and glucose supplementation of wheat flour doughs in fermentation experiments with the lactic acid bacteria *Bifidobacterium bifidum* 12.

The results of this thesis were published in three original articles (one ISI indexed journal with IF 3.390 – in *International Journal of Environmental Research and Public Health* Journal; the second ISI indexed journal with IF 4.329 – in *Polymers* Journal; the third ISI indexed journal with IF 5.079– in *Biology* Journal). In addition, one review articles (ISI indexed journal with IF 3.334 – in *British Journal of Nutrition* Journal) was published.

The PhD thesis is structured in two main parts: state-of-the-art comprising of two literature reviews (**Chapter 1, 2**) and original research comprising of the working hypothesis/objectives and the general methodologies, followed by the original research articles (**Chapter 3-5**), general conclusions and recommendations (**Chapter 6**), respectively the originality and innovative contributions of the thesis (**Chapter 7**).

In what concerns the **first part** (literature review articles), studies were identified by conducting PubMed, Web of Science Core Collection, Scopus, and Google Scholar electronic searches. The state-of-the-art regarding the biotechnological production of XOS, a first pretreament method of autohydrolysis is recommended for wheat straw fractionation, in order to break down the recalcitrance of the wheat straw cell wall and increase the accessibility to the hemicellulose. Wheat straw is a renewable and cheap agricultural biomass that has a good xylan/lignin ratio, making it suitable for XOS extraction

As far as the **second part** of this thesis is concerned, the experiments that involved the testing of methods in order to find an optimal strategy for the production of XOS from wheat straw by biotechnological means, such as autohydrolysis, alkali and enzymatic treatment, and the combinations thereof, and the purification experiments were conducted in accordance to the literature screening and with the help of the members' expertise from the Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB) in Potsdam, Germany . In addition, the assessment of the rheological and physicochemical effects of XOS in wheat flour doughs was possible with the great help of Bernadette-Emőke Teleky from UASVM Cluj-Napoca.

**Chapter 3** investigates the current status of prebiotics research in Romania and explores the awareness and knowledge on this topic among Romanian consumers and how socio- demographic characteristics influence their behavior regarding dietary

choices. The work could potentially help the interested stakeholders to address consumer needs and preferences regarding food applications with claimed effects.

The statistical analyses for the first study were performed using IBM SPSS Statistics 19. Descriptive statistics and crosstabulation were used to identify the influence of socio-demographic characteristics, such as gender, age, and education, on diet choice, prebiotics' perception, and knowledge. The data are reported as the mean ± standard deviation (SD), and differences between means at the 5% level were considered statistically significant.

**Chapter 4** presents an integrated biotechnological approach for production of XOS and fermentable sugars. This chapter demonstrates that an integrated strategy consisting of autohydrolysis, alkaline and enzymatic treatment with xylanase followed by a refining process by anion and cation exchange chromatography could be efficient for XOS production from wheat straw.

For the second study, the same program was used. All tests/experiments were conducted in triplicate, and the results were expressed as the means ± standard deviation (SD). Data normality was studied using the Shapiro-Wilk test [54], and the homogeneity of variances (homoelasticity) was studied using Levene test. A one-way ANOVA test was applied for normally distributed and homogeneous data. For $p < 0.05$ corresponding to statistical F, calculations with the Tukey post hoc test were used to study whether the treatments had significant differences. For non-homogeneous data, the Welch test was used, followed by the Kruskal–Wallis test, in which, if $p < 0.05$ was obtained, the calculations were continued with the Mann–Whitney test to verify which treatments had significant differences. At the parameters where the data were not homogeneous and we used Mann–Whitney, the Bonferroni correction was applied: 0.05/6 = 0.008 and 0.01/6 = 0.001; for 3 treatments, 0.05/3 = 0.016 and 0.01/3 = 0.003.

**Chapter 5** presents the application for potential food applications of wheat straw derived-XOS via alkali and enzymatic treatment and their rheological and physicochemical effects in wheat flour doughs. The work showed the efficiency of XOS supplementation of the wheat flour doughs in fermentation with BB-12, by positively influencing the viability of the bacteria and the rheological properties of the dough such as better elasticity, due to the formation of organic acids.

For the third study the statistical evaluation was carried out through the use of Graph Prism Version 8.0.1. (GraphPad Software Inc., San Diego, CA, USA), along with a one-way ANOVA test (Tukey multiple comparisons tests) [55]. Statistically significant differences of means were considered at a level of $p < 0.05$.

## The general conclusions were:

1. The online survey emphasized a limited knowledge and scattered confusion on the prebiotic concept among the sample of the Romanian consumers. Even if most of the responders were familiar with the term (74% of total responders), when assessing their knowledge, the results were contradictory (40% understood the term, 24% were not familiar and had little or no knowledge, and 20% were familiar but they had little or

no information).Future avenues of research should struggle more to confirm the causality between the health effects of emerging prebiotics and microbiota-mediated mechanisms so that healthcare providers can develop evidence-based recommendations.

2.Xylo-oligosaccharides are considered novel “prebiotics” by the scientific literature, showing beneficial health outcomes in “in vitro” and “in vivo” studies. However, substantiated scientific evidence of the XOS prebiotic efficacy will be needed to obtain health claims approvals.

3. Wheat straw is a promising feedstock for XOS production.

4. The highest XOS production (X3+ X4) (1.48±0.2 g/L, 1.33 ± 0.13 g/L) was observed after hydrothermal preatreatment at 140 °C and 180°C, followed by alkaline and enzymatic treatment with endo-b-1-4-xylanase from *T. viride*, at concentrations of (1.48±0.2 g/L, 1.33 ± 0.13 g/L), after 48 hours hydrolysis, at dosages of 2 mL per 100 g residue and pH 4.6.

5. The highest sugar recovery (64.86%), was observed after the hydrothermal pretreatment at the highest severity combined with enzymatic hydrolysis with xylanase.

6. The treatments applied conducted to degradation of monosaccharides in acetic acid, furfural and hydroxymethylfurfural, that increased with the severity of the treatment applied and could hinder the enzymatic hydrolysis. Furfural had a content of 209 ± 15.8 mg/L and HMF 14.81 ± 1.1 mg/L in the hydrolysate at log $R_0$ = 2.94, while in the hydrolysate at log $R_0$ = 3.53, furfural was detected in an amount of 136 ± 10 mg/L and HMF 9.54 ± 0.7 mg/L, due to the conversion of xylose and glucose.

7. The polymer adsorber resin MN-502 showed efficient removal of salts, cations, phenols and furan derivatives from the hydrolysate resulting after alkaline and enzymatic hydrolysis of the residues treated at the severity factor of log $R_0$ = 3.53. However, further optimization treatments targeting a more efficient fractioning of the wheat straw are needed to obtain higher XOS yields in a purified form.

8. XOS supplementation of the wheat flour doughs in fermentation experiments with BB-12, positively influenced the viability of the bacteria and the rheological properties of the dough.

9. The production of acetic acid was observed only in the XOS-enriched doughs and positively influenced its rheological properties by improving elasticity behavior before and after frozen storage. The highest acetic acid quantity of 0.87 ± 0.03 mg/L was obtained in 10% XOS.

10. The quantity of glucose, maltose, XOS and xylose decreased till the end of fermentation. However, dough enrichment with XOS or glucose did not significantly improve the viability of BB-12.

## Future perspectives:

1. Future research should be carried out for the use of synthetic biology tools to create microorganisms that will be capable to directly convert the lignocellulosic biomass into XOS

2. Further optimization of treatments to obtain higher XOS yields in a purified form

3. Safety evaluation is required for the food enzymes used in the production process of XOS

4.Studies on XOS as potential food ingredient (stability testing, complete compositional analysis, detection of contaminants (mycotoxins, heavy metals, process contaminants), exposure assessment for population groups, toxicological studies) should be carried out

5. Health-benefit analysis of XOS replacement in food products (e.g., bakery products)

6. Studies on the causality between the health effects of XOS and impact on the gut microbiota

7. Educational campaigns to inform consumers on the relationship between certain food ingredients and health outcomes in a clear way and based on a rigorous assessment of the scientific evidence.

## Originality and personal contributions

The results presented in this thesis might be considered helpful for various stakeholders, from the scientific community to entrepreneurs in the food industry and food biotechnology, to policy makers. This research can be considered a comprehensive study presenting the current advances with regards to XOS production and a model encompassing the recovery of biomass (e.g., wheat straw) and its utilization to produce value-added compounds such as XOS via biotechnological processes. Furthermore, the investigation of the effect of XOS addition in a food application model like wheat flour doughs that could be used for bakery food products presents an efficient application in the food industry, as alternative sweeteners.

Since the scientific literature classifies XOS as emerging “prebiotics”, the first aim of the thesis was to investigate the current status of prebiotics research in Romania and explore the awareness and knowledge on this topic among a sample of Romanian consumers through an online survey and observe how socio- demographic characteristics influence the behaviour of consumers regarding dietary choices. This was the first study carried out in Romania that explored the knowledge of Romanian consumers on this concept, highlighting the scattered confusion referring to the meaning of prebiotics, probably linked to the lack of a common consensus and terminology regarding this topic among the involved stakeholders, which leads to misinformation and confusion among consumers. Our work highlighted the need of more research to confirm the causality between the health effects of emerging prebiotics and microbiota-mediated mechanisms so that healthcare providers can develop evidence-based recommendations.

A further objective was to explore an integrated biotechnological approach for production of XOS and fermentable sugars from lignocellulosic biomass such as wheat straw, thus contributing to the sustainable goal of waste recovery, by valorizing a by-product resulted from the agricultural sector. The results showed that a strategy consisting of autohydrolysis, alkaline and enzymatic treatment with xylanase followed

by a refining process by anion and cation exchange chromatography could be efficient for XOS production from wheat straw. The downstreaming process showed for the first time the efficiency of the strategy employed for wheat straw derived-XOS, consisting of decolorization with the ion exchange resin MN-502 followed by an- and cation exchange chromatography.

Finally, our last objective to explore the effect of XOS addition in a food application model like wheat flour doughs that could be used for bakery food products in fermentation experiments with BB-12 highlighted promising results of the XOS behaviour in food models. Owning a QPS status in the EU, BB-12 was chosen for the fermentation experiment due to its affinity to use XOS as carbon source and its characteristics to be tolerant to hear, oxygen and acidic pH. The effectiveness of XOS addition as replacer to glucose was demonstrated by showing good rheological characteristics such as improved viscoelasticity with the increase of XOS amount. The organic acids produced (acetic acid and lactic acid) further improved the elasticity behaviour of the doughs, an addition of 10% XOS showing an elastic behaviour, rather than viscous. To our knowledge this is the first study to explore the rheological behaviour of XOS addition in sourdoughs fermented with the lactic acid bacteria BB-12.