Unlocking Nutrient Accessibility through Sourdough Fermentation

The effect of sourdough on antinutrient reduction, protein digestibility, and fibre functionality in bread

Ellinor Littorin

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Swedish University of Agricultural Sciences, SLU
Department of Molecular Sciences
Agricultural Programme - Food Science
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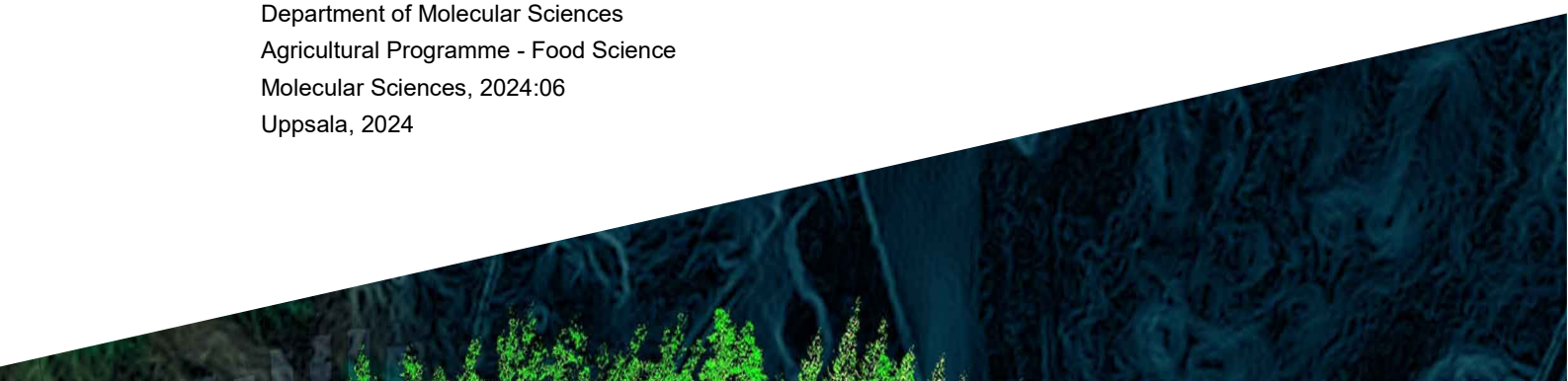

Unlocking nutrient accessibility through sourdough fermentation.

How sourdough fermentation affects reduction of antinutrients, digestibility of protein and functionality of fibre in bread

Ökad näringstillgänglighet genom surdegsfermentering

Surdegsfermenterings påverkan på reduktion av anti-nutrienter, proteiners nedbrytbarhet och fiberfunktionalitet i bröd

Ellinor Littorin

Supervisor:	**Klara Nilsson, SLU, Department of Molecular Sciences**
Assistant supervisor:	Saeid Karkehabadi, SLU, Department of Molecular Sciences
Examiner:	Galia Zamaratskaia, SLU, Department of Molecular Sciences

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Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences (NJ)

Department of Molecular Sciences

Abstract

Sourdough fermentation is a traditional bread leavening method based on the synergistic, metabolic activity of lactic acid bacteria (LAB) and yeast in the dough. This complex process is highly dependent on various extrinsic factors such as fermentation duration and intrinsic factors such as endogenous enzyme activities. The aims of this literature review are to deepen the knowledge on the effect of sourdough fermentation on nutrient accessibility, with particular focus on the antinutrient quantities of phytic acid and tannin, as well as protein and fibre digestibility within the bread.

To conduct the literature research keywords like wholegrain, antinutrients, digestibility and sourdough fermentation were used when searching for relevant scientific articles using databases such as Google Scholar, Web of Science and SLU library Primo. Key findings suggest that sourdough fermentation provides significant biochemical transformation of the dough through microbial enzymes and acidification by LAB, which activates grain endogenous enzymes. These changes result in reduction of phytic acid and tannin, which improves the bioavailability of minerals and protein. Protein digestibility increase due to reduction of antinutrients and because of solubilisation and depolymerisation of gluten. Additionally, the amount of fibre increases because of exopolysaccharide production by LAB and resistant starch formation. Conversion of insoluble to soluble fibre occurs, thereby fibre functionality is increased. Sourdough fermentation can improve the nutritional quality of bread. However, producing sourdough bread with a specific nutritional profile requires more research. It is necessary to understand how individual and combined fermentation factors influence nutrient quality and bioavailability within the bread.

Keywords: sourdough fermentation, digestibility, fibre, protein, phytic acid, endogenous enzymes

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Abbreviations

FODMAP	Fermentable, oligosaccharides, disaccharides, monosaccharides, and polyols
GABA	Y-aminobutyric acid
FAA	Free amino acid
IBS	Irritable bowel syndrome
IVPD	In vitro protein digestibility
LAB	Lactic acid bacteria
NI	Nutritional index
SCFA	Short chain fatty acid

1. Introduction

Bread is a staple food with cultural importance in many diets worldwide. The required basic ingredients in any bread formula are grain flour, water, salt and a leavening agent. Sourdough fermented bread has been consumed in Europe for 5000 years (Corsetti & Settanni 2007). However, during the 20th century, traditional sourdough was overlooked to baker's yeasts advantage as a leavening agent, because of yeasts short fermentation time and consistent result. Conversely, there is recent interest in optimising nutrient availability has embarked a renaissance of sourdough fermentation, since it offers numerous nutritional benefits (Couch 2016).

Consumption of wholegrains adds fibre to the diet and is associated to decreased mortality from cardiovascular disease, prevention of type II diabetes and a reduced risk of colon cancer (Tieri et al. 2020). Nonetheless, wholegrains contain antinutrient factors which decrease bioavailability of macro and micronutrients. Sourdough has the potential to reduce the effect of antinutrients (Salim et al. 2023) and increase the utility of fibre in human nutrition (Fernández-Peláez et al. 2020). Additionally, sourdough fermentation improves protein digestibility by reducing antinutrients and depolymerising proteins. This makes absorption of amino acids easier (Rizzello et al. 2019). Consequently, sourdough bread can be more digestible and nutritionally beneficial compared to bread made with baker's yeast, thus highlighting its growing relevance in today's nutrition.

2. Background

2.1 Extrinsic and intrinsic factors in baking

Even though bread contains few ingredients it has a complex matrix that affect the fermentation and final product. Flour composition, sugar availability, enzymes, minerals, amount of wholegrain flour and salt percentage are intrinsic factors that determine how the final bread performs (Struyf et al. 2017). Extrinsic factors are temperature, pH, water activity and fermentation time (De Vuyst et al. 2017).

Wholegrain flour contains the entire seed with endosperm, bran and germ, thus containing antioxidants, minerals and fibres (Allai et al. 2022). Refined, white flour, is solely containing the endosperm which is the largest morphological component of the grain. The endosperm consists of approximately 80% starch and the rest is storage proteins (Evers et al. 1999).

2.2 Bread leavening methods

2.2.1 Fermentation

Fermentation is an essential part of bread making as it both develops the flavour of the bread and creates leavening, which gives the bread its soft airy texture. According to the strictly biochemical definition fermentation is a process where microorganisms convert carbohydrates present in the flour into alcohol or acid and CO_2 (Maicas 2020).

2.2.2 Sourdough fermentation

Sourdough fermentation is a traditional bread leavening method which is based on a starter culture consisting of yeast and lactic acid bacteria (LAB) originating from the flour and house flora. The metabolism of sugars results in production of organic acids, which result in the drop of pH in the dough (Rizzello et al. 2019). The starter culture is obtained by adding one part of water to one part of flour (1:1) (Linko et al. 1997). The natural microflora of the flour contributes with varying microbial strains and therefore gives a unique sourdough starter culture (Corsetti & Settanni 2007). LAB to yeast ratio can vary between 1:1 to 1000:1 in sourdough microbial composition (Sagdic et al. 2023).

When the starter culture is prepared and left to ferment for 2-3 days at room temperature the synergistic enzymatic activity of yeast and LAB result in the

breakdown of nutrients to be metabolised. LAB produce acid which leads to a drop in pH, this activates grain endogenous enzymes, which further increase accessibility of the nutrients (Linko et al. 1997). When the sourdough has been active after some days it is suitable to use as s leavening agent (Sagdic et al. 2023a). The microbial activity in the starter depends on the available substrate in the dough (Corsetti & Settanni 2007). Microbial interaction and factors such as inoculating method, ingredients within the dough, environmental circumstances affect both the starter microbial composition and activity of the starter. However, the relative significance of these processes among different starters remains uncertain (Landis et al. 2021).

Both LAB and yeast impact the flavour profile, as well as leavening of the dough by creating CO_2 during respiration (De Vuyst et al. 2017). Typically, 20 h is preferred for sourdough fermentation in Sweden and Finland (Linko et al. 1997) making sourdough baking a time-consuming process in comparison to baker's yeast fermentation, which ferments for 40-100 min (Birch et al. 2013). From a production efficiency perspective there is a great interest in shortening the required sourdough fermentation time (Linko et al. 1997).

2.2.3 Lactic acid bacteria

Depending on the sorts of LAB different substrate are produced.

- Obligate homofermentative LAB produce lactic acid
- Facultative heterofermentative produce lactic acid and acetic acid
- Obligate heterofermentative produce lactic- and acetic acid, ethanol and CO_2

LAB can grow in temperature between 8-55°C, however the preferred temperature is 30-35°C (Å. S. Hansen 2004).

2.2.4 Type of sourdough

Another way to categorise sourdough is based on its characteristics.

- Type 0 is a predough
- Type I is firm sourdoughs used in artisan bakery
- Type II is semiliquid industrial sourdoughs
- Type III is dried industrial sourdoughs

Type II and III sourdoughs often need the addition of baker's yeast for proper function when baking, otherwise the naturally present microflora yeast will be prevented by the too acidic condition created by LAB (De Vuyst et al. 2017).

2.2.5 Yeast

Most commercial bread is fermented with baker's yeast (Rosell 2011) *Saccharomyces cerevisiae*, as it is convenient and provides homogenous fermenting

results (Trivedi et al. 1986). *S. cerevisiae* consumes fermentable sugars and produces CO_2 and ethanol (Trivedi et al. 1986). The gas is retained in the dough when a well-developed viscoelastic gluten-network expands, which makes the dough rise (Struyf et al. 2017).

2.3 Bioavailability

Bioavailability describes the efficiency of which the body absorbs and utilises nutrients after ingestion. A series of physiological events affect the bioavailability including digestion, solubilisation, absorption, tissue uptake and release, enzymatic effects, secretion and nutrient utilisation. The bioavailability is further affected by the cooking and processing methods applied to the food products, thereby altering the structure, composition and the presence of antinutrients which impedes proper uptake within the body (Schonfeldt et al. 2016).

2.4 Antinutrients

Antinutrients are compounds within the food that interfere with nutrient uptake in digestion. However, in the plant antinutrients serve as protection against herbivores, diseases as well as they chelate important nutrients in plant tissue (Salim et al. 2023). In the grain they are primarily found in the bran. Strategies used to reduce antinutrient content are fermentation, soaking, germination, debranning and cooking (Samtiya et al. 2020). Antinutrients can be categorised into heat-stable and non-heat-stable antinutrients.
These antinutrients can occur in cereal grain:

- Phytic acid, tannins, saponins, non-protein amino acids are heat stable.
- Lectins, cyanogenic glycosides, protease inhibitors, toxic amino acids are not heat stable (Thakur et al. 2019).

This review will focus on the antinutrients phytic acid and tannins because they are heat stable and interfere with protein digestibility. The heat labile antinutrients are diminished in the oven during baking process of the bread.

Phytic acid/phytic acid

Phytic acid, phytic acid, is present in cereal bran and partly in the endosperm and possesses the property of chelating micronutrient cations to form phytic acid. Additionally, it can bind positively charged proteins via electrostatic interactions, thereby affecting the activity of the protein (Bektaş & Ertop 2021). The phytic acid-protein complex is favoured by lower pH (Gilani et al. 2005). Phytic acid has six phosphate groups and is therefore highly charged (De Angelis et al. 2003).

Monogastric animals and humans lack phytic acid degrading enzymes in the digestive tract, thus cannot break down phytic acid (Gupta et al. 2015)

Tannins

Tannins belong to the category of water-soluble polyphenols (Gilani et al. 2005) and exist as either hydrolysable tannins such as tannic acid, and condensed tannins (Ram et al. 2020). Tannins have antinutrient properties as they chelate proteins, starch and digestive enzymes, thus decreasing digestibility (Chung et al. 1998). A significant decrease in amino acid digestibility have been noted due to tannin interacting with proline rich proteins (Gilani et al. 2005).

2.5 Protein in bread

2.5.1 Gluten

Gluten is the main storage protein in wheat as it constitutes 80-85% of the total protein content in wheat (Ooms & Delcour 2019). The two constituents are gliadin and glutenin (Žilić et al. 2011). Gliadin forms intramolecular disulphide bridges, while glutenin forms intermolecular disulphide bridges. This is why they have different solubility (Rasheed et al. 2020). Disulphide bonds reduce water solubility and digestibility of gluten (Hadidi et al. 2023).

The content and ratio between gliadin and glutenin is deciding the gluten quality, thus it is a considerable factor for dough quality as well as the final product (Bonilla et al. 2022). Gluten is a complex combination of homologous proteins with varying molecular mass and charge (Ooms & Delcour 2019). The gluten network in the dough is formed as the wheat flour is hydrated and kneaded. Glutenin forms the polymeric protein network which gives the dough strength and cohesive properties, while gliadin network, a heterogenous group of monomeric proteins, provides viscosity and extensibility to the glutenin network by acting as a plasticizer. Multiple changes happen during baking as protein surface hydrophobicity is changed, new disulphide bridges are formed and thiol disulphide interchanges (Goesaert et al. 2008).
The gluten network determines the viscoelastic properties of the dough, which is important to retain the CO_2 produced by microorganisms during fermentation (Ooms & Delcour 2019).

2.5.2 Protein quality and digestibility

Complete protein contain all nine essential amino acids, which the body cannot produce, thus needs to be ingested via diet (Westerterp-Plantenga et al. 2009).

Protein quality is decided upon amino acid composition, the digestibility and absorption in the gastrointestinal tract. The protein digestibility can be measured by laboratory simulated gastrointestinal tract, called in vitro digestibility. Its purpose is to mimic the *in vivo* processes, which entails analysis of food material before ingestion and later analysis of faeces (Pontonio & Rizzello 2024).

Gluten protein does not provide nutritionally important amino acids such as lysine, tryptophan and methionine, but is usually rich in the non-essential amino acids asparagine, glutamine, arginine and proline (Žilić et al. 2011). If an incomplete amino acid source, such as bread, is combined together with a complementary amino acid source, it can become a complete protein as all essential amino acids are included (Young & Pellett 1985).

2.6 Dietary fibre in bread

Dietary fibre cannot be digested by humans, nevertheless it is an important part of human nutrition. Dietary fibre aids in improving gut motility, regulating plasma glucose levels, binding low density lipoprotein cholesterol, and providing a prebiotic effect (Csatári & Kovács 2022). Understanding the factors influencing fibre utilisation is crucial in comprehending and upgrading its benefits.

2.6.1 Solubility of dietary fibre

Wholegrain cereal flours are a source of dietary fibres of both soluble and insoluble fibres. A general simplified definition is that higher solubility fibres are more easily digested by gut microbiota than insoluble fibre. The main dietary fibre functionality in the digestive tract are nutrient digestion and rate, passage rate and fermentation products for gut microbes (Williams et al. 2019).

The bran contains the majority of fibre in the cereal kernel, primarily cellulose and pentosans (Rosell 2011). Arabinoxylan is a form of hemicellulose which can be both soluble and insoluble. It is the main dietary fibre in both rye and wheat bran. Fructan and beta-glucan are soluble fibre, while cellulose is insoluble (Kamal-Eldin et al. 2009). For gut microbial fermentation the major substrates are non-digestible carbohydrates, soluble and insoluble fibre and non-digestible oligosaccharides (Gråsten et al. 2002).

2.6.2 Fermentable sugars

Fermentable sugars in dough are either added to the dough or broken down from starch or maltose units by cereal endogenous enzymes. The concentration of free saccharides is higher in wheat wholegrain in comparison to refined flour (Struyf et

al. 2017). Fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) are considered to have prebiotic effect but are related to gut issues for people suffering from irritable bowel syndrome (IBS). Symptoms of IBS are stomach pain, bloating, diarrhoea and constipation (Canesin & Cazarin 2021).

3. Aim

The overall aim of this review is to broaden and deepen the understanding of how sourdough fermentation impacts the reduction of antinutrients, the digestibility of protein and fibre functionality.

This overall aim is divided into two sub-aims:

-Deepen the knowledge on how sourdough influences the presence of phytic acid and tannin in the bread

-Analyse and compare results on the impact of sourdough fermentation on protein digestibility and fibre functionality

4. Method

This study has been performed as a literature study and the report is based on scientific material found on the following databases: Google Scholar, Web of Science, SLU library Primo as they have been suggested by SLU library to use as credible databases. The search tool Scispace has been used to find suitable sources obtained through the mentioned databases.

During research these keywords have been used in combination or individually to find relevant scientific articles: wholegrain, antinutrients, bioavailability, sourdough fermentation, phytic acid, phytate, phytase, tannin, tannase, protein solubility, protein digestibility, fibre solubility, fibre fermentability, gluten degradation, arabinoxylan solubility, fibre functionality, endogenous enzymes, IVPD, FODMAP, microbial strain

5. Literature review

The following section describes, compares and discusses different research studies that have analysed the effect of sourdough fermentation on the functionality of antinutrients and the bioavailability of proteins and fibres. Sourdough is a highly diverse product with varying microbial composition and is affected by multiple extrinsic and intrinsic factors.

5.1 Sourdoughs influence on functionality of antinutrients

Reducing anti-nutrient activity can enhance the absorption of nutritious components from food. Increased acidity, endogenous enzymes, extensive fermentation time and LAB are factors related to sourdough fermentation which can influence the antinutrients functionality (Wang & Wang 2024).

5.1.1 The effect of phytic acid on protein digestibility

Protein-phytic acid complex are highly prevalent in wheat. The polyanionic phytic acid molecule forms strong electrostatic bonds with cationic lysine, arginine and histidine residues at pH below the isoelectric point of protein. Protein aggregates continuously and may also precipitate, which can lead to formation of an insoluble complex. Since pH in the stomach is 2.5, protein-phytic acid complexes form thus reducing the protein digestibility. Additionally, phytic acid can hinder the conversion of the digestive stomach enzyme pepsinogen to pepsin by binding the basic amino acid residues of pepsinogen (Humer et al. 2014). Thus, it is necessary to degrade phytic acid before ingestion of food. Since the protein solubility is reduced the digestion in the small intestine is reduced.

5.1.2 Phytase

Phytase is an enzyme that degrades phytic acid. It is categorised into 3-phytases and 6-phytases, named after its cleavage site. The 3-phytases are of microbial origin, while the 6-phytases are of plant origin. There exists acidic phytases and alkaline phytases. Acidic phytases release five out of six phosphate groups when hydrolysing phytic acid. The optimum temperature for phytases is 50-60 °C (Humer et al. 2014), thus phytase activity is not ideal in sourdough since it is not fermented at such high temperature. Thereby it might be difficult to completely inactivate all phytic acid in the dough.

5.1.3 Sourdough fermentation and grain endogenous phytase

Upon soaking grains or flour in water, endogenous phytase is activated. This initiates the enzymatic degrading process of phytic acid present in the flour (De Angelis et al. 2003). The phytase is activated when the grain prepares itself for germination, because it requires accessible phosphate and micronutrients for growth, which are bound to phytic acid (Courtin et al. 2023). For proper enzymatic degradation the optimum pH is 4.0 (De Angelis et al. 2003). Sourdough bread doughs obtain this pH by LAB fermentation (Gupta et al. 2015) as pH in sourdough varies between 3.4-4.9, median value 4.1 (Arora et al. 2021). Rye and wheat possess endogenous phytase activity, but it differentiates between crop, year and sort. Therefore, solely depending on soaking and activation of endogenous enzyme activity for phytic acid reduction is not a viable solution, instead soaking should be combined with another method for improved reduction (De Angelis et al. 2003). During microbial fermentation the complex between nutrients and phytic acid is weakened, thereby improving nutrient digestibility (Couch 2016). The weakening effect is caused by gradual hydrolysis of phytic acid in solution. Phytic acid is more soluble in acidic condition (Humer et al. 2014). The acidic pH is one of the reasons why phytic acid degradation is successful in sourdough fermentation.

5.1.4 Impact of microbial activity in phytic acid reduction

Karamam et al. (2018) investigated the phytic acid degradation capability of LAB and yeasts. Multiple phytase active LAB and yeast strains were incorporated in the starter culture. The result showed a significant decrease of phytic acid with the combination of *S. cerevisiae* and *Pediococcus pentosaceus*. It was a 43.4% decrease in phytic acid content in whole wheat bread, which was the most prominent reduction according to the study (Karaman et al. 2018).

Buddrick et al. (2014) researched how the fermentation processes resulted in phytic acid content reduction but did not specify if the phytase activity was grain endogenous or of microbial origin. Not either was there a mention of a selection of microbes with phytase properties. The study found that varying temperature of fermentation (23, 30 and 37 °C) did not affect the reduction of phytic acid markedly different. However, different proofing times affected the result as 100% wheat bread fermented for three hours showed a reduction, while five and seven hours resulted in greater reduction. Maximum phytic acid reduction in rye sourdough bread was 85.5% (30°C), compared to wheat which was 63% (30°C) and wheat oat blend showed 53% reduction (30°C) (Buddrick et al. 2014). Phytic acid hydrolysis improves protein digestibility (Hassan et al. 2008).

When comparing the results 43.4% (Karaman et al. 2018) and 85.5%, 63% and 53% (Buddrick et al. 2014), it does not seem relevant to intentionally incorporate a phytase positive microbe, if the random sourdough microflora can result in 85.5% reduction of phytase in rye sourdough bread. Through this result it is apparent that there are multiple factors that determine phytic acid reduction success. This includes type of grain, fermentation time, temperature, activity of sourdough.

The varying result between rye and wheat-oat bread indicates that the specific circumstances were preferable in the rye dough, possibly due to higher phytase potency or that other extrinsic factors that were more beneficial in the rye bread. This shows that it is necessary to use different strategies to manipulate the extrinsic factors depending on the raw material of grain flour for adequate phytic acid reduction. Additionally, the microflora intereffects itself depending on microbial composition and available substrate, thereby exhibiting different properties depending on combination of fermentation factors. This means that the same microbial combination might exhibit a different result in phytic acid reduction in another type of flour.

Arora et al. (2021) reviewed 1230 peer-reviewed articles on sourdough fermentation and found that most studies did not specify or incorporate microbes with phytase activity. This indicates that endogenous enzymes, activated by acidification, are the primary contributors to phytase reduction in sourdough. However, the review emphasized that sourdough fermentation can be considered a unique tool to enhance mineral bioavailability and reduces phytic acid content. Given the limited evidence of bacterial phytase activity, it might be more relevant to optimise endogenous enzyme activity through proper acidification, than relying solely on microbial activity.

Furthermore, the degree of phytic acid reduction and its result is dependent on the original level of phytic acid, which varies between species, harvest point and year. Focusing solely on the percentage reduction of phytic acid does not indicate the amount of active and potent phytic acid in the flour. So, it would be relevant to look at the total amount of phytic acid per gram of flour and its effect on bioavailability of proteins and minerals. Also, there needs to be a distinguish between wholemeal and refined flour, as the phytic acid is primarily present in the bran.

5.1.5 Impact of microbial activity on tannin reduction

Tannase refers to several enzymes that can hydrolyse tannins. The efficiency of a tannase is substrate dependant (Aguilar et al. 2007). Therefore, the tannase activity of a sourdough starter vary depending on the microbial strain composition of the starter, as well as the type of flour in the dough.

Some LAB produce tannase which hydrolyse bonds in tannins (Gänzle 2014), thereby reducing its functionality. *Lactococcus lactis* produce tannase both intracellular and extracellular. Fermentation at 37 °C for four hours resulted in 100% tannin reduction (Mukherjee et al. 2014). However, it was performed on sourdough with black bean and not wheat or rye.

5.1.6 The effect of particle size of wheat bran flour on reducing phytic acid and tannin during sourdough fermentation

Hassan et al. (2008) investigated how the two parameters particle size and sourdough fermentation affect reduction of phytic acid and tannins in wheat bran from three different types of wheat. The results can be seen in table 1. The fermentation time was four hours.

The decrease in phytic acid in fermented flour was more than halved in the fine milled flour in comparison to the coarse, unfermented flour. The total level of phytic acid was the lowest in fine milled, fermented flour. However, the greatest reduction in tannin content was in the coarse milled bran and the total level of tannins was lowest in the coarse milled, fermented flour (Hassan et al. 2008).

Summarised, there is reduction of phytic acid and tannin in all particle sizes after fermentation. The highest level of phytic acid was in coarse milled, conversely tannin levels was highest fine milled flour.

Table 1. Antinutrient content before and after sourdough fermentation of wheat bran depending on different particle size of the flour (Hassan et al. 2008)

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Wheat bran	Particle size	Phytic acid mg/100 g	Tannin mg/100 g
Non-fermented	Coarse	626.12	0.03
	Medium	740.36	0.06
	Fine	795.20	0.07
Fermented	Coarse	572.79	0.01
	Medium	367.13	0.05
	Fine	301.63	0.06

Sourdough fermentation decreases the levels of phytic acid and tannin. However, one can partly rely on the endogenous activity caused by acidification. Additionally, there are more parameters than just the fermentation itself that influences the level of antinutrients. In fine milled flour the phytic acid reduction is more than half reduced. Flour particle size is therefore highly important to consider

when optimising phytic acid reduction. Interestingly, the greatest reduction of tannin is observed in coarse milled flour, which is opposite of result in phytic acid reduction. The dough was fermented for four hours, which was less time than other fermentation tests which has exhibited better reduction performance with longer fermentation time than four hours, such as in Buddrik et al. (2014).

5.2 The effect of sourdough fermentation on protein digestibility

5.2.1 Digestion and absorption of dietary protein

Protein digestion begins in the stomach, where the enzyme pepsin cleaves peptide bonds. Pepsin is activated by gastric acid, which also denatures proteins, making them more accessible. The partially digested protein then moves to the small intestine, where pancreatic enzymes further break down the proteins. The enzymes in the small intestine function at a higher pH due to presence of bicarbonate. Transport proteins in the microvilli then absorb single amino acids and small peptides, which diffuse into the capillary system (Goodman 2010).

5.2.2 Factors affecting digestibility and absorption of dietary protein

True amino acid digestibility is up to 80% for food proteins (Loveday 2022). Plant proteins have lower digestibility (75-80%) than animal protein (90-95%). This is partly due to rigid cell walls of plant cells that impair proper enzyme accessibility and presence of antinutrients (Sá et al. 2020).

During digestion a portion of the protein and peptides may pass into the large intestine if dietary fibre has increased the viscosity (Loveday 2022). Proteins in the bran form complexes with lignin, beta-glucan and arabinoxylan, thus hindering digestion and proper absorption in the digestive tract. Another problem is that bran proteins might be confined within cells that are rich in dietary fibre, thus limiting digestion (Hadidi et al. 2023).

Proteins are prone to chemical reactions in extremes of temperature and pH, as this changes the amino acid side chains. This can lead to perpetuate lost bioavailability (Loveday 2022). The amino acid composition of a protein determines its folding pattern and structure which depends on electrostatic interactions. Tight protein aggregation slows down hydrolysis, since the accessibility to hydrolyse peptide bonds is reduced, thereby affecting digestibility negatively. Gluten is a proline rich

protein, providing rigidity and has high resistance to peptidase hydrolysis. The solubility is also a factor influencing digestibility (Loveday 2022). Wheat bran protein is significantly solubilised by acidic condition (Arte et al. 2015). Gluten protein is partially resistant to digestion, but are somewhat deamidated when exposed to acid hydrolysis (Kroghsbo et al. 2014).

Protein digestibility is decreased by the presence of antinutrients, which was described in the antinutrient section (5.1.2) of the report.

5.2.3 Effects of sourdough fermentation on gluten proteins

In the process of sourdough fermentation, gluten proteins are proteolyzed. Glutenin is generally more easily hydrolysed by protease than gliadin. There are two pathways for gluten protein proteolysis. Primary proteolysis is performed by endogenous enzymes from the flour, including aspartic proteases, cysteine protease and serine carboxypeptidase II. The enzymes are activated during acidification by LAB activity (Ogilvie et al. 2021). Aspartic proteases are the most abundant proteinase in resting wheat and rye (Gänzle 2014).

Secondary proteolysis takes place when proteases are produced by LAB and yeasts from the sourdough culture. The types of excreted enzymes are strain specific and depend on the microflora in the culture. Some strains produce prolyl endopeptidase, which degrades proline-X bonds (Ogilvie et al. 2021). This is relevant as gluten is a proline rich protein (Loveday 2022). The cyclic structure of proline imposes structural hindrance for degradation. The microbial prolyl endopeptidases are able to effectively cleave Pro-rich gluten peptides in comparison to human gastric proteases (Nionelli & Rizzello 2016).

Enzymatic or acid hydrolysis have the ability of altering structure and shape of gluten proteins, which results in protein hydrolysates (Sagdic et al. 2023b). The microstructure of the depolymerised gluten macromolecules becomes a fibrous network together with a rise in antiparallel beta-sheets. This happens when LAB interacts with the disulphide bonds and aromatic amino acids. Every different kind of LAB have a distinct influence on gluten and it is the strain specific acidification that it depends upon (Wang & Wang 2024). Gluten has higher solubility in acidic condition and its depolymerisation happens as more hydrophobic parts are exposed due to intensified intramolecular forces (Sagdic et al. 2023b). Gliadin and glutenin proteins are extensively hydrolysed during sourdough fermentation. However, the proteolytic and rheological gluten changes are primarily mediated through acidic activation of cereal enzymes (Thiele et al. 2004).

Thiele et al. (2004) compared protein degradation between a sourdough fermented dough, an acid aseptic dough and a neutral aseptic dough. The result showed that the gluten macropolymer was not solubilised and depolymerised in the neutral aseptic dough in comparison to the two acidic doughs (Thiele et al. 2004). Another study by Thiele (2003) stated that the proteolytic activity on gluten was primarily performed by endogenous wheat enzymes, which was mediated through LAB acidification (Thiele 2003). This shows that it is the acidification from either LAB activity or an added acid, that causes solubilisation and degradation of the protein, rather than specific microbial action.

Acidifying the dough results in the endogenous enzyme activation and LAB fermentation is a slow process causing dough acidification. A more time effective method to obtain the result of solubilised and degraded gluten is to acidify the dough chemically. The gluten network is altered when there is net positive charge at pH below 4.0, its isoelectric point. Thereby the electrostatic repulsion increase, which makes the gluten more soluble and effectively hinders new bonds to form, besides the reduction of disulfide bridges (Arendt et al. 2007). This facilitates proteolysis of the solubilised glutenin subunits (Thiele et al. 2004).

5.2.4 Changes in total protein after fermentation

The total protein content increased with sourdough fermentation, which was due to yeast reproduction (Hassan et al. 2008). In table 2 the results of change in protein in sourdough fermented bran are presented.

Table 2. Protein content before and after sourdough fermentation of wheat bran on different particle size

Wheat bran	Particle size	Protein (%)
Non-fermented	Coarse	20.35
	Medium	18.36
	Fine	21.07
Fermented	Coarse	21.65
	Medium	20.79
	fine	22.40

(Hassan et al. 2008)

Terrazas-Avila et al. (2024) concluded that there was a reduction in total protein in sourdough fermented for 4, 6 and 16 h, with the exception of 8 hours of fermentation, which showed an increase in protein, (seen in table 3). The wheat flour held a protein content of 10.24 ±0.03%. The protein reduction was mentioned to be due to proteolytic activity and LAB metabolism. With longer fermentation time the peptide fractions are hydrolysed into amino acids (Terrazas-Avila et al. 2024).

Table 3. Protein content at different times during sourdough fermentation 20% wholemeal wheat flour

Time	4h	6h	8h	16h
Protein (%)	8.48 ±0.04	7.17 ±0.32	8.76 ±0.27	5.05 ±0.06

Terrazas-Avila et al. (2024)

Inconsistent result regarding increase or decrease in protein content is seen when comparing (Hassan et al. 2008) and (Terrazas-Avila et al. 2024). This might show a difference in how bran in comparison to wholemeal responds to fermentation. It could also be due to how efficiently the specific microflora is metabolising proteins, as well as extrinsic factors.

5.2.5 Impact of sourdough mediated proteolysis on protein digestibility

Rizzello et al. (2019) compared the *in vitro* protein digestibility (IVPD) and *in vivo* trial of refined flour bread prepared using three fermentation methods: baker's yeast (BYB), sourdough (SB) and traditional sourdough (tSB) method, both being type I sourdoughs, but SB contained added *S. cerevisiae*. All three breads were fermented at 30°C. BYB was fermented for two hours. The sourdough breads were fermented according to a two-step protocol. SB was fermented for 4+1.5 h, while t-SB was fermented for 24+4 h. The study concluded that the IVPD was 8% higher for SB and 16% higher for tSB compared to BYB. Yeast as leavening agent causes minor degradation of protein in comparison to sourdough, thus resulting in lower protein digestibility (Graça et al. 2021).

Furthermore, the total amount of gluten decreased significantly due to LAB proteolysis in sourdough fermentation compared to yeast fermentation. The correlation between degree of proteolysis and time was proportional. Soluble degradation products of protein were observed. Additionally, the nutritional index (NI), representing the ratio of essential amino acids and the total digestible protein fraction, was measured to be 21% higher for SB and 62% higher for tSB in comparison to the yeast leavened bread (Rizzello et al. 2019). The fermentation time is highly significant to obtain higher digestibility, which is seen in the difference of t-SB and SB, where longer fermentation time is preferable. However, the level of amelioration depends on multiple factors as sourdough bread is a complex product to evaluate.

Costantini et al. (2022) found that IVPD was lower in bread baked with dried sourdough (type III) in comparison to firm sourdough (type I). This was thought to be due to moderate enzymatic activity in comparison to the firm sourdough (Costantini et al. 2022). The difference between the types of sourdoughs shows how the microflora, as well as extrinsic factors, makes the digestibility variable. In this case the drying probably has impaired the function of the sourdough.

There is a significant increase in total free amino acids (FAA) and protein digestion through protein proteolysis and polypeptide solubilisation by sourdough fermentation. The total FAA is an indicator of the degree of proteolysis. After ingestion of sourdough bread the content of FAA in the blood plasma was notably higher and remained for longer time than bread baked with *S. cerevisiae* (Rizzello et al. 2019). There is a higher blood absorption of FAA from sourdough bread compared to yeast leavened bread, which indicates better digestibility in sourdough. Y-aminobutyric acid (GABA) is produced from decarboxylated L-glutamic acid and is highly increased by fermentation. GABA is a neurotransmitter which induces hypotension, is diuretic and possesses tranquilising effect in humans (Graça et al. 2021). Fungal proteases activity increased GABA levels more than four times (Costantini et al. 2022).

5.3 Functional enhancements of fibre through sourdough fermentation

5.3.1 Functionality and utilisation of fibre in human nutrition

The functionality of fibre is influenced by various factors, particularly its solubility and fermentability (Dikeman & Fahey Jr. 2006; Rizzello et al. 2019). Phytic acid has been shown to minimally affect the fermentability of fibre (Nyman & Björck 1989). Soluble fibre, characterised by its viscosity, is fermented in the colon, leading to the production of short chain fatty acids (SCFA), which modulates appetite regulating hormones and induces satiety (Salleh et al. 2019). Arabinoxylans create viscous solutions with water and are highly fermentable (Williams et al. 2019). Insoluble fibre aids in defecation by absorbing water (El-Habashy 2017). Rapidly fermented fibre will be converted to SCFA by gut microbes, while slowly or non-fermentable fibre are incorporated in the stool facilitating defaecation (Dikeman & Fahey Jr. 2006).

5.3.2 Changes in fibre amount, structure and solubility through sourdough fermentation

Sourdough fermentation has potential to enhance fibre functionality. It can decrease the levels of FODMAP by up to 30% (Canesin & Cazarin 2021). IBS patients have reduced gastrointestinal symptoms when consuming a diet which is low in FODMAP (Halmos et al. 2014). The reduction in FODMAP in sourdough bread might explain why some individuals experience fewer gut problems when consuming sourdough bread compared to regular bread. However, there has not been clinical evidence that sourdough fermented bread leads to less IBS related symptoms even though FODMAP levels are reduced (Laatikainen et al. 2017).

Sourdough fermentation impacts fibre through enzymatic hydrolysis processes. Flour intrinsic enzymes, activated during hydration, hydrolyse hemicellulose. LAB produce glycolytic enzymes that modify fibre properties. This results in an increase in both soluble and insoluble fibre content in rye sourdough, through conversion of insoluble to soluble dietary fibre by intrinsic enzymes (Fernández-Peláez et al. 2020). These changes are facilitated by a combination of activated endogenous enzymes and LAB activity during sourdough fermentation. The acidic pH in the dough favours the creation of resistant starch (Canesin & Cazarin 2021) and the microorganisms in sourdough fermentation can influence the starch retrogradation. Thereby the resistant starch increase (Kanazawa et al. 2021) as well as total fibre content.

Furthermore, sourdough fermentation enhances fibre solubilisation, as observed in the increased water solubility of arabinoxylan and the depolymerisation of arabinoxylans through enzymatic hydrolysis (Girard & Awika 2021). This process is proportional to fermentation time and leads to a reduction in cellulose content and significant alterations in the chemical and physical properties of the fibre. This leads to reduced insulinemic and glycaemic reaction (Păucean et al. 2024).

Additionally, some LAB strains produce exopolysaccharides through sucrose metabolism, to protect itself against environmental factors. This contributes to the increase in dietary fibre content in sourdough bread (Girard & Awika 2021). The amount of maltose in the matrix influences the production of exopolysaccharides, with the substrate and acceptor carbohydrate varying depending on the microbial strain. Traditional sourdough microbiota often contains one or more exopolysaccharide producing strains (Gänzle 2014).

Sourdough fermentation enhances fibre availability and functionality by increasing solubilisation and altering fibre properties.

5.4 Complexity of evaluating sourdough fermentation

Despite sourdoughs ingredient simplicity of flour mixed with water, sourdough fermentation constitutes a complex system influenced by various extrinsic and intrinsic factors. Fermentation time is a vital extrinsic factor as it dictates the duration of yeast and LAB metabolic activity. Which significantly affects intrinsic properties such as the leavening power, gas retention capacity, gluten development and degradation, pH, reduction in antinutrient contents and solubilisation of arabinoxylan. These factors influence the characteristics of the bread, such as loaf volume, taste and acidity or digestibility. Additionally, the microflora composition of sourdough determines what specific metabolic enzymes are excreted and how fast the pH reduces, which in turn affect the activation of grain endogenous enzymes.

Moreover, intrinsic dough changes are dependent on fermentation time and temperature, since varied conditions stimulate different response from the microflora, grain enzymes, fibre and protein within the dough matrix, affecting digestibility and functionality. The ratio of total amino acids and total digestible protein fraction increases significantly with longer fermentation time. This implies a controlled combination for desired outcome, which is dependent on the specific circumstances such as starter strain combination and cereal species. Gluten degradation, fibre solubilisation, optimal digestibility and phytic acid reduction are interconnected phenomena. Since phytic acid reduction is time dependant, this is affecting bioavailability of protein. Protein degradation and solubilisation is time dependant as well, and relates on acidification of the dough, which relies on proper LAB activity.

Additionally, fibre can act as an antinutrient and hinder nutrient uptake when it increases viscosity of chyme. Simultaneously, the higher chyme viscosity that is associated with fibre is positive because it helps reduce plasma lipids and a lower glycaemic response. Questions for further research could therefore be: How can protein digestibility be evaluated if looking at fibre from an antinutrient point of view? What level of fibre would be preferable to incorporate without affecting protein uptake? Is it possible to reduce its antinutrient properties, while still retaining the positive health effects? Does it matter if it is bran that is incorporated in dough or refined fibre?

Optimising the sourdough circumstance when looking at one flour component, such as phytic acid, and a single external factor, such as flour particle size, it is relatively straightforward. However, specifying the effect of the different extrinsic factors on the entire nutrient matrix when evaluating digestibility is more challenging. From a consumer point of view bread properties such as taste, texture, volume and

appearance may be prioritised over nutritional optimisation. Therefore, it is important to understand how sourdough fermentation can optimise the nutritional profile of the bread without reducing the bread quality.

6. Conclusion

The four most influential factors in sourdough fermentation that reduce phytic acid and tannin and increase the protein digestibility and fibre digestibility functionality in bread were: activation of endogenous enzymes, long fermentation times, lactic acid bacteria and acidic conditions.

The acidic condition in sourdough, which is provided by LAB, activates endogenous grain enzymes. This is the most important factor for gluten degradation and digestibility. The digestibility is significantly increased with longer fermentation time.

Fibre functionality is improved by increased solubility through acidified dough and endogenous enzyme activation. The reduction of FODMAP in sourdough bread cannot be connected to eased IBS symptoms. Total fibre in the dough can increase through the microbial exopolysaccharide production and formation of resistant start.

To reduce the antinutrients tannin and phytic acid the endogenous phytase activity is necessary and is enabled through acidification of the dough. Particle size determines how effectively tannin and phytic acid content is reduced. Phytic acid is best removed in fine milled flour and tannin is better removed in coarse flour.

In conclusion, the acidification in sourdough fermentation which activates the endogenous enzymes of the flour, plays a pivotal role in reducing antinutrients, increasing protein digestibility and fibre functional in sourdough baking. Additionally, the specific microbial strain combination together with extrinsic factors, where fermentation time is critical, significantly impacts nutritional matrix in sourdough bread.

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