

PhD THESIS

PÉTER PENKSZA

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Hungarian University of Agriculture and Life Sciences

Technofunctional properties of xylo-oligosaccharides

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1. BACKGROUND AND OBJECTIVES

One of the most significant factors in the development of many civilized, non-infectious diseases, for example: diabetes, colon cancer, obesity, is inadequate, low-fiber nutrition. As a result, there is an increasing effort in the food industry to produce functional foods that contain added fibers in such a quantity that they exert their positive physiological effects and, at the same time, preferably do not differ significantly from the taste, smell and texture of traditional foods.

Food fibers include cellulose, lignin, hemicellulose, pectin, vegetable gums and mucus, which are polysaccharides, and some carbohydrates with a lower degree of polymerization, collectively called non-digestible oligosaccharides (NDO). More and more non-digestible oligosaccharides are well known in Europe and Hungary, such as fructo-oligosaccharides (FOS) produced by the hydrolysis of inulin, or isomalto-oligosaccharides (IMO) produced by the partial hydrolysis of starch. These molecules function not only as fiber, but also as prebiotics, which pass through the human alimentary canal unchanged and selectively promote the growth of beneficial intestinal bacteria in the human intestine (Gibson and Roberfroid 1995). From the point of view of food technology, it is exciting to examine the dosage of small-molecule oligosaccharides as fiber, because in contrast to large polysaccharides (e.g. cellulose, pectin), which can significantly modify the appearance and structure of the product, non-digestible oligosaccharides can even "inadvertently" increase the content of the given food, so they can be more attractive to consumers.

One of the components of lignocelluloses found in plant by-products (corn cobs, bagasse) is xylan. At the beginning of my research work, XOS was a new food ingredient not yet known in Hungary and Europe, the authorization of which took place in 2018 (EU Regulation 2018/1648), and whose use in the Far East (Japan, Korea, China) goes back two decades. XOS is mainly used in dietary supplements, functional foods, and dairy products containing synbiotic probiotics and prebiotics. However, little is known about how it can be used in traditional European foods. The advantage of XOS over other NDOs is that it can be produced from cheap, abundant and renewable agricultural plant residues (Vazquez et al. 2000) and that it exerts its beneficial physiological effects even in small doses (1-4g/day). (Xiao, Ning, Xu, 2012)

The aim of my PhD thesis is to map the basic technological properties of xylo-oligosaccharides (XOS), as new non-digestible oligosaccharides, in order to prepare product and production technology development works planned using XOS. I also aimed to contribute with my research to the foundation of the scientific background of XOS Novel Food licensing.

1. My goal was to learn the basic physical properties (solubility, color, turbidity) of XOS as a new food ingredient.
2. Since we did not have any knowledge, my goal was to clarify what rheological properties XOS has in an aqueous medium and what effect these may have on the product development and production technology of fiber-enriched foods.
3. My goal was to investigate whether the presence of XOS modifies the gelation properties of the stock formers used in industry in any way.
4. My aim was to investigate the effect of XOS dosing in a staple food of the European diet (biscuits) and how it affects its physical-sensory properties.

2. MATERIALS AND METHODS

My doctoral thesis is based on a series of multi-stage, interdependent experiments, in which I used 3 XOS products (Longlive Shandong Co. Ltd, China):

- 95P (powder-based, contains 95% XOS),
- 70P (powder-based, contains 70% XOS),
- 70L (concentrate, contains 70% XOS).

In the three series of experiments, I investigated the following topics:

- Experiment 1: Examination of the basic physical properties of XOS
- Experiment 2: Examination of the interactions of XOS with stock formers in aqueous solutions
- Experiment 3: The effect of XOS as a dietary fiber on the physical and sensory characteristics of biscuits

During the examination of rheological properties, the measurements were made with a Physica MCR 51 rheometer (Anton-Paar, Anton-Paar Hungary Ltd, Veszprém, Hungary). I used the Rheoplus v32 software to evaluate the data. I investigated the rheological behavior of each type of XOS and its concentration and temperature dependence by rotational measurement technique, by recording the flow curve of the samples (shear stress as a function of shear rate). During the investigation of the interaction between stock formers and XOS, I used the amplitude sweep method with the oscillation technique.

The texture of the biscuits was characterized using the Brookfield LFRA Texture Analyzer (LFRA 4500 Texture Analyzer; Brookfield, Middleboro, USA). The data was recorded and the stock profile analyzed using the TexturePro Lite v1.1 Build 4 software.

3. RESULTS

The introduction of XOS into the European Union novel food category was recently completed (Commission Implementing Regulation (EU) 2018/1648 of October 29, 2018); as a result, only limited information was available on the effect it exerts in foods common in European culture. Therefore, in my dissertation, I wanted to map the properties of XOS in a series of three experiments.

In my first series of experiments, I mapped the basic properties of XOS. In this framework, I examined basic physical characteristics, such as color, solubility, turbidity, pH, dry matter content, water activity and refraction. Based on my results, I have not discovered any properties that would prevent the widespread use of XOS in food. This was followed by rheological tests using the rotation method. I compared the flow properties of XOS with sucrose and fructo-oligosaccharide, as the most frequently used carbohydrates and oligosaccharides. With increasing concentration, I evaluated the viscosity of the carbohydrates and was able to draw the following conclusions: Studying the consistency values, I observed three different areas and thus behavior depending on the concentration. In the first interval, at low concentrations (between 0.5 and 4% dry matter), the consistency of FOS was significantly higher than the three types of XOS and sucrose, and there was no significant difference between the latter. In the second interval, between 4–40% dry matter content, the consistency of XOS powder type products (95P and 70P) increased more intensively than the other three saccharides (FOS, 70L and sucrose). In the third interval, at high concentrations (40–70%), the consistency of XOS 70P and 95P solutions was significantly higher than that of the other saccharides. When examining the viscosity of carbohydrates with increasing temperature, I can state that the viscosity of all oligosaccharides, with the exception of XOS 70P, was constant between 4 and 10°C, and then decreased until the end of the measurement. The decrease in viscosity was more intense at 10–50°C than between 50–90°C. The viscosity of XOS 70P was the highest and that of FOS was the lowest in the tested temperature range.

In my second series of experiments, I examined XOS in addition to three stock formers to see if it could modify the formation of the gel structure. I examined the samples using the amplitude sweep method, where I evaluated the obtained rheograms. During the examination of the samples made with gelatin, I discovered that the complex viscosity of gelatin gels containing 1% XOS as a function of shear stress was higher in the shear stress interval 0.01–1 Pa than that of samples with 3% XOS. Above a shear stress of 1 Pa, the complex viscosity of the samples containing 1% XOS and the control sample showed a decreasing trend, while the

complex viscosity values of the other samples had approximately constant values with increasing shear stress. So, XOS was able to increase the mechanical stability of the gels, that is, a I got a stronger gel. During the examination of samples made with two carbohydrate-based thickeners, xanthan and carob seed flour and their mixture, I came to the conclusion that XOS did not significantly affect the formation of the gel in the case of xanthan. due to the polarization of its side chains, it dissolves easily, the various oligosaccharide concentrations do not affect its gelling ability. In the case of samples made with carob seed flour, it had a significantly negative effect on the formation of the gel structure, since XOS presumably inhibited the gelling properties of carob seed flour with its water-binding capacity.

In the third experiment series, I used XOS in a convenience product, in a dose corresponding to the recommended daily consumption amount (0.12 g/kg body weight/day), at 1.4%. In one sample series of experiments with biscuits, I used various XOS products as flour substitutes, in the other series as sugar substitutes. I prepared the biscuits according to American standards and subjected them to both sensory and instrumental tests. I examined the appearance of the biscuits, i.e. their geometric properties: volume, height, color, and the baking loss. I recorded the chewing profiles of the biscuits with a stock profile analysis and evaluated the characteristic parameters. I compared the measured values with the results obtained by the sensory reviewers and made the following conclusions: XOS had an effect on the color, surface and crunchiness of the biscuits. The xylose in XOS affected the browning of the biscuits, giving the product a more baked appearance. Sensory evaluation scores show that all biscuits made with XOS were judged by the judges to be darker and more intensely colored. This result is consistent with the results of ΔE^{*ab} , that is, that the change of L^* , a^* and b^* compared to the control is to such an extent that consumers can easily distinguish it. The diameter of the cookies increased with each addition of XOS, and this was confirmed by my measurements by both instrumental and sensory methods. Due to the water-absorbing ability of XOS, the shape sensory properties score decreased significantly for all types of XOS, indicating that the biscuits became more uneven and irregular due to the addition of XOS. The homogeneity of the surface scored significantly lower when XOS was added, as more holes, pores and cracks appeared, so the biscuits became more inhomogeneous. The hardness did not change or increased slightly, the cohesiveness decreased, which was also reported by the reviewers. Crispness and chewiness increased. This means that in the presence of XOS, the biscuits were characterized by more intense crumbling. The oral cavity coating ability increased due to the addition of XOS. This may be due to the fat or the increased amount of water in the biscuit, which the XOS has displaced during the

interaction with the starch. Compared to the control sample, XOS increased the intensity of the baked, sweet and caramel flavor and aroma of the biscuits.

As a result of my research, I can state that XOS can be used well during product development to increase the fiber content while maintaining consumer acceptance.

4. NEW SCIENTIFIC RESULTS

1. With my results, I proved that the viscosity-modifying ability of xylo-oligosaccharides differs significantly ($\alpha=0.05$) from that of the carbohydrates used as control samples (fructo-oligosaccharides and sucrose) in the concentration range between 40-70%. The consistency index of the XOS 70P and 95P solutions was significantly higher compared to the other samples.
2. During my tests, I proved that in the temperature range between 4-60°C there were significant differences between the viscosity of the investigated carbohydrates (xylo-oligosaccharides, fructo-oligosaccharides and sucrose) than at higher temperatures (60-90°C). Thus, below 60°C, xylo-oligosaccharide 70P has a significant viscosity-increasing effect compared to sucrose and fructo-oligosaccharides.
3. I verified with my tests that the addition of xylo-oligosaccharides in the tested concentration (1-3 m/m%) did not significantly reduce the strength of the gelatin gel, which was shown by the ratio of the initial storage modulus (G') and the loss modulus (G'') values. Furthermore, it increased the stability of the gelatin gel against mechanical stress, which was indicated by the fact that the complex viscosity values of the control sample decreased with the most intensively increasing shear stress.
4. With my tests, I proved that the addition of xylo-oligosaccharides at a concentration of 1-3 m/m% did not significantly affect the stability and hardness of the xanthan gel, however, it significantly reduced the stability and hardness of the gels made with carob seed flour alone or in combination with xanthan.
5. During the investigation of the use of xylo-oligosaccharides in biscuits, XOS dosage caused the biscuits to brown significantly in the presence of oligosaccharides after baking for 10 minutes at 205 °C. Based on the evaluation of trained sensory evaluators, I found that xylo-oligosaccharides significantly increased the "baked character" of the biscuits, as indicated by the enhanced caramel aroma, darker color and crisper texture.

Furthermore, the addition of xylo-oligosaccharides had a sensory significant effect on biscuit sweetness, biscuit flavor and global flavor intensity.

5. CONCLUSIONS AND PROPOSALS

The XOS products did not have any physical or sensory characteristics that would have prevented their use in food after the first experiences. As a result, I see no obstacle to testing foods present in domestic culture.

XOS can become a potential sugar substitute due to its mild sweetening power and the same amount-forming effect as sucrose, and its combination with other sweeteners can lead to exciting results in the future. The flock-forming ability of the two XOS products at high concentrations, which was significantly higher than both FOS and sucrose, is remarkable. However, due to its negative health effects (bloating, diarrhea) above the daily intake value (0.12 g/kg body weight/day), its use in high concentrations in the food industry is not advisable.

The results I obtained during the effect of temperature changes were the same as those found in the literature (Park et al. 2001). The ability of the sample 70P to form clusters at low temperatures can make it suitable for use as a sugar substitute in perishable foods that require refrigeration, such as milk-based drinks or other dairy products, or to replace FOS. And its significantly higher capacity to form stock compared to FOS, experienced at higher temperatures, may enable XOS to replace it in foods that are typically served hot. From the results of the concentration and temperature change tests, I can draw the conclusion that XOS can primarily replace FOS, but also the most popular carbohydrate, sucrose, in certain food groups. It is advisable to use additional rotational measurements to investigate whether it is capable of increasing the consistency of my results in more complex systems such as milk, plant-based drinks or yogurt.

The results of my rheological tests using the oscillation method are in line with the results found in the literature regarding gelatin (Kasapis and Al-Marhoobi 2003, Mumtaz et al. 2008). While I did not experience the phenomenon of positive interaction with the other two stock developers. From this I draw the conclusion that XOS should be subjected to further tests to see if, for example, it is able to strengthen the gel structure of gelatin even in higher concentrations. Increasing the dosage of XOS may affect the interaction with stock builders differently from

what I have experienced, but with this, a larger amount of XOS corresponding to the daily intake value can potentially enter the body.

Although in the Far East XOS is more commonly used in various beverages, the use of XOS in biscuits has shown promising results, as a more intense mouth-coating texture with a more baked color intensity was created. The taste and smell properties were not negatively affected, the reviewers perceived a more intense caramel and sweet smell and taste. Its physical parameters were slightly affected by XOS, so based on my research it can be concluded that using it in higher concentrations would probably show a significant deterioration, but in order to avoid exceeding the daily intake value, it is not recommended to use it in higher concentrations anyway. It is advisable to carry out further sensory tests on a representative sample of consumers, and with a multi-stage iteration process, recipe development can be created in addition to maximizing the physiological effect of XOS, the most popular product. To conduct this, of course, it is worth taking the recommended examinations of international consumer tests as a basis (ISO 11136:2014).

When using it in different products, you should definitely focus on the foods you consume on a daily basis. It would be advisable to choose products that consumers already associate with a healthy label, such as yogurts and other fermented milk products, jellies with a high fruit content, jams and functional drinks.

6. PUBLICATIONS RELATED TO THE TOPIC OF THE THESIS

Journal articles with Impact Factor:

Juhász, R., Penksza, P., Sipos, L. (2020) Effect of xylo-oligosaccharides (XOS) addition on technological and sensory attributes of cookies FOOD SCIENCE AND NUTRITION 78 (10).5452-5460 (Q2, IF=1,747)

Penksza, P., Juhász, R., Szabó-Nóti, B., Sipos, L., (2020) Xylo-oligosaccharides as texture modifier compounds in aqueous media and in combination with food thickeners FOOD SCIENCE AND NUTRITION 8 (7) 3023-3030 DOI: 10.1002/fsn3.117 (Q2, IF=1,747)

Penksza, P., Boda, B., Szabó-Nóti, B., Németh, Cs., Juhász R. (2020) Utilization of xylo-oligosaccharides as prebiotics in soy milk JOURNAL OF HYGIENIC ENGINEERING AND DESIGN 30 pp. 53-57., 5 p. (Q4 IF=0,17)

Penksza, P., Banka, Zs., Kun, Sz., Pásztor-Huszár, K.; Németh, Cs., Tóth, A., Juhász, R. (2018) Utilization of xylo-oligosaccharides as prebiotics in yoghurt. JOURNAL OF HYGIENIC ENGINEERING AND DESIGN 22 pp. 66-71. , 6 p. (Q4 IF=0,17)

Penksza, P., Sárosi, R., Juhász, R., Manninger-Kóczán, K., Szabó-Nótin, B., Szakács, L., Barta, J., (2013) Jerusalem Artichoke Powder as Food Additive in Dairy Products and Fat Replacers ACTA ALIMENTARIA: AN INTERNATIONAL JOURNAL OF FOOD SCIENCE 42 : Supplement pp. 53-62. , 10 p. DOI: 10.1556/aalim.42.2013.suppl.7 (Q4 IF=0,462)

Sárosi, R., Manninger-Kóczán, K., Penksza, P. Juhász, R., Szabó-Nótin B., Szakács, L., Barta, J. (2013) Jerusalem Artichoke Powder as a Food Additive in Bakery Products ACTA ALIMENTARIA: AN INTERNATIONAL JOURNAL OF FOOD SCIENCE 42: Supplement pp. 73-80. , 8 p. DOI:10.1556/aalim.42.2013.suppl.9 (Q4 IF=0,462)

Conference full paper:

Penksza, P., Banka, Zs., Kun, Sz., Pásztor-Huszár, K.; Németh, Cs., Tóth, A., Juhász, R. (2017) Utilization of xylo-oligosaccharides as prebiotics in yoghurt In: Vladimir, Kakurinov (szerk.) Food quality & safety, health & nutrition Skopje, Macedónia: Consulting and Training Center KEY, pp. 109-109. , 1 p.

Conference proceedings (abstracts):

Juhász, R., Penksza, P., Szabó, D., Manninger, K., (2019) Comparison of dietary fiber enriched cookies In: M., Beatriz P.P. Oliveira; Joana, S. Amaral; Manuel, A. Coimbra (szerk.) Book of Abstracts of the XX EuroFoodChem Congress Lisszabon, Portugália : Sociedade Portuguesa de Química, p. 32

Juhász, R., Penksza, P., Stéger-, Máté, M. (2018) Xilo-oligoszacharidok engedélyeztetése az Európai Unióban új élelmiszer összetevőként MTA, Kertészeti és Élelmiszertudományi Bizottság, Élelmiszertudományi Albizottság workshop, Szent István Egyetem, Budai Campus, Budapest, 2018. december 6.

Penksza, P., Szabó-Nótin, B., Stéger-Máté, M., Juhász, R., (2018) Effect of xylo-oligosaccharides a new non-digestible oligosaccharide on fruit products In: Zoltán, Kende (szerk.) 17th Alps-Adria Scientific : Abstract book Gödöllő, Magyarország : Szent István Egyetem Egyetemi Kiadó, pp. 26-27. , 1 p.

Juhász, R., Penksza, P., Stéger-, Máté M., (2018) Effect of xilo-oligosaccharides a new non-digestible oligosaccharide on fruit products In: István, Dalmadi; László, Baranyai; Quang, Duc Nguyen Third International Conference on Food Science and Technology Budapest, Magyarország : Szent István Egyetem, Élelmiszertudományi Kar,

Penszka, P., Kun, S., Steger-Mate, M., Juhász, R. (2015) Preliminary Study on prebiotic effect of Xylo-oligosaccharides in comparison with Fructans In: Anon (szerk.) International Scientific Conference on Probiotics and Prebiotics, IPC2015: Proceedings, Budapest, Magyarország : [s. n.], p. 125