# BSc THESIS

Kawtar Zaazai BScThesis

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2023

Hungarian University of Agriculture and Life Sciences
Institute of Food Science and Technology

Program name: BSc Food Engineer
Bakery and pasta technologies and quality

Place of thesis preparation: Department of Grain and Industrial Plant Processing

# Effect of kneading on Mixolab performance of gluten free products

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Bakery and pasta technologies and quality

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# 1- *Introduction*

One of the most widely grown grains in the world is wheat, which is in favor of many stakeholders, including farmers, bakers, and customers. Wheat production requires a relatively small quantity of resources and input, but it is also convenient for bakers, businesses, and aggregable to consumers.

The interest in wheat-based products was, is and will always have spotlights, this is due to the different possibilities that wheat offers in terms of innovation and nutrition. However, in the last decade, the interest in gluten free grains and products increased drastically. The intolerance to gluten aka Celiac disease cases is one of the main influences of this augmentation of interest. Since gluten causes severe damage to the small intestine, which is where the immune system attacks its tissues, the cornerstone treatment for this autoimmune disorder is a lifelong complete avoidance of gluten. (Source, n.d.)

Moreover, promoted with a bad reputation, food containing gluten have been linked to depression, persistent fatigue, skin rashes, headaches, and digestive problems; and so, gluten-free diet became more of a trend, symbol of a healthy and more balanced lifestyle. Some of the most significant justifications would be to enhance gastrointestinal health, achieve a healthy weight, and improve the mood. (Henry, 2022)

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The question asked here, gluten free products are more of a necessity or a chosen way of living? In both cases, the demand for gluten-free products has increased dramatically over the past five years, by roughly 26%.

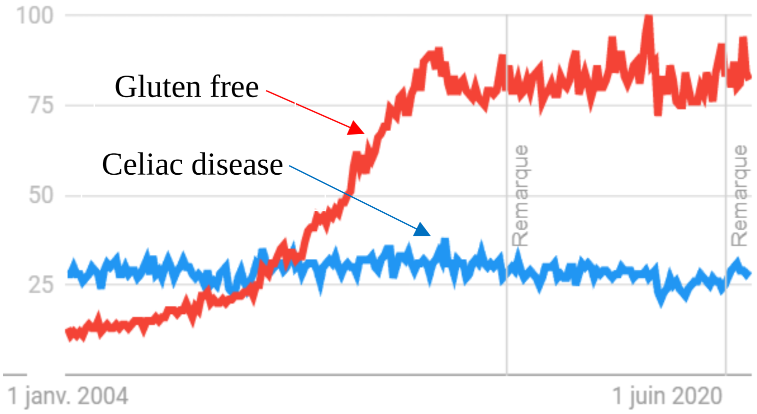

Figure 1. Research of celiac disease vs gluten free diet (Internet 1)

As demonstrated in Figure 1, interest in gluten-free diets is far outpacing research into Celiac disease. This can only indicate that individuals are motivated to change their lifestyles, not because they are ill or are aware of the true benefits of the gluten-free diet. As a result, the industries are pressured to produce gluten-free goods and to come up with new, appetizing ones. Since the product is gluten free, which means the removal / or not usage of the protein responsible for elasticity, chewiness and many other sensory effects, the industries struggle to create goods with the same qualities of gluten-based products, but in a gluten-free method. Consequently, research started to take amplitude and industries invested money and time into the gluten free area, looking at what makes gluten so special, and how it can be replaced all in the frame of gluten free products. Accordingly, Mixolab becomes crucial in determining the rheological characteristics of various doughs, including gluten- and non-gluten-based ones.

By simulating the process of mixing and baking, Mixolab measures the quality of the gluten-free dough and the dough's behavior, which can provide valuable information for bakers and food manufacturers. Essentially, it helps ensure consistent and high-quality baked goods by giving insight into how the dough will perform. But how accurate and trustworthy is the Mixolab? And how precise are the gluten-free dough measurements? By providing some answers to these questions, researchers and industries may make sensible choices about the Mixolab and the flours.

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## *2- Goals of the thesis*

As the interest in gluten free products increases, manufacturers and scientists must fill in the market to satisfy the consumer in need or desiring to adopt a more sophisticated diet. The production of gluten free products comes across many challenges, mainly being gluten free, which disables the production of quality products.

By analyzing the composition of the flours, and the characteristics of the dough, we can predict how the dough reacts with other ingredients like water, other flours, salt and additives. Consequently, we will have a better predictability of the final product in terms of sensory analysis, shelf life, acceptance by the consumers…, and optimize the final product.

Using the Mixolab, this groundbreaking instrument, the dough is put to the test for quality and rheological characteristics like stability, deformation, elasticity, and viscosity. Many tests, such as the farinograph and falling number test, are replaced by this instrument, which enhances many of their functions. It enables the investigation of various flours, including those containing gluten, like wheat, and others missing it, such as rice, sorghum, teff, amaranth, and oat. It is the ideal tool for assisting us in better comprehending this relatively recent topic.

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The fact that corn and rice are the two main gluten-free staple grains consumed throughout the world motivates scientists and engineers to advance their research in the gluten free area and create products that are safe for people with celiac disease and gluten allergies. Rice noodles, corn chips, polenta, maize tortillas, and many other dishes are among the most well-known recipes. The importance of this field has been demonstrated over the past 10 years by research publications that have concentrated on using gluten-free flour, analyzing them, and researching their final product.

In this paper, I will be using Mixolab to analyze corn and rice flour. Within my experiments, I focus on some aspects of Mixolab including repeatability of the protocols and the given data, to emphasize the reliability of the device and the previous studies. Additionally, I will go beyond the instructions of the Mixolab Handbook, using a different hydration and study its impact on the calculated data, the kneading and cooling process and comparing it to other hydrations (suggested by the Mixolab). Consequently, it will allow us to draw a picture of the importance of hydration on kneading and other characteristics.

# *3- Literature overview:*

## *3.1- Gluten free diet and celiac disease*

Due to increased public awareness, the gluten-free diet has seen remarkable growth over the past few years, whether for lifestyle enhancement or as a main treatment for those with celiac disease, nonceliac gluten sensitivity, or those with irritable bowel syndrome, numerous articles about gluten alternatives, celiac illness, and gluten-free products have recently been published. (Niland, 2018)

Gluten-free goods were first created for patients who were allergic to certain peptides found in gluten proteins (Catassi, 2008). Nonetheless, an increasing number of consumers are interested in wheat-free meals due to health concerns or a desire to eliminate wheat from their diet. In fact, studies identified several motivations, including health concerns, weight management, and perceived benefits of gluten-free products.

Engineers, on the other hand, face considerable technological challenges when making bread-like goods without gluten. Indeed, many gluten-free items on the market are of poor technological quality, with low volume, poor color, and disintegrating crumb, as well as a wide range of nutrient compositions, including low protein and high fat content (Matos Segura, 2011).

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The gluten-free diet used to treat celiac disease and non-celiac gluten sensitivity, is reviewed in "The Gluten-Free Diet: Safety and Nutritional Quality" by Saturni et al. explaining that gluten-free diet can be difficult to adhere to because everyday foods contain gluten, like wheat, barley, and rye. The safety of the gluten-free diet is discussed in this study, which points out that while it is usually seen to be safe for most individuals, it can also be linked to specific nutritional deficiencies, such as insufficient consumption of fiber, iron, and B vitamins. The authors stress the need for additional study on the long-term health implications of this dietary approach as well as the significance of a varied and balanced diet for people who follow the gluten-free diet. (Saturni, 2010)

Parallelly and despite this interest and the necessity of those products, Arias-Gastelum et al. painted in their research the sad reality, emphasizing the lack of many aspects of this area. In fact, and taking Northwestern Mexico as an example, (that can apply on many other countries), not only the paper showed the difficulties to have access to those products (economically, geographically…), but also the difficulties met as a gluten free intolerance in terms of travelling, socializing and access to information. This paper showcases the significant challenges for gluten free diet and the huge economic burden of this disease and points out that products without gluten are frequently more expensive than products with gluten, and higher production costs, a declining market, and more strict labeling regulations are all contributing factors. Therefore, the author points out that this price difference may make it difficult for people with low incomes to obtain gluten-free goods. (Arias-Gastelum M, 2018)

### *3.2- Rice and corn*

Corn and rice are two of the most important crops in the world, with a significant impact on global consumption and production. These two staple grains are consumed by billions of people around the world and play a crucial role in food security.

Corn, also known as maize, is the most widely grown crop in the world, with over 1 billion tons produced annually. It is a versatile crop that can be used for a variety of purposes, including food, animal feed, and biofuel. (Schafer, 2022)
Rice, on the other hand, is the third most widely grown crop in the world after corn, with over 700 million tons produced annually. It is a staple food for more than half of the world's population and is a crucial component of the diet in many Asian and African countries. In addition to being a source of carbohydrates, rice is also a good source of vitamins and minerals. (Wallach, 2022)

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Rice, corn, buckwheat, and other grains are all gluten-free grains that can be used in a variety of ways in gluten-free cooking and baking. They can be used alone or in combination with other gluten-free flours to create delicious and nutritious gluten-free products.
The research by Zhongkai Zhou et al. describes rice as one of the most significant foods in the world and a significant source of protein, carbs, and other nutrients. The article also discusses rice's useful features, such as its capacity to gelatinize and produce gels, its function in retaining and absorbing water, and its capacity to emulsify and foam. Additionally, the paper reviews the health benefits of rice, noting that it is a good source of complex carbohydrates, dietary fiber, and micronutrients such as iron and zinc, and discuss the potential health benefits of phytochemicals found in rice, including antioxidants and anti-cancer compounds. (Zhou Z. a., 2002)
Moreover, and since corn plays a significant role as a raw material in gluten-free products, research has consistently pushed to learn more about the characteristics of the leading substitute for wheat. Jeffrey A. Gwirtz’s research provides an in-depth review of the processing methods

used in the production of maize flour and corn meal food products. The article highlights the importance of maize as a major staple food in many countries, and discusses the various steps involved in maize processing, including cleaning, milling, and fortification.

Gwirtz discusses the composition of maize, which is primarily made up of starch, protein, oil, and fiber, and describes the various types of maize, such as dent, flint, and waxy maize, and their suitability for different types of food products (Figure 2). Besides, to attain the appropriate qualities in the finished product, Gwirtz also emphasizes the significance of regulating the particle size of the milled maize.

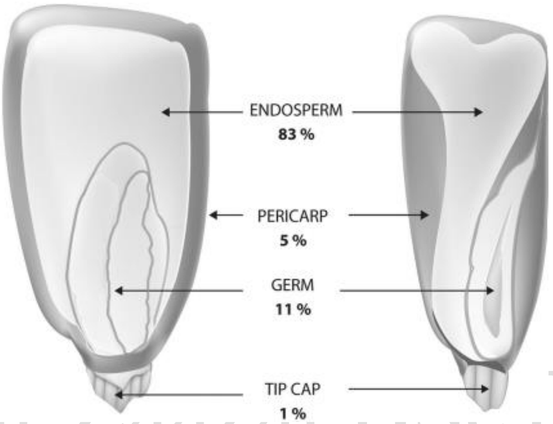

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Figure 2. Corn kernel (Gwirtz, 2014)

Both rice and corn are commonly used in gluten-free flours due to their natural lack of gluten. While rice flour has a low protein content and can produce poor baking properties, corn flour has a high protein content but can produce a dry and crumbly texture in baked goods.

Studies have compared the use of rice and corn in gluten-free flours and have found that the addition of other gluten-free flours or starches can improve the baking properties of both types of flour. The use of rice flour in combination with other flours or starches has been found to improve the texture and volume of baked goods (Mancebo, 2015), while the use of corn flour in combination with gums has been found to improve the texture and elasticity of baked goods (Anil, 2020). The choice of flour ultimately depends on the desired sensory attributes and nutritional profile of the final product.

### *3.3- Starch (amylose and amylopectin)*

As a substitute for wheat flour, which contains gluten that gives baked goods their elasticity and structure, starch plays a crucial role in gluten-free products. Starches can provide structure and texture to gluten-free products. There are several types of starches commonly used in gluten-free baking, including cornstarch, tapioca starch, potato starch, and rice starch.

When these starches are mixed with other gluten-free flours, such as almond flour or coconut flour, they can help to create a more cohesive dough or batter. This can result in a lighter, fluffier texture in baked goods, as well as improve chewiness and moistness.

Amylose, a type of linear polymer of glucose molecules, together with amylopectin, are the two main components of starch (Figure 3). Amylose is an important component in food production due to its role in determining the texture and functionality of starch-based products. Figure 3 shows its composition of a long, unbranched chain of glucose units connected by alpha-1,4 glycosidic linkages.

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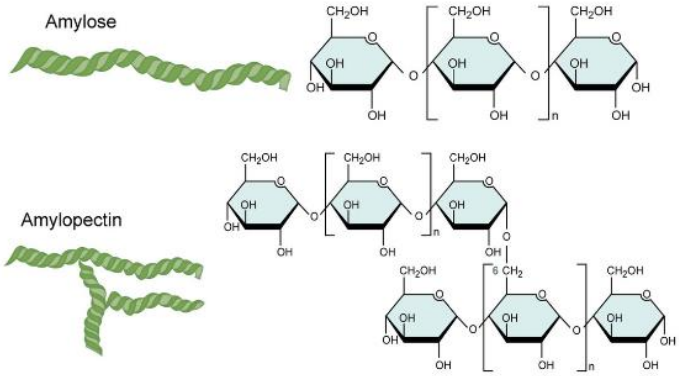

Figure 3. Starch composition (Internet 2)

Amylose plays a crucial role in the manufacture of food because it enables starch to gel when heated in the presence of water. Pastries, puddings, and sauces are just a few of the food products that are made using this gelation feature. Amylose not only contributes to texture but also to the viscosity and stability of food products.

Gluten-free products require suitable starch to provide the desired texture and structure. Stefan W. Horstmann et al. identify several key starch characteristics that impact the quality of gluten-free products, including amylose content, amylopectin structure, and gelatinization temperature.

The authors explain that the amylose content of a starch is a critical factor in gluten-free baking, as it affects the viscosity and gelation properties of the starch. High amylose starches are ideal for gluten-free products, as they improve the structure and reduce crumbliness. The amylopectin structure also plays a role in gluten-free baking, as it affects the texture and stability of baked goods. Starches with a high proportion of long chains of amylopectin have been found to produce superior gluten-free products due to their increased viscosity and gel strength. Additionally, the gelatinization temperature of a starch is also important in gluten-free baking, as it determines the optimal cooking temperature for the starch. The authors note that starches with a lower gelatinization temperature are more suitable for gluten-free products, as they can be cooked at lower temperatures without affecting the structure or texture of the final product. (Horstmann, 2017)

Starch gelatinization and retrogradation has been the subject of extensive investigation over the past 50 years, mostly because it affects the sensory and storage qualities of many starchy foods. However, numerous starchy food items benefit from starch retrogradation because of their textural and nutritive properties.

In their research, “The Principles of Starch Gelatinization and Retrogradation”, Masakuni Tako et al. discuss the importance of starch gelatinization and retrogradation in various food applications, including the manufacture of baked goods, pastries, and confectionery products. The degree of starch gelatinization is crucial to the quality and texture of these products, as it affects the properties of the starch and its interactions with other ingredients.

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The starch type, concentration, temperature, and pH are some of the elements that the author also emphasizes as it influences starch gelatinization and retrogradation. The ideal circumstances for gelatinization and retrogradation will depend on the particular application and intended product. (Tako, 2014)

By evaluating it from the standpoint of a raw product and demonstrating the relationship between the amylase ratio and the stickiness of the rice granules, Hongyan Li et al. reinforced earlier studies that revealed that the amylose concentration plays a significant influence in the texture of rice. Additionally, it has been shown in an earlier edition of Jeffrey D. Klucinec's case study using high amylose starch (HAS) that the ratio of amylose to amylopectin is still required to clearly comprehend the behaviors of starches, as there is a clear interactions between the two molecules influencing the final products . (Li, 2016) (Klucinec, 1999)

Parallelly, using purified (defatted) waxy and regular corn starch, removing all internal errors, revealed that not only water (used in different percentages) is a key component within this reaction, but also the relative presence of amylopectin and amylose to each other. This was with

Xing Zhou's extensive focus within his research on carbohydrate polymers, which highlighted the importance of various factors in terms of the gelatinization properties of starch related to amylose and amylopectin. (Zhou X. B., 2010)

H. Fredriksson et al. on the other hand, took a different approach to the starch analysis, using a small amount of water (roughly 50%). They supported their research with several significant prior experiments, which focused on the retrogradation and gelatinization behavior, the chemical characteristics, or how the molecular size influences the results, and the wide characteristics of amylopectin: using Gel permeation chromatography (GPC) and iodine staining, and comparison. (Fredriksson, 1998)

In general, studies on carbohydrate polymers and food chemistry all took an inventive approach to understand the behavior of starch molecules that are the basis of gluten-free products and to establish the significance of both amylose and amylopectin in the gel-forming process.

### *3.4- Gluten and starch*

It is crucial to avoid the confusion between gluten and starch, they belong to different categories, react differently in the dough, and have different sensory attributes when baked. This is especially true when it comes to gluten-free products, where it is more essential to understand the accurate distinctions.

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As previously said, one of the biggest challenges facing the gluten-free sector is the creation of products that share the same qualities as gluten-containing ones while remaining gluten free. The lack of gluten in the dough gives a lack of sensory characteristics that makes bread and other products pleasant to the consumers. The only and most obvious way to solve this issue is to establish the optimal conditions for starch found in many grains, including rice and corn, to somewhat resemble the gluten structure in the dough.

Mariusz Witczak et al. examined this insoluble protein, which has been researched from various perspectives and presents a major hope in producing quality gluten free products. This article supports Anne Van Der Borght et al.’s research but only this last one provides more detailed information by explaining the different constituents of the flour/ kernel and walking us step-by-step through the various methods used for the separation of gluten and starch. This article brings together various previously made research helping us to understand the origins of the first preparation, which dates back hundreds of years ago, and spotlighting the reasons for this intense attention toward gluten. (Witczak, 2016) (Van Der Borght, 2005)

In addition, almost all of the above-mentioned characteristics of starch —retrogradation, functionality, separation method, and the different constituents—, and more, like the starch digestibility from a dietary and medical standpoint, are discussed in an MDPI review titled “Starch Characteristics Linked to Gluten-Free Products”, summarizing previews research and great achievement. (Stefan W. Horstmann et al. 2017)

Hand in hand work to develop the gluten free area even more, and working together with reviews and research targeting to find a component to play the role of gluten in gluten free products, Alessandra Marti’s work tries to understand the importance of gluten in gluten free pasta, regardless of the small amount of information given about the starch arrangement, she tried to understand the behavior and the working mechanism of the main component (amylose and amylopectin), and the association with different factors (presence of gluten, temperature and so on). Furthermore, by employing diverse gluten-free cereals and their flours (such as rice, corn, sorghum, and pseudocereals) rather than refined gluten flours, the study shows the benefits from the lipids, proteins, and nutritional benefits that these gluten-free flours may provide. (Marti, 2013)

Elke K. Arendt et al. discuss the challenges and advancements in the production of gluten-free breads. The authors first provide an overview of celiac disease, gluten sensitivity, and wheat allergy, which are the primary reasons for the increasing demand for gluten-free products. Moreover, they discuss the importance of gluten in bread-making and the challenges of producing bread without gluten and highlights the use of alternative flours and ingredients such as rice flour, potato flour, and tapioca starch to improve the sensory qualities of gluten-free bread. The addition of hydrocolloids such as xanthan gum, guar gum, and psyllium husk have also been shown to improve the texture, structure, and volume of gluten-free bread.

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The research further explores the use of enzymes and sourdough fermentation to improve the flavor and texture of gluten-free bread and discusses the importance of sensory evaluation and consumer acceptance in the development of gluten-free breads. The review highlights the importance of developing gluten-free products that are comparable to traditional wheat-based products in terms of sensory qualities, such as taste, texture, and aroma. (Arendt, 2008)

### *3.5- Mixolab*

"Modern," "efficient," and "reliable” can be the words used to describe this ground-breaking dough measuring device, the Mixolab, which assesses the effectiveness of cereal flours. It is crucial to be able to describe the rheological behavior and pasting properties of flour/water doughs and starch/water mixes when they are subjected to the simultaneous action of mixing and heat transfer, characterizing the sample with water absorption, stability and elasticity, starch gelatinization, and other properties.

Traditional approaches, such as the amylograph, farinograph, falling number, and others, have been used for years to anticipate the behavior of various doughs. These machines take up a great deal of space in the lab, but they also require various setups and methods. While Mixolab offers a single device that combines all these analyses in 45 minutes of measurement. As a result, several studies have been done to contrast the Mixolab with other machines to demonstrate the efficiency of the Mixolab.

The most pertinent papers were those that were published in central Europe, such as those by Daniel vizitiu et al. University of Sibiu, Tamara Dabčević et al., University of Novi Sad, and Georgiana Gabriela Codină et al., University Dunarea de Jos, where they had a more or less similar objective of demonstrating a connection between the results of the Mixolab and those of the other instruments. While some described how the results have a strong correlation and can highly rely on the Mixolab, or somewhat different results were found but complementary, others found that the Mixolab has less prediction skills of mixing compared to farinograph. (Vizitiu, 2011) (Dabčević, 2009) (Codină, 2010)

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In her paper, Georgiana Gabriela Codinaha et al. demonstrated the link between some analytical features of wheat flour and Mixolab parameters during the initial kneading process (1st stage) at various mixing speeds. Contrary to recent research papers that are limited in their focuses on the effect of mixing circumstances on the dough at a specific point and do not provide an overview, Georgiana Gabriela Codinaha et al. chose another approach.

In fact, the Mixolab's ability to change mixing speed allows for a highly complex evaluation of changes in dough rheological properties during mixing and its behavior during bread making, and in this study, the effect of mixing speed at 80, 160, and 250 rpm, at different processing times, were analyzed at comparable levels of work input.

That being the case, the dough consistency and time point parameters provide the best explanation for the variation in work input. The mixing speeds used greatly influenced the patterns obtained during mixing, pasting, and gelling. As a result, changes in mixing speed affect all of the parameters recorded by the Mixolab on the curve. (Georgiana Gabriela Codină et al. 2010)

The Mixolab's various rheological qualities go beyond mixing speed. In practice, they are linked to something more fundamental and important in baking technology.

It is necessary to comprehend the structure, activity, and quality features of various flour streams before selecting them for different end-use products. Furthermore, granularity, damaged starch, protein content, ash level, and fat content, as well as enzymatic activity and fiber content, differ depending on the millstream type, which influence the way the flour should be approach in terms of kneading and heating, and has consequence on the final product. (Prabhasankar, 2000)

### *3.6- Mixolab and Flours*

Mixolab is a powerful tool for analyzing the rheological properties of wheat flour and its dough. This device measures the resistance of the dough to deformation and the energy required to maintain a constant deformation rate. The Mixolab test provides valuable information on the quality of wheat flour, including its gluten strength, starch behavior, and water absorption capacity. By analyzing these properties, bakers can better understand the functional properties of different wheat flours and optimize their use in bread-making.

In Tamara Dapčević Hadnađev et al research "Rheological properties of wheat flour substitutes/alternative crops assessed by Mixolab", showcases the different results of the grains based on climate factors and their influence on the characteristics of the components of the kernel, allowing the Mixolab to do its work at its finest. Additionally, the final product's baking attributes depend on the rheological characteristics of the wheat flour, which completely supports Iuliana Banu et al. research's employing Mixolab on the basis of the previous literature, such as its dependability compared with classical instruments. The use of the Mixolab goes further, in fact it is mainly used to find out the characteristics of the wheat flour, and yet the final major goal is more ambitious, which can be summarized in the rheological and sensory properties of the final products after mixing and baking. (Hadnađev, 2011) (Stoenescu, 2010)

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The variety of the finished bakery product is quite broad, and still provides this field with a lot of room for innovation. From bread to cakes to cookies, Kevser Kahraman et al. relied on Mixolab evaluation points (C2, C3, and C5, explained in Chapter 5) to determine the suitability of the flour in the cake batter and a prediction of the final products. Additionally, Hamit Koksel et al. used these rheological properties for baking quality purpose of bread from different wheat genotypes and stressed its importance. The modification of starch properties was shown to be critical for the structure of the final products. Thus, the wide range of wheat flour properties, from physicochemical to rheological, are a rich source of opportunities for product innovation, and highlights the importance of going deeper into the constituents of the flour. (Kahraman, 2008) (Koksel, 2009)

Even though Mixolab's major purpose is in wheat flour features, this helped us to gain a more comprehensive understanding of the various properties and traits of gluten generally. Mixolab might be a terrific instrument to boost the current progress because the trend is moving toward gluten-free products, which is encouraging professionals to delve deeper into this field.

While using pseudocereals like amaranth, quinoa, and buckwheat, are used in gluten-free breads with increased amounts of essential nutrients like protein, fiber, calcium, iron, vitamin E, and polyphenols, the industry still acknowledge that the properties of wheat are distinctive and difficult to mimic.

In light of this, the experiment conducted by Tamara Dapcevic Hadnadeva et al. aims to substitute wheat flour for gluten-free flour in the manufacturing of bread. Investigating the behavior of pure non-wheat flours during mixing and heating utilizing Mixolab. Their rheological characteristics—including water absorptions, stabilities, and levels of mechanical weakening—were compared with those of wheat flour. It was observed that rice and buckwheat flour mixes would produce the optimum rheological profile view, meanwhile, Mixolab profile of wheat flour was found to be in the middle of the profiles of rice and buckwheat flour. (Dapcevic, 2011)

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Notably, the purpose of using the Mixolab started changing, moving toward more advanced, powerful and innovative products like Nixtamalization (Traditional maize preparation involving cooking and steeping dried kernels in an alkaline solution, typically water), forecasting the degree of starch pre-gelatinization and starch damage (Espinosa-Ramírez, 2020), and creative ways using new protocols of the Mixolab to push the boundaries of this device even further.

In conclusion, the gluten-free diet has gained increasing popularity in recent years due to the rise of celiac disease and gluten sensitivity. As a result, there has been a growing demand for gluten-free products and a need for gluten-free grains and flours. The development of gluten-free products presents challenges for bakers due to the absence of gluten, which plays a critical role in the structure and texture of baked goods.

The above-mentioned literature showed the use of Mixolab technology for being a valuable tool in the analysis of gluten-free flours. It can measure the rheological properties of gluten-free dough and provide key information about the quality and functional properties of different gluten-free flours.

There are several gluten-free grains and flours available, including rice, corn, sorghum, quinoa and many more. However, the functional properties of these gluten-free flours can vary significantly, making it essential to understand their rheological properties.

To sum up, the development of gluten-free products presents challenges for bakers, but the use of Mixolab technology can provide valuable insights into the rheological properties of gluten-free flours. By understanding the behavior of different gluten-free flours, bakers can develop high-quality gluten-free products with improved texture and structure.

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## *4- Materials and Methods:*

### *4.1- Materials*

Mixolab (figure 4)

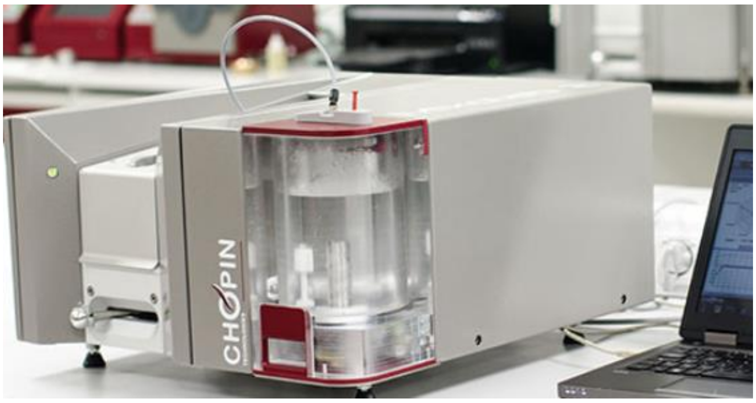

Figure 4. Mixolab - Chopin Technology (Internet 3)

- Balance: To weigh the flour calculated by the Mixolab
- Moisture Analyzer: Analyzes the moisture content of floor, which is introduced within the data of the Mixolab to allow the calculation of the amount of water required to reach the desired hydration level.
- Distilled water
- Excel / Data analysis pack: to analyze data with ANOVA
- Samples: rice and corn flours:

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For the commonly used gluten free flours, corn in rice were top listed around the world, not only the nutritional value is high but also it is wildly available due to high production especially in Hungary.

The amount used of each flour is different according to the calculation of the Mixolab and the moisture content of each flour.

Rice flour

Corn flour, Type 1

Corn flour, type 2

### *4.2- Method*

1. Select the desired protocol.
2. The moisture content is measured using a couple of grams of the flour in the Moisture Analyzer.
3. After receiving the moisture content of the flour, we enter the necessary data into the Mixolab "test preparation sheet".
4. Select a hydration base ("As-is", "15% base", "14% base", "dry matter"). The recommended Hydration value depends on the grain type and the suggestion of the Mixolab. For our samples, b14 was the recommended hydration.

   The water absorption capacity of a flour is the hydration required to bring a dough to a given maximum consistency.

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Table 1. Suggested hydrations by the Mixolab Handbook

| Flours | Suggested Hydration |
|---|---|
| White flour | 55% |
| Corn flour | 60% or 115% - b14 * |
| Rice flour | 55% - b14 |

* Reference system used to express hydration, most commonly use bases are: The 14% base, The 15%, The dry matter base (Dubat, 2016)

5. Position the dough mixer in its housing, close the lid (Figure 5)
6. Weigh the quantity of samples calculated by the Mixolab software.
7. Start the test.

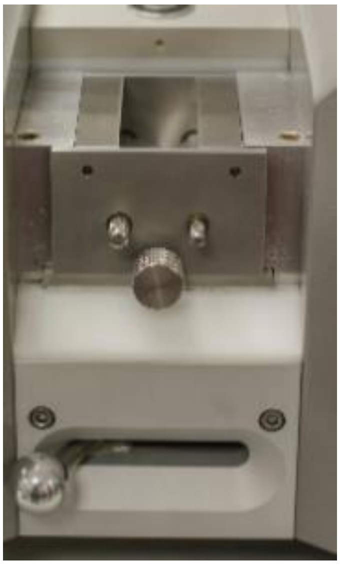

Figure 5. Mixing part (Internet 4)

Table 2. Settings used for Corn and Rice flours.

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| | | Corn | Rice |
|---|---|---|---|
| 1 | Dough mass | 75g | 100g |
| 2 | Kneading speed | 80 rpm | |
| 3 | Tank Temperature | 30 °C | |
| 4 | Temperature of the 1st step | 30 °C | |
| 5 | Duration of 1st step | 8 min | |
| 6 | 1st temperature gradient | 4 °C/ min | |
| 7 | Duration of 2nd step | 7 min | |
| 8 | 2nd Temperature gradient | -4°C /min | |
| 9 | Temperature of 3rd step | 50 °C | |
| 10 | Total analysis time | 45min | |

After 45 min (necessary time for the analysis of the dough), a thorough washing of the mixer between each experiment is required (not to influence the next experiment).

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# *5- Results and discussion*

## *5.1- Protocol vs experiment:*

By conducting a series of tests using rice flour with a hydration level of 55%, we can evaluate the degree of correlation between the experimental results and the expected outcomes presented in the official Mixolab Handbook. To obtain a reliable dataset, we conducted three repetitions of the rice protocol measurement following Mixolab instructions and calculated the mean value, to obtain a single set of data, represented in Figure 8 and Table 3.

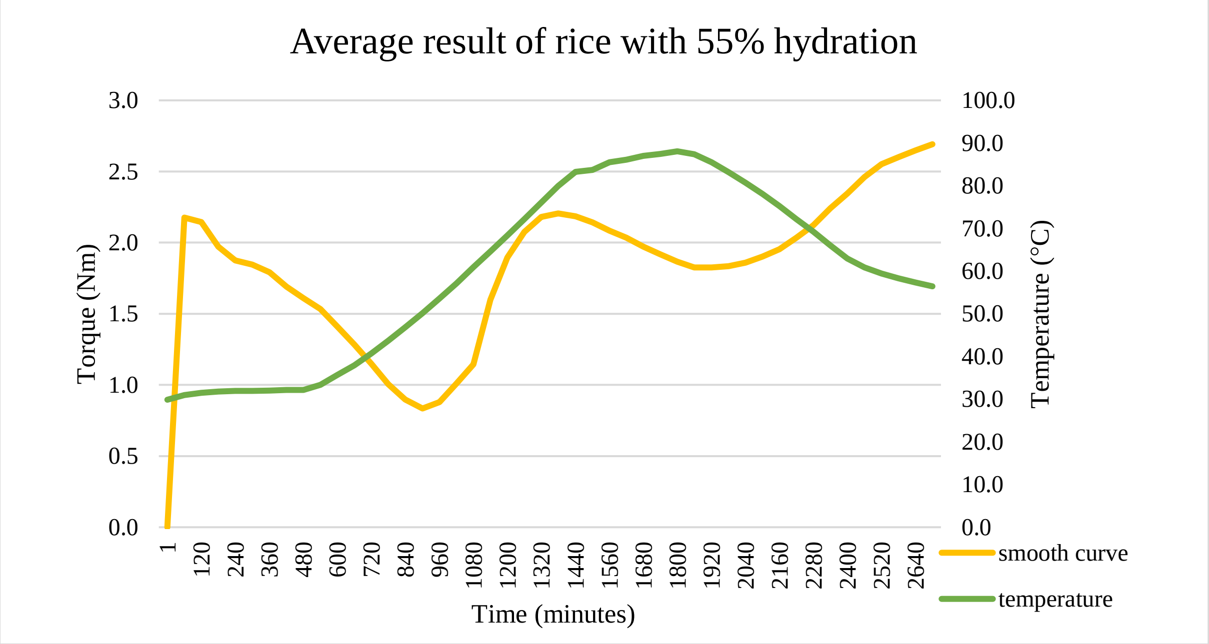

Figure 6. Plot of Rice flour experiment

Table 3. Key points of rice flour experiment with 55% hydration (mean value of 3 repetitions)

| | Time (Min) | Torque (Nm) | Dough Temperature(°C) | Amp. (Nm) | Stability (Min) |
|---|---|---|---|---|---|
| C1 | 0.970 | 2.607 | 30.867 | 0.153 | 1 |
| CS | 8.000 | 1.609 | 32.133 | | |
| C2 | 15.440 | 0.811 | 51.633 | | |
| C3 | 19.223 | 1.452 | 65.500 | | |
| C4 | 22.430 | 1.261 | 69.100 | | |
| C5 | 45.007 | 2.693 | 56.467 | | |

Being aware of the composition (proteins, starch, lipids, moisture content…) difference of the flours, Mixolab offers 4 potential outcomes, which 4 samples when experimenting with rice flour. (Figure 9, Table 4)

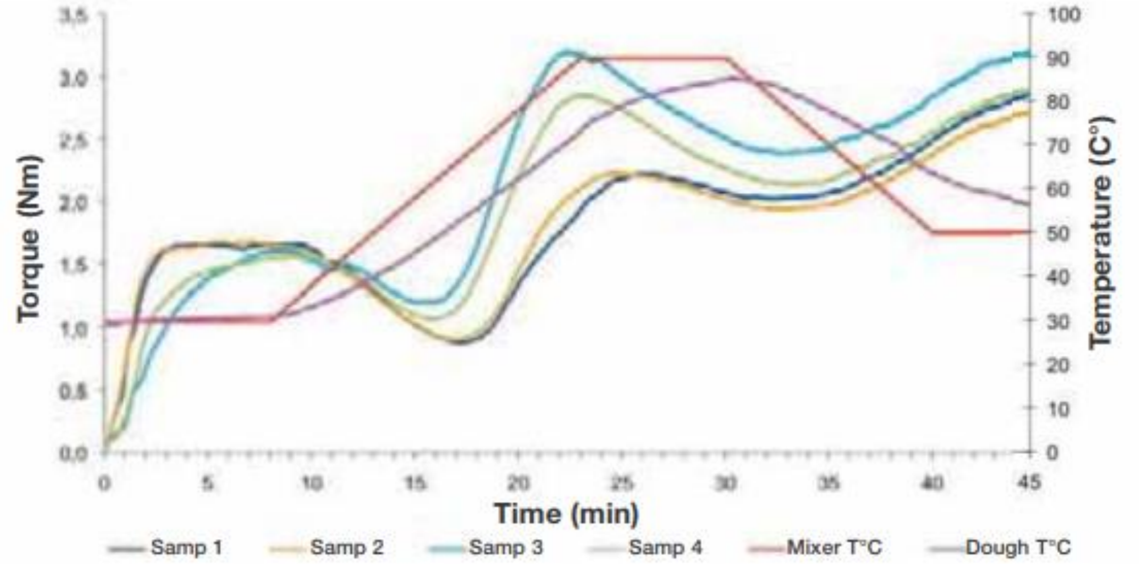

Figure 7. Test conducted with Chopin+ on Rice flour with 55% Hydration. (Internet 5)

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Table 4. Rice samples outcome results by Mixolab.

| | WA (% b14) | C1 (Nm) | C2 (Nm) | C3 (Nm) | C4 (Nm) | C5 (Nm) | Stability (min) | C3-C2 (Nm) | C3-C4 (Nm) | C5-C4 (Nm) |
|---|---|---|---|---|---|---|---|---|---|---|
| sample 1 | 55 | 1.66 | 0.88 | 2.22 | 2.01 | 2.87 | 9.50 | 1.34 | 0.21 | 0.86 |
| sample 2 | 55 | 1.68 | 0.90 | 2.24 | 1.93 | 2.70 | 9.30 | 1.34 | 0.31 | 0.77 |
| sample 3 | 55 | 1.61 | 1.19 | 3.20 | 2.38 | 3.19 | 7.70 | 2.01 | 0.82 | 0.81 |
| sample 4 | 55 | 1.57 | 1.07 | 2.86 | 2.13 | 2.87 | 8.50 | 1.79 | 0.73 | 0.74 |

C1, used to determine water absorption, and beginning of stability, we observed a higher torque usage indicating increased water absorption at the beginning of the experiment, even earlier than the protocol. Additionally, as the temperature of the dough rises, the torque consistency decreases, depending on the quality and heat resistance of the proteins. C2, marking the end of protein weakening and the start of starch gelatinization, indicates a lower dough residency with increasing temperature. This could be due to the absence of gluten and only the presence of other proteins such as glutelin, prolamin, and α-globulin, which offer no significant resistance when kneading the dough.

At a particular temperature, the starch gelatinization phenomena take over and lead to an observed increase in consistency. The degree of this increase is influenced by the starch quality and amylase activity. This effect is evident with C3, which marks the onset of amylase activity, and produces a torque of 1.45 Nm.

Furthermore, the temperature values reach their maximum between C3 and C4, peaking at 88.07°C, and as amylase activity increases, degrading starch molecule, the consistency decreases between these two points. However, the difference in consistency is only slightly significant, with a torque difference of merely 0.191 Nm, which proves a low amylase activity and no increase of the consistency of the dough.

During the cooling process (Figure 10), the starch undergoes retrogradation, leading to a higher consistency of the product, as evidenced by the results of C5 at the end of our experiment. The torque measurement of 2.69Nm is equal to the torque suggested in sample 2 by the handbook.

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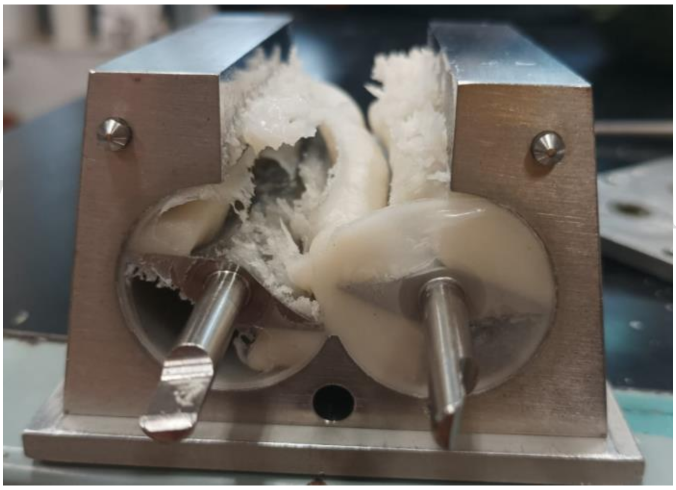

Figure 8. Cooled rice flour dough after kneading process.

The rice flour experiment differs from the protocol in a number of ways as the expected torque and temperature values were not reached, which could be explained by a number of reasons, including mistakes made during the loading stage, the content of the flour, environmental humidity levels, and others. Beyond these differences, there are some clear parallels between our experimental rice dough and the suggested samples by the Mixolab Handbook, in terms of the behavior of the dough, the alignment of the curves, and the final torque. This suggests that while the kneading process can be irregular, the final product is going to be reasonably consistent with what was expected.

### *5.2- Review of the repeatability*

Repeatability is used to describe how consistently Mixolab results are produced. It is crucial to adhere to defined processes for sample preparation, loading, and measurement in order to guarantee reproducibility in Mixolab tests.

To get an average result that can give a more accurate indication of the true value of the dough qualities, it is crucial to repeat the measurements numerous times. To estimate the degree of measurement variability, we can also calculate the standard deviation of the data.

Repeatability and reproducibility allow us to have trust on the experiment and precise results used in advanced research. The official Mixolab book mentions repeatability and reproducibility and how the results difference is not significant.

To ensure the consistency of the results, we conduct experiments using two types of corn flour, both subjected to a uniform hydration percentage of 60%. Each type of flour is tested three times, enabling us to oversee the coherence of the outcomes (Table 5).

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Table 5. Key points of corn type 1 with 60% hydration repeated 3 times.

| corn1 | Time (perc) | Torque (Nm) | dough Temperature :(°C) |  | corn2 | Time (perc) | Torque (Nm) | dough Temperature :(°C) |  | corn2 | Time (perc) | Torque (Nm) | dough Temperature :(°C) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C1 | 0.8 | 0.608 | 29.3 |  | C1 | 0.83 | 0.416 | 30.7 |  | C1 | 0.85 | 0.434 | 30.8 |
| CS | 8 | 0.199 | 29.8 |  | CS | 8 | 0.267 | 30.8 |  | CS | 8 | 0.254 | 30.9 |
| C2 | 13.9 | 0.145 | 44.8 |  | C2 | 1.23 | 0.113 | 30.6 |  | C2 | 1.52 | 0.124 | 30.8 |
| C3 | 15.13 | 0.881 | 48.1 |  | C3 | 4.47 | 0.311 | 30.6 |  | C3 | 9.65 | 0.31 | 33.9 |
| C4 | 18.07 | 0.626 | 59 |  | C4 | 5.2 | 0.207 | 30.7 |  | C4 | 11.18 | 0.22 | 37.6 |
| C5 | 45 | 2.95 | 56.7 |  | C5 | 45.02 | 3.021 | 57.2 |  | C5 | 45.02 | 3.08 | 57.3 |

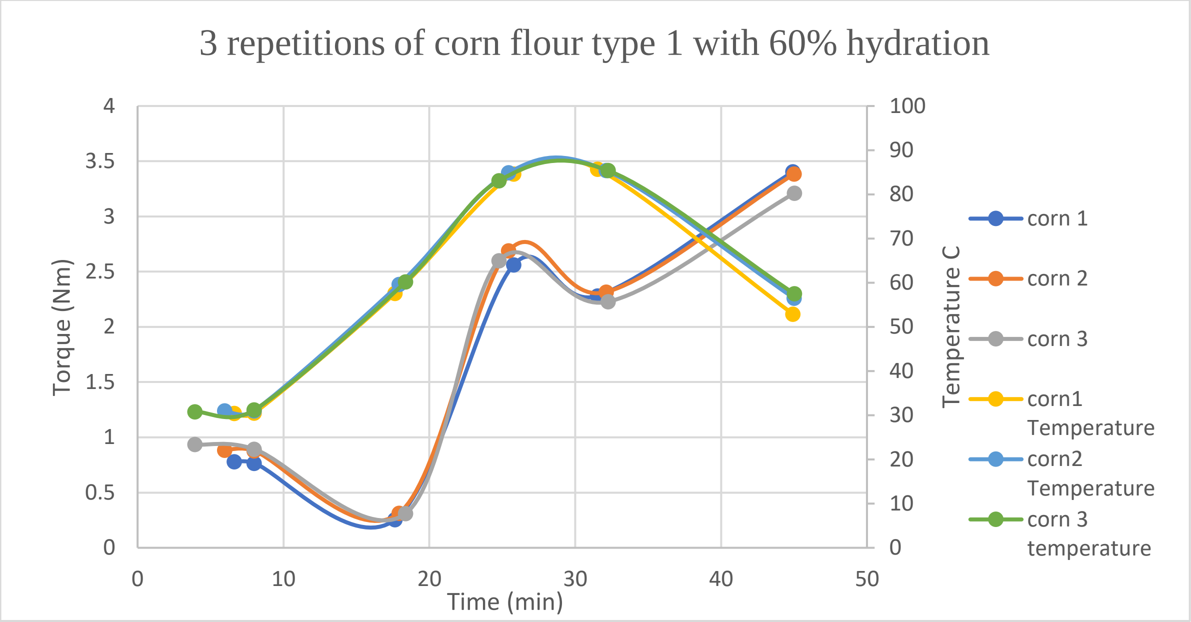

Figure 9. Plotting of the main points of Corn flour Type 1 experiemnt with 60% hydration

The first type of flour, subjected to 60% hydration, demonstrated a strong correlation across the 3 repeated experiments. As shown in Figure 9, we are able to observe the amount of torque involved and the temperature variation during the 45-minute kneading process. Notably, the five primary points on the Mixolab graph (C1, C2, C3, C4, and C5) displayed closely aligned data, signifying the experiment's good repeatability, and highlighting the Mixolab accuracy.

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In contrast, when utilizing corn flour type 2 (a different brand) with the same hydration profile of 60%, the results did not exhibit the desired level of coherence. Despite utilizing the same methodology, Figure 10 indicates an obvious difference between the three repetitions. Notably, there was variance in both the torque applied by the Mixolab and the temperature achieved.

While the blue, grey, and orange curves all exhibited a positive, smooth slope towards the end, samples corn b and corn c displayed an undefined start, with an unpredictable and disordered kneading force. This observation could be justified by the formation of lumps within the dough or water concentration in specific parts of the mixture. However, after 20 minutes of kneading, the torque stabilized, and the rhythm became closer to the first sample (corn a), indicating that the dough had been homogenized, allowing all three samples to reach a nearly identical final force (average of 3 Nm).

Temperature is a crucial factor in dough making, in addition to kneading force. It affects various aspects such as gelatinization process, carbohydrate and enzyme degradation, and dough development (if yeast is present). In our experiments, the temperature of the first corn dough reached its peak (around 60°C) at the mid-time of the kneading process (22 minutes) and then slightly decreased. This corresponds to starch gelatinization and hot gel stability. However, the second and third experiments (corn b and c) took a different path, with the temperature gradually increasing until it reached 60°C at the end of the experiment. This unexpected temperature trend did not correspond to starch gelatinization and retrogradation.

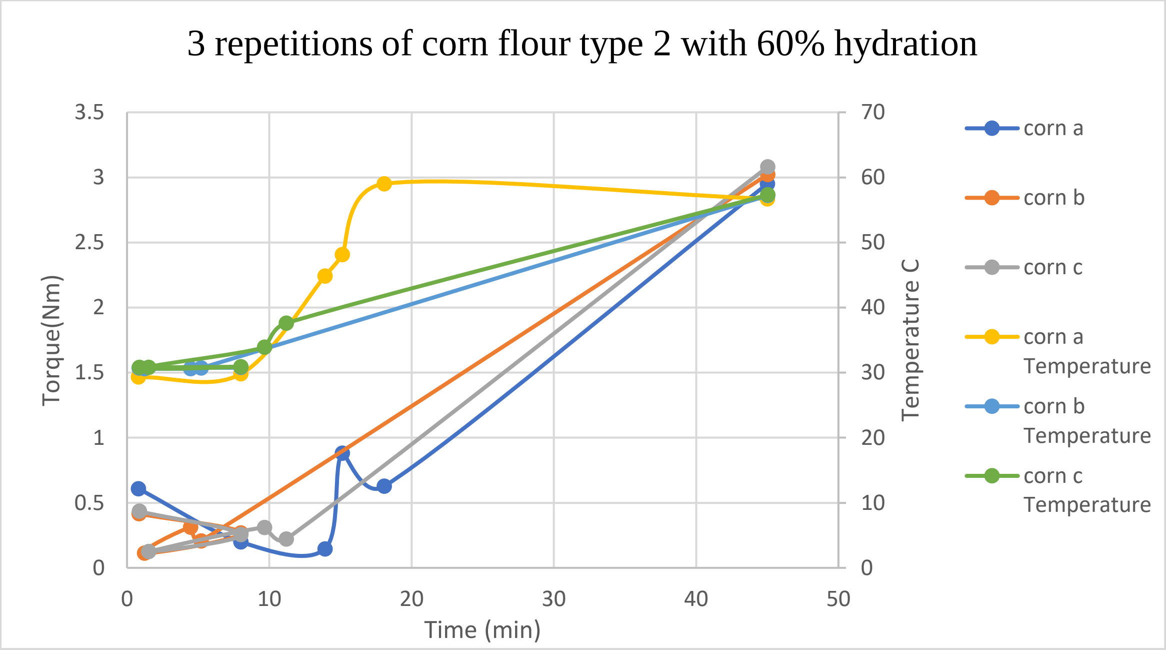
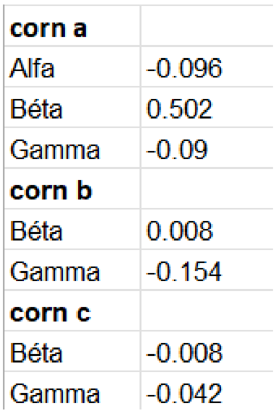


Figure 10. Plotting of the main points of Corn flour Type 2 experiemnt with 60% hydration.

Due to the confusing data in the second and third experiments, the calculation of alpha (Slope of curve between end of period at 30°C and C2 corresponding to the Protein weakening speed under the effect of heat) was unsuccessful. Only beta and gamma were measurable, and had different results as shown in the data of Table 6.

It is obliviously clear that the data measured by Mixolab are far from being close to each other, which demonstrates the incoherence of the 3 experiments made in a row and supposed in the same conditions.



| | |
|---|---|
| **corn a** | |
| Alfa | -0.096 |
| Béta | 0.502 |
| Gamma | -0.09 |
| **corn b** | |
| Béta | 0.008 |
| Gamma | -0.154 |
| **corn c** | |
| Béta | -0.008 |
| Gamma | -0.042 |

Table 6. Slopes calculated by Mixolab for Corn flour Type 2 with 60% hydration.

### *5.3- Corn flour- Comparison between 2 hydrations*

In theory, Mixolab protocol for corn suggests 2 hydration percentages: 60% and 115%, with 100 g flour. In practice, we encountered 2 obstacles:

1- When calculating the moisture content of the flour (using the moisture analyzer) and entering the data into the Mixolab setting, for a Hydration of 60%, it suggested a flour amount of 60.94g, an amount less than the recommended one by the official protocol (100g).

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2- With a moisture content of 10,6%, asking for hydration 115% (hydration suggested by the Mixolab Handbook), showed an error message. In fact, when Mixolab calculated the water needed, an amount higher than 50ml (maximum amount of water that the Mixolab can provide), which was not possible to deliver by the device. As a result, we looked for another hydration percentage. 92% was the closest percentage which calculated the need of 49,90ml water and 50,10g corn flour.

After 3 repetitions of each hydration protocol (60% and 92%), of corn flour type 2, we calculate the mean values of each 3 repetitions, which will enable us to get two sets of data, and to compare the outcomes of the torque calculated by Mixolab.

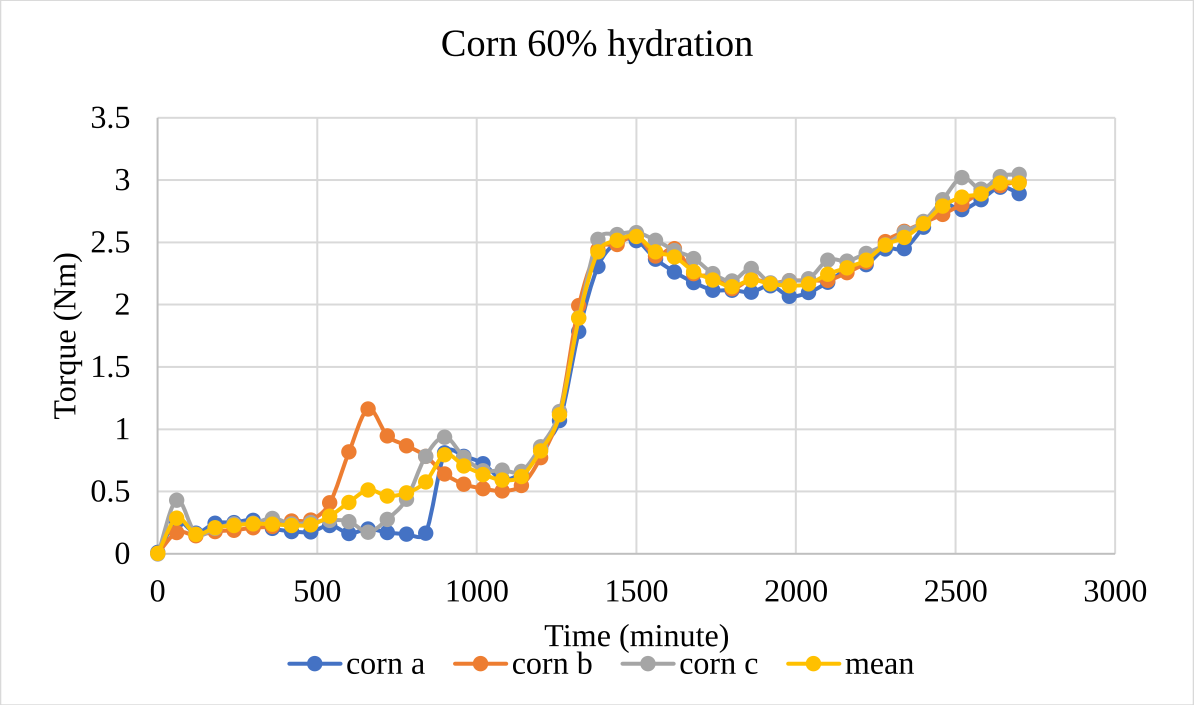

Figure 11. Plotting of the torque of corn dough hydrated with 60% and its mean.

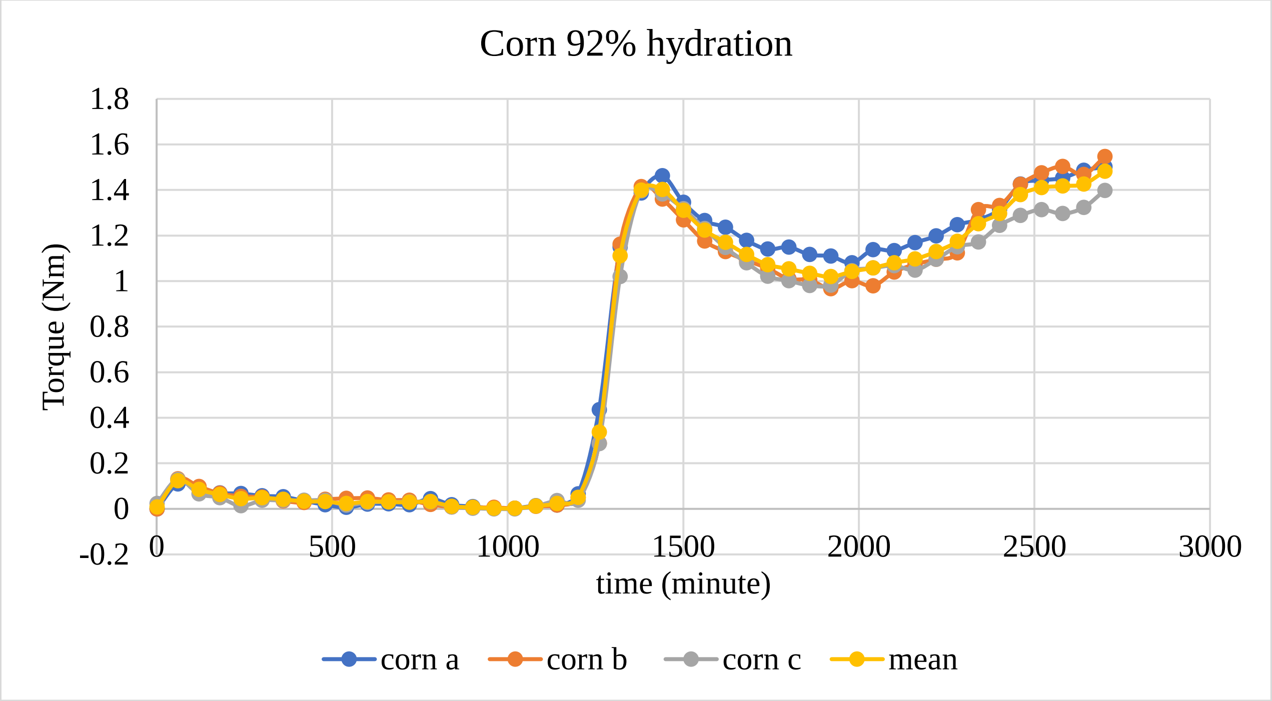

Figure 12. Plotting of the torque of corn dough hydrated with 92% and its mean.

Table 7. Corn flour type 2, with 60% and 92% hydrations and calculated torque (Nm)

| Time (in min) | Torque (Nm) | |
|---|---|---|
| | 60% | 92% |
| 1 | 0.005333 | 0.008 |
| 60 | 0.287 | 0.123333 |
| 900 | 0.796667 | 0.006333 |
| 1500 | 2.549667 | 1.310333 |
| 1800 | 2.146 | 1.053333 |
| 2400 | 2.650333 | 1.296667 |
| 2700 | 2.976667 | 1.481667 |

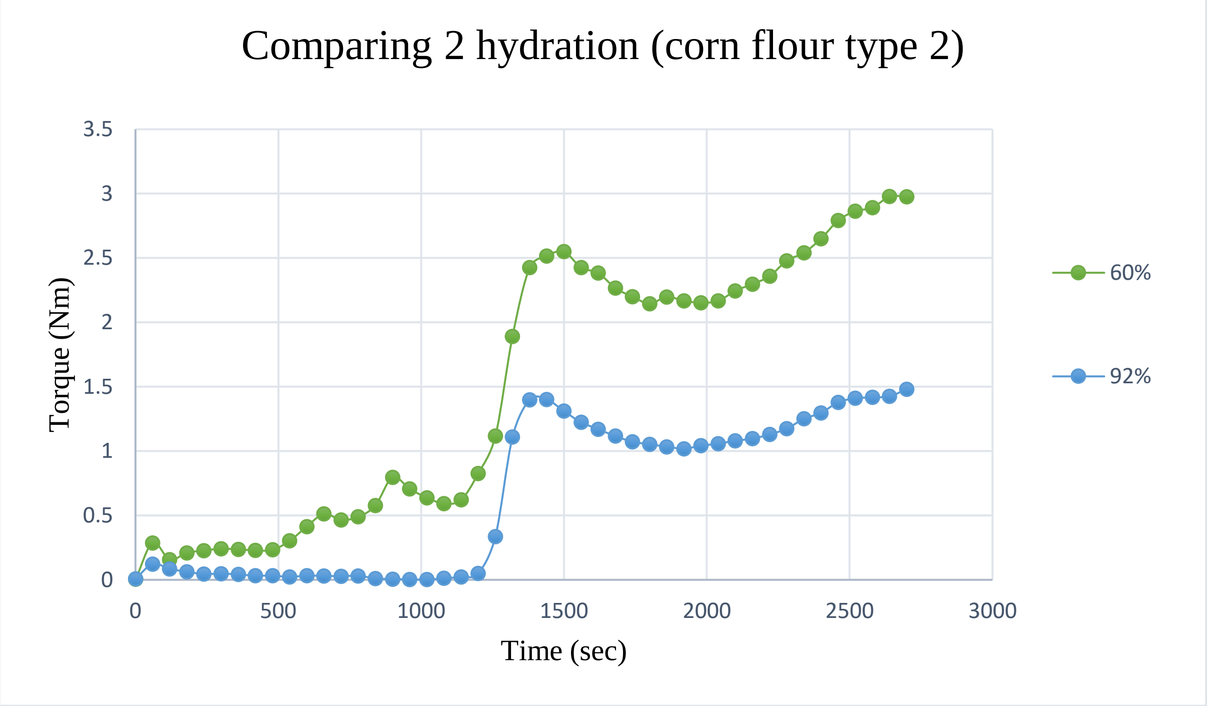

Figure 13. Comparing the mean values of corn flour type 2, with 60% and 92% hydrated

Analysis of the data: using one way ANOVA, we study if there is a significant difference between the hydration percentages.

Initial hypothesis: the difference between the mean values of the 2 sets of data is not significant.

Alternative hypothesis: there is a significant difference between 2 sets of Data.

Table 8. Analysis by ANOVA

Anova:

Single Factor

SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Column 1 | 46 | 68.44733 | 1.487986 | 1.08839 |
| Column 2 | 46 | 30.22233 | 0.657007 | 0.36125 |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 15.88207 | 1 | 15.88207 | **21.9115** | 1E-05 | **3.94687** |
| Within Groups | 65.2343 | 90 | 0.724826 | | | |
| Total | 81.11637 | 91 | | | | |

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The critical F-value at the 0.05 level of significance is 3.946, which is less than the computed F-statistic F= 21.911, evidence that the difference between the means of the 2 data sets is statistically significant.

Therefore, based on the ANOVA results (Table 8), we can conclude that there is a significant difference between the values of the torque reached at 60% and 92% hydrations. Consequently, the dough consistency, the kneading effect, and the resulting cooked product will all be different.

### Comparing Mixolab computed parameter

From the 3 repetitions, we calculate the average values for each hydration (60% and 92%), allowing us to compare the key points of the kneading process of the dough.

Table 9. Key points calculated (torque Nm) during kneading process (corn 60% hydration)

| corn AVG (60%) | Time (min) | Torque (Nm) | Dough Temperature: (°C) | Amp. (Nm) | Stability (perc) |
|---|---|---|---|---|---|
| C1 | 0.827 | 0.486 | 30.267 | 0.077 | 0.733 |
| C2 | 5.550 | 0.127 | 35.400 | | |
| C3 | 9.750 | 0.501 | 37.533 | | |
| C4 | 11.483 | 0.351 | 42.433 | | |
| C5 | 45.013 | 3.017 | 57.067 | | |

Table 10. Key points calculated (torque Nm) during kneading process (corn 92% hydration)

| corn AVG (92%) | Time (min) | Torque (Nm) | Dough Temperature: (°C) | Amp. (Nm) | Stability (perc) |
|---|---|---|---|---|---|
| C1 | 23.57 | 1.44 | 81.77 | 0.05 | 3.20 |
| C2 | 32.08 | 1.01 | 84.93 | | |
| C5 | 45.01 | 1.46 | 55.70 | | |

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From the two tables 9 and 10, listed above, we can clearly compare the effect of the two different hydrations and their impact on the dough by analyzing the key points of the results of the Mixolab.

Water absorption is an important parameter to consider when making corn-based products such as tortillas, tamales, and pupusas. The amount of water added to the corn flour affects the dough's texture, elasticity, and handling properties.

In fact, we can observe that the C1, used to determine the water absorption, has a much higher torque in the dough hydrated at 92%. However, obtained after a much longer time (23 min), compared to the dough hydrated at 60%, which has a smaller torque (0.4 Nm), but calculated within the first seconds of the experiment (0,827 min). Additionally, the amplitude of C1 at the hydration of 60% is higher than the hydration at 92% (0.077 Nm > 0,05 Nm), proving that the dough when hydrated at 60%, during the first minutes, demonstrates a better elasticity than when hydrated at 92%.

C2 measures the protein weakening as a function of mechanical work and temperature, which reaches 1.01 torque at 32.08 min when the hydration is 92%, compared to 0,12 torque measured at 5.5 min when the dough hydrated at 60%. The difference of the time when C2 was measured explains the huge difference of the toque calculated. In Fact, it shows that within 92% hydration, the weakening of the mechanical strength happens later than when the dough is hydrated at 60%.

C3, that measures starch gelatinization and C4, that measures hot gel stability, were only measured when the dough hydrated at 60%, and was not measured at the hydration of 92%, this could be explained by the fact that the 92% hydration is not a protocol suggested by the Mixolab book.

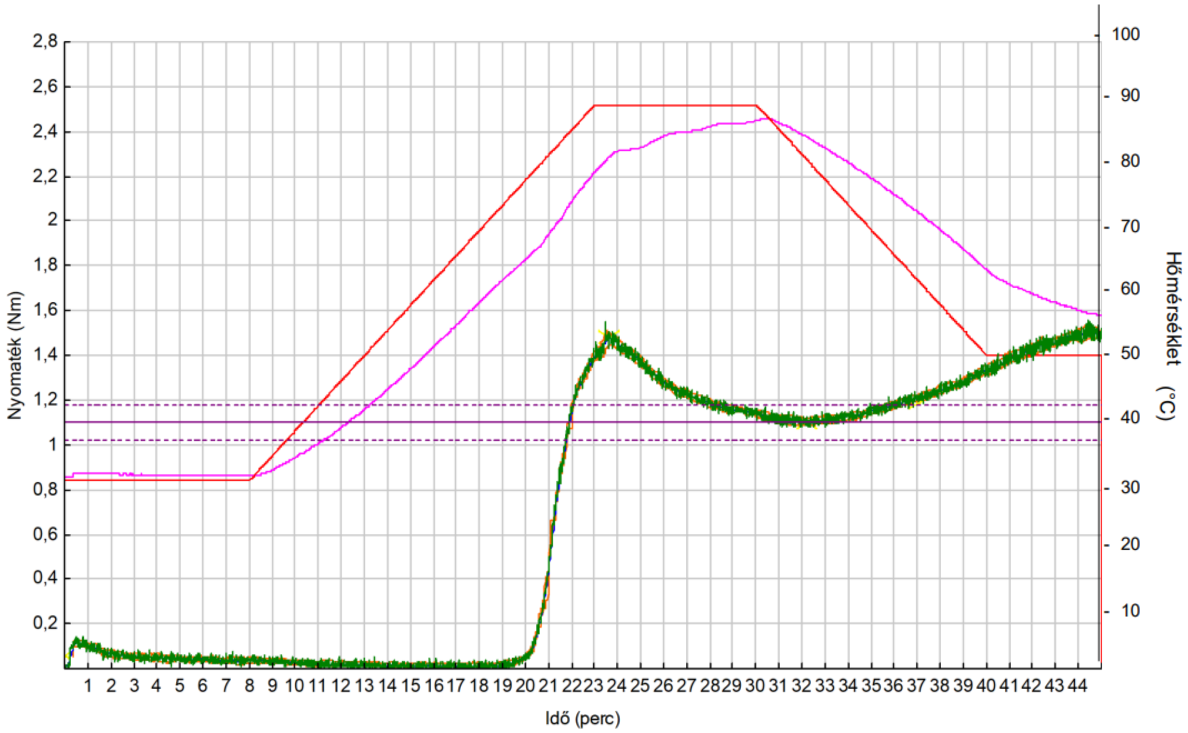

Figure 14. Mixolab curve of Corn flour, hydration of 92%

In fact, from the extracted data and the graph drawn by Mixolab, we can clearly see that the curve, in Figure 14, does not follow the expected value and the 5 stages of the kneading process. (As shown in the graph below)

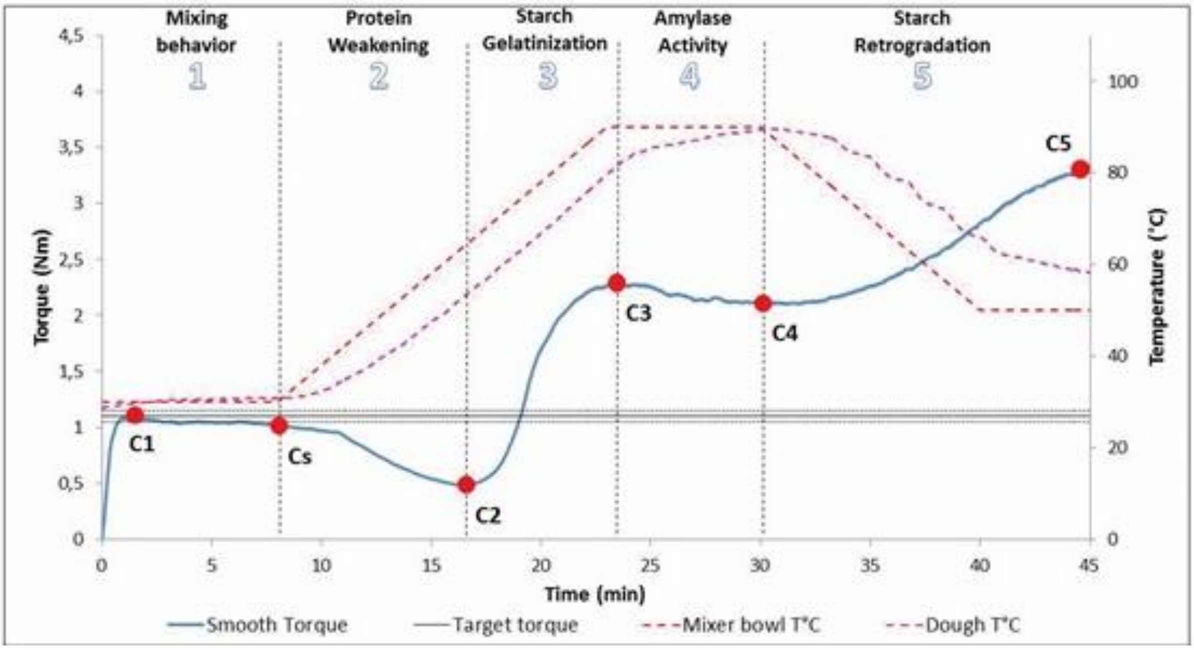

Figure 15. Typical Mixolab Curve (Internet 6)

Starch retrogradation is a process that takes place while gelatinized starch cools. Gelatinization, a process that causes swelling and the release of amylose and amylopectin molecules, occurs when starch is heated in water. These molecules begin to reassociate and re-crystallize as the starch solution cools, generating a more organized structure. This is calculated by C5, which was calculated at the last minute of the experiment (45 min), with a significant difference. In fact, the torque shown for the 60% hydration is much higher than the torque at 92% hydration (3,017>1,46 Nm).

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It is crucial to pay attention to how much water is added when preparing the dough since too much water can produce an excessively wet and sticky dough. This might make it difficult to handle and shape the dough, which can make it tough to get the texture or consistency wanted. Besides, kneading is an important step in the preparation of corn dough as it helps to develop the gluten-like protein: zein protein. The amount of water in the dough can affect the texture of the final product, and therefore, it can also affect the way the dough behaves during kneading.

The purpose of kneading corn-based dough (gluten free dough in general) is mainly to equally distribute the components and moisture, resulting in a homogeneous texture. Furthermore, excessive kneading might make the dough overly dry, which can lead to crumbles or splits when the dough is cooked, and this is one of the main technological challenges within the gluten free area.

When kneading corn-based dough, it is important to strike the right balance (Figure16). The dough should be kneaded just enough to ensure that the moisture and ingredients are evenly distributed, but not so much that the dough becomes tough or dry.

Figure 16. Picture of Corn flour Type 2 after kneading with adequate amount of water.

Furthermore, using too much water can lengthen the cooking time. The excess water must evaporate before the dough can cook correctly, leading to a lengthier cooking process than usual. This can be frustrating when aiming for a specific cooking time and may cause the final product to be either overcooked or undercooked.

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On the other hand, if corn-based dough is made with too little water, the dough is challenging to work with. In fact, if the corn dough has a low water content, it is more difficult to knead and will be dry and crumbly (Figure17). Moreover, the flavor and texture might be altered, producing an undesirable end product.

Figure 17. Picture of Corn flour Type 2 after kneading, showing a crumbly texture

## *6- Challenges:*

Mixolab is frequently used in the food business to evaluate the flour's quality and the attributes of dough, including how well it mixes and bakes. For the test to be accurate and relevant, Mixolab’s capacity to produce results consistently and repeatedly is essential.
Research has demonstrated that the Mixolab is a trustworthy tool for determining the rheological characteristics of dough and flour. Many studies that assessed the repeatability of Mixolab measurements found that the instrument may deliver reliable and coherent data when evaluating repeatability. According to our experiments, Mixolab measurements typically have low levels of variability, and the instrument can deliver reliable results even when analyzing several samples, confirming many previous studies.

It is crucial to remember that a variety of variables, including the caliber of the samples being tested, the temperature and humidity levels at the time of testing, and the operator's skill, might have an impact on how reliable the Mixolab is. Therefore, it is important to mention the number of errors that might occur during an experiment, which requires an understanding of each and every step to be taken.

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The Mixolab Applications Guide, draw attention to different factors causing the following errors to occur during an experiment, here are some of them:

Sample preparation: If the flour is not precisely weighed, fully mixed, or given the necessary amount of rest time, measurement inaccuracies may result.
Instrument calibration: Inaccurate measurement findings may occur from improper or infrequent Mixolab calibration.
Timing: Results may be incorrect if the sample is placed into Mixolab too soon or too late.
Sensor’s placement (personal point of view): I noticed that the placement of the sensor permits contact with the dough while kneading it, which provides useful information. One sensor, however, will only provide a small amount of info to work with.

Figure 18. Piece of corn dough stuck on the lid at the beginning of kneading.

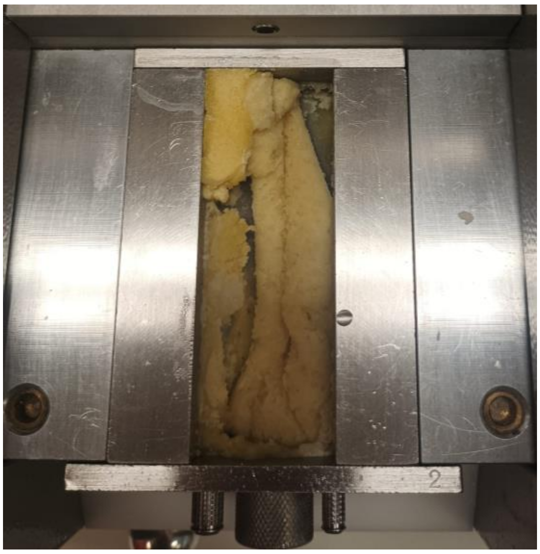

Figure 19. Piece of corn dough stuck on the edges of the kneading bowl.

During my repeatability measurement of corn type 2, it is obvious that something went wrong within the loading or temperature or other factors, which was reflected on the curves within the first 10 minutes of the graph drawing (Figure 10), this could be explained by Figure18 and 19 which represent a typical problem encountered within the experiments.

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After multiple usage, I personally felt more confident using such an advanced device which helped me to get other experiments perfectly done. I wanted to share those results not only to show how the errors can be made easily but also how reproducibility can be reached perfectly as well.

## *7- Summary*

The past ten years have seen many engineering, research labs, and major businesses dive headfirst into gaining a deeper understanding of the gluten-free area. Today, it is still a developing sector, welcoming research, and innovative ideas to help overcome the challenges it is facing.

In this progression, Mixolab turns out to be a crucial instrument. In fact, it helps study the quality of the flour and the characteristics of the dough, such as how well it mixes and bakes. According to our research, Mixolab assessment helps us better comprehend gluten free flour, corn and rice flour in our case, in a laboratory setting while adhering to procedures and guidelines and anticipating a range of outcomes. Specific data points, including kneading torque, protein weakening, starch gelatinization, dough temperature, and others, were provided to show the instrument's accuracy. Additionally, the cohesiveness of the instrument was evaluated using rice flours, allowing us to confidently rely on earlier studies and apply their findings to ongoing research.

In the last part of our experiments, we had the chance to juggle between hydrations and analyze their effect on the corn dough, where we observed the importance of the water in the final products as a key factor. Furthermore, Mixolab allows us to go beyond the protocols and test different set up and hydrations, yet, sometimes unfortunately, providing incomplete results, when going beyond the protocols and calculations.

In addition to the multitude of promising opportunities that Mixolab presents, it is crucial to be mindful of the potential errors during the kneading process. The inconsistent results shown with corn flour give an example of how these inaccuracies can arise either from human error or mechanical malfunctions.

In conclusion, Mixolab is an innovative instrument that has significantly advanced and succeeded in the food sector. By mimicking the kneading process as in industrial and home production, it allows bakers and food engineers to better understand the rheological characteristics of the dough and how to optimize the recipes and produce high-quality products. Thanks to the Mixolab, producers now have a clearer idea of how their products will turn out.

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# *10- Statement*

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