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Exploring the Functionality of *in situ* Produced Dextran in High-Protein or Wholegrain Enriched Wheat Bread

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ACADEMIC DISSERTATION

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ABSTRACT

Utilization of grain legumes (such as faba bean) and minor cereals (such as sorghum and millet) is constantly growing since consumers are increasingly interested in sustainable, plant based and health promoting foods. Grain legumes and minor cereals are raw materials with high nutritional quality due to high content of protein, dietary fibre and other bioactive compounds. Utilization of these grains is challenging as they have negative impact on product texture and flavour. Sourdough technology is one of the "clean label" options to improve technological functionality of these grains.

The aim of this thesis was to study the influence of faba bean, sorghum and millet on technological and nutritional properties of composite wheat bread. Wheat flour was replaced with faba bean (30%), sorghum or millet flours (50%), which were either native or fermented. Utilization of native flours had detrimental effect on the rheological properties of dough as well as the volume, texture and sensory properties of bread in comparison to 100% wheat control breads. In contrast, mildly acidified and dextran-containing flours improved all properties of composite breads.

The functionality of sourdough was based on sufficient production of dextran and mild acidification. Faba bean sourdough fermented with *Weissella confusa* VTT E-143403 (dextran content of 5.2% dry weight) improved the specific volume (21%) and texture of breads, especially softness (12%). However, faba bean sourdough fermented with *Leuconostoc pseudomesenteroides* DSM 20193 (dextran content of 3.6% d.w.) decreased bread volume and increased crumb hardness, probably due to the higher acidification. Furthermore, efficacy to improve shelf-life (delay staling rate) was shown to be linked to slower starch retrogradation and improved water retention. Sourdough fermentation also increased the level of free phenolic compounds in millet and sorghum. Fermentation of millet decreased starch *in vitro* digestibility (lower predicted glycemic index), while improving the *in vitro* digestibility of proteins. These changes may be attributed to the production of organic acids and concomitant activation of hydrolytic enzymes like glycoside hydrolase, cellulases, esterases and proteases.

Utilization of tailored sourdough technology had a significant impact on the sensory properties of sorghum breads. Sourdough fermentation of sorghum without dextran increased unpleasant flavour properties such as acidic, bitter flavour and aftertaste, probably due to increased content of acids and small molecular weight polyphenols (e.g. caffeic acid). This study showed that dextran containing sorghum breads had less intensive acidic and bitter flavour and milder aftertaste even though the actual acidity and polyphenol compositions was the same as in control breads. This revealed the exceptional ability of dextran to mask acidic and bitter flavour notes. This observation was further verified by adding purified dextran (0.12−0.96% bread weight) to white wheat bread together with acid and bitter flavour compounds

(lactic/acetic acid and caffeine). When the amount of dextran was sufficient (> 0.43% b.w.), the perceived intensity of acidic and bitter flavours in the bread decreased.

This thesis demonstrated efficient production of dextran *in situ* in faba bean, sorghum and millet flours during sourdough fermentation, which facilitates production of nutritionally high quality composite breads without additives. Additionally, this thesis revealed for the first time the ability of dextran to modify sensation of acidic and bitter flavours, which allows future product innovations in plant based foods.

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When life kicks you, let it kick you forward.
– Kay Yow

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I. Wang, Y., Sorvali, P., Laitila, A., Maina, N. H., Coda, R., & Katina, K. (2018). Dextran produced *in situ* as a tool to improve the quality of wheat-faba bean composite bread. *Food Hydrocolloids, 84*, 396-405.

II. Wang, Y., Compaoré-Sérémé, D., Sawadogo-Lingani, H., Coda, R., Katina, K., & Maina, N. H. (2019). Influence of dextran synthesized *in situ* on the rheological, technological and nutritional properties of wholegrain pearl millet bread. *Food Chemistry, 285*, 221-230.

III. Wang, Y., Trani, A., Knaapila, A., Hietala, S., Coda, R., Katina, K., & Maina, N. H. (2020). The effect of *in situ* produced dextran on flavour and texture perception of wholegrain sorghum bread. *Food Hydrocolloids*,*106.*

The publications are referred to in the text by their roman numerals.

Author's contributions

I. Yaqin Wang planned the study with other authors and performed most of the experiments. The bread baking and Haake oscillatory measurement was done by Päivi Sorvali. Yaqin was the major contributor to the results interpretation and manuscript writing.

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III. Yaqin Wang planned the work together with Antti Knaapila, Kati Katina, Rossana Coda, and Ndegwa Henry Maina and she carried out the sensory evaluations and prepared the manuscript. The polyphenolic compound analysis was done by Antonio Trani. The rheological characterisation of dextran aqueous solutions was performed by Sami Hietala and Yaqin.

Other related publications of the author:

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ABBREVIATIONS

ANF	anti-nutritional factors
c*	critical overlap concentration
cfu	colony-forming unit
CAB	chemically acidified faba bean wheat bread
CMC	carboxymethyl cellulose
CWB	control wheat bread
CFWB	control faba bean wheat bread
CFSB	control faba bean sourdough bread
CMWB	control millet wheat bread
CMSB	control millet sourdough bread
CSWB	control sorghum wheat bread
CSSB	control sorghum sourdough bread
Da	Daltons
DFSB	dextran-enriched faba bean sourdough bread
DMSB	dextran-enriched millet sourdough bread,
DPPH	2,2-diphenyl-1- picrylhydrazyl radical
DSSB	dextran-enriched sorghum sourdough bread
DY	dough yield
EPS	exopolysaccharides
FQ	fermentation quotient
GAE	gallic acid equivalent
GG	guar gum
GI	glycemic index
GS	glucansucrase
HI	starch hydrolysis index
HPAEC-PAD	high performance anion exchange chromatography with pulsed amperometric detection
HPMC	hydroxypropylmethyl cellulose
IMO	isomaltooligosaccharides
IVPD	*in vitro* protein digestibility
LAB	lactic acid bacteria
LBG	locust bean gum
M_v	viscosity average molecular weight
Mw	weight average molecular weight
NMR	nuclear magnetic resonance
SD	sourdough
SEC	size-exclusion chromatography
TPA	texture profile analysis
TTA	total titratable acidity
UHPLC-PDA–MS	ultra high performance liquid chromatography with photodiode array and mass spectrometer

1 INTRODUCTION

The recent lifestyle trends toward a more sustainable and healthy diet has aroused efforts to develop new functional grain foods that are rich in protein, dietary fiber or other bioactive constituents. Faba bean (*Vicia faba* L.) is high in protein (30%) and a potential ingredient to partially replace animal-based protein in human diet (Jezierny et al. 2010; Multari et al. 2015). Sorghum and millet are important food sources in arid and semi-arid tropics of Africa and Asia, which are rich in phenolic compounds, dietary fiber, and minerals (FAO 1995). Consumption of wholegrain sorghum and millet has been related to the reduced risk of cardiovascular diseases, cancer, heart disease, type II diabetes and obesity (Taylor et al. 2014). From both a food security and agricultural sustainability perspective, promoting industrial production and consumption of these alternative grains is an important target for the near future. Composite flour which is a blend of wheat with other cereal or legume flours for making baked products, could be a promising strategy (FAO 1995). Replacing wheat with faba bean flour can be a sustainable and economical way to increase bread protein content (Coda et al. 2017b) while, wheat substitution with indigenous grains like sorghum and millets was reported to be desirable to stimulate the agricultural sector and decrease wheat imports in many developing countries (FAO 1995).

Despite their recognized benefits, the incorporation of high levels of alternative flours leads to significant detrimental effects on technological and sensory quality as compared to refined wheat products (Aprodu and Banu 2015; Lucy et al. 2017). The flavour challenges of using alternatives grains include, for instance, the intense "beany" flavour of faba bean (induced by lipid oxidation) and bitter taste of sorghum (phenolic compounds) (Heiniö et al. 2015; Jiang et al. 2016). Furthermore, wheat gluten proteins are the main structure forming components, which form a three dimensional gluten network with starch granules embedded, creating viscoelastic dough matrix that allows water retention and gas holding (Wieser 2007). In composite breads, the lower loaf volume, harder crumb and higher staling rate are mainly ascribed to the "dilution" of the gluten matrix (different protein functionality in nonwheat flours) and to disruption of the gluten network formation (Ferrero 2017). To overcome the texture deficiency, hydrocolloids improve the viscoelastic properties necessary for the texture development of composite breads (Ferrero 2017). However, those hydrocolloids are designated as food additives and require label statement as such on the product packaging, which are considered as unnatural, artificial and unhealthy by consumers (van Gunst and Roodenburg 2019).

Sourdough is an important group of traditional fermented foods that develops from a mixture of flour and water and fermented by cereal originated microbiota, typically a symbiosis of lactic acid bacteria (LAB) and yeast (Hammes and Gänzle 1998). The symbiotic metabolic performance of the microbial habitant determines the characteristics of the sourdough and quality of the subsequent product. An important metabolite is dextran, which is synthesized by many strains, particularly the species

of *Weissella*, *Leuconostoc*, and *Lactobacillu*s genus (Kothari et al. 2014). Dextrans produced by LAB are generally high molar mass long-chain polymers consisting of d-glucopyranose repeating units, which exhibit similar physiochemical properties and functionalities as hydrocolloids, e.g. water-binding and thickening (Kothari et al. 2014). In cereal and bakery industry, dextrans are increasingly recognized for their beneficial effects on rheological and textural characteristics of the products (Galle et al. 2012a; Katina et al. 2009). The production *in situ* through the use of sourdough technology represents a natural alternative to commercial hydrocolloids (Lynch et al. 2018). Furthermore, it might be accompanied by a number metabolic beneficial impacts, such as the increment of bioavailability of nutrients and the reduction of antinutritional factors (Gobbetti et al. 2014).

The successful application of *in situ* produced dextran depends on a number of factors, including 1) the composition of the flours, 2) the adaptation of the dextran producers in the fermented substrate, 3) the metabolic traits of the strain such as the acidification potential, and 4) the chemical structure, macromolecular properties (e.g. molar mass and conformation) and the yield of the dextran. Studies are necessary to optimize the baking performance and to understand the techno-functional role played by dextran and other components in the composite formulations. Furthermore, the use of texturing agents may modify the flavour perception of the final product. Research dealing with the effect of dextran addition on flavour perception of bread was, however, very limited. This thesis offers for the first time a comprehensive investigation on the influence of *in situ* produced dextran on rheology, texture, nutrition, and flavour perception of composite bread formulations. The literature review provides a general description of alternative flours (i.e. faba bean, sorghum, and pearl millet) and their flavour and texture challenges in bakery product applications, followed by the introduction of sourdough bioprocessing technology and an in-depth depiction of the functional metabolites exopolysaccharides (EPS) with dextran being the focus (i.e. biosynthesis, structure, and macromolecular properties). The art of flavour perception and the texture-taste-aroma interactions that determine flavour perception are summarized. In the experimental part, an outline of materials and methods used in three publications (**I-III**) are presented followed by a summary of the most important results. Finally, a general discussion, conclusion and future prospects are provided.

2 LITERATURE REVIEW

2.1 Applications of composite flours in wheat bread

2.1.1 Composite flours from the past to the future

Wheat crop grows in a temperate climate which is less well adapted in most tropical countries. The increasing consumption of bread products following the urbanization in many developing countries facilitates the huge increase of wheat importation, which is harmful to the local economy and a risk to food security (Nwanekezi 2013; Ohimain 2014). The use of composite flours was initiated in the developed countries during the world wars when wheat supplies were deficient. In 1964, the Food and Health Organization (FAO) of the United Nations launched a Composite Flour Programme targeting at identifying indigenous alternatives that could be used in developing countries to partially replace wheat in bread production (CAT 1988). Composite flour is defined as "a flour mixture of non-wheat cereals or pulses with or without wheat flour that is used for making bread and other baked products traditionally produced from wheat flour" (FAO 1995). The research interest of composite flours was evoked by their economic advantages for developing countries: 1) the saving of foreign exchange on wheat importation, 2) the promotion of indigenous grains in industrial use, 3) the development of local agriculture sector and contribute to the country's GDP, and 4) the increase of nutritional value (Noorfarahzilah et al. 2014). In 1970's, several laboratory trials were done in European countries and field work has been carried out in Latin America, Asia and Africa under joint projects by FAO and UNDP. The International Association for Cereal Science and Technology (ICC) subsequently promoted the research and investigation in composite flours and numerous publications were released. Governments of several developing countries such as India, Nigeria, Peru, and Philippines have taken the initiative of programs to examine the feasibility of using local grains as a substitute for wheat flour. Studies have shown that a high level of substitution such as 40% in composite bread could create a large demand of alternative crops, which would boost farm income, increase employment, and contribute to rural development (Ohimain 2014). They revealed that starch rich ingredients (e.g. cassava and sweet potato), cereals (e.g. sorghum, millet and maize), and legumes (e.g. soy bean, chickpea, and lupin) can be used to partially replace wheat in making bread or biscuit products. These alternatives have been selected mainly based on their availability and the suitability/compatibility for end use.

In the last few years, meat substitutes or plant-based eating is growing fast and moving more mainstream (Innova Database 2019). The environmental change and sustainability of the planet are the main concerns of consumers and the driving force for meat alternatives (Innova consumer survey 2018). The increasing rates of chronic

and degenerative diseases, such as obesity, diabetes, cardiovascular disease (CVD), and certain cancers, in many Western countries due to the diet and lifestyle have also gradually changed the attitude of consumers toward food (Cencic and Chingwaru 2010). Nutraceutical and functional foods that possess prophylactic or therapeutic properties will be an important or even a major fraction of total food in the near future. Composite flours that contain legume proteins and dietary fiber have therefore gained renewed interest and are highly promoted for their functional and nutritional characteristics in making/designing novel food products (Coda et al. 2017b; Rangaraju 2014; Udachan 2018).

Legumes or legume proteins can be included in baked goods to obtain a high protein product with enhanced amino acid balance. Legumes are rich source of lysine (an essential amino acid) but deficient in sulphur-containing amino acids, making them a good complement to wheat having a low content of lysine but a relatively high amount of sulphur amino acids (Singh and Singh 1992). Faba bean (*Vicia faba* L.) is a widely cultivated legume crop, which has been a food staple and major supplier of dietary protein in many regions in the world (Singh et al. 2013). The global average annual production of faba bean showed a general trend of decline from 5.2 million tons between 1961-65 to 3.4 million tons between 1991-2000, followed by an increase to 4.5 million tons during 2010-2017 (FAOSTAT 2017). Asia accounted for the largest part of production share (39.3%) followed by Africa (28.1%) and Europe (20.5%) (FAOSTAT 2017). Faba bean has mostly been used for livestock feed or as human edible seeds (Singh et al. 2013). During recent years, the consumption shift towards sustainable alternative proteins has brought great attention to faba bean due to the high nutritional value of its seeds, which contain approx. 40 and 31% of starch and protein, respectively (Duc et al. 1999). They also contain fair amounts of vitamins, minerals, dietary fiber, and bioactive components such as phenolic compounds and γ-aminobutyric acid (Jezierny et al. 2010). Furthermore, faba bean is a versatile crop compared with other pulses. When faba bean has been used for crop rotations it contributes to the sustainability of cropping system by fixing nitrogen, reducing fossil energy consumption, and providing protein-rich food and feed (Singh et al. 2013).

In spite of its popularity, the presence of several antinutritional factors in faba bean may lead to health issues (Crépon et al. 2010) and represents one of the main reasons for the limited use. For instance, the raffinose family oligosaccharides (RFOs)–raffinose, stachyose and verbascose, which are the major soluble sugars in faba bean, have been reported to cause gastrointestinal discomfort (Salunkhe and Deshpande 1991). Phytic acid, which is the main storage form of phosphorus of faba bean seeds, may reduce the bioavailability of minerals due to the formation of complexes and decrease the protein digestibility by inhibiting the activity of protease enzymes in the digestive tract. Tannins, present in the seed coats, are able to precipitate proteins and thus inhibit their absorption. However, phytic acid and tannins also hold some positive health effects, such as decreasing the toxicity of

heavy metals and reducing plasma LDL-cholesterol levels (Crépon et al. 2010). Faba beans are also rich in two glucosidic aminopyrimidine derivatives, vicine (V) and convicine (C), which are concentrated in the cotyledons. The presence of V and C is a significant cause of favism, an acute haemolytic anaemia (prematurely destruction of red blood cells), in individuals having an X chromosome-inherited glucose-6-phosphate dehydrogenase (G6PD) deficiency (Arese and De Flora 1990).

Sorghum (*Sorghum bicolor* L. Moench) and pearl millet (*Pennisetum glaucum*) have been important cereals in the arid and semi-arid tropics of Africa and Asia for centuries (FAO 1995). They are the major sources of macronutrients, energy, vitamins and minerals for millions of people. Sorghum and pearl millet are cultivated in harsh climate conditions with limited water resources and agricultural input, mainly by small farm-holders. The annual production of sorghum in the world showed an increase from 44 million tons in 1961-1965 to 61 million tons in 2007-2017 (FAOSTAT 2017). The three largest production regions are Africa (47.3%), Americas (34.8%), and Asia (14.5%) in 2017. The FAO data on millet production include different millets such as pearl millet and minor millets. The global millet production also expanded form 25 million tons in 1961-1965 to 32 million in 2007-2017. The top two production regions were Asia (51.7%) and Africa (45.2%) in 2017. Sorghum and pearl millet are mainly consumed as wholegrains in making porridges, flatbreads, couscous and alcoholic beverages (FAO 1995). Sorghum wholegrains contain 75% carbohydrates, 11% protein, 3% lipids, 10% dietary fiber, and a considerable amount of B-complex vitamins and minerals such as calcium and iron (Dias-Martins et al. 2018). Pearl millet contains 72% carbohydrates, 12% protein, 6% lipids, 8% dietary fiber, and 3% minerals, particularly iron and zinc (Dias-Martins et al. 2018). Sorghum and millets are well recognized for their health promoting profiles, namely the dietary fiber and phenolic compounds. Consumption of dietary fiber reduces the risk of coronary heart disease, stroke, diabetes, obesity and certain gastrointestinal disorders (Anderson et al. 2009).

Phenolic compounds are concentrated in the pericarp. Numerous phenolic acids and flavonoids have been identified in sorghum and millet grains, where they occur largely in bound form associated with the cell walls through ester or ether bonds (Taylor et al. 2014). The major phenolic compounds in sorghum are ferulic acid and anthocyanin, whilst in pearl millet the ferulic and cinnamic acids, apigenin, and myricetin are dominant (Shahidi and Chandrasekara 2013). Phenolic compounds are natural antioxidants which have been constantly shown to prevent or reduce oxidative stress, prevent cardiovascular disease, and possess anti-cancer, anti-diabetic, anti-inflammatory, and anti-hypertensive properties (Taylor et al. 2014). Furthermore, sorghum and pearl millet are known to have lower starch digestibility and glycemic index (GI) than wheat (Annor et al. 2017). The main antinutritional compounds present in sorghum and pearl millet are tannins and phytic acid.

2.1.2 Product-specific texture and flavour challenges

Most of the studies reported that wheat can be replaced by 5 to 10% without significant alternation of the final bread quality (Ohimain 2014). A higher percentage inclusion of alternative flours improves the nutritional functionality and health benefits of the end products as well as the use of locally grown grains, but are challenged by the poor product quality. Compared to wheat, alternative grains such as faba bean, sorghum and millet are often considered as "inferior grains" for bakery applications due to their absence of gluten functionality. Wheat flour contains the unique gluten proteins (80-85% of total wheat protein), which are divided according to their solubility in alcohol-water solution into the insoluble polymeric glutenin (contributes to dough elasticity) and the soluble monomeric gliadin (confers viscosity) (Goesaert et al. 2005). Gluten proteins are the key structure forming proteins, which are able to absorb water and form a continuous network and thin films around the gas cells. They provide dough with viscoelastic properties which allow the retention and expansion of gas bubbles during fermentation and oven rise. The gas retention in turn contributes to the loaf volume and texture of the final bread (Goesaert et al. 2005). The replacing of wheat flour with high levels of gluten-free flours thus produce inadequate products with respect to loaf volume, crumb structure, mouthfeel and staling rate (Aprodu and Banu 2015; Mariera et al. 2017). Apart from the different technological functionalities of the proteins, the presence of a considerable amount of dietary fibers in the alternative flours disrupts the gluten network formation and also influences starch gelatinization and retrogradation characteristics.

Coupled with the texture deficiencies, composite bread making is also challenged by the lower consumer acceptance of the product flavour. Flavour, a simultaneous perception of smell, taste and chemical stimuli, is the most important factor determining food choice. Legume and cereal raw materials each have their characteristic flavour components and flavour precursors. The flavour of the untreated native grains or flours is rather mild. The specific flavour of the resulting products is, however, mostly formed during food processing due to the process-induced modifications of the flavour active components (Heiniö et al. 2015). In bread making, for instance, fermentation and the following baking process are critical steps for flavour formation (Heiniö 2014). Flavour active volatile compounds are mainly associated with the perceived odor as such, whereas flavour active non-volatile compounds affect directly the perceived taste or act as flavour precursors to form new flavours (Heiniö et al. 2015). The most important volatile compounds are aldehydes, ketones, and alcohols, while the important non-volatile compounds are free fatty acids and lipids, phenolic compounds, amino acids, small peptides, free sugars, and organic acids (Heiniö et al. 2008). Lipid hydrolysis and subsequent fatty acid oxidation have a profound impact on flavour stability of the flour and the final products, which is often perceived as off-notes. For example, the unpleasant "beany flavour", which is the main barrier to greater human consumption of faba bean, soybean, and other legume based products, is caused by oxidation of fatty acids such

as linoleic and linolenic acids catalyzed by its endogenous lipoxygenase or peroxidase (Jiang et al. 2016). The further oxidation of hydroperoxides induced by heating or enzymes thus creates off-flavour compounds. Phenolic compounds are the key factor for the challenging bitterness flavour of wholegrain cereal products (Heiniö et al. 2008; Heiniö et al. 2015). In wholegrain sorghum foods for instance the bitterness is correlated to the total polyphenol content, and especially condensed tannins contained in the external layers (Kobue-Lekalake 2009). The bitter taste becomes more intense after baking. Both free and bound phenolic acids and flavonoids may cause bitterness. However, the free soluble phenolic compounds are more flavour-active than the bound compounds since they are readily dissolved in saliva and can adhere to the taste receptors (Heiniö et al. 2015).

Hydrocolloid additives have been used to minimize the undesirable/detrimental texture changes when wheat is substituted at higher dosage levels above 20% with non-wheat flours. For example, Angioloni and Collar (2012) formulated highly nutritious wheat-legume (chickpea/pea/soybean) breads (with wheat flour substitution up to 42%) by using carboxymethylcellulose (CMC, 6% flour basis) as the structuring agent and found acceptable dough viscoelasticity and bread sensory quality. Previtali et al. (2014) used CMC and guar seed flour (guar gum) at a concentration of 2% (flour basis) in wheat bread enriched with 25% lentil flour and observed softer crumb and higher sensory properties. The use of these additives would add to the cost of the final product and necessitate declaring on the ingredient label, which is not appreciated by consumers requiring more natural food products containing minimum or no additives.

2.2 The potential use of dextrans from sourdough LAB

2.2.1 Sourdough bioprocessing technology

Sourdough fermentation is one of the oldest food biotechnologies (>5000 years) widely employed in the manufacture of baked goods. It is regarded as a natural, sustainable and effective way to enhance sensory, microbial safety and shelf life of the final products. Sourdough is a mixture of flour and water fermented by spontaneous or inoculated lactic acid bacteria and/or yeasts (Hammes and Gänzle 1998). The bacteria activities result in organic acid production and activation of a number of cereal endogenous/bacteria enzymes while the yeasts are the main responsible for carbon dioxide production (Hammes and Gänzle 1998). Sourdough was originally utilized as a natural leavening and acidifying agent in the commercial and household production of wheat and rye bread (Siepmann et al. 2017). Current research has been dedicated to utilizing sourdough in alternative grains and flours such as quinoa (Rizzello et al. 2016), teff (Wolter et al. 2014), buckwheat (Wolter et al. 2014), barely (Rieder et al. 2012), sorghum (Galle et al. 2012b), millet (Adebiyi

et al. 2017) and faba bean (Coda et al. 2017b), to improve their nutritional- and/or techno-functionality and thus to enrich or completely replace wheat flour.

Numerous results have appeared in the literature showing how sourdough fermentation would affect the nutritional properties of alternative flours and their related baked goods. For instance, fermentation of faba bean flour was found an effective tool to decrease antinutritional factors such as vicine and convicine (>91%), trypsin inhibitor, and condensed tannins (>40%) (Coda et al. 2015). The fermented faba bean also showed a significantly higher amount of free amino acids (e.g. essential amino acids and γ-aminobutyric acid) and improved protein digestibility (Coda et al. 2015). Fermentation of pearl millet flour was observed to reduce the phytate content by more than 50% due to the action of cereal/bacteria phytases, which are able to dephosphorylate phytate and are activated in acidic environment (optimum pH 4.5) (Omoba et al. 2015). Adebiyi et al. (2017) showed that fermentation increased the crude fiber, crude protein, and majority of the essential and non-essential amino acids of pearl millet flour and biscuit products. Furthermore, fermentation was shown to improve the bioavailability of minerals such as iron, calcium, and manganese in millet and sorghum flour (Makokha et al. 2002). Fermented pearl millet and sorghum flour also exhibited higher phenolic content and substantially increased antioxidant activity (Omoba et al. 2015; Zaroug et al. 2014). Additionally, sourdough fermentation also reduced the *in vitro* starch digestibility and increased content of resistant starch, lowering the predicted glycemic index (GI) of gluten-free sorghum bread (Wolter et al. 2014).

Microbial metabolism during sourdough fermentation may also produce some functional secondary metabolites such as texture-enhancing exopolysaccharides (EPS). LAB synthesize a diverse range of polysaccharides including intracellular storage polysaccharides such as glycogen, cell wall structural polysaccharides (WPS) such as peptidoglycan and lipoteichoic acids, and exocellular polysaccharides which together with a few glycoproteins are grouped within the term “glycocalyx” or “sugar coat” (Reitsma et al. 2007). The exocellular polysaccharides can be subdivided into two groups according to their location relative to the bacteria cell. Capsular polysaccharides (CPS) are linked to the cell surface via covalent bond (form a thick outer layer named capsule) and exopolysaccharides are loosely attached to the cell surface or released into the environment (form slime) (Madigan et al. 2006). The biological role of EPS in their natural environment is still unclear. It was suggested that EPS may play a role in cellular recognition, protection of bacteria cell integrity under adverse conditions (desiccation, osmotic shock, pH shifts), antibiotic resistance, surface adhesion, and biofilm formation (Chapot-Chartier et al. 2011; De Vuyst and Degeest 1999; Dertli et al. 2015; Looijesteijn et al. 2001). EPS being high molar mass natural polymers have a wide range of industrial applications ranging from pharmaceutical to food industry as thickening, gelling or stabilizing agents and cosmetic and chemical industries (Mishra and Jha 2013). LAB EPS have been well recognized for their important role in the rheological and organoleptic

attributes of fermented dairy products such as yogurt, fermented cream and milk based desserts, and as a fat replacement in the production of low-fat cheeses (Duboc and Mollet 2001). In recent years, LAB EPS have gathered increasing interest from bakery industry showing the potential to replace or reduce hydrocolloids utilized as bread improvers.

2.2.2 Types of exopolysaccharides produced by LAB

EPS produced by LAB can be classified into two categories according to the biosynthesis mechanism and structural features: the homopolysaccharides (HoPS) that are composed of single type of monosaccharide and the heteropolysaccharides (HePS) comprised of three to eight repeating units of different monosaccharide combinations where D-glucose, D-galactose, and L-rhamnose are most often presented, and, in few cases, N-acetyl-aminosugars, glucuronic acid, and non-carbohydrate substituents (De Vuyst and Degeest 1999). The majority of EPS produced by LAB are HePS. The HePS are produced by a great variety of mesophilic and thermophilic LAB belonging to the genera: *Lactobacillus*, *Lactococcus* and *Streptococcus*. The monosaccharide compositions and structure of HePS show very few common features and are not species-specific (De Vuyst and Degeest 1999). Conversely, HoPS demonstrate more structural similarities, which have a main backbone structure with variable degrees of branching and can be clustered into four groups: (i) α-D-glucans [dextran: > 50% α-D-Glc(1→6) with less frequently α-(1→2), α-(1→3) and α-(1→4) branching points; mutan: > 50% α-D-Glc(1→3) with α-(1→6) linked branches; alternan: alternating α-D-Glc(1→3)/(1→6); and reuteran: α-D-Glc(1→4)/(1→6)], (ii) β-D-glucans [β-D-Glc(1→3) with β-(1→2) side chains], (iii) β-D-fructans [levan: β-D-Fru (2→6); and inulin type: β-D-Fru(2→1)], and (iv) others [polygalactan: α-D-Gal/β-D-Gal with a pentameric repeating unit of galactose; and glycogen-like: α-D-Glc(1→4)] (Monsan et al. 2001; Ruas-Madiedo et al. 2002). *Streptococcus*, *Pediococcus*, *Lactobacillus*, *Leuconostoc* and *Weissella* species among LAB strains are the most frequent HoPS producers.

The biosynthesis of HePS is a rather complex process involving four key stages: sugar transportation, intracellular sugar nucleotide precursors and repeating units synthesis in the cytoplasm, repeating units translocation across the membrane, and eventually extracellular polymerization (De Vuyst and Degeest 1999). Energy is intensively required during most of the HePS synthesis steps. The biosynthesis of HoPS, in contrast, is a relatively more simple process which happens extracellularly and requires the presence of a specific substrate such as sucrose. The biosynthetic pathway does not involve any active transportation and energy expenditure. The molar mass of HePS ranges from 10 to 6,000 kDa which is generally lower than HoPS (up to 400 MDa) (De Vuyst and Degeest 1999; Zarour et al. 2017). The yield of HePS reported, varying between 0.15 and 0.6 g/L, is also lower than HoPS

usually 1−10 g/L (De Vuyst and Degeest 1999). In dairy products, the effective concentrations observed for HePS are very low. In fermented milk, for instance, the HePS amounts reported ranged from 0.03 to 0.4 g/L (Amatayakul et al. 2006). However, in cereal and bakery industry, few studies have reported the applications of HePS due to the very limited production. Thus, the main focus has been given to HoPS in baked products possibly owning to their larger production quantities and the dominance of HoPS producing strains in cereals (Galle and Arendt 2014; Lynch et al. 2018). In this thesis, we focus on dextran, which is by far the most investigated HoPS in cereal products.

2.2.3 Dextrans

2.2.3.1 Biosynthesis

Dextran occurs naturally in small proportions in foods, such as refined crystalline sugar, maple syrup, sauerkraut juice, and honey, and also as a component of dental plaque (Kothari et al. 2014). Pasteur was the first to discover dextran in the form of slime production by small cocci in cane sugar syrup (Pasteur 1861). The slime was assigned the name dextran afterwards by Scheibler (1874), who described the nature of the product to be a carbohydrate of empirical formula ($C_6H_{10}O_6$) with a positive rotatory power. The microorganism responsible for the slime production was later isolated by Van Tieghem (1878) and given the name *Leuconostoc mesenteroides*. In 1930, Hucker and Pederson did systematic studies on the genus of *Leuconostoc* and reported the dextran formation from sucrose (Hucker and Pederson 1930). Subsequently, Hehre (1941) reported the first cell-free synthesis of dextran using sucrose as the substrate and enzyme prepared from the culture supernatant of *Leuconostoc mesenteroides*. Afterwards, the corresponding extracellular enzyme was named dextransucrase (Hestrin et al. 1943).

Dextransucrase (sucrose: 1,6-α-D-glucosyltransferase; E.C. 2.4.1.5) is the key enzyme that catalyzes the biosynthesis of dextran and requires the presence of sucrose as the substrate. Dextransucrase has been purified and characterized from various strains including the members of *Weissella*, *Leuconostoc*, *Lactobacillu*s, and *Streptococcus* genus (Schmid et al. 2016). The biosynthesis can be *in vitro* by using purified dextransucrase (cell-free) with sucrose as a substrate, or *in situ* by cultivating LAB strains on substrates supplemented with sucrose (Leemhuis et al. 2013). The optimum reaction conditions for the isolated enzyme are strains dependent, with pH ranged from 5.0 to 6.5 and temperature from 30 to 45°C (Ullrich 2009). Small proportions of calcium (such as 0.005%) are needed for optimal enzyme activity and dextran production. The sucrose concentration also affects the dextransucrase activity. Hehre and Sugg (1942) showed that an increase of sucrose concentration from 0.5 to 5% corresponded to increased yields of dextran in the cell-free extracts containing dextransucrase from *L. mesenteroides*. Kim et al. (2003)

studied *L. mesenteroides* dextransucrase at even higher sucrose concentrations from 0.1 to 4 M. They observed a reduction of high molar mass dextran (>10^6 Da) and a proportional increase of low molar mass dextran (<10^5 Da) with an increased sucrose amount. Furthermore, the degree of branching increased accordingly from 5% to 16.6% (Kim et al. 2003). They also reported that the degree of dextran branching increased from 4.8 to 14.7% with increasing temperature from 4 to 45°C. *In situ* formation of dextran on the agar plate is detected by the glistening and slimy appearance of colonies (Figure 1). In commercial fermentative production, the *L. mesenteroides* strains have been used. The practical operation conditions are set at initial pH 6.7-7.2, temperature 25°C, initial sucrose concentration approx. 2%, and time 24-48 h (Vandamme et al. 2002). With decreasing temperature form 25 to 4°C, the high molar mass dextran reduces with a concomitant increase of the low molar mass dextran, leading to less viscous solutions (Belder 2000).

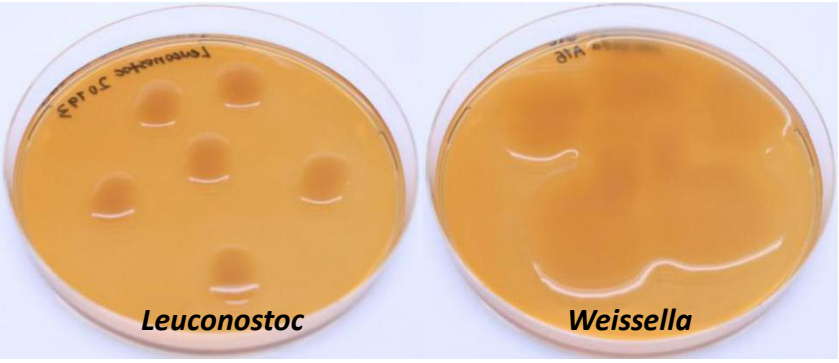

Figure 1. Slimy colonies (dextran production) of *L. pseudomesenteroides DSM 20193* and *W. confusa* A16 on MRS agar plates supplemented with 5% sucrose.

The reaction mechanism of dextransucrase has also been studied and yet not fully clarified. Efforts have been put in elucidating the primary and three-dimensional structures of the enzymes and understanding the functions of their catalytic domains (Leemhuis et al. 2013). In a double-displacement (or retaining) mechanism (Figure 2), the glycosidic bond of sucrose is firstly cleaved (glycoside hydrolase activity), resulting in a covalent β-glucosyl-enzyme intermediate and a free fructose fraction; in the second stage, the glucosyl moiety is transferred to a series of acceptors by transglycosylation reactions (transglycosylase activity) (Leemhuis et al. 2013; Monsan et al. 2001). The primary catalytic amino acid residues in the first stage are comprised of two aspartic (a nucleophile and a stabilizer of the glucosyl intermediate) and one glutamic residue (a general acid/base catalyst as proton donator) (Monchois et al. 1999). Whether the dextran chain is elongated from the non-reducing end or the reducing end is still under debating. The reducing end mechanism involves two nucleophilic reaction sites that allows formation of two covalent β-glucosyl-enzyme intermediates from two sucrose molecules (Monsan et al. 2001). The C-6 hydroxyl group of one of the glucosyl intermediates makes a nucleophilic attack onto the C-1

group of the other, resulting in the formation of α-(1→6) glucosidic linkage. The released active site attacks another sucrose molecule and forms a new glucosyl-enzyme intermediate which is subsequently attached to the reducing end (C-1) of the isomaltosyl unit (or growing dextran chain) until a complete reaction cycle. Conversely, the non-reducing end mechanism involves a single active site and only one glucosyl-enzyme intermediate. Much evidence supports for the non-reducing end elongation (or one active site) based on the amino acid sequence and crystal structure analysis, labelled glucosyl-enzyme intermediate, and also biochemical and mutagenesis studies (Ito et al. 2011; Mooser and Iwaoka 1989; Moulis et al. 2006; van Hijum et al. 2006; Vujicic-Zagar et al. 2010).

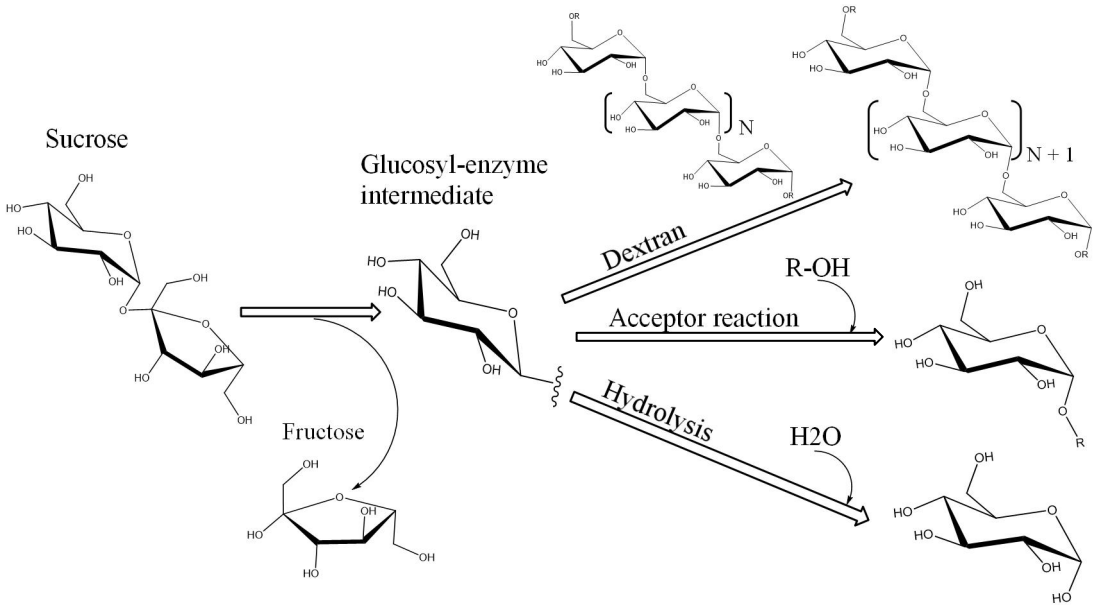

Figure 2. The enzymatic catalysis of dextran synthesis and acceptor reaction from sucrose (modified from Leemhuis et al. 2013).

In the presence of alternative glucosyl acceptors (e.g. maltose, isomaltose, and lactose), small *Mw* oligosaccharides are formed. The acceptor sugars may compete with dextran formation and terminate the polymerization of dextran by liberating it from the enzyme-complex active site, thus decrease the yield and *Mw* of the final dextran product. In 1983, Robyt and Eklund studied the influence of 17 different saccharide acceptors and reported that maltose was the strongest alternative acceptor, followed by isomaltose, D-glucose, lactose, raffinose, melibiose, D-galactose, and D-fructose (Robyt and Eklund 1983). The acceptor reaction of maltose results in the formation of predominantly a linear series isomaltooligosaccharides (IMOs) with a degree of polymerization (DP) of up to 8 and a minor homologous series of α-(1→2) branched IMOs (Shi et al. 2016). IMOs are promising prebiotics with a higher DP (> 3) preferred for lower digestibility in the gastrointestinal tract and longer persistence in the colon (Iwaya et al. 2012). The α-(1→2) branched IMOs also present high prebiotic effects and have been applied in commercial prebiotic products (Plou et al.

2002). Fructose, a major hydrolysis product in the dextransucrase-catalyzed synthesis of dextran from sucrose, acts as a weak acceptor and results in the production of a disaccharide leucrose in small quantities and isomaltulose in some cases (Korakli and Vogel 2006; Shi et al. 2016). Furthermore, water is used as a minor acceptor which gives the hydrolysis of sucrose.

Previous studies stated that branches of different length are formed by the action of an acceptor dextran chain on the reducing end of the glucosyl- or dextranosyl-dextransucrase covalent complexes. To be more specific, the C-1 hydroxyl group of the glucosyl moiety or the dextranyl chain are attached to the secondary hydroxyl positions such as the C-3–OH group on an exogenous dextran chain and results in the formation of a new α-(1→3) branch linkage (Robyt and Taniguchi 1976). Bozonnet et al. (2002) investigated the bi-functions of dextransucrase from *L. mesenteroides* NRRL B-1299 using molecular cloning, which contains two catalytic domains displaying both polymerase activity and α-(1→2) branch formation. More recent studies, however, suggested that dextransucrase is not the major catalyst for branch linkage synthesis. They revealed the presence of separate α-(1→2) branching sucrase *in vivo* for the branching of dextran (Moulis et al. 2016; Passerini et al. 2015). An α-(1→3) branching sucrase has also been identified, which exhibits no polymerase activity but catalyzes efficiently the branch formation by transferring the glucosyl residue from sucrose to linear α-(1→6) dextran acceptor (Vuillemin et al. 2016). The dextransucrase and branching enzymes might work in a synergistic manner and further studies to elucidate the mechanisms are still required.

2.2.3.2 Structure and physicochemical properties

Dextran consists of predominate α-(1→6) main linkage and side chains attached to the 2- 3- or 4-positions of the backbone glucose units as shown in Table 1 (Monsan et al. 2001). The chain length of the branches are often short, of which approximately 85% consists of one to two glucose residues and the remaining 15% may have an average of 33 unevenly distributed glucose residues. Most of the studies have been done on structural analysis of dextrans from *Leuconostoc* species. Pharmaceutically, the most important dextran is synthesized by *L. mesenteroide*s NRRL B-512, which is characterized by 95% α-(1→6) linkages and 5% α-(1→3) branch linkages. Dextrans synthesized by *Weissella* species feature a very linear structure with only 3-4 % α-(1→3) branches (Netsopa et al. 2018).

Due to the presence of branches, dextrans may exhibit comblike, laminated, or ramified structures, which has been a matter of debate (Figure 3). Different branching distributions may occur, such as random or regular distributions along the main chains or form clusters (Vettori et al. 2012). The comblike structure was firstly proposed since a vast fraction of the side chains of dextran seems to consist of single α-glucosylpyranosyl units (Kenne and Lindberg 1983). Sabatie et al. (1988) later

Table 1. An overview of the linkage pattern and molar mass analysis techniques of native dextrans produced by LAB strains.

Microoganisms	α-(1→6)	α-(1→3)	α-(1→2)	α-(1→4)	Molar mass (kDa)	Structural studies	References
W. confusa Cab3	97	3			18000	NMR, HPSEC analysis	Shukla et al. 2014
W. confusa E392	97.3	2.7			6400	^{1}H and ^{13}C NMR, HPSEC	Maina et al. 2008
W. confusa R003	97.4	2.6			10	^{1}H, ^{13}C and 2D NMR, DLS	Netsopa et al. 2018
W. cibaria Sj1b	95.9	4.1			2452	NMR, HPSEC	Xu et al. 2018
W. cibaria CMGDEX3	96.6	3.4			2000	^{1}H and ^{13}C NMR, FTIR	Ahmed et al. 2012
W. cibaria YB-1	95.7	4.3			3890	NMR, FTIR	Ye et al. 2018
W. cibaria RBA12	97	3				^{1}H and ^{13}C NMR, FTIR	Baruah et al. 2017
L. mesenteroides NRRL B512F	95	5			9000–500000	GLC-MS	Lindberg et al. 1968
L. mesenteroides AA1	100				10000–40000	NMR, HPSEC, FTIR and SEM	Aman et al. 2012
L. mesenteroides FT045B	97.9	2.1			91	NMR, FTIR	Vettori et al. 2012
L. citreum E497	74.8	3.5	11		11000	^{1}H and ^{13}C NMR, HPSEC	Maina et al. 2008
L. citreum NM105	67.6		32.4		100000	^{1}H, ^{13}C and 2D NMR, HPSEC, FTIR	Yang et al. 2015
L. pseudomesenteroides YB-2	96.8	3.2			767	NMR, FTIR	Ye et al. 2018
L. pseudomesenteroides DSM 20193	94.2	5.8			4379	NMR, HPSEC	Xu et al. 2018
L. carnosum CUPV411	91.7	6.8		1.5	358000	NMR, FTIR, SEC-MALLS, X-ray	Llamas-Arriba et al. 2019
Lb. mali CUPV271	94.9	3.6		1.5	123000	NMR, FTIR	Llamas-Arriba et al. 2019
Lb. curvatus TMW 1.624	95	5			37500–45670	NMR, FTIR and SEM	Rühmkorf et al. 2013
Lb. reuteri TMW 1.106	86			14	6530–7020	NMR, FTIR and SEM	Rühmkorf et al. 2013
P. pentosaceus CRAG3	75	25			293	NMR, FTIR and SEM	Shukla et al. 2013
S. sobrinus GTF-S1	68	32				NMR	Taylor et al. 1990
S. mutans GS-5	69	31				NMR	Shimamura et al. 1994

W. = *Weissella*, *L.* = *Leuconostoc*, *Lb.* = *Lactobacillu*s, *P.* = *Pediococcus*, *S.* = *Streptococcus*.

suggested that the ramified structure based on the acceptor reactions. The techniques for assessing the side chain distributions are still limited. However, the great variation in the type and degree of branches present in dextrans, a less regular distribution would be expected.

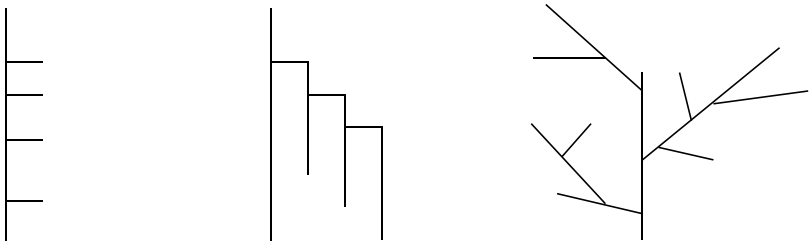

Figure 3. Proposed structures of dextrans (Kenne and Lindberg 1983).

Like other hyper-branched macromolecules, native dextrans have a wide molar mass distribution ranging from 10 to 10^6 kDa and a high degree of polydispersibility. The molar mass of native dextran can be reduced by acid/enzyme hydrolysis and fractionation to produce clinical dextrans (40-70 kDa). The polydispersity index (PDI), the ratio between weight average molecular weight (*Mw*) and number average molecular weight (*Mn*), reflects the width of molecular weight distribution of a polymer (Rogošić et al. 1996). For example, a polymer with PDI value 1.0 indicates a monodisperse which displays uniform chain lengths. The radii (R_g = radius of gyration, R_h = hydrodynamic radius) values are useful parameters to estimate the size of the dextran molecules in solutions and increase with *Mw* (Ioan et al. 2000). The structure sensitive parameter ρ (ρ = R_g /R_h) of dextrans is generally lower than β-glucan with similar *Mw*, indicating a more compact structure of dextrans in solutions (Kirkwood and Riseman 1948).

The properties (solubility and viscosity) of dextran aqueous solutions are largely dependent on their structure and molecular weight and are independent from pH or salt concentration (Kothari et al. 2014). Generally, the solubility increases with increased branches (Belder 2000). Some dextrans are readily soluble in water to give a clear and stable solution. Some may have a certain degree of crystallinity or form aggregates in solutions (e.g. lyophilized dextran) and need to be strongly heated to dissolve (Belder 2000). Dextran exhibits a compact coil conformation in poor solvents such as ethylene glycol and an expanded conformation in good solvents like methyl sulphoxide or formamide (Belder 2000). Dextran is insoluble in monohydric alcohols (e.g. methanol and ethanol) and ketones (e.g. acetone and 2-propanone).

The viscosity (η) of dextran solution is related to the solvent viscosity (η_o) which leads to the relative viscosity (η_r) and specific viscosity (η_{sp}) (Mezger 2006). The specific viscosity represents the viscosity increment due to the dissolved polymer in the solvent.

$$\eta_r = \frac{\eta}{\eta_0} \quad (1)$$

$$\eta_{sp} = \eta_r - 1 \quad (2)$$

The inherent viscosity (η_i), the viscosity increment per unit concentration (c) of the polymer, indicates the specific capacity of the polymer to increase relative viscosity:

$$\eta_i = \frac{\eta_{sp}}{c} \quad (3)$$

By extrapolating value of η_{sp}/c to zero concentration, the intrinsic viscosity [η] may be calculated (Mezger 2006):

$$[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c} \quad (4)$$

The intrinsic viscosity, representing the hydrodynamic volume occupied by a polymer, is a measure of the intrinsic ability of the polymer to increase viscosity in a given solution. It is affected by a number of factors such as the type of solvent, percentage and length of side chains, number of intermolecular bonds and temperature (Jeanes 1966). Intrinsic viscosity is often determined by dilute polymer solution viscosity measurements over a range of concentrations. For an ideal Newtonian fluid, the solution viscosity is independent of the shear rate and thus the plot of shear stress versus shear strain rate is linear with slope η (Mezger 2006). The Newtonian liquid can be measured using any suitable simple viscometer. One example is the rotational rheometer, in which the test fluid is sheared under controlled stress or rate conditions in certain measuring geometries such as parallel plates, cone and plate, and concentric cylinder (double gap). For a non-Newtonian fluid, the viscosity varies with shear rate. It can behave "shear shinning" that the viscosity drops at high shear rate due to structure breakdown of the polymer solution enabling easier flow (Mezger 2006). In contrast, the viscosity increases at a high shear rate due to the formation of new structures under deformation is referred to as "shear thickening". Therefore, the non-Newtonian viscosity is often measured at shear rate relevant to the specific processing or application operations.

The viscosity average molecular weight (M_v) of a polymer is related to its intrinsic viscosity [η] (mL/g) by Mark-Houwink-Sakurada equation with the empirical constant K (mL/g) and the molecular parameter a (Mezger 2006):

$$[\eta] = KMv^a \quad (5)$$

The constant K describes the polymer-solvent interactions while the exponent a reflects the conformation of the polymer chain. For values $0<a<0.5$ a rigid sphere is expected in an ideal solvent, $0.5<a< 0.8$ a flexible coil, and $0.8<a<2$ a rigid rod like shape (stiff chain). The Mark-Houwink parameters have been measured and recorded in various dextran polymer solutions with different molecular weights (180-5900000Da) and solvents, in a temperature ranged from 20 to 50°C (Masuelli and Chemistry 2013). The values of a obtained for dextrans varied from 0.27 to 0.56, indicating a compact sphere form or a random coil conformation (Masuelli and Chemistry 2013). Furthermore, the a values decrease with increased Mw likely due to the increased branches.

Dextran solutions exhibit Newtonian flow characteristics at low concentrations and non-Newtonian shear shinning behaviour at high concentrations. Depending on the polymer concentration, dextran solutions undergo a transition from dilute to semi-dilute and concentrate. The critical overlap concentration, c*, corresponds to the transition from a dilute solution where polymer molecules are isolated coils to a sime-dilute where individual polymer chains interpenetrate with each other and form an entangled network. The overlap concentration depends on the number and space occupied by the polymer molecules and is related to an abrupt increase in viscosity. Values of c* can be estimated from a log-log plot of the specific viscosity (η_{sp}) as a function of concentration (c). The c* measured in aqueous dextrans showed a great variability which might be explained by the structural diversity of dextrans. The second critical concentration, c**, corresponds to the transition from the semi-dilute region to a concentrated region where the polymer chain dimensions are independent of polymer concentration. In previous studies, dextrans produced by *P. pentosaceus* and *L. mesenteroides* NRRL B-640 showed non-Newtonian shear shinning behaviour at concentrations above 0.5 g/L (Purama et al. 2009). Dextran produced by *L. mesenteroides* NRRL B-523 demonstrated viscoelastic behaviour at 25 g/L (Padmanabhan et al. 2003). Dextran from *W. confusa* R003 showed liquid-like behaviour at concentrations below 2.5% (w/v) and viscoelastic at 5%, and gelling behaviour at 10% (Netsopa et al. 2018).

2.2.3.3 Characterization

Full characterization of dextrans requires information on the dextran producing LAB, dextran yield, monosaccharide composition and linkages, the degree of branching and length of the branches, and macromolecular parameters related to the viscosity-intensifying properties such as *Mw* and *Rg* (Ruas-Madiedo and de los Reyes-Gavilán 2005). The characterization of dextrans may be conducted by a number of physical and chemical techniques (Table 1).

Isolation of pure dextran

The structural analysis starts from the isolation of pure dextrans in a way that the chemical and physical properties are not affected (Leemhuis et al. 2013). The recovery or purification of dextran from the culture medium involves multiple steps: (1) cell removal via centrifugation or filtration, (2) dextran precipitation from the cell-free supernatant using a cold solvent such as ethanol and acetone, (3) dextran redissolved in distilled water by stirring and heating, (4) dextran reprecipitation and dialysis (optional), and (5) lyophilization (Ruas-Madiedo and de los Reyes-Gavilán 2005). Except for solvent precipitation, other methods have also been utilized to purify dextran including membrane filtration techniques, ion-exchange chromatography, and SDS-PAGE (for protein removal) (Ruas-Madiedo and de los Reyes-Gavilán 2005). Furthermore, size exclusion chromatography (SEC) and gel-filtration chromatography are often used in

the end process of purification to obtain a highly pure dextran product (Shukla and Goyal 2013; Ye et al. 2018). After the isolation process, the lyophilized dextran is weighed to calculate the dextran yield. The production output of dextran is expressed as the equivalent milligrams of dextran per milliliter of culture media. Some impurities might present in the isolated dextran fractions such as proteins and low *Mw* carbohydrates.

Molecular weight analysis

The isolated dextran fractions are usually heterogeneous mixtures of molecules of varying size. Therefore, the average molecular weights are determined, and results can be altered from different methods. Numerous techniques for determining the molecular weight of dextrans are available, of which high performance size exclusion chromatography (HPSEC) is the most often used and can be coupled with refractive index detection and multi-angle laser light scattering (MALLS) (Leemhuis et al. 2013; Ye et al. 2018). In HPSEC analysis, high molecular weight dextrans might form aggregates in aqueous solutions and cause problems in filtration and may give inaccurate results (Maina et al. 2014). Dimethyl sulfoxide (DMSO) may be the solvent of choice to avoid the presence of aggregates. Alternative methods such as asymmetric flow filed-flow fractionation (AsFIFFF) and diffusion-ordered NMR spectroscopy (DOSY) have also been applied for size estimation of high molar mass dextrans (Maina et al. 2014).

Composition and glycosidic linkage analysis

The monosaccharide compositions of dextrans can be determined by acid or enzyme hydrolysis in combination with high performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) (Maina et al. 2008). The absolute configuration (D or L) of the glucose residues of dextrans can be established using gas chromatography (GC) (Gerwig et al. 1979). The linkage analysis of dextrans are performed by one-dimensional (^{1}H and ^{13}C) or two-dimensional nuclear magnetic resonance (NMR) spectroscopy (Ahmed et al. 2012; Vettori et al. 2012; Yang et al. 2015), which is the most powerful technique providing detailed information on the primary structure of dextran. For instance, the chemical shifts reflect the type of linkage and the area of peaks reflects intensities of each linkage.

2.2.4 Application of LAB originated dextrans in bread system

2.2.4.1 *In situ* versus *ex situ*

LAB dextrans can be applied in baked goods through the application of sourdough biotechnology, namely the use of sourdoughs started with well characterized dextran-producing strains. Dextran enriched sourdough is usually added at 10 to 40% based on dough weight, to obtain a product with enhanced quality such as higher loaf volume and

softer crumb (Galle et al. 2012b; Katina et al. 2009; Wolter et al. 2014). The production of *in situ* functional dextran by sourdough technology can escape the rigorous toxicological testing and circumvents the labeling requirement on the list of ingredient, which results in a natural or "clean-label" product (De Vuyst and Degeest 1999). Furthermore, low molecular weight IMOs are formed during the *in situ* production when maltose is present as an acceptor. The synthesized IMOs and dextran itself may present prebiotic potential. Dextran has been shown via *in vitro* methods to be fermentable substrates for human gut bacteria such as bifidobacteria and stimulate their growth (Olano-Martin et al. 2000; Tingirikari et al. 2014). Therefore, the *in situ* production may be adjusted to design health promoting functional products. Additionally, the nutritional benefits, such as elimination of antinutritional factors, associated with sourdough bioprocessing add value to the final product. Nevertheless, the *in situ* dextran synthesis can be affected by numerous factors such as pH, temperature and composition of the cereal substrate.

As an alternative to being produced *in situ*, dextran has been added directly to food matrices during processing as an ingredient in many studies (Rühmkorf et al. 2012; Zhang et al. 2018). Indeed, dextran has a long history of use in medicines and in an indirect way (e.g. packaging materials) in food (FASEB 1975). In 2001, the European Commission authorized the commercialization of a dextran prepared from *L. mesenteroides* as a bread improver up to utilization level of 5%. The direct use of dextran as food additives in the United States, however, has not yet been permitted. The advantage of using dextran as a pure ingredient is that the desirable molecular weight, structure and amount of the polymer can be added in food processing. This can be more controlled and ensure the consistency of the positive technological effects of dextran on the end products (Lynch et al. 2018). Furthermore, the *ex situ* application may provide a possibility for employing dextrans that are produced with limited amounts in sourdough fermentation. Nevertheless, the *ex situ* application of dextran may not deliver the same technological multifaceted functionalities in food products as the *in situ* production, which is a dynamic process progressing along with sourdough fermentation. Additionally, the *ex situ* application may increase the production cost related to isolation and purification of food-grade dextran and necessitate to submit to food additive regulations.

2.2.4.2 The techno-functional role of *in situ* produced dextran

Research on LAB dextrans in the baking industry is focused on their functionality as texture improvers to replace commercially employed hydrocolloids such as hydroxypropyl methylcellulose (HPMC) and xanthan gum (Lynch et al. 2018). When applied *in situ*, the interactions between the various components of the dextran containing dough system are complex (Figure 4); the effect of dextran on dough structure, the direct impact of sourdough acidification, and the influence of acid on flour endogenous or microbial enzymes. In dough systems, parameters that are most affected

include water absorption capacity, dough consistency, strength, elasticity and gas retaining ability. In bread systems, the crumb moisture content, crumb firmness, loaf volume, and staling rate are often modified. Several fundamental and empirical rheological techniques such as farinograph, extensigraph, dynamic oscillation, rheofermentometre, alveograph, and TA texture analyzer have been used for determining the mechanical properties of dough as well as for establishing relations between these properties and the final product quality (Dobraszczyk and Morgenstern 2003; Galle et al. 2012b; Rieder et al. 2012; Ross et al. 1992).

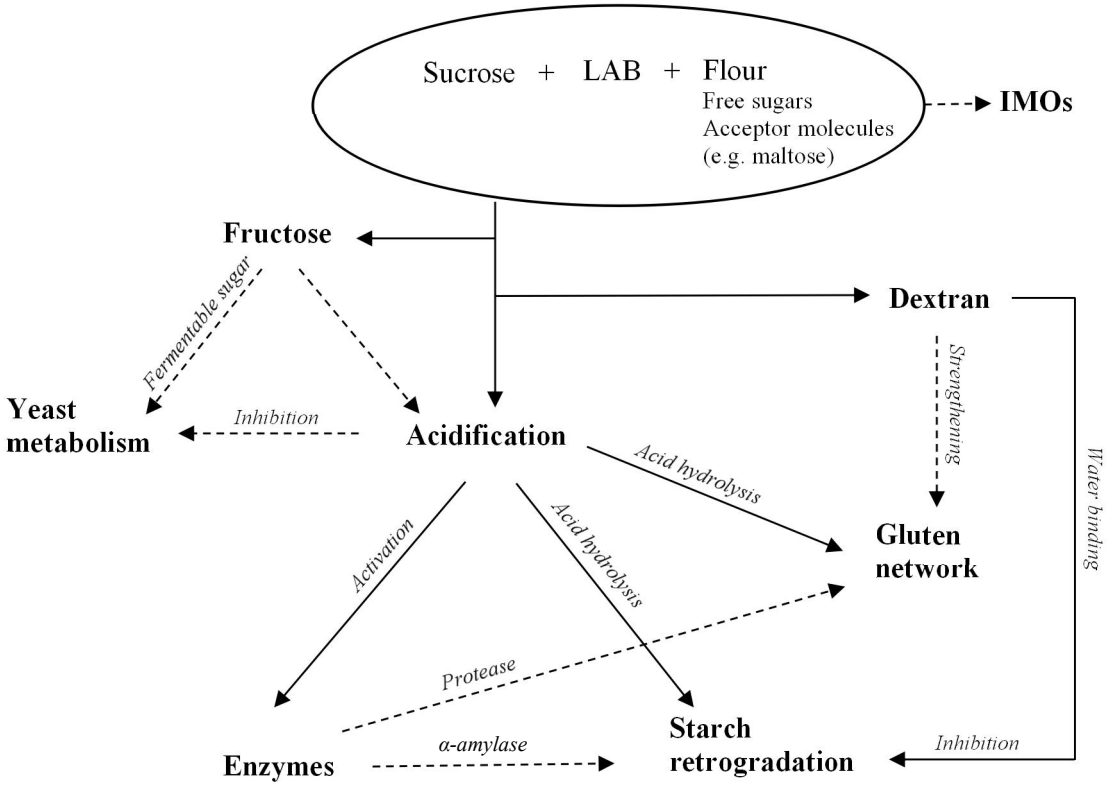

Figure 4. Interactions between the various components in the dextran containing dough system. Solid arrow: definite interaction; dashed arrow: possible interaction (adapted from Lynch et al. 2018).

The effect of dextran on dough rheology and bread textural quality

Dextran has been constantly reported for its multiple positive effects on dough rheology and bread quality including (1) increased water absorption and thus reduced water mobility, (2) modification of dough viscoelastic behaviour, (3) increased bread volume and crumb softness, (4) anti-staling, and (5) decrease in starch retrogradation (Zannini et al. 2014; Zhang et al. 2018). For example, Di Cagno et al. (2006) demonstrated that *W. cibaria* WC4 and *Lb. plantarum* LP9 produced approx. 2.5 g/kg dextran in wheat sourdough which outperformed the externally added xanthan gum, increasing significantly the bread volume and crumb softness. Katina et al. (2009) showed that

dextran formation from *W. confusa* E392 in wheat sourdough reached levels up to 16 g/kg, which resulted in a wheat bread with increased specific volume (up to 10%) and crumb softness (25-40%) over a 6-day storage period. Galle et al. (2012a) demonstrated that *W. cibaria* MG1 produced 5.1 g/kg dextran in wheat sourdough, which notably decreased dough elasticity (higher phase angle and lower complex modulus), increased bread volume (35%) and delayed staling over 5 days of storage. Similarly, Wolter et al. (2014) showed increased specific volume (29%) and reduced crumb hardness (122%) in wheat sourdough bread containing dextran produced by *W. cibaria* MG1. In respect to gluten-free products, Galle et al. (2012b) showed dextran production (8 g/kg) in sorghum sourdough, led to softer crumb and prolonged shelf-life of the subsequent sorghum bread. Wolter et al. (2014) also demonstrated a decrease in crumb hardness in buckwheat (122%), teff (29%), and quinoa (21%) sourdough breads enriched with dextran formed by *W. cibaria* MG1. However, the loaf volume was not improved in gluten-free breads by dextran application.

The mechanism of the beneficial effects induced by dextran in wheat and gluten-free systems is not fully understood. The high water-binding and retention capacity of dextran is considered to play an important role in both matrices. Furthermore, it has been suggested that in wheat-based matrix, dextran could interact with the gluten proteins, for instance by hydrogen bonding or steric interactions, and increase the dough stability and gas retention capacity (Ross et al. 1992). Improved gas retention can further lead to higher bread volume. This might also explain why a similar positive effect in specific volume that dextran imparted in wheat bread was not observed in gluten-free breads. Additionally, the fructose released from sucrose hydrolysis by dextransucrase activity might stimulate yeast metabolism during proofing and foster gas production (Galle et al. 2012a, 2012b).

The improvement of bread textural quality is most likely a function of the structure of dextrans. The structure-function relationship was proposed by Lacaze et al. (2007) that dextran with linear chain structure exhibited better effects on bread volume than dextran with more branches. The author speculated that the linear dextran chains could line up and interact with each other via hydrogen bonding thus provides stronger support for the dough structure. Rühmkorf et al. (2012) suggested that dextran with high *Mw* and α-(1→3) branched linkages displayed superior structural effects in bread, particularly the moisture retention, compared to dextrans with lower *Mw* and α-(1→4) branches. The high *Mw* α-(1→3) branched dextran presented a more compact conformation, which might bind water in a more tight way. Furthermore, Zhang et al. (2018) compared dextrans with a range of *Mw* from 10 to 2000 kDa and showed that dextran with the highest *Mw* (T2000) had the strongest retarding effect on wheat bread staling.

Staling of bread reduces product shelf-life and negatively affects consumer acceptance, generating significant food waste. Starch retrogradation is considered to be the major contributor of bread staling (Gray and Bemiller 2003). It is often accompanied by a number of physical changes such as increased amylopectin associations and crystallinity

with the formation of B-type crystalline polymorphs, and reduced water mobility by incorporation into the crystallites (Gray and Bemiller 2003). The authors studied the thermal behaviour by differential scanning calorimetry (DSC) and the crystallization pattern by X-ray diffraction (XRD) of the breads during storage and concluded that bread with dextran T2000 had the lowest retrogradation enthalpy value and lowest degree of B-type crystallites (Zhang et al. 2018). The analysis of the water mobility by low-field nuclear magnetic resonance (LF-NMR) in a follow-up study suggested that less water molecules were involved in the amorphous starch when dextran was present (Zhang et al. 2019). It was also stated that dextran could reduce water migration and restrict the swelling and gelatinization of starch granules, thus suppress amylopectin recrystallization. Additionally, Lynch et al. (2018) studied the scanning electron micrograph of dextran containing sourdough wheat bread. They showed that dextran was closely associated with the starch granules and formed a 'film' encompassing the starch, thereby preventing starch water uptake and gelatinization.

The possible effect of sourdough acidification on dextran functionality

The technological features of the bread containing *in situ* formed dextran might also be affected by the simultaneously produced acids as has been shown in previous studies using different types of EPS. For instance, Kaditzky et al. (2008) demonstrated that *ex situ* addition of levan increased the loaf volume and retarded crumb firming of wheat bread, whereas the *in situ* production of levan from *Lb. sanfranciscensis* did not show the same beneficial effects due to the enhanced acidification. Similarly, the increased acidity in reuteran-enriched wheat sourdough masked the positive effects of reuteran, resulting in reduced gas production of the dough and decreased volume and increased firmness of the bread (Galle et al. 2012a).

Acidification due to sourdough application may affect the dough structural components such as gluten proteins and starch. The major effect on gluten proteins is the increased solubility and water uptake due to their net positive charge at acidic pH values (protonation of the carboxylic side chains of the gluten proteins) (Arendt et al. 2007). Increased intramolecular electrostatic repulsion results in an unfolding of the gluten proteins and an increasing exposure of protein hydrophobic regions. This forces disentanglement of the gluten network and thus reduces its stability, resulting in a softer gluten network as indicated by the increased phase angle and reduced complex modulus (Clarke et al. 2004). The less entangled gluten network also reduces the dough extensibility and energy necessary for deformation when elongated (Komlenić et al. 2010). Further to the direct influence on gluten proteins, secondary effects of acidification are the activation of cereal endogenous or bacterial proteolysis enzymes. Flour proteases for example demonstrate an optimum activity at around pH 4 (Kawamura and Yonezawa 1982). As a consequence of the softened/weakened gluten network, the gas retention capacities are improved, which results in a higher loaf volume and less crumb firmness.

However, intensive acidification leads to over hydrolysis and degradation of gluten proteins and thus a highly softened gluten network with reduced gas retention ability, which is detrimental to bread textural quality. Thus, the beneficial effects of *in situ* produced dextran may be reduced due to the adverse effects of excessive acidification on gluten network. Acids production might also cause partially hydrolysis of the starch granules, which affects the pasting properties and starch retrogradation (Wang and Copeland 2013). Mild acidic environment favors the α-amylase activity, leading to the degradation of the crystallizable amylopectin side chains and inhibition of the inclusion of water molecules into the crystallites, thus reduces retrogradation (Goesaert et al. 2009; Zhang et al. 2019).

Moreover, studies have demonstrated similar effects of sourdough application on rheological properties of gluten-free dough and bread quality, depending on the levels of acidification (Mert et al. 2014). The effects are mainly attributed to the breakdown of non-gluten proteins which resulted in weakened protein-protein and protein-starch interactions. Therefore, when applying the *in situ* dextran production technique, both the acidification activity of the starters and the structural properties of the synthesized dextran should be taken into account.

2.2.5 Influence of LAB fermentation on bread flavour

Sourdough has a well-established role in improving the aroma of wheat and rye breads (Hansen and Schieberle 2005; Katina et al. 2006). To date, numerous studies have been performed to clarify the attractive flavouring compounds in sourdough and the resulting breads where hundreds of volatile and non-volatile compounds have been identified (Hansen 1989; Hansen and Hansen 1996; Pétel et al. 2017). Although the types of flour is a key factor for flavour formation, the processing techniques are equally important. Three mechanisms have been suggested for the flavour generation of sourdough breads: sourdough fermentation, Maillard reaction, and lipid oxidation (Figure 5). In sourdoughs, the profiles of flavour compounds are affected by the activity of the dominating microorganisms, namely the production of acids, the formation of flavour precursors (e.g. free amino acids), and the production of active flavor compounds such as volatile compounds (e.g. alcohols, aldehydes, esters and ketones) (Pétel et al. 2017).

Homofermentative LAB convert hexoses mainly into lactic acid whereas hetero-fermentative LAB produce lactic acid, acetic acid, ethanol and CO_2 (Hammes and Gänzle 1998). The concentration of these acids is considered to be an important factor in bread flavour and shelf-life. The fermentation quotient (FQ) describing the molar ratio between lactic and acetic acids, has been used for the measurement of wheat and rye sourdoughs (Corsetti 2013). It is calculated as FQ = (g of lactic acid in 100 g of dough/*Mw* of lactic acid) : (g of acetic acid in 100 g of dough/*Mw* of acetic acid). Lactic acid is described as “fermented sour taste associated with dairy products” which gives the bread a yogurt or milky like flavour whereas acetic acid gives a vinegar like and

slightly astringent flavour (Belz et al. 2019; Lotong et al. 2007). The sour characteristics can be adjusted by tailoring the fermentation conditions (e.g. specific starters, fermentable sugar, temperature, dough yield, etc.) to achieve optimal results. For instance, the production of lactic acid can be increased by increasing temperature (e.g., 35-37 ℃) and that of acetic acid by addition of fructose or by aeration with the presence of heterofermentative LAB (Gobbetti et al. 1995). Furthermore, acetic acid not only contributes to bread flavour but plays an important role in anti-microbial activity which is effective against fungal growth and rope-forming bacteria (Corsetti 2013).

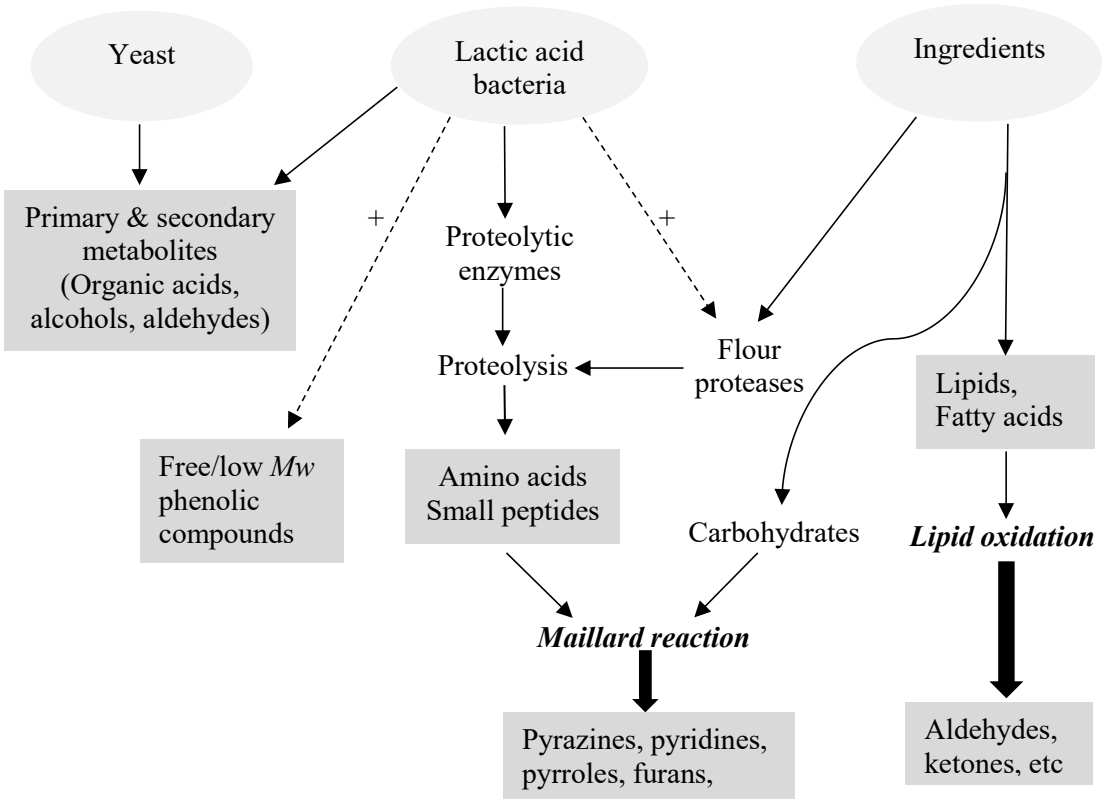

Figure 5. Formation of bread flavour during sourdough fermentation and baking process (Adapted from Pétel et al. 2017).

The proteolysis activity by flour associated proteases and/or bacteria proteinase and peptidase liberates flavour precursors such as free amino acids and small-sized peptides, which have been shown to increase after sourdough fermentation (Hansen and Schieberle 2005; Katina et al. 2004). Amino acids can be degraded during the fermentation or baking process leading to the formation of aldehydes and alcohols (Katina et al. 2004). Free amino acids or small peptides together with free sugars are also important flavour precursors in Maillard reactions, which form a number of volatile compounds, such as pyrazines, pyridines, pyrroles, furans, and sulphur-containing compounds. These compounds are important for the characteristic flavour of bread crust which are described as roasted or toasted, caramelized and sweet (Heiniö et al. 2015). In contrast, the small *Mw* peptides released from proteolytic activity are perceived as less

accepted. They are considered to contribute to the bitter taste in cereal products (Heiniö et al. 2015).

The large number of concurrent biochemical reactions during sourdough fermentation release aroma/taste compounds, or hydrolyze, oxidize endogenous compounds from the flours, which form both desired and undesired flavour attributes. The utilization of sourdough in baking does not always improve the sensory quality of products, which depends largely on the raw materials and the fermentation conditions (Meignen et al. 2001). For example, sourdough fermentation of legumes like faba bean (Varis 2017) and wholegrain flours (Heiniö et al. 2015) often increase the intensity of off-flavours and aftertaste of the subsequent bread probably due to (1) intensive acidification, (2) intensive proteolysis producing bitter taste small peptides due to the activation of bacteria or cereal endogenous protease, and (3) release of free phenolic compounds due to acid or enzyme hydrolysis. Addition of sugar in large quantity or other sweeteners/flavouring agents might be an approach to mask/inhibit the undesirable off-notes present in these products (Heiniö et al. 2015; Selvamuthukumaran and Pathak 2019). However, this may cause deleterious effects on health and is contrary to consumer expectations for reduced sugar baked goods. It is necessary to find a more natural or healthful approach to minimize the off-flavours and make composite (sourdough) bread more appealing to consumers.

2.3 The role of hydrocolloids in flavour perception

2.3.1 Oral processing and dynamic flavour perception

The perception of flavour is a multisensory process involving the senses of smell, taste and chemesthesis (sensation initiated by chemical stimuli, also called irritation) (Spence 2015). The sense of smell or olfaction contributes to the majority of our food experience. Olfactory stimuli can be sensed through two pathways, the orthonasal pathway which involves aromas that are sniffed and detected by the receptors in the nose and the retronasal pathway which involves aroma release and delivery via the top of the throat to the nasal cavity. The taste sensation also plays a key role in the multisensory flavour perception. At least five primary taste qualities have been recognized and discriminated by humans: bitterness, sourness, sweetness, saltiness and umami (or savoriness). Around 50-100 polarized neuroepithelial cells (the primary taste cells) are clustered into taste buds and approximately 2000-5000 taste buds are distributed in the human oral cavity, which are in contact with their surrounding diffusion media such as saliva (Fábián et al. 2015). When the taste stimuli interact with receptors through the small taste pores at the tip of the taste buds, the chemical information carried by the stimuli are converted to electric impulse and are transmitted by afferent nerve to neurons in the central nervous system (particularly the nucleus of the solitary tract) and eventually to cortical regions of the brain (Fábián et al. 2015). The taste receptors include several sorts of G protein-coupled receptors (GPCRs) and ion channels (Figure 6).

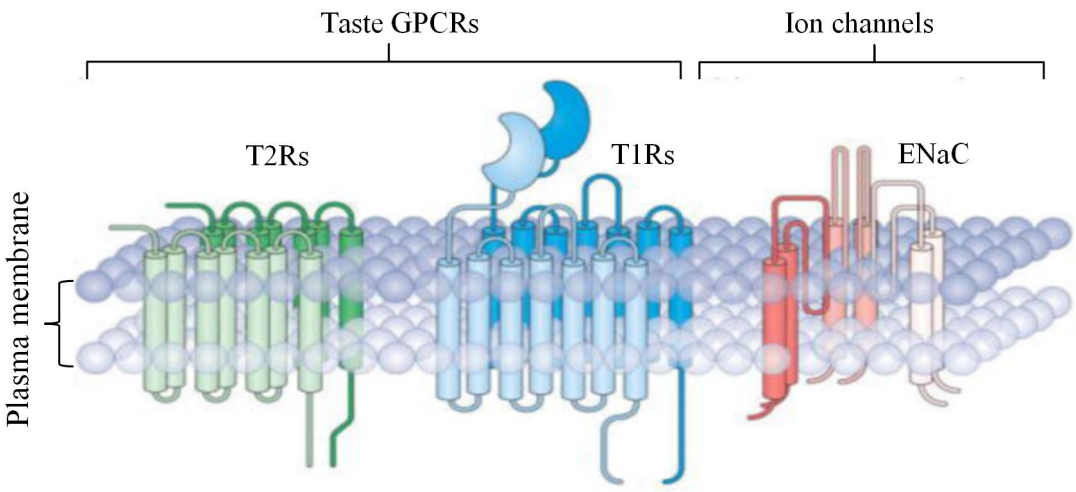

Figure 6. Plasma membrane proteins of taste cells that transduce taste. T2Rs (type 2 taste receptors), bitter taste receptors, are G protein-coupled receptors (GPCRs); T1Rs, sweet and umami taste receptors, are also GPCRs; ENaC (the epithelial Na channel), salty taste ion channels (adapted from Roper and Chaudhari 2017).

Sweet taste perception due to sugars or artificial sweeteners, is transduced by the binding interactions between the sweet stimulus and taste receptor type 1 (T1Rs) (Roper and Chaudhari 2017). Bitter taste is stimulated by a great variety of compounds (e.g. quinine and caffeine), most of which are toxic. The bitter taste receptors T2Rs have a broad response to a numerous range of bitter tasting chemicals and are therefore important for humans in detecting toxic or harmful foods. Umami taste is elicited by amino acids especially glutamate and aspartate which are detected by multiple receptors mainly belonging to the T1Rs family. The transducer for salty taste has been suggested to be the epithelial Na^+ channels (ENaCs). Sour taste is generated by intracellular acidification of the taste cells. Organic acids such as lactic and acetic acids are weak acids which are present mostly in the undissociated form. They exhibit higher membrane permeability than strong acids like HCl which are readily dissociated to protons. The undissociated acids permeate cell membrane and acidify the cytoplasm, thus activate downstream reactions (Roper and Chaudhari 2017). Whereas the extracellular protons need to be transported through a proton conductance. The taste of sour is linearly related to the concentration of protons and acids which is based on the titratable acidity of the stimuli rather than its pH (Neta et al. 2007).

Perception of flavour is a dynamic phenomenon that changes over the time during eating depending on the processes of mastication (or chewing), breathing, salivation, tongue movements, temperature and swallowing (Lawless and Heymann 2010). During the oral processing, taste compounds are dissolved in saliva, either directly by dilution in terms of a liquid or semi-solid food, or progressively during the mastication of solid food. Regarding the solid food, the flavour perception is determined by two aspects, the nature and relative amounts of the flavour-active compounds present in the matrix and

the availability of these compounds to the sensory system as a function of time (Overbosch et al. 1991). The latter is a combined effect of food breakdown through chewing which fosters the flavour release to the surrounding saliva or vapour phase and the subsequent transportation of the released volatiles to the olfactory receptors or the non-volatiles to the taste receptor cells (Figure 7).

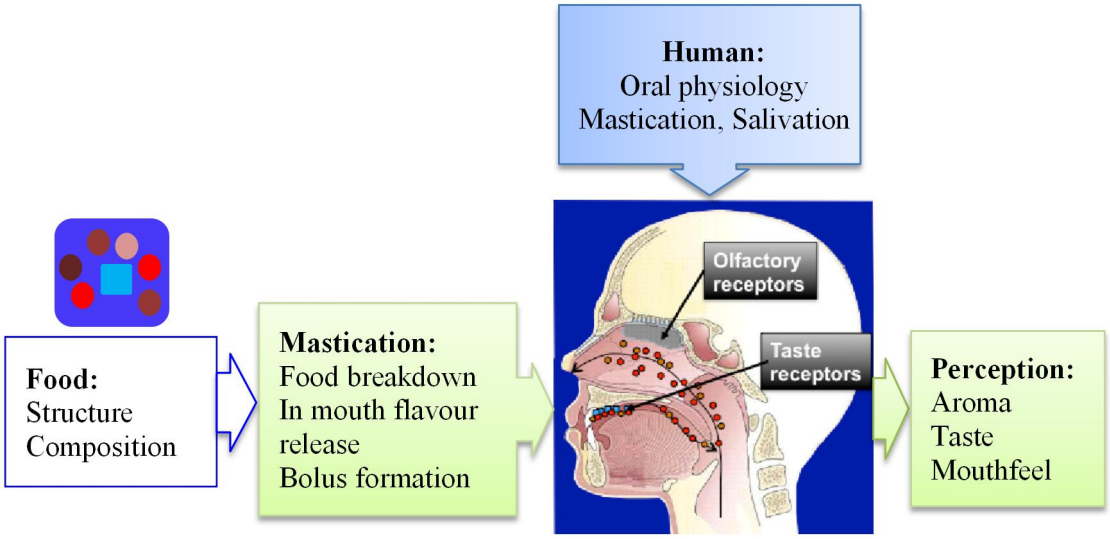

Figure 7. Schematic representation of oral processing and food breakdown, leading to release and perception of aroma and taste (adapted from Feron and Salles 2018).

Mastication is a multimodal process involving the jaw movements whereby the particle size of food pieces are reduced and lubricated by salivation to form a bolus suitable for swallowing (Foster et al. 2011). The particle size reduction during chewing has been suggested to be the result of selection and breakage functions (Voon et al. 1986). Selection function is the probability of a food particle being broken by the teeth. Whereas breakage function represents the degree of size reduction and is closely related to the fracture characteristic of a specific food.

Structure and composition of a food matrix significantly influences the release kinetics of aroma and taste stimuli due to the interactions between different components (Feron and Salles 2018). The changes in texture properties lead to changed particle size distribution during chewing and thus altered the surface area exposed for exchange, dilution and dissolution of flavour compounds in saliva, which consequently increase or reduce the levels of flavour stimuli accessing receptors in the oral cavity or the nasal cavity through the retronasal pathway (Feron and Salles 2018).

2.3.2 Effect of texture on flavour perception

The texture/structure influences not only our visual and tactile senses, but also the diffusion and release of flavour compounds, which further affects the flavour perception. Over the past decades, texture-flavour interactions have been the topic of numerous studies, initiated from simple model systems to model food systems with increasing complexity, which are close to real food formulations (Tournier et al. 2007). Studies have been carried out by addition of texturing agents to various model systems containing fixed concentrations of flavour compounds to achieve a desired texture and mouthfeel of the food system. The texturing agents applied are mainly hydrocolloids such as gums (arabic, guar, gellan, and xanthan), cellulose derivatives (carboxymethyl cellulose (CMC), methyl cellulose (MC), and HPMC), carrageenan, pectin, gelatin, and starch (Saha and Bhattacharya 2010). Hydrocolloids are able to modify the rheology properties of food systems, which have two fundamental functionalities in foods namely, the flow behaviour (viscosity) and the mechanical solid property (texture) (Saha and Bhattacharya 2010). Thickening or viscosity enhancing occurs at polymer concentrations above its critical overlap concentration (c*). Some hydrocolloids are able to form gels like pectin, gelatin, carrageenan, and gellan. The gel formation comprises the association or cross-linking of polymer chains and the formation of a three dimensional network, which traps and stabilizes the water molecules within the matrix resulting in a rigid structure resistant to flow (Saha and Bhattacharya 2010).

Studies of texture-flavour interactions have been focused on thickened solutions (characterized by viscosity) and gel systems (characterised by gel firmness or strength). Generally, an increase of hydrocolloid content resulted in a reduction of aroma or taste intensity perception (Tournier et al. 2007). For solutions, it has constantly been shown that the perceived intensity of aroma (volatile compounds) and taste (non-volatile compounds) decreases with increased viscosity. For example, Moskowitz and Arabie (1970) used magnitude estimation to study the relation between viscosity imparted by CMC and taste intensity of glucose, citric acid, sodium chloride and quinine sulfate. They suggested that taste intensity (T) followed a power function of the apparent viscosity (V) with a negative slope: $T = kV^{-n}$, where n ranged from 0.05 to 0.2. The exponent n is an index of taste masking ability, which varied between different thickeners and taste attributes. Pangborn et al. (1973) studied the impact of different hydrocolloids (HPC, CMC, alginate, and xanthan) on the basic taste intensities and reported that sourness of citric acid was the most affected which was suppressed by all hydrocolloids, followed by bitterness of caffeine. Pangborn and Szczesniak (1974) continued the studies with those hydrocolloid thickeners and volatile compounds using a sniffing method, which showed that the aroma intensities of dimethyl sulfide and butyric acid were notably reduced. In more recent studies, the flavour masking behaviour of hydrocolloids in solutions has been investigated exhibiting a “yield” concentration. Namely, the reduction of flavour intensity occurred at hydrocolloid concentrations higher than its c* which coincides with a sharp increase in viscosity (Baines and Morris 1987; Cook et al. 2002; Hollowood et al. 2002). Above this value, a steady decrease with increased polymer concentration was observed. Whereas below this point, the perceived flavour intensity remained unchanged.

For hydrocolloid gels, a similar trend was observed as for thickened solutions that an increase in the polymer level or gel strength resulted in a decrease in perceived intensities of aroma and taste. For instance, the intensity of overall aroma, sweetness of sucrose and glucose, and sourness of citric acid of pectin gel reduced with increased pectin concentration, while gel strength increased at the same time (Lundgren et al. 1986). Similar results were reported in another study that high strength carrageenan gels were perceived as less sweet, salty, sour and bitter than their low strength counterpart (Costell et al. 2000). Koliandris et al. (2008) classified gels prepared from acetyl and gellan, carrageenan, and locust bean gum (LBG) into three types according to fundamental rheological measurements: brittle (low strain at rupture), intermediate and elastic (high strain at rupture). They concluded that the brittle gels exhibited a higher release of volatiles than elastic gels.

The influences of texture on flavour perception are indeed relying on the nature of the flavour compounds and the nature of the hydrocolloids. For example, the sourness intensity of pectin jam was found to decrease with increased pectin content while the sweetness was not affected (Guichard et al. 1991). Furthermore, the nature of hydrocolloids seems to have more effect on flour perception than viscosity. Ferry et al. (2006) compared three types of starch (wheat, waxy maize and modified waxy maize) with HPMC at identical viscosity and reveal that HPMC was the strongest flavour masker. Similarly, Arancibia et al. (2013) studied semisolid dairy desserts prepared with CMC (1.1% and 1.3%) and modified starch (3.5% and 4.0%) and reported that the starch-based samples were perceived to have a higher milk flavour intensity than CMC. In modal fermented milk beverages, samples containing CMC and propylene glycol alginate were perceived as less intense in yoghurt and acid flavour than samples thickened by high-methoxy pectin and xanthan gum, which also presented the lower release of volatile compounds in the headspace as detected by proton-transfer reaction mass spectrometry (Gallardo-Escamilla et al. 2007). Additionally, the ability of CMC and different food gums at their critical concentrations c* to mask the astringency taste of tannic acid followed the order: CMC (~56% reduction) > guar gum (~38%) > xanthan gum (~30%) > arabic gum (~12%) (Troszyńska et al. 2010).

2.3.3 Mechanisms at the origin of the flavour masking effect

Various mechanisms have been suggested for the flavour masking phenomena of hydrocolloids in food systems. These mechanisms can be generally divided into three categories: (1) modification of the structure of the food matrix which affects the release kinetics of flavour compounds; (2) molecular interactions between hydrocolloids and aroma/taste compounds; (3) adhesion of hydrocolloids to the mucosa in the oral cavity.

Effect of structure

Flavour molecules are released to the saliva or vapor phases when food are diluted or breakdown into small pieces during mastication. The rate of release is mainly affected by the diffusion or mass transfer coefficient, the speed at which the molecules transfer across the matrix-saliva or matrix-air interface (Juteau et al. 2004). The presence of

texturing agents results in a denser or more rigid/stable three dimensional network, which reduces the diffusion coefficient of flavour molecules and slows down their migration to the interface. For hydrocolloid solutions, the suppressing effect in flavour perception above the critical concentration has been postulated to be attributable to the restricted mixing with saliva due to the formation of entangled polymer network (Koliandris et al. 2008). This also explains the difference in masking ability between starch and other hydrocolloids that polymeric structure mix less efficiently than granular structure as a consequence of increased intermolecular associations (Ferry et al. 2006). Another hypothesis is that the high water binding capacity of hydrocolloids results in reduced water mobility/availability in the food matrix particularly at concentrations higher than c*, thus lead to decreased flavour perception (Hollowood et al. 2002).

For gelled systems, the behaviour in mouth as a function of the structure has been proposed. For a melting in mouth gel such as gelatin, the entire structure is destroyed and flavour molecules are completely released, showing excellent mixing with saliva (Koliandris et al. 2008). Whereas for gels that require a strain at break (or mechanical breakdown), the flavour release mechanism is more complex. The brittle gels (low strain at break) exhibited enhanced flavour release than the elastic gels as a result of greater exposure of surface area for exchange following breakage during chewing. The variability in flavour reduction capacity among different types of hydrocolloids is most likely related to their physicochemical properties such as the degree of chain entanglement or cross-liking.

Molecular interactions

At a molecular level, hydrocolloids might interact with flavour compounds in various ways due to the heterogeneity of these compounds, such as hydrogen bonding with hydrophilic compounds, hydrophobic interactions with non-polar compounds, van der Waals apolar interactions (London dispersion forces), steric interactions, and molecular inclusion. For example, Yven et al. (1998) observed reduced mushroom aroma (1-octen-3-ol) in xanthan solution and revealed hydrogen bonding interactions between xanthan and 1-octen-3-ol by exclusion chromatography. Braudo et al. (2000) proposed van der Waals interactions between the flavour compounds and hydrophobic groups of pectinate. Samavati et al. (2012) suggested that the retention of aroma compounds in the xanthan gum solution was linked to steric interactions. Rutschmann and Solm (1900) stated that the unbranched amylose fraction (helical) of starch could form molecular inclusion complexes with flavour compounds. Furthermore, Lubbers et al. (2007) studied the interactions of nine aroma compounds with modal dairy gels (starch, pectin, and LBG) by a quantitative structure property relationship technique and reported that the surface-weighted negatively charged partial surface area of the molecules are correlated with flavour retention. The larger the surface of a molecule, the more interactions can be involved such as hydrophobic binding. However, studies concerning the physico-chemical interactions between taste compounds and hydrocolloids are rather limited. The physical inhibition of mobility of the tastants is considered to be the dominating factor for the reduction in perceived taste other than binding mechanism.

Mucoadhesion

Many hydrocolloids are adhesive to the mucosal membrane in the oral cavity which has been widely used in drug delivery to prolong the contact with the mucosal surface (Cook et al. 2017). The major interactions between these polymers and the mucin chains include hydrogen bonding, Van der Waals forces, and hydrophobic interactions. More recently, the adhesive or mouthcoating nature of hydrocolloids has been stated to be a factor in the modifications of flavour release and perception of food products. For instance, Mälkki et al. (1993) studied the impact of CMC, oat fiber gum and guar gum on sweetness and aroma perception. They revealed that oat gum solution exhibited the highest sweet taste but lowest aroma release which was likely linked to the adherence of the matrix to taste buds. It is, however, difficult to make conclusions about the role played by mucoadhesion in flavour perception since the mucoadhesive strength are not measured in those studies.

Despite the huge attention that texture–flavour interaction has received from researchers, the utilization of these results in industrial food development is still limited. To date, publications have been focused on rather simple model systems such as aqueous solutions and gels which lack complexity compared to real food systems. The food matrix may include hundreds of flavour compounds and its perception imply reciprocal interactions. Furthermore, few studies in the literature dealt with flavour perception in solid products as a function of texture. The phenomenon occurred in liquid and semi-solid systems might be not applicable for solid matrix since it displays different oral movements, a longer processing time and a slower mixing with saliva. Additionally, previous studies have mainly conducted with a trained sensory panel. Studies concerning consumer preference/acceptance are necessary to understand the application of texture-flavour interactions knowledge in flavour design/controlling of food products.

3 AIMS OF THE STUDY

This study focuses on bioprocessing of faba bean, wholegrain pearl millet, and sorghum with dextran produced *in situ* as a tool to improve the nutritional, textural, and sensory quality of composite bread containing 50-70% of wheat flour. The impact of dextran on the perception of flavour intensity of bread was also studied. A sensory scaling technique and multiple concentrations (above and below c*) of dextran was employed to follow the perceptual changes as a function of dextran concentration.

The specific objectives were:

i. To assess the ability of potential strains to produce sufficient dextran *in situ* in faba bean, millet and sorghum flours supplemented with sucrose (I-III)
ii. To characterize the synthesized dextran and determine the metabolic traits (e.g. polyphenolic profiles, organic acids and sugars) of the strains (I-III)
iii. To study the influences of dextran and acidification on rheological and textural properties and starch retrogradation of composite dough and bread (I-III)
iv. To evaluate the impact of sourdough bioprocessing on bread nutritional and sensory quality and assess consumer liking (II-III)
v. To investigate the relationship between texture and flavour perception of bread enriched with dextran (III)

4 MATERIALS AND METHODS

This section combines the materials and methods used in the Studies **I-III** with detailed information being presented in the original publications. This thesis also contains relevant methods and results that are not published elsewhere. A simplified outline of the raw materials, sample preparation and analysis methods used in this thesis is shown in Figure 8. In Studies **I-III**, sourdough fermentation was carried out with faba bean, millet and sorghum flour with selected strains to produce sufficient dextran *in situ*. The rheological properties of dough and textural quality of bread were evaluated in Studies **I and II**. The nutrition and sensory quality of the bread were determined in Studies **II and III**, respectively. Furthermore, a strategy of using magnitude estimation test was adopted in Study **III** to investigate the relationship between dextran concentration and flavour perception of bread.

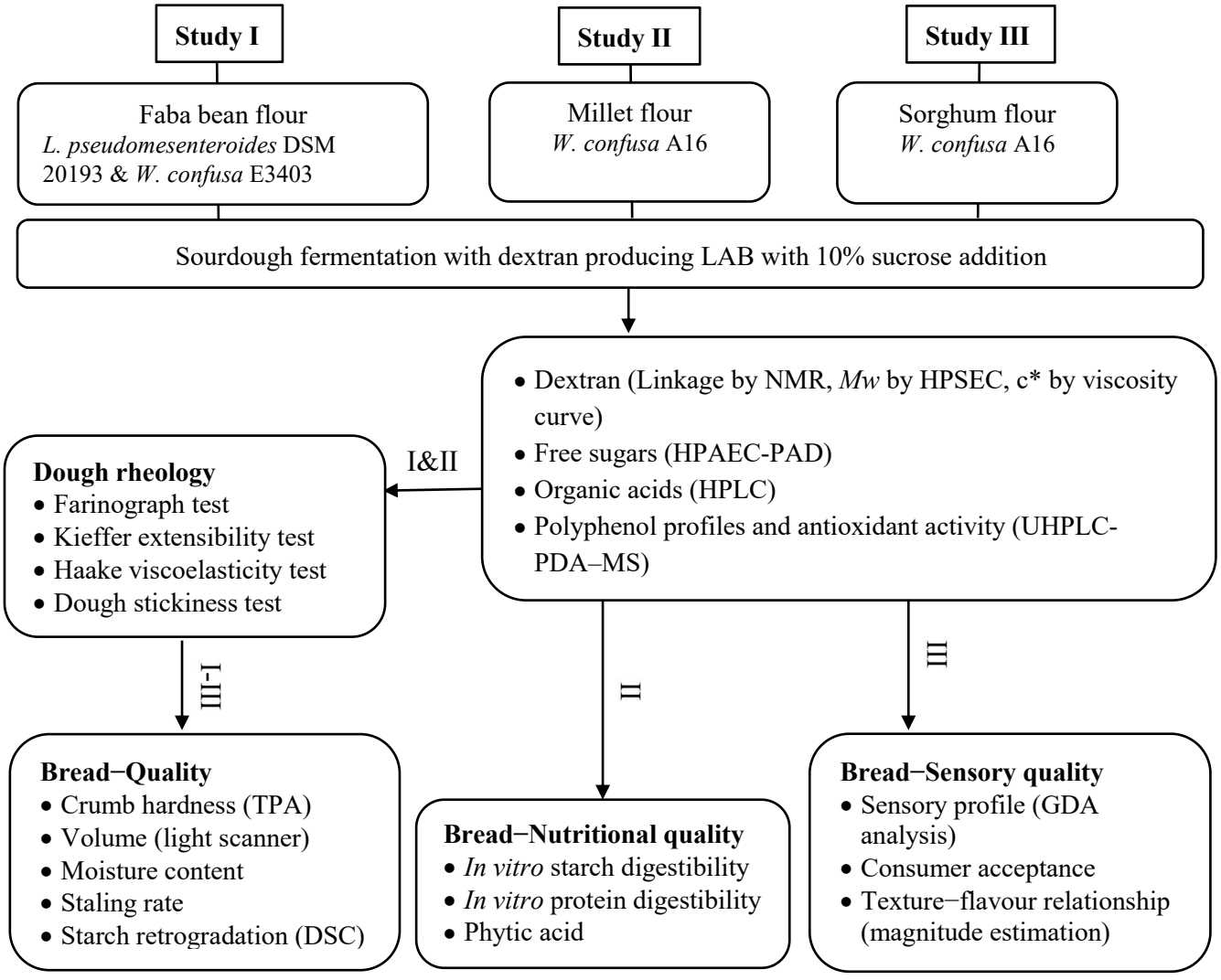

Figure 8. Schematic summary of the experiments in Studies **I-III**.

4.1 Materials

The ingredients common to all breads prepared were wheat flour (Finland), fresh yeast (Suomen Hiiva Oy, Finland), sucrose (Dansukker, Finland), salt (Finland), and rapeseed oil (Bunge Oy, Finland). In Study **I**, faba bean flour was purchased from Italy (San Martino di Lupari, PD) and wheat flour was from Fazer Mill & Mixes (protein 14.0%, fat 1.7%, moisture 14.0%). In Studies **II and III**, yellow pearl millet and red sorghum grains were purchased from the local market in Burkina Faso while wheat flour was purchased from Helsingin Mylly Oy (protein 12.5%, fat 2.1%, moisture 13.6%). The millet and sorghum whole grains were sorted manually and rinsed thoroughly with cold potable water to remove dirt and foreign materials. The grains were spread on oven racks with aluminium foil and allowed to dry in a fan-assisted oven at 30°C for 2 d. The dry grains were subsequently ground into flour by a rotor mill (Ultra Centrifugal Mill ZM 200, Germany) with high speed (10,000−15,000 *g*). The obtained wholegrain flour had a particle size of ≤ 0.5 mm and was stored at 4°C prior to use. In Study **I**, two dextran-producing strains, *W. confusa* VTT E-143403 (E3403) obtained from the VTT Culture Collection (Finland) and *L. pseudomesenteroides* DSM 20193 bought from the Leibniz Institute DSMZ (Braunschweig, Germany) were used. Studies **II and III** employed a potential dextran producer *W. confusa* A16 as described under section 4.2.

4.2 LAB screening and identification (II)

Strains were isolated from Massa (Compaoré-Sérémé 2016), a typical pancake made from fermented millet flour in Burkina Faso (Sawadogo-Lingani et al. 2007). The sample was streaked on mMRS-glucose agar (Sigma Aldrich) and colonies of presumptive LAB were isolated. Preliminary screening was conducted on LTV and MRS-sucrose agar (Lab M, Heywood, UK) plates, from which highly viscous slimy colonies were selected (Sawadogo-Lingani et al. 2008). The second screening was carried out *in situ* by inoculating the selected strains in sourdoughs prepared from millet flour and distilled water at a ratio 40/60 according to Xu et al. 2017. The strains exhibiting the highest viscosity enhancement as measured by an Anton Paar rotational rheometer (Rheolab QC, Germany) at 20 °C (shear rate ranged from 2 to 300 s^{-1} and 300 to 2 s^{-1}) were selected. The candidate isolates were further subjected to taxonomic strain identification by analysis of the 16S rRNA gene sequence (De Angelis et al. 2006). Partial *pheS* gene was also sequenced to attribute the isolate to the species *Weissella confusa* (Naser et al. 2005). More detailed information can be found in Study **II** supplementary materials.

4.3 Dextran characterization

4.3.1 Dextran isolation and purification (II&III)

The native dextran used for structure characterization was produced by striking the selected strain on MRS agar supplemented with 2% (w/v) sucrose (Merck). The plates were incubated for 5 d in anaerobic conditions at 30°C. After incubation, the cell mass on the agar surface was collected and suspended in sterile sodium phosphate buffer saline (PBS, 0.01 M, pH 7.4, Sigma). Food grade dextran was prepared by cultivating the same strain in general edible medium (GEM, 20 g dextrose, 20 g sucrose, 30 g soy peptone, 7 g yeast extract, 1 g $MgSO_4 \bullet 7H_2O$ in 1 L 0.01M potassium phosphate buffer, pH 6.3) supplemented with 5% sucrose and incubated for 7 d in anaerobic conditions at 30°C. Subsequently, an equal volume of PBS buffer was added to the GEM medium. Dextran was recovered and purified from the suspensions according to Maina et al. (2008). The isolated dextran was weighed and its purity evaluated by high performance anion exchange chromatography with pulse amperometric detection (HPAEC-PAD) according to Xu et al. (2018). The purity of the obtained dextran was calculated by dividing the value of the released glucose content after acid hydrolysis (10 mg dextran in 2 mL 1 M sulfuric acid hydrolyzed at 100°C for 2 h) by the weighed dextran content and multiplying by 100%.

4.3.2 Structure elucidation and molar mass determination (II)

The ^1H nuclear magnetic resonance (NMR) spectra of the dextran was recorded as previously published using a Bruker Avance III NMR spectrometer (Bruker BioSpin, Rheinstetten, Germany) operating at a frequency of 600 MHz with a QCI cryoprobe (Maina et al. 2008). Samples (10 mg/mL) were prepared as solutions in D_2O (99.8%), filtered (0.45 μm), and transferred into 5 mm NMR tubes (Wilmad NMR tubes, ultra-imperial grade, Sigma Aldrich) prior to analysis. The measurements were performed at 50℃ and acetone (^1H = 2.225 ppm) was used as the reference for adjusting the chemical shifts.

Weight average molecular weight of the isolated dextran was determined with high performance size exclusion chromatography (HPSEC) (Maina et al. 2014). The lyophilized samples were completely dissolved in 0.01 M LiBr/DMSO eluent for 4 d at a concentration of 2.0 mg/mL. A refractive index increment ($d\eta/dc$) value of 0.072 ml/g was used for concentration determination (Basedow and Ebert 1979). Data were collected with OmniSEC 4.5 software (Viscotek Crop.).

4.3.3 Rheological characterization of dextran aqueous solutions (III)

Milli-Q water was heated to 60°C and the lyophilized dextran was added and the solution was mixed at 60°C for 2 h and 40°C for overnight with constant magnetic stirring at 200 rpm. Concentration range investigated was 0.1−2.0% (w/w). The flow curves of dextran aqueous solutions were determined using a DHR2 rheometer (TA) with a double wall concentric cylinders geometry (operating gap 0.5 mm) at 25°C, for a range of shear rates (10-500 s^{-1}).

4.4 LAB growth and sourdough preparation (I-III)

The LAB strains were routinely cultivated in MRS broth (Oxoid, Basingstoke, UK) anaerobically at 30°C. Strains were subcultured in GEM for 24 h at 30°C for sourdough preparation. Cells were obtained from the incubated culture medium by centrifugation (15,000 *g* x 15 min), washed once with PBS buffer, resuspended in distilled water and inoculated to sourdoughs at an initial cell density of 10^6 cfu/g. The recipe for sourdoughs in Studies **I-III** are presented in Table 2 and dough yield (DY) was the same 250. Briefly, two types of sourdough were used in this study: dextran-enriched sourdoughs (DSD) prepared by substituting 10% (w/w) of the flour with sucrose to support dextran production and control sourdoughs (CSD) prepared with the same strains but without sucrose supplementation. Sourdough fermentations were carried out at 25°C for 24 h. Sourdough fermentation was performed at lower temperature than that for strain cultivation to avoid intense acidification (Salovaara & Valjakka 1987). In Study **I**, two chemically acidified faba bean doughs (CAD) were also prepared by addition of lactic and acetic acids to achieve similar acidity levels as in the *W. confusa* E3403 and *L. pseudomesenteroides* DSM 20193 dextran-enriched sourdoughs and incubated at 25°C for 1 h before use.

4.5 Determination of cell density, pH and total titratability (I-III)

Cell counts of presumptive LAB were determined at 0 h and after 24 h by serial dilutions in sterile saline solution and subsequent plating on MRS agar. The plates were incubated in microaerophilic conditions for 2 d at 30°C. Total mesophilic bacteria were determined on plate count agar (Lab M) under aerobic conditions at 30 °C for 2 d. The pH values at fermentation time 0 and 24 h were followed using a portable pH meter (HI99161, Hanna Instruments, Woonsocket, USA). Total titratable acidity (TTA) values were measured by an EasyPlusTM Titrator (Germany) as the volume (mL) of 0.1 M NaOH required to adjust the pH of 10 g sourdough in 100 mL Milli-Q water to 8.5.

4.6 Determination of sugars, oligosaccharides, dextran, and acids (I-III)

Sourdoughs were freeze-dried, ground and sieved through a 0.5 mm screen to obtain powder samples. Free sugars, oligosaccharides, and dextran were determined with HPAEC-PAD as previously described (Katina et al. 2009; Xu et al. 2017). Organic acids were analyzed by injecting the water-soluble extracts of sourdoughs to high performance liquid chromatography (HPLC). More details about HPAEC-PAD and HPLC analysis can be found in Studies **I-III**.

4.7 Sourdough viscosity (I-III)

Viscosity flow curves of sourdoughs at 0 h and after 24 h of fermentation were measured by an Anton Paar rotational rheometer as described in section 4.2.

Table 2. Formulations (% flour weight) for sourdoughs and different bread doughs.

		Study I Faba bean					Study II Millet			Study III Sorghum		
	CWB[1]	CFWB	E3403 CAB	20193 CAB	CFSB	DFSB	CMWB	CMSB	DMSB	CSWB	CSSB	DSSB
Sourdough												
Non-wheat flour[2]			30	30	30	27		42.7	38.4		42.9	38,6
Water			45	45	45	45		64	64		60	60
Sucrose						3			4.3			4,3
Starters					* [3]	*		*	*		*	*
Acetic acid			0.1	0.2								
Lactic acid			0.2	0.3								
Bread												
Sourdough			74.9	74.9	74.9	74.9		106.7	106.7		106.7	106.7
Non-wheat flour		30					50	7.3	7.3	50	7.3	7.3
Wheat	100	70	70	70	70	70	50	50	50	50	50	50
Water	60-63	63	18.1	18.1	18.1	18.1	64			60		
Yeast	5	5	5	5	5	5	5	5	5	5	5	5
Sugar	2	2	2	2	2	2	2	2	2	2	2	2
Salt	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Fat	5	5	5	5	5	5	5	5	5	5	5	5

[1] CWB = control wheat bread, CFWB = control faba bean wheat bread, E3403 CAB = E3403 chemically acidified bread, 20193 CAB = 20193 chemically acidified bread, CFSB = control faba bean sourdough bread, DFSB = dextran-enriched faba bean sourdough bread, CMWB = control millet wheat bread, CMSB = control millet sourdough bread, DMSB = dextran-enriched millet sourdough bread, CSWB = control sorghum wheat bread, CSSB = control sorghum sourdough bread, DSSB = dextran-enriched sorghum sourdough bread.

[2] Non-wheat flour corresponded to faba bean, millet and sorghum flour.

[3] Starters in Study **I** were *W. confusa* E3403 and *L. pseudomesenteroides* DSM 20193 and in Study **II & III** was *W. confusa* A16.

4.8 The fate of polyphenols and antioxidant activity (II&III)

In Study **II**, soluble and bound phenolic compounds from native millet and lyophilized millet sourdoughs were extracted using ultrasound-assisted 80% (v/v) aqueous methanol and alkaline hydrolysis (2 M NaOH) (Svensson et al. 2010). The phenolic content of the extracts was analyzed with Folin–Ciocalteu (FC, Merck) method using a UV spectrophotometer (UV1800, Shimadzu, Japan) (Singleton et al. 1999). Antioxidant activity of the soluble fractions was determined based on the free-radical scavenging activities against 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Cuendet et al. 1997). In Study **III**, polyphenols of the native sorghum and lyophilized sorghum sourdoughs were determined using ultra high performance liquid chromatography coupled with photodiode array and mass spectrometer in series connected detectors (UHPLC-PDA–MS). Samples were extracted with a solvent mixture of methanol-water-formic acid (80:19.9:0.1). The identification was done by comparing the obtained λ_{max}, $[M-H]^-$ and MS/MS fragmentation patterns with published data (Kang et al. 2016). The semi-quantification was achieved by multiply the area percentage of each peak (calculated from the chromatogram) with total polyphenol content (obtained from Folin Ciocalteu assay). Phenolic content was expressed as mg/100g of gallic acid equivalent (GAE).

4.9 Bread making (I-III)

Five different types of bread were used in this study (Table 2):

i. Control 100% wheat bread (**I-III**).
ii. Control composite bread including faba bean-wheat (30:70) (**I**), millet-wheat (50:50) (**II**), and sorghum-wheat (50:50) (**III**).
iii. Control chemically acidified faba bean wheat bread (**I**).
iv. Control sourdough bread containing fermented faba bean (43% dough basis), millet (59%), and sorghum (59%) with different strains (**I-III**).
v. Dextran-enriched sourdough bread (**I-III**).

The replacement level (30%) of faba bean flour was determined by calculating the nutritional composition to obtain a 20% protein energy content of the total energy value (Table 2 and 3 in Study **I**). The European Parliament and Council (Regulation No 20/12/2006) declared that the nutrition claim “high in protein” can only be made where at least 20% of the energy value of the food products is provided by protein. The substitution level (50%) of millet and sorghum was determined to provide 28 g of whole grains per 100 g of bread. The definition of wholegrain foods is different across the EU and no legislation considering labelling has been made (EFSA 2010). In Denmark (DTU 2008) and Sweden (SNF 2007), a food recognized as wholegrain should contain at least 50% (dry matter) of wholegrain ingredients. The Healthgrain Forum (2017) recommended that the labelling of “whole grain” on food package can be allowed when the product contains 30% (dry matter) wholegrain ingredients. In US, the Dietary

Guidelines for Americans (DGA) and American Association of Cereal Chemists International (AACCI) announced that a food providing at least 8 g whole grains per 30 g serving (27 g/100 g) can be characterized as a wholegrain food (Ferruzzi et al. 2014).

Baking trials were conducted using a straight-dough baking process. In general, ingredients were mixed in a Diosna spiral mixer (Dierks & Söhne GmbH, Germany) for 3 min at low speed and 4 min at fast speed. The dough was rested for 15 min (35°C, RH 75%), divided (250 g), molded, and put into baking pans for proofing for 45 min (35°C, RH 75%). The breads were baked in an oven (Sveba Dahlen, Fristad, Sweden) at 200°C for 15 min with 15 s steaming at the beginning. The baked breads were allowed to cool for 1 h (room temperature) and then packed with plastic bags for subsequent analysis. Two to three independent bakings were performed and 9-12 loaves were made in one individual baking for each type of bread.

4.10 Dough rheological measurements

4.10.1 Farinograph mixing characteristics (I&II)

The mixing characteristics of bread doughs were determined using a Brabender Farinograph (Brabender GmbH & Co.KG, Germany) at 30°C, according to AACC method 54-21 (AACC 2000). The doughs were prepared according to the baking recipes without yeast addition to ensure the reproducibility of the measurements. The water absorption (WA) value, percentage of water required to yield a standard dough consistency of 500 BU (Brabender Units), was recorded from the Farinograph curves.

4.10.2 SMS/Kieffer dough and gluten extensibility rig (I&II)

The extensibility of bread doughs was evaluated using an SMS/Kieffer dough and gluten extensibility rig on a texture analyzer (TA, TA-XT2i, Stable Micro Systems Ltd., UK) with a 5 kg load cell. Briefly, the dough after kneading was rested for 20 min (35°C, RH 75%) and molded manually into a ball and then a cylinder shape. The dough was pressed into a Teflon mould which was pre-greased with paraffin oil and loaded with strips in the grooved base. The form was covered and allowed to rest for 40 min (35°C, RH 75%) and samples were subsequently removed by lifting the strips. During the test, the hook probe of the Kieffer rig stretched the dough centrally with a speed of 2.0 mm/s until rupture. The maximum resistance to extension (R_{max}), extensibility (Ext), and dough strength value (A_{tot}) were recorded.

4.10.3 Haake dynamic oscillatory test (I)

The kneaded bread doughs were rested for 30 min at room temperature and oscillatory measurements were performed using a Haake RheoStress rheometer (RS 50, Haake Rheometer, Karlsruhe, Germany) with a parallel plate geometry (diameter 35 mm, gap

2.5 mm) at 20°C. Dough samples (3.3 g) were rounded manually and placed in the center of the bottom plate, which was surrounded by water drops to prevent moisture loss. The amplitude sweep test was first applied to determine the linear viscoelastic region (LVR) and frequency sweep test was then conducted at 0.05 to 10 Hz.

4.10.4 SMS/Chen-Hoseney dough stickiness (II)

The dough stickiness was evaluated using the TA texture analyzer with the SMS/Chen-Hoseney dough stickiness cell (A/DSC) and a 25 mm perspex cylinder probe (P/25P) as previously described (Chen and Hoseney 1995). Bread doughs were measured directly after mixing and after 60 min of resting (35°C, RH 75%). The peak positive force of the plot was recorded as dough stickiness.

4.11 Bread texture and volume (I-III)

Texture Profile Analysis (TPA) of bread crumbs was performed using the texture analyzer with a 5 kg load cell and 36 mm diameter cylinder probe on days 1 and 4 of storage. Samples were cut into 25 mm cubes from the central part of three slices per bread and a total 3 breads per type. The test speed was 2 mm/s with 40% deformation. Staling rate was calculated based on the increment in crumb hardness: staling rate = hardness (day 4 – day 1) / days of storage. Crumb moisture content was evaluated by a two-stage moisture-air oven method, according to AACC method 44-15A (AACC 1995). Loaf specific volume (mL/g) was measured by a laser-based scanner (Volscan Profiler 300, Stable Micro Systems, UK).

4.12 Bread aging analysis by differential scanning calorimetry (II)

Starch retrogradation of bread after 1 and 4 d of storage was measured using a differential scanning calorimeter DSC823e (Mettler-Toledo Inc., Switzerland). The parameters obtained from the DSC curves were the onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and enthalpy (ΔH) value.

4.13 Bread nutritional characterization

4.13.1 Phytic acid analysis (method not published elsewhere)

Phytic acid content in bread was determined using Megazyme kits (K-PHYT). The sample was extracted with 0.66 M HCl followed by a number of enzymatic reactions to release the inorganic phosphorus, which was then quantified by colourimetric determination.

4.13.2 *In vitro* starch digestibility (II)

Starch content of bread was measured with a Total Starch Assay kit (Megazyme, K-TSTA) and *in vitro* starch digestibility was determined according to Germaine et al. (2008). The bread was chewed in the mouth and then treated with pancreatic α-amylase at 37°C. Reducing sugar content of the dialysate was measured at time 0, 30, 60, 90, 120 and 180 min. The starch hydrolysis indexes (HI) and predicted GI were calculated from the obtained starch hydrolysis curves (**II**).

4.13.3 *In vitro* protein digestibility (II)

Protein content of bread was measured with the Dumas combustion method by a Vario Max CN element analyzer (Elementar Analysensystem GmbH, Germany) and *in vitro* protein digestibility (IVPD) was determined as previously described (Akeson and Stahmann 1964). Samples were subjected to a two-stage *in vitro* gastric digestion model, namely the pepsin (3 h) and pancreatin (24 h) treatment (**II**).

4.14 Sensory descriptive analysis (III)

Panel Selection: Seventeen panelists were recruited from staff and graduate students at University of Helsinki (8 men and 9 women, ages 20 to 50 yr). Panelists were selected based on their sensory acuity (odor recognition and identification of basic taste). Quantitative descriptive analysis (QDA) was used to profile the sensory attributes of bread (Lawless and Heymann 2010).

Samples: Control wheat bread, control sorghum-wheat bread, sorghum sourdough bread, and dextran-enriched sorghum sourdough bread were prepared one day before the training or evaluation as described in section 4.9. Samples were presented (in randomized order) as slices (crust and crumb) with 1 cm thickness in lidded plastic boxes with 3-digit codes. Water and corn snacks were provided to clear the palate.

Panel training: In session 1, the four types of bread were served to generate the list of descriptors and a specific vocabulary of bread associated sensory terms was provided (U.S. Wheat Associates and Lesaffre 2017). The selected descriptors, including 8 flavour attributes and 8 texture and mouthfeel attributes, are presented in Study **III**. In session 2, the reference standards, evaluation method, and order were determined. In session 3, panelists assessed the four breads by rating the attributes on graphic line scales (0−10) with endpoints anchored with verbal definitions.

Formal evaluation: Panelists participated in four separate sessions on different days to evaluate flavour and texture (2 for each). Flavour attributes of the three sorghum breads were first evaluated and commercial rye bread, roasted bread (10% sucrose), and caffeine solution (0.05%) were provided as reference. Wheat control was excluded in the flavour session since wheat and sorghum products exhibit different taste profiles and are therefore not comparable. In the subsequent assessment of texture and mouthfeel attributes, wheat control and the three sorghum breads were all included and commercial rye and wheat bread were served as reference. Data was collected by the Fizz 2.74 Acquisition 2.51 software **(**Biosystemes, Courternon, France).

4.15 Consumer acceptance (method not published elsewhere)

A scaled acceptance test was performed over 3 d in the sensory laboratory to assess consumer preferences for flavour and texture of the bread. Voluntary frequent bread consumers (n = 50) were recruited to the test; 34 women and 16 men between 20 and 60 year-old. In the form questionnaire, 56% stated they consumed more than two bread slices/day, while the rest consumed half to two slices/day. White wheat bread was rated as the most consumed bread, followed by wholegrain wheat bread and rye bread. In the evaluation, the four breads were presented to the consumers in the same manner as described in section 4.14. Consumers were first asked to rate overall, odor, taste, and texture liking for each bread using a 9-point hedonic scale, ranging from 1 (dislike extremely) to 9 (like extremely). They were then requested to assess purchase intention using a 5-point scale, where 1= definitely would not buy, 2 = would not buy, 3 = may or may not buy, 4 = would buy, and 5 = definitely would buy.

4.16 The effect of dextran concentration on taste perception (III)

Panel training: Seventeen assessors (14 from the QDA panel) were recruited based on their ability to correctly rank in order of taste intensity of basic tastants (e.g. citric acid, caffeine) with a series of concentrations in water. In session 1, bitter taste wheat breads prepared with a concentration series of 0.2, 0.3, and 0.5% (f.w.) caffeine, and sour taste wheat breads prepared with lactic/acetic acid at concentrations of 0.3/0.04, 0.45/0.06, 0.6/0.08 % were provided to panelists. The best sour (0.6/0.08%) and bitter (0.2%) tastant concentration was selected, which was detected as the predominate taste and moderately strong in bread. In session 2, panelists were instructed to use the scaling method of magnitude estimation.

Samples: Wheat model breads were prepared with the isolated food-grade dextran at a range of concentrations of 0, 0.1, 0.2, 0.35, 0.5, and 0.9% dough weight, which were selected below and above the c* and included the dextran amount presented in sorghum sourdough breads. All model breads incorporated a fixed amount of bitter or sour tastants, namely 0.2% caffeine and 0.6/0.08% lactic/acetic acid. Samples were served as described in QDA analysis without the crust.

Magnitude estimation test: The trained panel evaluated the sourness and bitterness intensity of bread samples using the magnitude estimation methodology with a standard modulus (or reference stimulus) (Lawless and Heymann 2010). The standard modulus was pre-scored as 100, which was the model wheat bread with 0.2% dextran (selected near the middle of the concentration range). Panelists assigned numerical values to the samples in comparison with the standard modulus conforming to a ratio principle; i.e. if the sour taste appears to be twice as strong in the sample compared to the modulus, the value assigned to the sample would be 200. Evaluation of sourness and bitterness were performed in 6 separate sessions in two independent days. Assessors first tasted the modulus and then the model bread samples. Panelists were asked to chew and hold the bread samples in the mouth for > 5 s before swallowing.

4.17 Statistical analysis

Results from all chemical and instrumental measurements represent the average value. Statistical analysis was performed on all results using one-way univariate analysis of variance (ANOVA) with SPSS Statistics 24.0 program (SPSS Inc., Chicago, IL, USA) and Turkey's test ($\alpha = 0.05$). Sensory results were subjected to normality test showing normal distribution. A one-way ANOVA was performed on sensory data derived from QDA and the magnitude estimation test. The correlation between sensory texture attributes and instrumental texture profiles was analyzed with Pearson's Correlation.

5 RESULTS

5.1 Identification of dextran producing strains

Strains used in Study **I**, *W. confusa* E3403 (source VTT Culture Collection) isolated from faba bean flour and *L. pseudomesenteroides* DSM 20193 (source Leibniz Institute DSMZ) isolated from cane juice, were previously shown to produce dextran (Xu et al. 2019). In Study **II**, a total of 186 LAB isolates from Massa was screened by the research team in Burkina Faso under the collaborative ERAAFRICA Project "FIBREPRO". The strong slime producers (17 strains) were subjected to the final screening for viscosity formation in the University of Helsinki and results were presented in Table S2 in Supplementary Material of Study **II**. The highest viscosity increment ($p < 0.05$) was observed in sourdough fermented with A16, which was therefore selected as the potential dextran producer in Studies **II** and **III**. The phylogenetic analysis of 16S rRNA and *pheS* gene sequences indicated that the strain A16 belonged to the genus *Weissella confusa* (98% identity), and thus the isolate was identified as *W. confusa* A16.

5.2 Structure, molar mass and c* of *W. confusa* A16 dextran

The purity of the isolated dextran from *W. confusa* A16 on agar plates was 80.32% according to the monosaccharide analysis. The ^{1}H NMR spectra of the dextran is shown in Figure 9 (A). The anomeric proton signal showed two spectral resonances at 4.97 and 5.32 ppm, corresponding to H-1 of the α-(1→6) and α-(1→3) glucosyl linkage, respectively (Maina et al. 2008). The ratio of the α-(1→6) to α-(1→3) linkages, calculated based on their relative peak intensities (measured by integration), was 97:3. The bulk region protons in the ^{1}H NMR spectra resonances between 3.97 and 3.52 ppm, corresponded to H-6b, H-5, H-6a, H-3, H-2, and H-4 of the α-(1→6) glucosyl linkage, respectively. Thus, the dextran from *W. confusa* A16 has a linear structure with predominantly α-(1→6) glycosidic bonds and a few α-(1→3)-linked branches (3%). A schematic representation of the *W. confusa* A16 dextran is presented in Figure 9 (B). The structure of dextran from *L. pseudomesenteroides* DSM 20193 was elucidated in previous studies and mainly consisted of α-(1→6) linkages and 5.8% α-(1→3) branches (Xu et al. 2018). The structure and macromolecular properties of dextran from *W. confusa* E3403 have not been reported to date.

The weight average molecular weight (*Mw*) of the *W. confusa* A16 dextran obtained from the HPSEC chromatogram was 3300 kDa and the polydispersity index (PDI) was 1.1. The gyration (R_g) and hydrodynamic (R_h) radii values of the *W. confusa* A16 dextran were 44 and 37 nm, respectively. The *Mw* of *L. pseudomesenteroides* DSM 20193 dextran was 4379 kDa and its PDI was 1.2 (Xu et al. 2018).

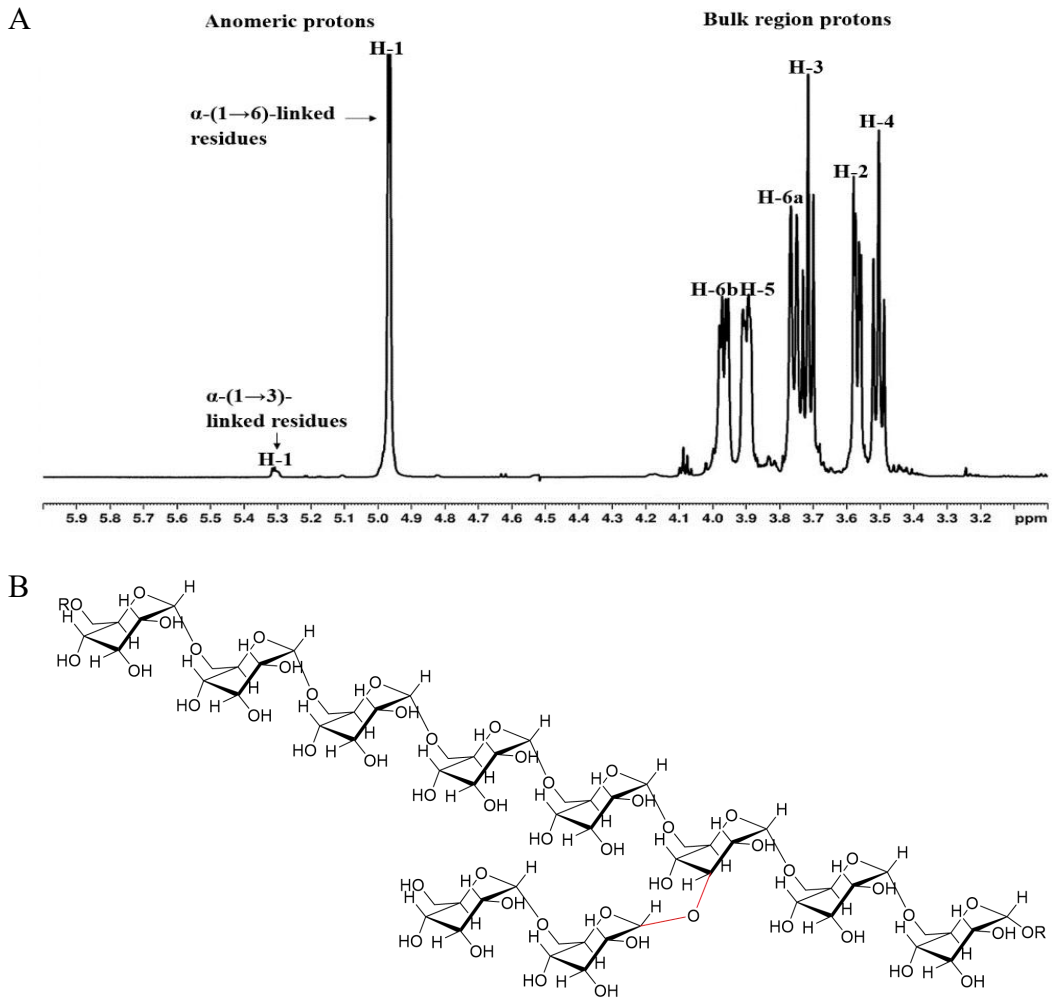

Figure 9. (A) 1D ^{1}H NMR spectrum of dextran produced by *W. confusa* A16 at 600 MHz in D_2O at 50°C. (B) Schematic structure of the dextran, with predominantly α-(1→6) linkages and less than 3% α-(1→3) branches.

The purity of the isolated food-grade dextran from *W. confusa* A16 on GEM medium was 82.09% and the production yield was 12.5 g/L of medium. The steady-shear flow measurements of the aqueous dextran solutions showed Newtonian viscosity at concentrations ranging from 0.1 to 2%. In this investigated range, the zero shear viscosity [η_0] at each polymer concentration was extrapolated and the specific increased viscosity (η_{sp}) was calculated. The double-logarithmic plot of specific viscosity against concentration is shown in Figure 10. The critical overlap concentration c* of the *W. confusa* A16 dextran was estimated to be 0.43% w/w, a point at which there was a discontinuity of the curve corresponding to an abrupt change of slope from 0.92 to 3.12. The intrinsic viscosity [η] of the *W. confusa* A16 dextran was 123.6 mL/g, which represent the effective hydrodynamic volume of the polymer coils and was obtained by extrapolating to zero concentration of the dextran solution. The viscosity average molecular weight (*Mv*) of the *W. confusa* A16 dextran, calculated from the Mark-Houwink-Sakurada equation, was 1900 kDa. The *L. pseudomesenteroides* DSM 20193 dextran had an intrinsic viscosity [η] of 109 mL/g (Xu et al. 2018).

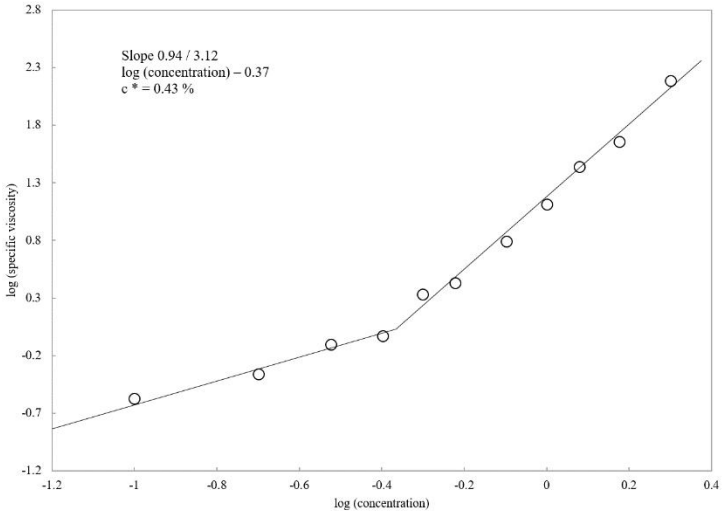

Figure 10. Determination of c* critical concentration of dextran synthesized by *W. confusa* A16, from a log–log plot of concentration versus the specific viscosity.

5.3 Microbial growth in faba bean, millet and sorghum sourdoughs

As shown in Table 3, at the beginning of the fermentation (time 0 h), the cell density of presumptive lactic acid bacteria in all sourdoughs was approximately 10^6 cfu/g. After 24 h of fermentation, a significant increase of cell density occurred in all sourdoughs ranging from 3.0 to 3.9 logarithmic cycles (Δlog). In general, higher Δlog values were observed in faba bean and millet than in sorghum sourdoughs. Regardless of the flour and strains used, the increase of LAB cell density in all dextran-enriched sourdoughs did not differ significantly from their corresponding control sourdoughs. Furthermore, the total mesophilic bacteria count showed comparable values to the cell densities of lactic acid bacteria before and at the end of fermentation (data not shown).

5.4 Acidity of sourdoughs and bread

The acidity values (pH and TTA) of sourdoughs and bread crumbs are presented in Table 3. In faba bean sourdoughs (Study **I)**, the initial pH (6.4−6.5) and TTA (3.7−4.2 mL) values were similar. After 24 h of fermentation, the pH values dropped and the TTA values increased. The dextran-enriched faba bean sourdough (DFSD) showed significantly higher TTA values than its control faba bean sourdough (CFSD) counterpart. Furthermore, sourdoughs fermented by *L. pseudomesenteroides* DSM 20193 exhibited lower pH and higher TTA (two folds) values compared to sourdoughs fermented by *W.confusa* E3403. The addition of faba bean sourdoughs (43% of dough weight**)** resulted in a significant decrease of bread pH and at the same time an increase of TTA compared to the control bread without sourdough. Bread prepared with faba bean sourdough fermented by *L. pseudomesenteroides* DSM 20193 showed lower pH and higher TTA values than bread prepared with faba bean sourdough fermented by *W. confusa* E3403. Nevertheless, the addition of dextran-enriched sourdough did not change significantly the TTA values compared to bread with control sourdough.

Table 3. Cell count of lactic acid bacteria and acidity (pH and TTA) of sourdoughs (SD) at 0 h and after 24 h of fermentation, and acidity of bread crumbs.

	Cell density (log cfu/g)			SD 0 h		SD 24 h		Bread crumb	
	SD 0 h	SD 24 h	Δlog	pH	TTA (ml)	pH	TTA (ml)	pH	TTA (ml)
Faba bean									
CWB[1]								5.7 ± 0.0^{f}	2.4 ± 0.1^{a}
CFWB								6.0 ± 0.0^{h}	4.2 ± 0.1^{d}
E3403 CFSD	6.1 ± 0.1^{a}	9.5 ± 0.1^{b}	3.4^{b}	6.5 ± 0.0^{b}	4.2 ± 0.1^{e}	5.6 ± 0.0^{e}	8.8 ± 0.3^{e}	5.3 ± 0.0^{d}	6.3 ± 0.1^{f}
E3403 DFSD	6.1 ± 0.1^{a}	9.5 ± 0.1^{b}	3.4^{b}	6.4 ± 0.0^{b}	3.8 ± 0.1^{cd}	5.1 ± 0.0^{d}	10.0 ± 0.3^{f}	5.0 ± 0.0^{c}	7.0 ± 0.2^{f}
20193 CFSD	6.1 ± 0.2^{a}	9.9 ± 0.1^{c}	3.8^{c}	6.5 ± 0.0^{b}	4.1 ± 0.0^{de}	4.7 ± 0.1^{c}	16.4 ± 0.4^{g}	4.7 ± 0.0^{a}	9.7 ± 0.6^{g}
20193 DFSD	5.9 ± 0.0^{a}	9.8 ± 0.0^{c}	3.9^{c}	6.5 ± 0.0^{b}	3.7 ± 0.2^{c}	4.6 ± 0.0^{c}	18.5 ± 0.2^{h}	4.6 ± 0.0^{a}	10.2 ± 0.6^{g}
Millet									
CWB								5.6 ± 0.1^{e}	2.2 ± 0.1^{a}
CMWB								5.9 ± 0.1^{g}	2.7 ± 0.1^{ab}
A16 CMSD	5.9 ± 0.1^{a}	9.3 ± 0.1^{ab}	3.4^{b}	6.4 ± 0.0^{b}	1.3 ± 0.1^{a}	4.2 ± 0.1^{a}	6.8 ± 0.1^{b}	4.7 ± 0.0^{ab}	5.3 ± 0.2^{e}
A16 DMSD	5.9 ± 0.0^{a}	9.4 ± 0.1^{ab}	3.5^{b}	6.4 ± 0.1^{b}	1.3 ± 0.1^{a}	4.4 ± 0.1^{b}	6.1 ± 0.2^{a}	4.8 ± 0.0^{b}	5.0 ± 0.1^{e}
Sorghum									
CWB								5.7 ± 0.0^{fg}	3.1 ± 0.1^{bc}
CSWB								5.8 ± 0.0^{fg}	3.4 ± 0.1^{c}
A16 CSSD	6.1 ± 0.1^{a}	9.3 ± 0.0^{ab}	3.2^{a}	6.2 ± 0.0^{a}	2.5 ± 0.2^{b}	4.1 ± 0.1^{a}	8.1 ± 0.2^{d}	4.7 ± 0.0^{a}	6.8 ± 0.1^{f}
A16 DSSD	6.2 ± 0.1^{a}	9.2 ± 0.1^{a}	3.0^{a}	6.2 ± 0.1^{a}	2.7 ± 0.0^{b}	4.1 ± 0.0^{a}	7.4 ± 0.1^{c}	4.7 ± 0.0^{ab}	6.5 ± 0.1^{f}

[1] E3403 = *W. confusa* E3403, 20193 = *L. pseudomesenteroides* DSM 20193, A16 = *W. confusa* A16, CWB = control wheat bread, CFWB = control faba bean wheat bread, CFSD = control faba bean sourdough, DFSD = dextran-enriched faba bean sourdough, CMWB = control millet wheat bread, CMSD = control millet sourdough, DMSD = dextran-enriched millet sourdough, CSWB = control sorghum wheat bread, CSSD = control sorghum sourdough, DSSD = dextran-enriched sorghum sourdough.

Different superscript letters in the same column indicate statistical significance ($p<0.05$).

In millet matrices (Study **II)**, the dextran-enriched millet sourdough (DMSD) and control millet sourdough (CMSD) fermented by *W. confusa* A16 showed similar pH (6.4) and TTA (1.3 mL) values before fermentation. After 24 h of fermentation, slightly higher pH and lower TTA values were measured in dextran-enriched millet sourdough than in control millet sourdough. However, the acidity level of bread prepared with dextran-enriched millet sourdough (59% of dough weight) was not significantly different from that prepared with control millet sourdough.

In sorghum systems (Study **III)**, the pH values decreased from 6.2 (0 h) to 4.1 (24 h), while TTA increased from 2.7 ml (0 h) to 7.4 mL and 8.1 mL (24 h) in dextran-enriched sorghum sourdough (DSSD) and control sorghum sourdough (CSSD) fermented with *W. confusa* A16, respectively. Likewise, bread with dextran-enriched sorghum sourdough (59% of dough weight) showed comparable acidity levels to bread with control sorghum sourdough.

5.5 Sugar, acid, and dextran formation and viscosity enhancement in sourdoughs

The added and flour endogenous sucrose (10 and 2.9% flour weight, respectively) was completely consumed in dextran-enriched faba bean sourdough (DFSD) during 24 h of fermentation (Table 4). The sucrose was mainly utilized for dextran production, which resulted in a high yield of 5.2% (dry weight) in dextran-enriched faba bean sourdough fermented by *W. confusa* E3403 and 3.6% in that fermented by *L. pseudomesenteroides* DSM 20193. A substantial amount of fructose accumulated in dextran-enriched faba bean sourdough fermented by *W. confusa* E3403 (4.6%) but not in the one fermented by *L. pseudomesenteroides* DSM 20193 (1.5%). Glucose was not detected at the end of fermentation. HPAEC-PAD analysis demonstrated the presence of a few water-extractable oligosaccharides in dextran-enriched faba bean sourdough (data not shown). Regarding to control faba bean sourdough (CFSD), a small amount of dextran (0.4−0.9%) was formed due to the presence of endogenous sucrose in the native faba bean flour. Fructose was detected only in trace amount in control faba bean sourdough after fermentation. Irrespective of the strains employed, the apparent viscosity values measured at a shear rate of 123 s^{-1} were significantly higher in dextran-enriched faba bean sourdough compared to their respective control faba bean sourdough and non-fermented faba bean sourdough (0 h). Moreover, dextran-enriched faba bean sourdough fermented by *W. confusa* E3403 showed significantly higher viscosity value than that fermented by *L. pseudomesenteroides* DSM 20193, corresponding to its higher content of dextran. The amount of lactic acid (0.8-0.9%) in all faba bean sourdoughs was comparable except for control faba bean sourdough fermented by *L. pseudomesenteroides* DSM 20193 (1.2%). All sourdoughs had the same concentration of acetic acid (0.3%) except for dextran-enriched faba bean sourdough fermented by *L. pseudomesenteroides* DSM 20193 (0.7%). The fermentation quotient (FQ) varied from 0.9 to 2.6.

In millet matrices, the added and flour endogenous sucrose (10 and 1.5% flour weight, respectively) was also completely utilized in dextran-enriched millet sourdough (DMSD)

Table 4. Sugar, organic acid, and dextran (% dry weight) formation and apparent viscosity (shear rate at 123 s^{-1}) of sourdoughs after 24 h of fermentation.

	Sucrose	Glucose	Fructose	LA[1]	AA	FQ	Dextran	Viscosity
	(%)	(%)	(%)	(%)	(%)		(%)	[Pa·s]
Faba bean								
Faba bean flour	2.9 ± 0.1^{b}	nd	nd					0.9 ± 0.0^{b}
E3403 CFSD[2]	nd	nd	0.8 ± 0.0^{b}	0.8 ± 0.0^{a}	0.3 ± 0.0^{b}	1.5 ± 0.0^{b}	0.9 ± 0.0^{a}	11.6 ± 0.6^{f}
E3403 DFSD	nd	nd	4.6 ± 0.0^{d}	0.9 ± 0.0^{a}	0.3 ± 0.0^{b}	1.8 ± 0.0^{b}	5.2 ± 0.0^{d}	15.3 ± 0.6^{g}
20193 CFSD	nd	nd	0.1 ± 0.0^{a}	1.2 ± 0.0^{b}	0.3 ± 0.0^{b}	2.6 ± 0.4^{c}	0.4 ± 0.0^{a}	3.5 ± 0.2^{d}
20193 DFSD	nd	nd	1.5 ± 0.0^{c}	0.9 ± 0.0^{a}	0.7 ± 0.0^{c}	0.9 ± 0.0^{a}	3.6 ± 0.1^{c}	11.8 ± 0.2^{f}
Millet								
Millet flour	1.5 ± 0.1^{a}	nd	nd					0.3 ± 0.0^{a}
A16 CMSD	nd	nd	0.7 ± 0.0^{b}	0.9 ± 0.0^{a}	0.2 ± 0.0^{a}	3.3 ± 0.3^{c}	0.4 ± 0.0^{a}	0.3 ± 0.0^{a}
A16 DMSD	nd	0.6 ± 0.0^{a}	5.5 ± 0.1^{e}	0.8 ± 0.0^{a}	0.2 ± 0.0^{a}	2.7 ± 0.3^{c}	3.5 ± 0.5^{c}	6.2 ± 0.3^{e}
Sorghum								
Sorghum flour	1.3 ± 0.1^{a}	nd	nd					0.2 ± 0.0^{a}
A16 CSSD	nd	nd	0.6 ± 0.0^{b}	0.9 ± 0.0^{a}	0.1 ± 0.0^{a}	4.2 ± 0.3^{d}	0.5 ± 0.1^{a}	0.2 ± 0.0^{a}
A16 DSSD	nd	3.1 ± 0.1^{b}	4.7 ± 0.1^{d}	0.9 ± 0.0^{a}	0.1 ± 0.0^{a}	4.5 ± 0.4^{d}	2.0 ± 0.1^{b}	2.6 ± 0.2^{c}

[1] LA = Lactic acid, AA = acetic acid, FQ = fermentation quotient, nd = not detected.

[2] E3403 = *W. confusa* E3403, 20193 = *L. pseudomesenteroides* DSM 20193, A16 = *W. confusa* A16, CFSD = control faba bean sourdough, DFSD = dextran-enriched faba bean sourdough, CMSD = control millet sourdough, DMSD = dextran-enriched millet sourdough, CSSD = control sorghum sourdough, DSSD = dextran-enriched sorghum sourdough.

Different letters in the same column indicate statistical significance ($p<0.05$).

after 24 h of fermentation with *W. confusa* A16, resulting in 3.5% of dextran (Table 4). Minor amount of resistant oligosaccharides (Figure 1C in Study **II**) and a significant amount of fructose (5.5%) were detected in dextran-enriched millet sourdough. In control millet sourdough (CMSD), only a small quantity of dextran (0.4%) was formed. The viscosity value measured at 123 s^{-1} in dextran-enriched millet sourdough was significantly higher than that in control millet sourdough. Additionally, the content of lactic and acetic acid and the values of FQ were comparable in dextran-enriched millet sourdough and its control millet sourdough counterpart.

In sorghum sourdoughs, *W. confusa* A16 synthesized 2% of dextran from the added (10%) and flour endogenous (1.3%) sucrose (Table 4). A considerable amount of fructose (4.7%) and glucose (3.1%) was detected in dextran-enriched sorghum sourdough (DSSD) at the end of fermentation. Without sucrose supplementation, only 0.5% of dextran was formed in control sorghum sourdough (CSSD). No significant levels of resistant oligosaccharides were detected in all sorghum sourdoughs (Supplementary material Figure S1 in Study **III**). A dramatic viscosity increase was observed in dextran-enriched sorghum sourdough compared to control sorghum sourdough. The organic acid content and FQ values in dextran-enriched sorghum sourdough did not differ significantly from its control sorghum sourdough counterpart.

5.6 Rheological properties of bread doughs

5.6.1 Farinograph water absorption

In faba bean containing bread doughs as shown in Table 5, the substitution of wheat with 30% of faba bean flour (control faba bean wheat bread dough (CFWB dough)) resulted in a vast decrease in water absorption compared to control wheat bread dough. The addition of control faba bean sourdough increased significantly the water absorption compared to the CFWB dough. The utilization of chemically acidified faba bean dough also increased slightly the water absorption but to a lesser degree than control faba bean sourdough. Remarkably, the use of dextran-enriched faba bean sourdough led to a dramatic increase in water absorption which exhibited values higher than wheat control ($p<0.05$). No significant differences were observed between the utilization of *L. pseudomesenteroides* DSM 20193 and *W. confusa* E3403.

In millet containing bread doughs (Table 5), the changes in water absorption showed a similar trend, following the order: dextran-enriched millet sourdough dough (65%) > control wheat bread dough (62.5%) > control millet sourdough dough (56.8%) > control millet wheat bread dough (55.6%).

5.6.2 Kieffer parameters

The replacement of wheat with 30% of faba bean flour (control faba bean wheat bread dough (CFWB dough)) generated a sharp decline of R_{max} (maximum resistance to extension) and A_{tot} (strength) compared to wheat control doughs (Table 5). The addition of control faba bean sourdough fermented by *W. confusa* E3403 led to higher R_{max} and

A_{tot} values than the CFWB dough. The use of chemically acidified faba bean dough also increased the R_{max} and A_{tot} values but to a lesser extent than control faba bean sourdough fermented by *W. confusa* E3403. The addition of dextran-enriched faba bean sourdough fermented by *W. confusa* E3403 showed the greatest improvement in R_{max} and A_{tot} values. In contrast, the use of control or dextran-enriched faba bean sourdough fermented with *L. pseudomesenteroides* DSM 20193 did not show any increment in R_{max} and reduced significantly the A_{tot} compared to the CFWB dough. The extensibility (Ext) values showed great variability among all dough samples.

In millet containing bread doughs, the incorporation of 50% millet flour (control millet wheat bread dough (CMWB dough)) substantially reduced the R_{max} and A_{tot} values compared to 100% wheat. The utilization of control millet sourdough fermented by *W. confusa* A16 increased slightly the R_{max} compared with the CMWB dough. Only the addition of dextran-enriched millet sourdough fermented by *W. confusa* A16 significantly increased the R_{max} and A_{tot} in comparison to the CMWB dough. The Ext values were similar among the composite dough samples (with and without sourdoughs) and were significantly lower than that of wheat control doughs.

Table 5. Water absorption (500 BU) obtained from the Brabender Farinograph and parameters from the Kieffer extensigraph for different bread doughs containing faba bean or millet flour.

Bread doughs	WA[1] (%)	R_{max} (g)	Ext (cm)	A_{tot} (mm^2)
Faba bean				
CWB[2]	70.9 ± 0.0^{g}	30.5 ± 2.0^{f}	4.7 ± 0.1^{fg}	611.5 ± 4.3^{g}
CFWB	68.3 ± 0.1^{e}	7.7 ± 0.7^{a}	5.7 ± 0.5^{h}	250.9 ± 2.2^{d}
E3403 CAD	69.6 ± 0.1^{f}	10.7 ± 0.8^{b}	3.4 ± 0.3^{de}	266.5 ± 2.8^{d}
20193 CAD	69.6 ± 0.1^{f}	10.9 ± 0.8^{b}	3.0 ± 0.5^{d}	254.8 ± 4.0^{d}
E3403 CFSD	70.7 ± 0.0^{g}	13.5 ± 1.1^{c}	4.4 ± 0.6^{ef}	357.0 ± 4.2^{e}
E3403 DFSD	72.8 ± 0.2^{h}	15.0 ± 1.1^{c}	3.2 ± 0.4^{d}	377.6 ± 4.5^{e}
20193 CFSD	70.9 ± 0.1^{g}	7.4 ± 0.4^{a}	2.6 ± 0.4^{cd}	167.6 ± 2.1^{b}
20193 DFSD	73.1 ± 0.1^{h}	7.9 ± 0.3^{a}	4.5 ± 0.6^{ef}	208.7 ± 2.2^{c}
Millet				
CWB	62.5 ± 0.0^{c}	41.9 ± 1.4^{g}	2.2 ± 0.1^{c}	481.3 ± 8.9^{f}
CMWB	55.6 ± 0.1^{a}	15.2 ± 0.6^{c}	1.4 ± 0.0^{a}	135.8 ± 7.3^{a}
A16 CMSD	56.8 ± 0.1^{b}	17.1 ± 0.6^{d}	1.5 ± 0.0^{b}	143.2 ± 4.4^{a}
A16 DMSD	65.0 ± 0.0^{d}	20.7 ± 0.7^{e}	1.5 ± 0.1^{ab}	161.8 ± 6.5^{b}

[1] WA = farinograph water absorption, R_{max} = maximum resistance to extension (g), Ext = extensibility (cm), A_{tot} = total area under the curve.

[2] E3403 = *W. confusa* E3403, 20193 = *L. pseudomesenteroides* DSM 20193, A16 = *W. confusa* A16, CWB = control wheat bread, CFWB = control faba bean wheat bread, CAD = chemically acidified faba bean dough, CFSD = control faba bean sourdough, DFSD = dextran-enriched faba bean sourdough, CMWB = control millet wheat bread, CMSD = control millet sourdough, DMSD = dextran-enriched millet sourdough.

Different letters in the same column indicate statistical significance ($p<0.05$).

5.6.3 Viscoelastic properties

The viscoelastic properties of faba bean containing bread doughs were measured with oscillatory test and results are reported in publication **I** Figure 2. All dough samples displayed a higher elastic modulus (G') than viscous modulus (G"), which indicates a solid, elastic-like behaviour. The lowest G' was obtained in the control faba bean wheat bread dough (CFWB dough) ($p<0.05$). Independent from the strain used, the G' values of dextran-enriched faba bean sourdough doughs and their respective chemically acidified faba bean doughs were similar. However, the control faba bean sourdough doughs showed significantly higher G' values than the dextran-enriched faba bean sourdough doughs, which were comparable to wheat control.

The changes in phase angle (δ) by replacing wheat flour with 30% non-fermented faba bean flour (CFWB dough) were frequency dependent. At medium and high frequency, wheat control exhibited a higher δ than the CFWB dough. However, the CFWB dough presented higher δ values than the chemically acidified faba bean doughs in the whole frequency range. The use of control faba bean sourdough increased significantly the δ compared to the wheat control dough, CFWB dough and chemically acidified faba bean doughs. Whereas the addition of dextran-enriched faba bean sourdough resulted in even higher δ values than its control faba bean sourdough counterpart.

5.6.4 Dough stickiness

In millet containing bread doughs, the dough stickiness values after kneading were in the range of 48-59 g, with the lowest value being observed in wheat control dough and the highest in the control millet wheat bread dough (Figure 11).

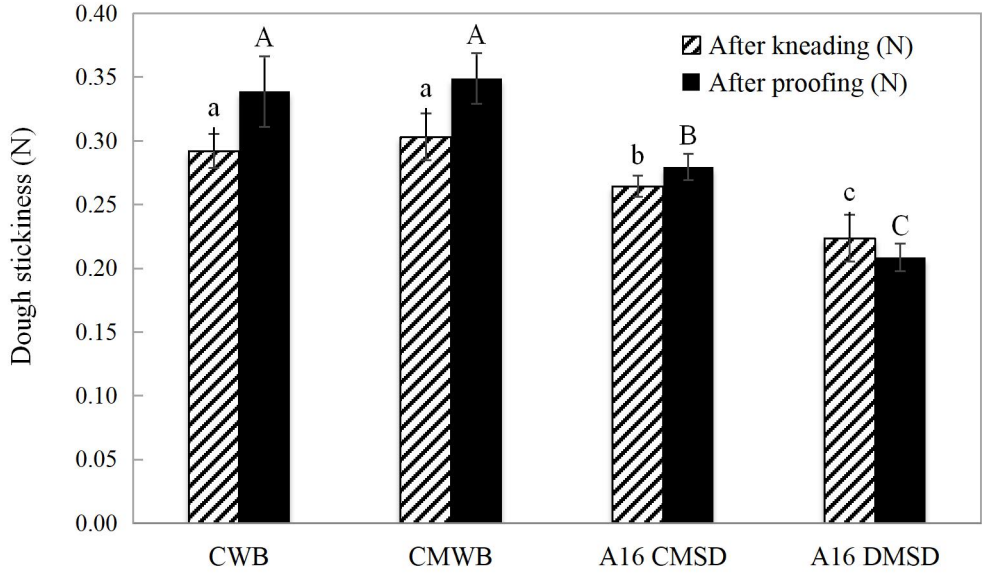

Figure 11. Stickiness of bread doughs (1% salt, 5% fresh yeast, 2% sucrose) directly after kneading and after 60 min proofing. A16 = *W. confusa* A16, CWB = control wheat bread dough, CMWB = control millet wheat bread dough, CMSD = control millet sourdough bread dough, DMSD = dextran-enriched millet sourdough bread dough. Different lowercase letters indicate significant difference ($p < 0.05$) among the four types of bread dough after kneading; the uppercase letters indicate significant difference ($p < 0.05$) after proofing.

The use of control millet sourdough did not change significantly the dough stickiness compared to the control millet wheat bread dough. In contrast, the utilization of dextran-enriched millet sourdough markedly reduced the dough stickiness to levels comparable to wheat control. The dough stickiness values measured after 60 min of proofing were between 61 and 70 g, showing a similar trend to the stickiness values after kneading.

5.7 Bread quality characterization

5.7.1 Specific volume

In faba bean containing breads, the substitution of wheat with 30% of faba bean flour (control faba bean wheat bread (CFWB)) resulted in a significant decrease of bread specific volume by 11% (Table 6). Incorporation of chemically acidified faba bean dough led to a decline of specific volume by 16-21% compared to wheat control bread. The application of control faba bean sourdough fermented by *L. pseudomesenteroides* DSM 20193 was not sufficient to improve the loaf specific volume, whereas the use of control faba bean sourdough fermented by *W. confusa* E3403 increased significantly the loaf volume to values comparable to wheat control bread. The application of dextran-enriched faba bean sourdough fermented by *L. pseudomesenteroides* DSM 20193 decreased the loaf volume by 24% compared to wheat control. In contrast, the inclusion of dextran-enriched faba bean sourdough fermented by *W. confusa* E3403 improved the loaf specific volume, resulting 8% higher than wheat control.

In millet containing breads, replacing wheat with 50% of millet flour (control millet wheat bread (CMWB)) generated a significant decrease of loaf specific volume by 29% (Table 6). Only the addition of dextran-enriched millet sourdough fermented by *W. confusa* A16 improved significantly the specific volume by 13% compared to the CMWB. A similar trend was observed in sorghum containing breads.

5.7.2 Textural properties of breads

In faba bean containing breads, wheat substitution with 30% faba bean flour (CFWB) resulted in an increase of crumb hardness of 23% compared to wheat control (Table 6). Incorporation of chemically acidified or control faba bean sourdough led to an increase of 10-32% of crumb hardness compared to the CFWB. Furthermore, the utilization of dextran-enriched faba bean sourdough fermented by *L. pseudomesenteroides* DSM 20193 increased the crumb hardness to the highest extent and more than 2-fold compared to the CFWB. Only the addition of dextran-enriched faba bean sourdough fermented by *W. confusa* E3403 showed a decrease of crumb hardness to levels identical to wheat control bread. The different values of crumb hardness persisted during 4 days of storage.

Table 6. Specific volume (mL/g) and crumb hardness (g) of breads contain faba bean, millet or sorghum flour.

Breads	Specific Volume	Hardness[1] (day1)	Hardness (day4)
Faba bean			
CWB[2]	3.8 ± 0.1^{b}	77.3 ± 6.2^{a}	90.1 ± 5.5^{a}
CFWB	3.4 ± 0.0^{c}	100.0 ± 11.1^{bc}	100.0 ± 7.5^{ab}
E3403 CAB	3.2 ± 0.1^{d}	116.5 ± 13.3^{cd}	132.4 ± 14.9^{cd}
20193 CAB	3.0 ± 0.1^{de}	131.5 ± 20.1^{d}	125.5 ± 15.7^{cd}
E3403 CFSB	3.8 ± 0.1^{b}	110.1 ± 8.5^{c}	118.3 ± 7.3^{bc}
E3403 DFSB	4.1 ± 0.1^{a}	88.2 ± 9.0^{a}	118.4 ± 7.6^{bc}
20193 CFSB	3.4 ± 0.1^{c}	130.7 ± 10.0^{d}	140.8 ± 5.6^{d}
20193 DFSB	2.9 ± 0.1^{e}	215.7 ± 9.4^{e}	239.7 ± 16.5^{e}
Millet			
CWB	4.5 ± 0.2^{a}	150.4 ± 25.0^{a}	250.9 ± 26.0^{a}
CMWB	3.2 ± 0.2^{c}	350.6 ± 34.1^{c}	469.1 ± 33.6^{c}
A16 CMSB	3.2 ± 0.1^{c}	450.4 ± 38.9^{d}	608.5 ± 36.4^{d}
A16 DMSB	3.6 ± 0.1^{b}	199.2 ± 23.4^{b}	306.3 ± 23.7^{b}
Sorghum			
CWB	4.3 ± 0.1^{a}	127.0 ± 9.8^{a}	253.4 ± 28.7^{a}
CSWB	2.8 ± 0.1^{c}	564.6 ± 39.3^{c}	855.0 ± 42.5^{c}
A16 CSSB	2.6 ± 0.0^{d}	655.4 ± 34.9^{d}	925.5 ± 57.7^{d}
A16 DSSB	3.2 ± 0.1^{b}	266.4 ± 32.6^{b}	414.4 ± 38.3^{b}

[1] The crumb hardness (%) of faba bean containing breads was calculated based on the percentage of the CFWB (100) due to variations in the baking dates.

[2] E3403 = *W. confusa* E3403, 20193 = *L. pseudomesenteroides* DSM 20193, A16 = *W. confusa* A16, CWB = control wheat bread, CFWB = control faba bean wheat bread, CAB = chemically acidified bread, CFSB = control faba bean sourdough bread, DFSB = dextran-enriched faba bean sourdough bread, CMWB = control millet wheat bread, CMSB = control millet sourdough bread, DMSB = dextran-enriched millet sourdough bread, CSWB = control sorghum wheat bread, CSSB = control sorghum sourdough bread, DSSB = dextran-enriched sorghum sourdough bread.

Different letters in the same column (in each flour matrices) indicate statistical significance.

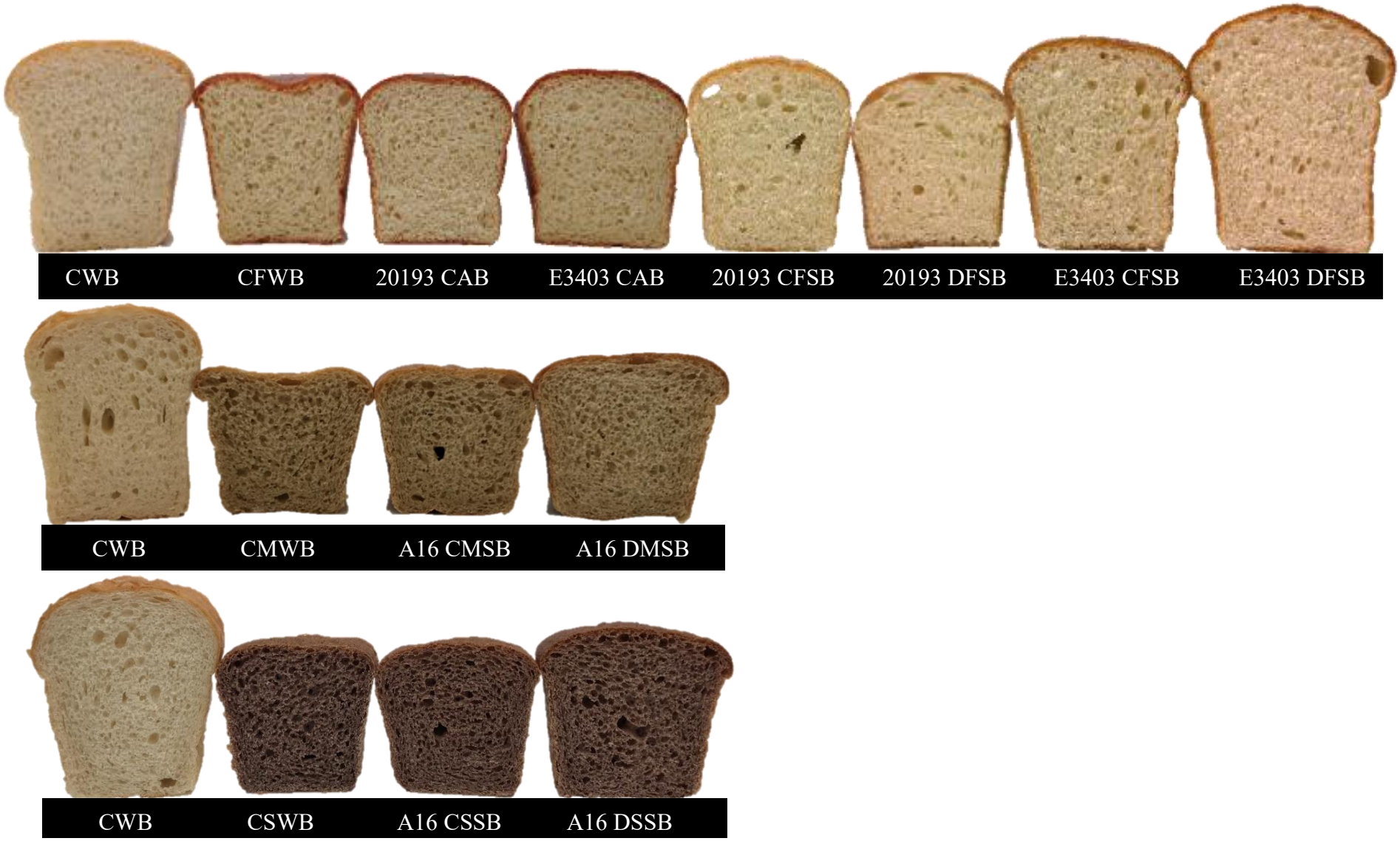

Figure 12. Cross-section images of (left) 100% wheat bread and (right) breads contain faba bean, millet, and sorghum flour (from top to bottom). E3403 = *W. confusa* E3403, 20193 = *L. pseudomesenteroides* DSM 20193, A16 = *W. confusa* A16, CWB = control wheat bread, CFWB = control faba bean wheat bread, CAB = chemically acidified bread, CFSB = control faba bean sourdough bread, DFSB = dextran-enriched faba bean sourdough bread, CMWB = control millet wheat bread, CMSB = control millet sourdough bread, DMSB = dextran-enriched millet sourdough bread, CSWB = control sorghum wheat bread, CSSB = control sorghum sourdough bread, DSSB = dextran-enriched sorghum sourdough bread

In millet containing breads, the inclusion of 50% millet flour (control millet wheat bread (CMWB)) significantly increased the crumb hardness by 2.3-fold in comparison to 100% wheat bread (Table 6). The bread prepared with control millet sourdough fermented by *W. confusa* A16 was 3-fold harder than wheat control. Conversely, the bread prepared with dextran-enriched millet sourdough fermented by *W. confusa* A16 was significantly softer, showing similar hardness values to wheat control. The crumb springiness and cohesiveness of CMWB was significantly lower than wheat control bread (Table 7). The use of control millet sourdough did not alter the springiness and cohesiveness compared to the CMWB. However, the inclusion of dextran-enriched millet sourdough significantly increased the springiness and cohesiveness. In sorghum containing breads, the modifications in crumb hardness, cohesiveness, and springiness followed the same trend as for the millet containing breads.

Table 7. Cohesiveness, springiness and moisture content of breads contain millet or sorghum flour.

Breads	Cohesiveness (day1)	Springiness (g, day1)	Moisture content (%, day1)
Millet			
CWB[1]	0.82 ± 0.03^{a}	2.91 ± 0.49^{a}	44.5 ± 0.0^{a}
CMWB	0.47 ± 0.05^{d}	0.95 ± 0.05^{c}	44.0 ± 0.0^{b}
A16 CMSB	0.61 ± 0.03^{c}	1.32 ± 0.57^{c}	43.6 ± 0.0^{c}
A16 DMSB	0.76 ± 0.04^{b}	1.85 ± 0.80^{b}	44.6 ± 0.1^{a}
Sorghum			
CWB	0.76 ± 0.01^{a}	0.96 ± 0.02^{a}	44.4 ± 0.0^{a}
CSWB	0.50 ± 0.04^{b}	0.92 ± 0.01^{c}	42.9 ± 0.1^{d}
A16 CSSB	0.56 ± 0.04^{b}	0.90 ± 0.02^{c}	43.2 ± 0.0^{c}
A16 DSSB	0.72 ± 0.07^{a}	0.94 ± 0.01^{ab}	43.6 ± 0.0^{b}

[1] A16 = *W. confusa* A16, CWB = control wheat bread, CMWB = control millet wheat bread, CMSB = control millet sourdough bread, DMSB = dextran-enriched millet sourdough bread, CSWB = control sorghum wheat bread, CSSB = control sorghum sourdough bread, DSSB = dextran-enriched sorghum sourdough bread.
Different letters in the same column (in each flour matrices) indicate statistical significance ($p<0.05$).

5.7.3 Moisture content

The replacement of wheat with 50% millet flour (control millet wheat bread (CMWB)) induced a dramatic reduction of crumb moisture content compared to wheat flour alone (Table 7). The application of control millet sourdough fermented by *W. confusa* A16 decreased the moisture content compared to the CMWB. In contrast, the addition of dextran-enriched millet sourdough fermented by *W. confusa* A16 significantly increased the crumb moisture content in comparison with the CMWB. The control sorghum wheat bread (CSWB) also showed markedly lower crumb moisture than wheat control (Table 7). The moisture content in bread prepared with control sorghum sourdough fermented

by *W. confusa* A16 increased compared to the CSWB, which further increased in bread prepared with dextran-enriched sorghum sourdough fermented by *W. confusa* A16.

5.8 Effect of dextran on bread staling and starch retrogradation

In millet containing breads, the incorporation of 50% millet flour (control millet wheat bread (CMWB)) caused a significant increase of bread staling rate by 18% compared to wheat control (Figure 13). No positive effect was observed when control millet sourdough fermented by *W. confusa* A16 was used. On the contrary, the addition of dextran-enriched millet sourdough fermented by *W. confusa* A16 showed an inhibitory effect on the staling rate, leading to values comparable to wheat control bread ($p<0.05$).

Likewise, the inclusion of 50% sorghum flour (control sorghum wheat bread (CSWB)) increased the bread staling rate by 130% compared to wheat control. The staling rate of CSWB was much higher (2.5-fold) than that of CMWB. However, the utilization of dextran-enriched sorghum sourdough fermented by *W. confusa* A16 effectively retarded the staling rate of sorghum containing breads to levels identical to wheat control bread.

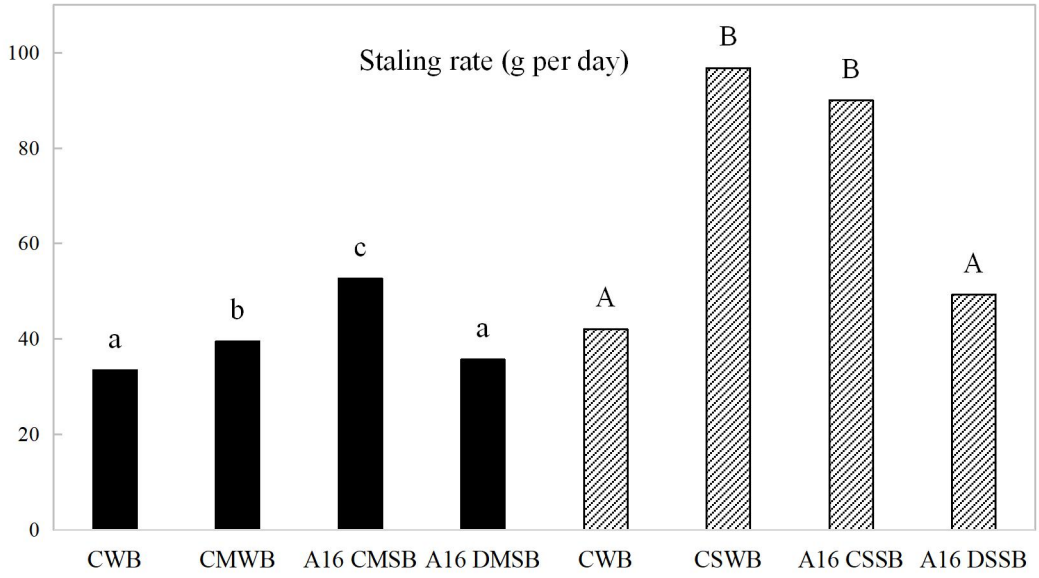

Figure 13. The staling rate [hardness (day 4 – day 1) / days of storage] of breads contain millet (solid) and sorghum flour (pattern). A16 = *W. confusa* A16, CWB = control wheat bread, CMWB = control millet wheat bread, CMSB = control millet sourdough bread, DMSB = dextran-enriched millet sourdough bread, CSWB = control sorghum wheat bread, CSSB = control sorghum sourdough bread, DSSB = dextran-enriched sorghum sourdough bread. Different lowercase letters indicate significant difference ($p < 0.05$) among the millet containing breads; the uppercase letters indicate significant difference ($p < 0.05$) among the sorghum containing breads.

In millet containing breads, the molecular basis of the impact of dextran on bread staling, such as the amylopectin retrogradation enthalpy, was investigated and the results are

illustrated in Table 8. The storage time (day 1-4) exhibited a significant effect on the transition temperatures (i.e. T_o, T_c, and T_p) and the enthalpy values (ΔH). Wheat flour replacement with 50% millet flour (control millet wheat bread (CMWB)) in bread making resulted in significantly higher T_c, T_p, and melting enthalpy in comparison with 100% wheat, irrespective of the storage time. Applying control millet sourdough fermented by *W. confusa* A16 in this study did not show any significant influence on the thermal values compared to the CMWB. Conversely, adding dextran-enriched millet sourdough fermented by *W. confusa* A16 substantially reduced the retrogradation enthalpy, which brought a shift of the endothermic peak and endset towards noticeably lower temperatures than the CMWB ($p<0.05$).

Table 8. Retrogradation parameters of wheat and millet-wheat breads on days 1 and 4 of storage.

Millet	T_o [1] (°C)	T_c (°C)	T_p (°C)	ΔH (J/g)
Day 1				
CWB[2]	37.88 ± 0.16^{d}	74.28 ± 0.25^{a}	55.66 ± 0.24^{a}	2.20 ± 0.21^{a}
CMWB	37.47 ± 0.03^{cd}	75.80 ± 0.25^{bc}	56.37 ± 0.04^{b}	3.38 ± 0.26^{bc}
A16 CMSB	37.26 ± 0.13^{c}	76.55 ± 0.45^{c}	56.45 ± 0.21^{b}	3.50 ± 0.40^{bc}
A16 DMSB	37.49 ± 0.20^{cd}	75.03 ± 0.08^{ab}	55.70 ± 0.25^{a}	2.92 ± 0.02^{ab}
Day 4				
CWB	36.51 ± 0.17^{b}	78.39 ± 0.05^{d}	57.95 ± 0.07^{c}	3.37 ± 0.05^{bc}
CMWB	36.08 ± 0.02^{a}	81.95 ± 0.16^{f}	58.68 ± 0.20^{cd}	4.47 ± 0.19^{e}
A16 CMSB	36.06 ± 0.03^{a}	81.81 ± 0.17^{f}	58.98 ± 0.44^{d}	4.39 ± 0.23^{e}
A16 DMSB	36.25 ± 0.11^{ab}	79.27 ± 0.09^{e}	58.10 ± 0.08^{c}	3.68 ± 0.05^{cd}

[1] To = onset temperature, Tc = conclusion temperature, Tp = peak temperature, ΔH = retrogradation enthalpy.

[2] A16 = *W. confusa* A16, CWB = control wheat bread, CMWB = control millet wheat bread, CMSB = control millet sourdough bread, DMSB = dextran-enriched millet sourdough bread.

Different letters in the same column indicate statistical significance ($p<0.05$).

5.9 Nutritional quality of millet enriched bread

The impact of sourdough fermentation on the nutritional properties of millet enriched breads was investigated. As revealed in Table 9, the phytic acid content in control millet wheat bread (CMWB) was double than that of wheat control bread. The level of phytic acid was not reduced by sourdough fermentation ($p > 0.05$). The starch and protein content of millet enriched breads with or without sourdough was slightly lower than wheat control bread. The CMWB also exhibited *in vitro* protein digestibility (IVPD) 23% lower than control wheat bread. The use of control or dextran-enriched millet sourdough fermented by *W. confusa* A16 improved significantly the IVPD by 8−13% compared to the CMWB. Nevertheless, the IVPD of the control millet sourdough bread and dextran-enriched millet sourdough bread did not differ significantly from each other.

Table 9. Nutritional characterization of wheat and millet-wheat breads.

Millet	CWB[1]	CMWB	A16 CMSB	A16 DMSB
Phytic acid (mg/100g)	317.1 ± 4.1[a]	600.7 ± 4.9[b]	587.3 ± 6.9[b]	595.4 ± 7.4[b]
Starch (% dry weight)	67.9 ± 2.6[a]	64.8 ± 2.7[b]	64.8 ± 2.7[b]	62.8 ± 2.8[c]
Protein (%)	13.0 ± 0.1[a]	11.5 ± 0.1[b]	11.6 ± 0.1[b]	11.1 ± 0.0[c]
IVPD[2] (%)	66.4 ± 3.9[a]	43.8 ± 2.1[c]	51.9 ± 2.3[b]	56.5 ± 1.4[b]
HI (%)	100 ± 0.0[a]	83.4 ± 3.1[b]	70.4 ± 1.2[c]	71.1 ± 2.8[c]
GI (%)	94.6 ± 0.0[a]	85.5 ± 1.7[b]	78.4 ± 0.7[c]	78.7 ± 1.5[c]

[1] A16 = *W. confusa* A16, CWB = control wheat bread, CMWB = control millet wheat bread, CMSB = control millet sourdough bread, DMSB = dextran-enriched millet sourdough bread.
[2] IVPD = *in vitro* protein digestibility, HI = starch hydrolysis index, GI = predicted glycemic index. Different letters in the same row indicate statistical significance ($p<0.05$).

The *in vitro* starch hydrolysis in all tested breads (Figure 14) demonstrated a gradual increase with increasing incubation time. During the 3 h of incubation, the CMWB underwent significantly lower starch hydrolysis rate, compared to 100% wheat bread. Furthermore, the bread containing control or dextran-enriched millet sourdough fermented by *W. confusa* A16 had significantly lower starch hydrolysis rate than the CMWB. The hydrolysis index (HI) and predicted glycemic index (GI) of tested samples are presented in Table 9. The 50% of millet flour inclusion decreased significantly the HI by 17% compared to wheat control. Using control or dextran-enriched millet sourdough resulted in a decrease of HI of approximately 30% compared to wheat control. Moreover, the reduction of HI in those millet enriched breads corresponded to a significant decrease of GI. No significant difference was observed in the HI and GI values of bread prepared with control millet sourdough and its dextran-enriched millet sourdough counterpart.

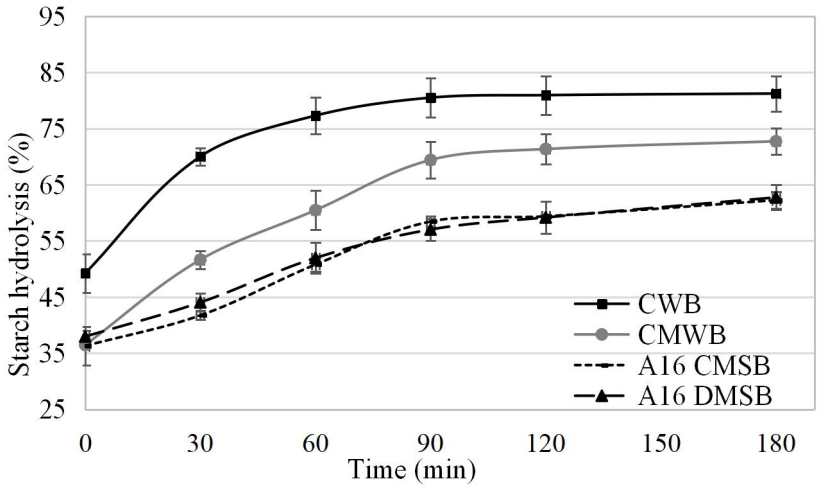

Figure 14. Starch hydrolysis curves of wheat and millet-wheat breads, expressing the percentage of starch (digested over total). CWB = control wheat bread, CMWB = control millet wheat bread, CMSB = control millet sourdough bread, DMSB = dextran-enriched millet sourdough bread.

5.10 Phenolic content and antioxidant activity

5.10.1 Soluble and bound phenolic content of millet extracts

The total (soluble and bound) phenolic content of millet sourdough extracts and native millet flour is shown in Figure 15. After 24 h of fermentation, the soluble phenolic content increased significantly of 21-30% while the bound phenolic content decreased of 22-24%. No significant difference was observed between control millet sourdough and dextran-enriched millet sourdough. The total phenolic content in non-fermented millet flour (443 mg /100g GAE, dry weight) was slightly higher compared to dextran-enriched millet sourdough (430 mg/100g) and control millet sourdough (417 mg/100g).

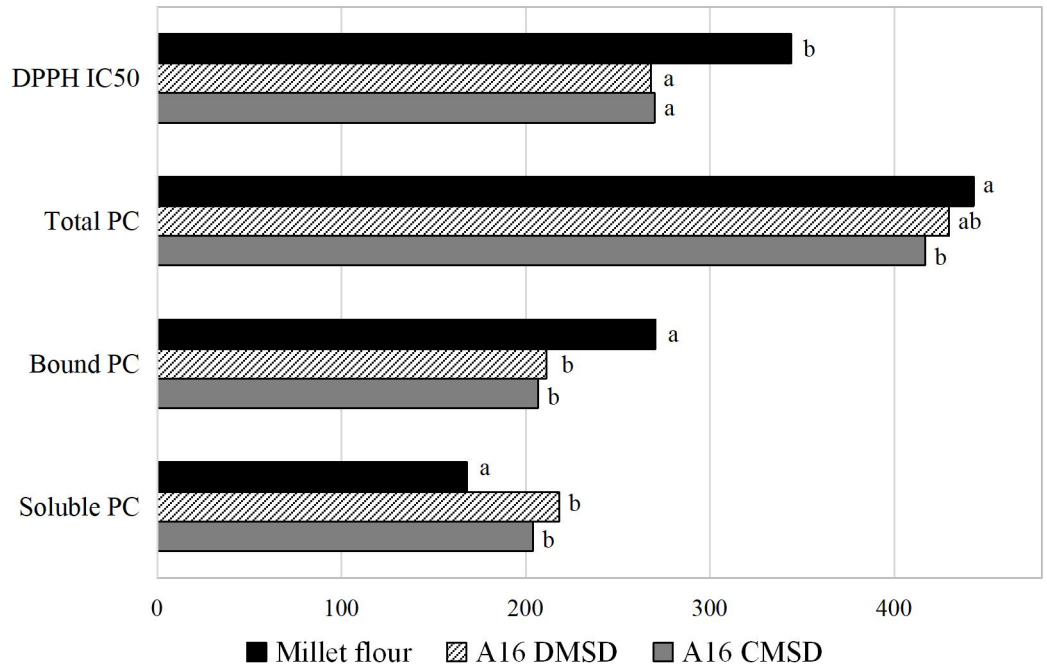

Figure 15. Phenolic content (mg/100g GAE) and IC_{50} (mg/100ml) of extracts derived from native and fermented millet flour. PC = phenolic content. DPPH radical scavenging activity, IC_{50} = the concentration of the sample required to scavenge 50% of free radicals, A16 = *W. confusa* A16, CMSD = control millet sourdough, DMSD = dextran-enriched millet sourdough. Different letters in the same filling pattern bars indicate statistical significance ($p<0.05$).

5.10.2 Antioxidant activity of millet extracts

DPPH free radical-scavenging activity (% inhibition) of the methanol extracts of millet flour, control millet sourdough, dextran-enriched millet sourdough, and standard positive control BHT (synthetic antioxidant) at a range of the tested concentrations is shown in Figure 1D in Study **II**. The extracts of control millet sourdough and dextran-enriched millet sourdough showed equal DPPH inhibitory activity at all concentrations and were significantly higher than that of non-fermented millet flour. The IC_{50} (the

concentration of the sample required to scavenge 50% of free radicals) of the methanol extracts of millet flour, dextran-enriched millet sourdough, and control millet sourdough was 3.44, 2.68 and 2.70 mg/mL, respectively (Figure 15).

5.10.3 Polyphenolic profile of sorghum extracts

The identification and characterization of the polyphenolic compounds of the methanol-formic acid extracts of sorghum sourdoughs and native sorghum flour were conducted by LC–MS/MS fragmentation data and by comparison with external standards and literature data. The chromatographic retention time (measured at 280 nm) and concentration of the identified polyphenolic compounds is summarized in Table 10. The work was done in collaboration with Dr. Antonio Trani in International Center for Advanced Mediterranean Agronomic Studies of Bari (CIHEAM-IAMB, Italy). A total of sixty compounds was found in the extracts, forty-four of which were tentatively identified as phenolic acids, flavonoids, phenylpropane glycerides, and phenolamides. In addition, a hydroxyl fatty acid (trihydroxy-octadecenoic acid) was also identified.

The results demonstrated that the total phenolic content in the methanol-formic acid extracts of sorghum flour and sourdoughs fermented with *W. confusa* A16 (dextran-enriched sorghum sourdough and control sorghum sourdough) was identical, ranging from 132.9 to 139 mg/100g GAE. In sorghum four, the dominant group detected was flavone and flavanone derivatives, followed by phenylpropane glycerides, and flavan-3-ol derivatives. The dextran-enriched sorghum sourdough and its control sorghum sourdough counterpart exhibited similar polyphenolic profiles (type of polyphenols) but significantly different individual polyphenol concentrations compared to non-fermented sorghum flour. For example, caffeic acid concentration in dextran-enriched sorghum sourdough and control sorghum sourdough (16.4 and 18.3 mg/100g, respectively) was significantly higher than that in sorghum flour (4.6 mg/100g). The two types of sorghum sourdough also showed a double amount of tetrahydroxy flavone compared to non-fermented sorghum flour. Additionally, procyanidin B was detected in higher concentrations in extracts of sorghum sourdoughs. However, some phenylpropane glycerides (i.e. 2-O-caffeoylglycerol isomer, 1,3-O-dicaffeoylglycerol isomer, 1,3-O-feruloyl-dihydrocaffeoylglycerol, and 1,3-O-coumaroyl-caffeoyl-glycerol isomer) and flavonoids (e.g. dihydroxyflavone and methyl catechin) were significantly lower in the two sorghum sourdoughs than sorghum flour.

Table 10. Polyphenolic coumpounds identified and quantified in native (non-fermented) sorghum flour and lyophilized sorghum sourdoughs.

Identity	Retention time (min)	Native sorghum flour (μg GAE g^{-1})	DSSD[1]	CSSD
Protocatechuic acid	2.38	6.2 ± 0.1[a]	6.3 ± 0.4[a]	7.2 ± 0.6[a]
Unknown	2.56	10.9 ± 0.3[a]	12.6 ± 1.0[ab]	13.3 ± 0.5[b]
Catechin hexoside	3.32	4.1 ± 1.3[a]	3.2 ± 1.0[a]	1.5 ± 0.1[a]
Unknown	3.48	5.6 ± 1.3[a]	5.2 ± 0.8[a]	5.6 ± 0.3[a]
Procyanidin B	4.19	22.9 ± 0.6[a]	28.6 ± 2.1[b]	28.2 ± 1.1[b]
1-O-caffeoyl-2-O-glucosylglycerol	4.58	11.5 ± 0.3[a]	13.3 ± 0.8[b]	11.3 ± 0.6[a]
Catechin	4.86	7.4 ± 0.2[a]	8.8 ± 0.4[a]	10.8 ± 0.5[b]
2-O-caffeoylglycerol	5.09	1.6 ± 0.1[a]	0.0[b]	1.1 ± 0.2[a]
Unknown	5.10	0.0[a]	3.9 ± 0.3[b]	5.7 ± 1.1[b]
1-O-caffeoyl-2-O-glucosylglycerol	5.27	2.9 ± 0.2[a]	3.5 ± 0.2[a]	0.0[b]
Caffeic acid	5.54	45.8 ± 1.2[a]	164.1 ± 10.3[b]	183.4 ± 10.5[b]
2-O-caffeoylglycerol isomer	5.81	18.4 ± 0.6[a]	6.7 ± 0.6[c]	9.8 ± 0.5[b]
2-O-caffeoylglycerol isomer	5.90	39.8 ± 1.4[a]	17.2 ± 1.1[b]	20.5 ± 1.0[b]
Unknown	6.80	4.4 ± 0.2[a]	4.0 ± 0.3[a]	3.5 ± 0.4[a]
Coumaroyl glycerol, N1-N4-dicaffeoyl spermidine, Taxifolin hexoside isomer	7.22	154.0 ± 5.6[a]	157.9 ± 11.9[a]	169.9 ± 9.5[a]
Catechin isomer	7.46	7.6 ± 0.3[a]	6.1 ± 0.6[a]	6.1 ± 0.5[a]
Quercetin hexoside	7.59	17.7 ± 0.6[a]	15.9 ± 1.3[a]	14.7 ± 0.8[a]
Unknown	7.69	5.0 ± 0.5[a]	5.3 ± 0.4[a]	4.6 ± 0.5[a]
Tetrahydroxy flavone	7.88	22.7 ± 0.8[a]	52.6 ± 4.2[b]	56.5 ± 3.3[b]
N1-N8-caffeoyl feruloyl spermidine, Trihydroxy flavone	8.11	44.0 ± 1.7[a]	25.9 ± 1.4[b]	21.5 ± 1.1[c]
Flavonoid hexoside, Nringenin hexoside	8.29	67.3 ± 2.7[a]	58.6 ± 3.5[ab]	54.6 ± 2.6[b]
Quercetin hexoside isomer, Dicaffeoyl glycerol	8.53	15.3 ± 1.3[a]	13.8 ± 1.0[a]	15.0 ± 0.8[a]
Luteolin hexoside	8.62	3.1 ± 1.0[a]	0.0[b]	0.0[b]

Unknown	8.73	14.5 ± 1.7[a]	11.1 ± 1.0[b]	9.9 ± 0.6[b]
Dihydroxyflavone	8.91	45.1 ± 2.3[a]	35.3 ± 2.3[b]	30.8 ± 1.6[b]
Naringenin, Taxifolin, Unknown	9.07	39.6 ± 1.9[a]	33.1 ± 1.6[a]	34.1 ± 2.3[a]
Gallic acid monohydrate, Unknown	9.24	242.3 ± 6.3[a]	222.3 ± 14.3[a]	228.5 ± 13.2[a]
Isorhamnetin hexoside	9.54	106.1 ± 2.7[a]	101.0 ± 6.4[a]	104.1 ± 8.1[a]
Methyl catechin	9.90	29.1 ± 0.5[a]	25.5 ± 1.5[b]	26.6 ± 0.6[b]
Pentahydroxy flavone	9.97	41.8 ± 2.5[a]	37.0 ± 2.7[a]	40.4 ± 4.5[a]
Methyl afzelechin	10.51	46.3 ± 4.0[a]	46.5 ± 0.7[a]	40.7 ± 1.8[a]
Unknown	10.83	6.4 ± 0.5[a]	6.3 ± 0.1[a]	8.5 ± 0.6[b]
Dicaffeoyl glycerol isomer, 5-methoxy-7,4'-dihydroxy flaven-3-ol	11.15	26.8 ± 1.2[a]	22.5 ± 2.5[a]	23.1 ± 1.8[a]
1,3-O-Dicaffeoyl glycerol isomer	11.36	58.5 ± 1.6[a]	23.7 ± 1.2[b]	24.6 ± 2.5[b]
Methyl afzelechin isomer	11.48	44.2 ± 0.8[a]	45.3 ± 4.9[a]	47.8 ± 5.4[a]
Unknown	11.57	8.4 ± 0.1[a]	0.0[b]	9.4 ± 3.3[a]
Quercetin, Luteolin	11.81	28.8 ± 0.8[a]	34.7 ± 2.6[a]	37.7 ± 4.7[a]
Isorhamnetin	11.89	11.3 ± 0.6[a]	13.2 ± 1.1[a]	15.3 ± 3.6[a]
1,3-O-Coumaroyl-caffeoyl-glycerol	12.31	2.0 ± 0.1[a]	0.9 ± 0.1[b]	0.0[b]
1,3-O-Feruloyl-dihydrocaffeoylglycerol, 1,3-O-Coumaroyl-caffeoyl-glycerol isomer	12.58	34.5 ± 1.3[a]	13.4 ± 0.7[b]	12.7 ± 0.6[b]
1,3-O-feruloyl-caffeoylglycerol	12.80	31.4 ± 1.2[ab]	29.0 ± 2.1[b]	34.2 ± 1.9[a]
Apigenin	13.47	3.6 ± 1.4[a]	4.8 ± 0.4[a]	5.8 ± 0.4[a]
Tricin	13.65	1.8 ± 0.1[a]	1.8 ± 0.2[a]	2.2 ± 0.2[a]
Unknown	13.79	1.2 ± 0.3[b]	2.1 ± 0.1[a]	2.5 ± 0.2[a]
1,3-O-Dicoumaroylglycerol	13.90	1.9 ± 0.5[a]	0.9 ± 0.1[a]	1.2 ± 0.1[a]
Trihydroxy-octadecenoic acid, 1,3-O-coumaroyl-feruloylglycerol	14.10	6.9 ± 0.5[a]	4.5 ± 0.4[b]	4.6 ± 0.3[b]
Total polyphenolic content		1344.1 ± 33.9[a]	1328.6 ± 85.5[a]	1389.9 ± 77.4[a]

[1] CSSD = control sorghum sourdough, DSSD = dextran-enriched sorghum sourdough. Different letters in the same row indicate statistical significance ($p<0.05$).

5.11 Sensory profiling of sorghum enriched bread

The flavour and texture profiles of the wheat and sorghum enriched breads are shown in Figure 16. The corresponding sensory data can be found in Study **III** Supplementary Table S4 online. According to the statistical analysis, a high level of consistency within the panelists evaluations and good repeatability across the different testing sessions was observed.

The control sorghum wheat bread (CSWB) was considered significantly different from the 100% wheat bread concerning all evaluated texture and mouthfeel attributes (Figure 16A). The bread containing control sorghum sourdough fermented by *W. confusa* A16 was found identical in texture to the CSWB except for resistance to pressure (hardness), which was significantly higher in control sorghum sourdough bread. In contrast, the bread prepared with dextran-enriched sorghum sourdough fermented by *W. confusa* A16 was similar to wheat control bread. To be more specific, bread with dextran-enriched sorghum sourdough received significantly higher scores for elasticity, foldability, moist mouthfeel, and cohesive/dough-like texture, and at the same time significantly lower ratings for crumb coarseness, resistance to deformation (rigidity), resistance to pressure, and resilience in mouth (toughness), compared to CSWB and control sorghum sourdough bread.

The flavour attributes varied significantly among the three types of sorghum bread except for the overall odor intensity (Figure 16B). The incorporation of control sorghum sourdough increased significantly the intensity of sour odor, sour taste, bitter taste, and aftertaste compared to the CSWB. On the contrary, the addition of dextran-enriched sorghum sourdough led to significantly reduced intensity of sour taste and odor in comparison to its control sorghum sourdough counterpart, showing scores similar to the CSWB. Furthermore, the inclusion of dextran-enriched sorghum sourdough decreased the ratings for bitter taste and aftertaste below those of CSWB. Additionally, the bread prepared with dextran-enriched sorghum sourdough was perceived as more sweet and roasted than the CSWB and control sorghum sourdough bread.

Figure 16. Texture (A) and flavour (B) profiling of wholegrain sorghum-wheat (with and without sourdough fermentation) and wheat bread samples. A16 = *W. confusa* A16, CWB = control wheat bread, CSWB = control sorghum wheat bread, CSSB = control sorghum sourdough bread, DSSB = dextran-enriched sorghum sourdough bread.

5.12 Consumer preference of sorghum enriched bread

The four types of bread (wheat control + 3 sorghum enriched breads) were further assessed by consumers to get a general idea of consumer's perception and acceptability (Figure 17). The control sorghum wheat bread (CSWB) was significantly less attractive in taste and texture and was less liked overall compared with 100% wheat bread. Bread prepared with control sorghum sourdough fermented by *W. confusa* A16 received similar liking ratings for all attributes compared to the CSWB. In contrast, bread prepared with dextran-enriched sorghum sourdough fermented by *W. confusa* A16 was highly appreciated with respect to all attributes evaluated, reaching scores comparable to wheat control bread ($P < 0.05$). Furthermore, the consumer's buying intention was significantly different among the bread samples. The CSWB and bread with control sorghum sourdough received the lowest scores for purchase intent, whereas the bread prepared with dextran-enriched sorghum sourdough was ranked the highest.

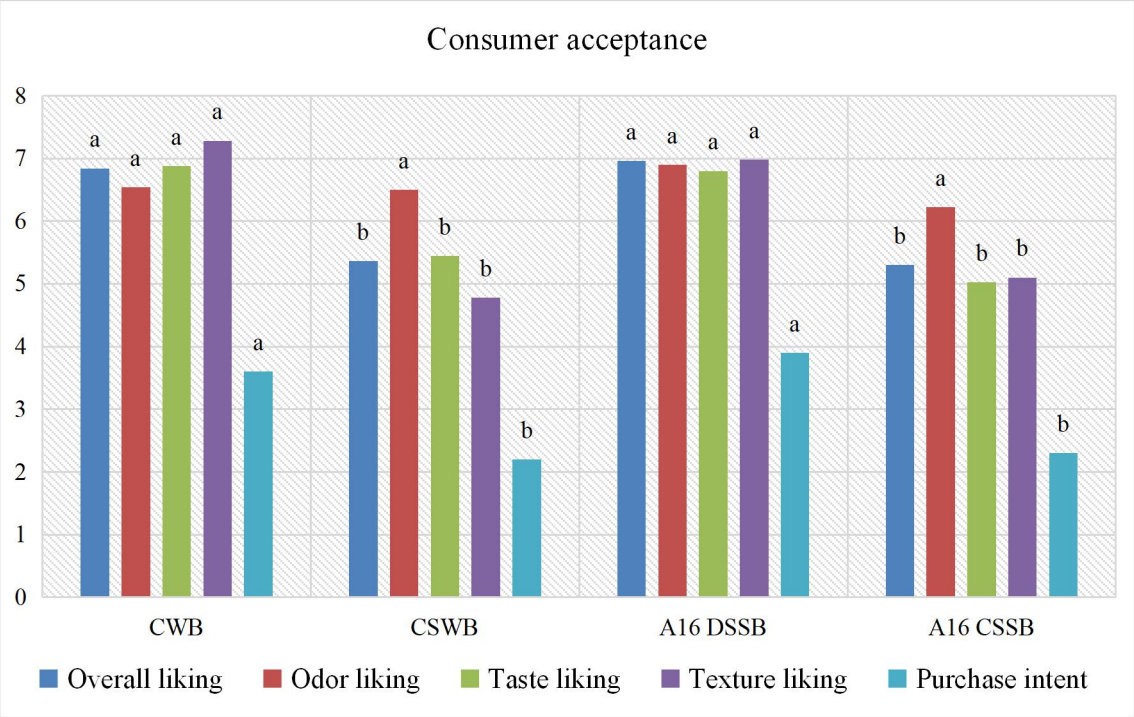

Figure 17. Overall liking attribute means from consumer acceptance testing of wheat and sorghum-wheat breads. Data represent 50 consumers. Liking attributes were scored on a 9-point hedonic scale, where dislike extremely = 1 and like extremely = 9. Purchase intent was scored on a 5-point scale where (definitely) would not buy = 1 or 2, may or may not buy = 3, and (definitely) would buy = 4 or 5. A16 = *W. confusa* A16, CWB = control wheat bread, CSWB = control sorghum wheat bread, DSSB = dextran-enriched sorghum sourdough bread, CSSB = control sorghum sourdough bread. Different letters in the same colour bars indicate statistical significance ($p<0.05$).

5.13 Dextran concentration and flavour intensity perception

Relationship between dextran concentration and bread texture

The impact of dextran on textural properties of bread was evidenced by a positive dose-response effect (Table 11 and 12). In other words, increasing the concentration of dextran in bread formulation led to a progressive improvement of loaf specific volume, cohesive texture, and crumb softness. According to the statistical relevance of the textural parameters measured, the six model breads containing sour tastants could be divided into two distinct segments: segment 1 including three sour breads with dextran application dosage at 0.12, 0.26, and 0.38% (bread weight); and segment 2 encompassing two sour breads with dextran addition amount of 0.57 and 0.96% (Table 11). These two segments were significantly different from each other and from the sour bread without dextran addition. It is worth noting that incorporating dextran at a dosage level of 0.57 and 0.96% resulted in a substantial magnitude of modifications in bread textural parameters, which showed ~29% increment in specific volume, ~8% rise in cohesiveness, and ~53% reduction in crumb hardness compared to the bread without dextran supplementation.

The model breads containing bitter tastants exhibited a similar trend as observed in sour breads even though a narrower set of difference among samples was detected (Table 12). Inclusion of dextran at concentrations of 0.57 and 0.96% significantly enhanced bread textural quality, showing a ~13% increase in specific volume and ~39% decrease in crumb hardness than bitter bread without dextran addition. With lower concentrations of dextran added (0.26-0.38%), bread texture was also improved but to a significantly lower level compared to higher dextran dosage. Addition of dextran at 0.12% level did not change significantly any measured texture parameters.

Table 11. Results of sensory evaluation for sour taste of model breads with dextran addition at varying concentrations (% bread weight) and the bread texture parameters.

Dextran	Sour taste intensity[1]	Specific volume (mL/g)	Hardness (g)	Cohesiveness
0	97.2 ± 5.0^{b}	3.4 ± 0.0^{d}	202.3 ± 18.0^{e}	0.75 ± 0.03^{c}
0.12	101.7 ± 4.0^{b}	3.8 ± 0.1^{c}	156.3 ± 9.8^{d}	0.76 ± 0.01^{bc}
0.26	94.5 ± 3.2^{b}	3.7 ± 0.0^{c}	135.6 ± 13.5^{cd}	0.79 ± 0.01^{ab}
0.38	93.4 ± 3.5^{b}	4.1 ± 0.0^{b}	122.3 ± 14.7^{bc}	0.78 ± 0.02^{bc}
0.57	74.9 ± 4.3^{a}	4.3 ± 0.1^{a}	107.2 ± 15.5^{a}	0.81 ± 0.01^{a}
0.96	72.2 ± 4.9^{a}	4.4 ± 0.0^{a}	94.5 ± 15.0^{a}	0.80 ± 0.01^{a}

[1] Sour taste of chemically acidified wheat bread prepared by addition of lactic and acetic acid (0.6 and 0.08% flour weight, respectively). The magnitude estimation scale was anchored with the 'reference modulus', the acidified bread with 0.26% dextran on bread weight which was pre-assigned a fixed score of 100. Values are a mean ± standard deviation.
Different letters in the same column indicate statistical significance ($p<0.05$).

Table 12. Results of sensory evaluation for bitter taste of breads with dextran addition at varying concentrations (% bread weight) and the bread texture parameters.

Dextran	Bitter taste intensity[1]	Specific volume (mL/g)	Hardness (g)	Cohesiveness
0	98.7 ± 5.1^{b}	3.9 ± 0.1^{d}	148.0 ± 14.2^{c}	0.77 ± 0.01^{bc}
0.12	90.1 ± 4.7^{b}	3.9 ± 0.1^{cd}	145.5 ± 15.1^{c}	0.77 ± 0.01^{c}
0.26	90.0 ± 4.3^{b}	4.2 ± 0.1^{bc}	121.9 ± 13.5^{b}	0.78 ± 0.02^{abc}
0.38	91.8 ± 4.1^{b}	4.1 ± 0.0^{bcd}	122.6 ± 13.5^{b}	0.78 ± 0.01^{abc}
0.57	71.2 ± 4.5^{a}	4.4 ± 0.1^{a}	99.6 ± 11.5^{a}	0.80 ± 0.04^{a}
0.96	69.2 ± 4.5^{a}	4.4 ± 0.0^{a}	90.7 ± 11.8^{a}	0.80 ± 0.02^{a}

[1] Bitter taste of wheat bread prepared from addition of caffeine at 0.2% (flour weight). The 'reference modulus' used to anchor the magnitude estimation scale was the caffeine bread containing 0.26% dextran on bread weight which was pre-assigned a fixed score of 100.
Values are a mean ± standard deviation.
Different letters in the same column indicate statistical significance ($p<0.05$).

The effect of dextran concentration on perception of taste intensity

To investigate the relationship between dextran and the suppressed perception of flavour intensity (sour and bitter notes) in the bread containing dextran-enriched sorghum sourdough fermented with *W. confusa* A16 in section 5.11, a sensory scaling technique (magnitude estimation) was employed to follow the perceptual taste intensity changes as a function of dextran concentration. The magnitude of sourness and bitterness taste perception of the model breads is illustrated in Table 11 and 12, respectively. For the sour model breads, the perception of sourness intensity was generally not affected when dextran was added at concentrations ranging from 0.12 to 0.38% (bread weight), compared to the sour bread without dextran. On the contrary, when the dextran utilization dosage reached 0.57%, there was a sharp decline of sourness perception intensity by 22% in comparison to the bread without dextran ($p < 0.05$). Increasing the dextran concentration to 0.96%, the perceived sourness intensity was further reduced by ~3% but not significantly different from the sour bread with 0.57% of dextran. Similarly, in the bitter model breads, an apparent decrease of bitterness perception was observed when dextran was used at higher concentrations of 0.57 and 0.96%, showing 28 and 30% less bitter taste than bread without dextran, respectively. However, bitter breads containing lower dextran concentrations (0.12 to 0.38%) exhibited constant ratings for bitterness intensity, which was not significantly different from the bread without dextran.

6 DISCUSSION

6.1 Dextran as a texture enhancing agent in high-protein wheat bread

The utilization of faba bean (protein-rich ingredient) in baked goods is limited by the poor textural/sensory properties compared to the most commonly consumed refined wheat products. Sourdough fermentation of faba bean flour with the simultaneous formation of dextran is a potential strategy to compensate for the quality loss. This study compared the effect of dextran produced by *W. confusa* E3403 and *L. pseudomesenteroides* DSM 20193 with different fermentation profile on the rheological and textural properties of wheat dough and bread containing 30% faba bean flour.

Metabolite formation

The LAB were found to be the predominant group of microorganisms after 24 h of fermentation, indicating a limited presence of other spontaneous groups, in agreement with previous studies (Coda et al. 2017a). Based on the sugar analysis, the added and flour endogenous sucrose was completely consumed by LAB. The released glucose was completely consumed in dextran-enriched faba bean sourdough by the bacteria during fermentation mainly for dextran synthesis and as a growth substrate. The released fructose was nearly unused in faba bean sourdoughs fermented with *W. confusa* E3403, while in faba bean sourdoughs fermented with *L. pseudomesenteroides* DSM 20193 most of the fructose was consumed. This might be attributable to the mannitol dehydrogenase activity of *L. pseudomesenteroides* using free fructose as an electron acceptor, resulting in formation of mannitol and acetate in a malor ratio 2:1 (Erten 1998; Wisselink et al. 2002). Mannitol production by *L. pseudomesenteroides* DSM 20193 in faba bean sourdoughs was shown in our previous study (Xu et al. 2017).

The different patterns of sugar metabolism led to the different amount of organic acids as can be seen from the higher values of acetic acid and TTA in dextran-enriched faba bean sourdough fermented by *L. pseudomesenteroides* DSM 20193 compared to the one fermented with *W. confusa* E3403. The accumulation of acetic acid in faba bean matrix fermented with strains of *L. pseudomesenteroides* has been reported in previous studies (Xu et al. 2017). Furthermore, the fructose conversion to mannitol enhanced the growth of *L. pseudomesenteroides*, which might explain its higher cell density and acidity at the end of fermentation (Wisselink et al. 2002). The amount of acids in sourdoughs is crucial in the taste and flavour of the resulting bread. The fermentation quotient (FQ), which is defined as the lactic/acetic acid molar ratio, is closely related to the aroma and texture of the product (Hammes and Gänzle 1998). Acetic acid is beneficial regarding its preservative effect (i.e. antimicrobial and antifungal properties) and sensory contribution (Corsetti 2013). Nevertheless, high concentrations of acetic acid may cause

strong acidic (or vinegar like) and pungent flavour in the final bread and are detrimental to the crumb texture (Salovaara and Valjakka 1987; Valjakka et al. 2003).

The *in situ* synthesis method used in this thesis resulted in a substantial amount of dextran (3.6–5.2% dry weight) in the sourdough, corresponding to 0.61-1% (bread weight) in the subsequent bread. Commercial hydrocolloids such as xanthan gum and HPMC were used as food additives in bread making to enhance textural quality at concentrations between 0.1−1% (Ferrero 2017). *W. confusa* E3403 outperformed *L. pseudomesenteroides* DSM 20193 in dextran production, which is in agreement with previous studies (Xu et al. 2019). Additionally, former studies employing dextran-forming *W. confusa* E392 in wheat sourdough was found to produce 1.8% dry weight under similar fermentation conditions (Katina et al. 2009). The difference in dextran yield could be ascribed to the flour matrix, which affected the activity of dextransucrase (Kaditzky and Vogel 2008). In fact, different flours might possess different buffering capacity due to their different content of fibers, proteins, and minerals. High buffering capacity could provide more favourable pH conditions for dextransucrase activity and thus facilitated dextran production (Kaditzky and Vogel 2008). Moreover, in wheat flour, the endogenous maltose acted as an acceptor molecule for dextransucrase leading to isomaltooligosaccharide synthesis, which lowered dextran yield (Koepsell et al. 1953; Galle et al. 2010). Whereas in faba bean fermentation, the added and naturally occurring sucrose was primarily used for polymeric dextran formation, with only minor amount of resistant oligosaccharides produced.

Viscosity as an indicator of dextran production

The synthesized dextran acted as a thickening agent in the fermented faba bean matrix leading to a more viscous sourdough compared to its control counterpart. The viscosity increment seemed to be positively related to the dextran concentration. However, the control faba bean sourdough fermented by *W. confusa* E3403 had low amount (0.9% dry weight) of dextran but exhibited similar viscosity to dextran-enriched faba bean sourdough fermented with *L. pseudomesenteroides* DSM 20193 after 24 h of fermentation, indicating that dextran synthesized by *W. confusa* E3403 was superior in viscosity enhancement than *L. pseudomesenteroides* DSM 20193. Previously, Xu et al. (2017) compared the thickening property of dextrans produced by six starters belonging to *Weissella* spp. and *Leuconostoc* spp. in faba bean sourdough showing that the extent of thickening depended not only on the content but on other properties of dextran. The common factors that affect the viscosity enhancing capacity of dextrans are molar mass and degree of branching (Lacaze et al. 2007; Rühmkorf et al. 2012).

Relationship between dough rheology and bread texture

The farinograph water absorption, the fundamental rheological measurements (dynamic oscillatory test) and the empirical measurements under a large deformation (Kieffer extensibility test) were performed to exploit the impact of dextran and sourdough acidification on the rheological properties such as viscoelasticity of the bread dough. Kieffer parameters (the maximum resistance to extension (Rmax), extensibility (Ext),

and dough strength value (Atot)) are important in evaluating dough extensibility and strength (Smewing 1995). Adequate extensibility is a prerequisite for proper dough handling and performance in the baking process. In the oscillatory test, the storage modulus (G') represents the elastic component of a material while the loss modulus (G") is regarded as the viscous part. The ratio between G" and G' gives the tangent of the phase angle (δ). The greater the phase angle, the more viscous is the material.

Faba bean flour incorporation reduced the dough elasticity (decreased G' and increased δ), R_{max}, and A_{tot}, indicating a flowy and frail dough lacking stability. It also led to decreased water absorption capacity at standard dough consistency. As discussed in the literature review section 2.1.2, wheat flour substitution led to a weakened (or "diluted") gluten network due to the different technological functionalities of faba bean proteins. The presence of a considerable amount of insoluble fiber in faba bean might disrupt the gluten network formation. Thus, the control faba bean wheat bread dough was less elastic and prone to rupture during stretching. The utilization of control or dextran-enriched faba bean sourdough significantly increased the viscous property (increased δ) and farinograph water absorption compared to the composite control without sourdough. In particular, dough containing dextran-enriched faba bean sourdough showed a higher increasing effect. The impact of control or dextran-enriched faba bean sourdough on Kieffer parameters depended on the strains employed. The control or dextran-enriched faba bean sourdough fermented by *L. pseudomesenteroides* DSM 20193 did not show any impact on R_{max} and A_{tot} whereas the control or dextran-enriched faba bean sourdough fermented by *W. confusa* E3403 increased both of them. This difference might be due to intensive acidification in sourdoughs fermented with *L. pseudomesenteroides*, which might counteract the positive effects of dextran on bread dough extension properties (Kaditzky et al. 2008). The use of chemically acidified dough also showed improvements in water absorption and Kieffer parameters but to a significantly lesser degree than control faba bean sourdough fermented by *W. confusa* E3403, suggesting that chemical acidification was not directly comparable with biological/microbial acidification.

Taken together, the rheological parameters most closely relevant to bread technological properties such as specific volume and crumb hardness are the Kieffer parameters (i.e. R_{max} and A_{tot}). According to literature data, R_{max} (Dobraszczyk and Salmanowicz 2008; Kieffer et al. 1998) and A_{tot} (Nash et al. 2006) were positively correlated with loaf specific volume which gave reproducible results and good discrimination of baking performance between different flours. The Kieffer test provides deformation settings/conditions closely resembling real deformation occurring during dough proofing and oven spring (large extensional deformation and slow strain rates) (Dobraszczyk and Morgenstern 2003; Dobraszczyk and Salmanowicz 2008).

The influence of dextran containing sourdough on the bread dough rheological properties was in fact a synergetic effect of acidification and dextran on development of the gluten network. As reviewed in section 2.2.4.2, the acidification and pH drop foster net positive charge of gluten proteins, which increases their electrostatic repulsion leading to force-induced unfolding and greater exposing of the hydrophobic areas. This

in turn forces disentanglement of the gluten network and thereby results in a softer gluten network and more viscous dough (Arendt et al. 2007). Apart from the direct influence on gluten proteins, microbial fermentation induces the actions of flour endogenous or bacterial enzymes such as proteases, which causes depolymerization and weakening of the gluten network (Clarke et al. 2004). The reduce of dough elasticity with the presence of dextran or other hydrocolloids has been investigated in former studies (Galle et al. 2012a, 2012b; Rosell et al. 2001; Wolter et al. 2014).

Food hydrocolloids are known to improve farinograph water absorption due to their hydrophilic nature and water binding capacity (Guarda et al. 2004). The increased farinograph water absorption might also be partially attributed to the increased solubility and water uptake of gluten proteins and cereal fiber under acidic environment (Arendt et al. 2007). The increased R_{max} and A_{tot} indicated a strengthened dough which required higher force and more energy for deformation when elongated. The possible interactions between dextrans and proteins, such as hydrogen bonding and steric interactions, may provide additional support to the gluten network and thus reinforce the dough structure (Ross et al. 1992). This further leads to increased stability and tolerance of the dough matrix or cell walls surrounding the expanding gas cells during yeast fermentation and baking, resulting in declined foam collapse, gas diffusion and losses and consequently a higher bread specific volume (Zannini et al. 2014).

Faba bean flour inclusion showed detrimental effects on bread textural attributes, such as smaller loaf volume and harder crumb compared to 100% wheat, leading to inferior quality of the faba bean containing bread. The utilization of control or dextran-enriched faba bean sourdough fermented by *W. confusa* E3403 compensated for the adverse effect of acidification and gluten network "dilution", increasing the bread textural quality to levels comparable to that of wheat control bread. The addition of dextran-enriched faba bean sourdough fermented by *L. pseudomesenteroides* DSM 20193 did not achieve the same beneficial effects on bread textural properties since excessive acidification negatively affect bread volume, crumb structure, and firming kinetics (Kaditzky et al. 2008). Nevertheless, the macromolecular properties of the synthesized dextran also need to be taken into account and should be further investigated to allow better understanding of the functionaility.

6.2 Nutritional and textural quality of wholegrain millet bread with dextran produced *in situ*

Utilization of wholegrain millet in baked goods will enhance the nutrition and health benefits and also promote the use of indigenous grains on an industrial scale, thus contributing to food security. Tailored bioprocessing of millet flour was used to enhance the textural and nutritional properties of millet bread containing 50% wheat flour. Based on the findings of Study **I**, *W. confusa* A16 giving mild acidification and producing sufficient dextran *in situ*, was selected to improve the textural properties of millet bread. To understand the mechanisms of the techno-functional performance in the bread matrix,

the chemical structure (branch linkage, molar mass) of the synthesized dextran was elucidated.

Yield, structure and macromolecular properties of W. confusa A16 dextran

The sucrose (11.5%) was completely consumed by the strain, leading to a substantial amount (3.5% dry weight) of dextran. Fermentation with *W. confusa* A16 resulted in mild acidification of the final bread (pH 4.8, TTA 5 mL). The dextran formed by *W. confusa* A16 exhibited higher intrinsic viscosity, lower critical overlap concentration c*, and linear structure with fewer branches (3%) compared with dextrans produced by *W. cibaria* Sj1b (4.1% branches) and *L. pseudomesenteroides* DSM 20193 (5.8% branches) (Xu et al. 2018). The viscosity average molecular weight (*Mv* = 2000 kDa) of the *W. confusa* A16 dextran as determined from the viscosity flow curves was lower than the weight average molecular weight (*Mw* = 3000 kDa) obtained with HPSEC. With a high molar mass polymer, a notable difference between *Mw* and *Mv* is often observed due to the different technique and solvent used for study. According to theory, the order of the average values should be *Mw* > *Mv* (Stepto 2010). In this thesis, the HPSEC analysis was performed with DMSO-based solvent whereas intrinsic viscosity was determined with water-based solution. Evidence of different dissolution and aggregation capacities of high molecular weight dextrans has been detected in aqueous and DMSO-based eluents, particularly the freeze-dried dextrans (Maina et al. 2014).

Dough rheology and bread texture

Dough stickiness is a combination of surface adhesion and rheological properties, which is important in the baking industry since excessive stickiness causes significant economic loss and problems in dough handling (Dobraszczyk 1997). Millet flour inclusion increased the dough stickiness, which was most likely related to the increased adhesion (force caused by interactions between the dough surface and contact material due to unbounded water) and decreased cohesion (force generated by interactions within the dough) (Ahmed and Thomas 2018). The utilization of dextran-enriched millet sourdough dramatically reduced the dough stickiness compared to the control millet wheat bread dough, which correlated well with the subjective evaluation during the baking process. Furthermore, the presence of dextran increased significantly the farinograph water absorption and dough strength (Kieffer R_{max} and A_{tot}), which is in line with the observations in Study **I**.

The positive effect of dextrans on dough rheology has been linked to the polymer concentration, chemical structure and molecular conformation, which determine their intermolecular interactions with dough structural components such as protein and starch (Rühmkorf et al. 2012). Linear dextrans with high molar mass (≥ 1000 kDa) are considered to be the most effective in improving dough rheology and bread baking quality (Rühmkorf et al. 2012; Zhang et al. 2018). Such dextrans are capable of binding a high amount of water (Ross et al. 1992), leading to decreased dough stickiness. The dextran produced by *W. confusa* A16 is therefore ideal for achieving desirable dough properties and bread making quality.

Millet incorporation diminished the bread quality, resulting in smaller loaf specific volume, harder crumb, less springiness and cohesiveness, and lower moisture content compared to 100% wheat, leading to inferior quality of the millet containing bread. The study demonstrated that control sourdough fermentation had no influence on loaf volume and even significantly increased the crumb firmness of fresh and stored millet enriched bread. On the contrary, the dextran production improved substantially all the measured textural parameters of the millet enriched bread, leading to a larger volume, softer crumb, and higher crumb moisture, springiness and cohesiveness.

Dextran as an anti-staling agent

Inclusion of millet flour or control millet sourdough accelerated the staling rate of bread crumb. In contrast, the rate of crumb hardening was significantly retarded with the addition of dextran-enriched millet sourdough, to levels identical to wheat bread. This is consistent with the higher moisture retention observed for these formulations during storage. Bread staling is a rapid irreversible and complex phenomenon encompassing multiple components and mechanisms through which amylopectin recrystallization (or starch retrogradation) and water redistribution take place simultaneously (Fadda et al. 2014; Gray and Bemiller 2003). The degree of amylopectin crystallinity during bread aging was monitored as melting enthalpy changes during heating by DSC curves. The control millet bread and control millet sourdough bread showed higher transition temperatures (T_c, T_p) and melting enthalpy (ΔH), indicating more crystallites formed in these bread samples during the storage period. The bread prepared with dextran-enriched millet sourdough, however, displayed significant lower enthalpy values and therefore deferred amylopectin recrystallization, corresponding to its slower firming rate. Similar results have been described in previous studies when adding purified dextrans with high molecular weight (1500-2800 kDa) to wheat bread (Zhang et al. 2018).

The mechanisms of the delaying effect of dextran on bread staling at the molecular level are still not fully understood. Dextrans may affect the retrogradation kinetics by (1) the high water binding capacity that decreases the moisture mobility, (2) inhibiting the starch crystallinity by interactions with gelatinized starches, and (3) decreasing the starch-protein interactions which has been reported to accelerate the staling of bread (Biliaderis et al. 1997; Fadda et al. 2014; Zhang et al. 2018). Starch retrogradation has shown to be water-dependent, meaning that a higher moisture content was correlated with less starch-starch interactions (Amigo et al. 2019). Dextran presence restricted water mobilization during bread storage, which resulted in less water incorporated into the amorphous starch and thus less degree of B-type crystallites formation (Zhang et al. 2019).

The fate of phenolic compounds and antioxidant activity

The impact of bioprocessing on the soluble and bound phenolic compounds and the *in vitro* antioxidant activity was followed to observe the changes of product health-promoting properties. The wholegrain millet flour had a high content of phenolic compounds but most of them (~61%) occurred in insoluble bound form (i.e. being

esterified or covalently bound to the cell wall structural components) with limited bioavailability (Călinoiu and Vodnar 2018). Sourdough fermentation was able to enhance the release of bound phenols and consequently increased the content of soluble phenols of millet, which is consistent with previous results (Shahidi and Chandrasekara 2013). Furthermore, the increased soluble fraction in millet sourdough extracts (dextran-enriched millet sourdough and control millet sourdough) was positively correlated to the enhanced DPPH radical-scavenging activity, which is in agreement with literature data (Shumoy et al. 2017). The above modifications might be attributed to the fermentation-induced structural breakdown of grain cell walls under the effect of acidification and action of hydrolytic enzymes (such as glycoside hydrolases, cellulases, and esterases), resulting in the liberation of bound phenols (Shumoy et al. 2017). The conversion of bound phenols to soluble form may improve the health-related functionality of the final products, since the free soluble phenols are accessible and rapidly absorbed in the small intestine, exerting their health beneficial effects (Călinoiu and Vodnar 2018).

Impact of bioprocessing on bread nutrition quality

The level of phytic acid in the millet enriched bread was not affected by sourdough fermentation, indicating a limited phytase activity. Future developments to reduce the level of phytic acid such as the addition of a phytase or a starter culture possessing phytase activity should be considered to improve the bio-accessibility of nutrients like minerals. The *in vitro* starch and protein digestibility were significantly reduced by the incorporation of millet flour. Millet-based food products are well recognized to have slower starch and protein digestibility (Annor et al. 2017). The factors contributing to this include the high content of dietary fiber and fatty acids in wholegrain millet flour and the presence of antinutritional factors/phenolic compounds (e.g. tannins) (Annor et al. 2017). Fatty acids may form complexes with starch and alter the conformation of starch polymer chains, resulting in slower digestion by starch hydrolytic enzymes (Annor et al. 2015). Fibers increase the viscosity of the digestion mixture and thus reduce the starch hydrolysis rate. Phenolic compounds from millet extracts inhibit the digestion by interacting with the α-glucosidase, pancreatic α-amylase or some protein degrading enzymes (Shobana et al. 2009). Tannins and phytic acid can bind proteins making them less available for enzymatic digestion (Ramachandra et al. 1977).

Inclusion of millet sourdoughs improved significantly the protein digestibility and reduced the starch digestibility (or predicted GI) compared to the control millet wheat bread, leading to improved nutritional benefits of the final product. Low GI cereal foods have been linked to improved glucose tolerance and reduced risks of heart disease and type II diabetes (Bjorck and Elmstahl 2003). The decrease of starch digestibility was likely due to the formation of acids and resistant starch (Wolter et al. 2014). Organic acids and especially acetic acid have been shown to decrease the postprandial metabolic (glucose and insulin) responses *in vivo* due to reduced gastric emptying rate (Bjorck and Elmstahl 2003). Lactic acid decreased the rate of starch hydrolysis in a dose-dependent manner, which created interactions between starch and gluten proteins and reduced the susceptibility of starch (Bjorck and Elmstahl 2003; Ostman et al. 2003). The improved protein digestibility might be linked to the actions of proteolytic digestive enzymes (e.g.

protease), which enhanced protein solubility and degradation resulting in more digestible peptides and amino acids (Annor et al. 2017; El Hag et al. 2002). Furthermore, the degradation of certain polyphenols such as tannins facilitated the liberation of bound proteins and thus increased their bioavailability and digestibility (El Hag et al. 2002). The presence of dextran did not seem to affect the starch and protein digestibility, indicating that dextran had limited effect on *in vitro* digestive enzyme activity. As a consequence, the dextran-enriched millet sourdough bread and its control millet sourdough bread counterpart showed comparable *in vitro* starch and protein digestibility.

6.3 Dextran as a flavour-masking agent in wholegrain sorghum bread

Similar to the findings in Studies **I** and **II**, the *in situ* produced dextran by *W. confusa* A16 in Study **III** demonstrated great potential in improving the textural quality and volume of wholegrain sorghum-wheat bread. However, Study **III** focused on the impact of dextran on sensory properties of the sorghum containing bread. Aside from the texture deficiencies, sorghum enriched bread is also challenged by the low consumer acceptance of product flavour (e.g. bitter taste). This study provides information for the first time on the relevance of dextran in flavour intensity perception in baked products.

Non-volatile flavour compounds (sugars, acids, and polyphenols)

The glucose released from the added and flour endogenous sucrose (11.3%) was partially consumed (2.5%) by the bacteria during sourdough fermentation for dextran synthesis (2% dry weight) and metabolism. The sucrose addition resulted in 2.7% (dry weight) of remnant sugars (glucose and fructose) accumulation in the dextran-enriched sorghum sourdough bread, which was higher than the control sorghum sourdough bread (1.1%) and control sorghum wheat bread (0.7%). Sucrose addition, however, did not affect organic acid production and the acidity level of bread.

The polyphenolic composition of sorghum extracts suggested the presence of a great number of polyphenols, such as free phenolic acids, phenolic acid esters, flavonoids (flavan-3-ol derivatives, flavone, and flavanone derivatives), phenylpropane glycerides, phenolamides, and a few hydroxy fatty acids, which is in line with literature (Kang et al. 2016). Similar to the millet matrix, the total polyphenol content of sorghum extracts remained unchanged after fermentation. However, there was a shift in concentrations of individual polyphenols towards the increasing content of those compounds with smaller molecular weight. For example, caffeic acid was significantly higher (4-fold) in sorghum sourdough extracts than the non-fermented control. The acid hydrolysis and enzyme action (e.g. cereal endogenous and/or bacterial esterases) might account for this modification, which degraded phenolic acid esters (e.g. 2-O-caffeoylglycerol isomer) to simple phenolic acids (Svensson et al. 2010). The health-promoting characteristics (e.g. antioxidant, antimicrobial, anti-diabetic, anti-obesity, and anti-cancer) might be enhanced by altering the polyphenolic compositions (Salazar-López et al. 2018). Additionally, the abundant polyphenols in sorghum might prevent dextransucrase activity (Goyal et al. 2013). Certain polyphenolic compounds such as caffeic acid have

shown antimicrobial activities, which could lead to slow bacterial adaptation and inhibition of specific activities (Lima et al. 2016; Sekwati-Monang et al. 2012).

Flavour and texture perception of the sorghum bread

As revealed in Study **III**, the sensory perception of bread texture attributes was highly correlated with the instrumentally measured parameters according to the Pearson's Correlation analysis, which also demonstrated a reasonable accuracy of prediction in respect to consumer responses. The instrumental crumb hardness was highly correlated with perceived resistance to pressure and resilience in mouth. The instrumental cohesiveness well predicted the sensory attribute elasticity. The moisture content was a good predictor of the moist mouthfeel. The moisture content and crumb softness were closely associated with the assessed dough-like texture.

The control sourdough fermentation increased significantly the off-notes of the sorghum bread. In particular, the high dosage (59% dough weight) of sourdough addition in this study resulted in intense sour flavour, bitter taste, and aftertaste. The increase of undesirable flavours after sourdough fermentation was likely due to: (1) the release of small size polyphenols exhibiting strong bitter taste. Bitterness of the polyphenolic compounds is cognate with their structure (Robichaud and Noble 1990). In general, larger size molecules such as polymeric fractions, dimers, and trimers taste less bitter compared to monomers, like gallic acid and caffeic acid; (2) the liberation of low molecular weight bitter peptides and amino acids under proteolytic and peptidolytic activities of endogenous and/or bacteria proteases in acidic pH; and (3) intensive acidification (Fallico et al. 2005; Zhao et al. 2016). In contrast, the use of dextran-enriched (0.56% bread weight) sorghum sourdough demonstrated a clear suppressing effect on those off-flavours compared to the control sorghum sourdough, leading to a hypothesis of flavour masking ability of dextran. It should be noted that the dextran-enriched sorghum sourdough bread and its control sourdough bread counterpart exhibited identical acid content and titratable acidity. The dextran-enriched sorghum sourdough and control sorghum sourdough also showed comparable polyphenol concentrations. Thus, theoretically, the two sourdough breads would be rated similarly regarding sourness and bitterness intensity. The dextran-enriched sorghum sourdough bread was perceived as more sweet and roasted which can be related to its higher sugar content. The increased sweet taste may suppress the perceived bitterness and sourness. Nevertheless, this does not appear to be the main contributor for flavour reduction concerning the limited amount of total free sugars (1.7% bread weight) in the final bread in comparison with the amount of sweeteners employed as flour-masking agents in industrial food production, e.g. over 10% sugars was used to mask bitter taste in bran-enriched cereal products (Heiniö et al. 2015).

Flavour intensity perception in bread with dextran above and below c*

The flavour masking ability of dextran in bread matrix was further confirmed by adding purified dextran to model bread formulations containing taste compounds, i.e. lactic/acetic acid and caffeine. The same trend was observed in sourness and bitterness

perception; dextran addition at concentrations of 0.57 and 0.96% (bread weight) induced a significant decrease in perceived taste intensity while at lower concentrations (0.12−0.38%) the flavour perception was not affected. This is in line with the observations in sorghum containing breads, the dextran-enriched (0.56%) sorghum sourdough bread showed markedly less sourness and bitterness than control sorghum sourdough bread containing 0.11% of dextran. The texture of bread seemed to affect the flavour perception and that an increase of dextran concentration led to an increase of crumb cohesiveness and soft texture coinciding with a decrease of taste perception. Additionally, the masking effect appeared to occur at concentrations above its critical coil overlap concentration (c* = 0.43%). Similar observations were reported in thickened solutions showing that flavour perception was modified only when the hydrocolloid concentrations reached the point of c* (Baines and Morris 1987; Cook et al. 2002). At or above c*, a sharp increase in solution viscosity and a substantial decrease in aroma and taste perception was detected.

As indicated in the review section 2.3.3, various physicochemical mechanisms have been proposed for the flavour-masking phenomenon in food systems with hydrocolloids. The flavour perception of food is a dynamic phenomenon consisting of two critical stages, the structure breakdown during chewing, which fosters liberation of aroma and taste molecules, and the subsequent transportation to taste receptor cells on taste buds in the oral cavity or gustatory receptor cells in the nasal cavity (Thomson 1984). The addition of hydrocolloids in food matrix modifies the structure (or the perceived/oral texture in the mouth), which may affect the diffusion coefficient of the flavour molecules. For thickened solutions, polymer chains start to overlap and interpenetrate at the critical point, leading to an inefficient mixing between the solution and the saliva in the mouth, thus suppressed the diffusion of flavour compounds to respective receptor cells (Cook et al. 2002). In addition, at a molecular level, the possible interactions (chemical or physical binding) between hydrocolloids and flavour molecules or taste buds may affect flavour perception (Elisabeth Guichard 2007; Voilley and Souchon 2006).

In this study, the texture properties of the bread were significantly changed when dextran was used above the critical concentration, which might have induced a different breakage function (degree of food particle size reduction) during chewing and thereby different flavour release kinetics (Lucas et al. 2002). The mechanical breakage of solid food upon chewing increases surface area for flavour release and allows the formation of a properly sized bolus (a ball-like food-saliva mixture). Bread containing high contents of dextran exhibited a more cohesive and soft texture. Once chewed and mixed with saliva in the mouth, the bread bolus with high apparent viscosity was formed, which might retain the flavour molecules within the structure and reduce their migration to the receptor cells (Figure 18). On the contrary, bread with dextran present at lower concentrations (< c*) showed a more brittle or crumbly texture generating intenser breakage during chewing and thus greater exposed surface area and more flavour stimuli released into the surrounding saliva or vapour phase to be sensed.

Another possible hypothesis is that dextran might interact with flavour molecules or taste receptor cells, for instance by hydrogen bonding or forming complex (molecular inclusion), and thereby reduced their availability for sensation (Braudo et al. 2000; Yven et al. 1998). Nevertheless, the molecular bindings were not considered to be the dominating mechanism in this study. The chemical nature of the two taste stimuli (acids and caffeine) are quite distinct from each other and should have exhibited different degrees of perceptual change due to significantly different taste-dextran molecular interactions involved. Furthermore, the addition of dextran at two different concentrations (0.57 and 0.96% bread weight) resulted in comparable perceptual reduction, which was theoretically expected to give different results in a binding process.

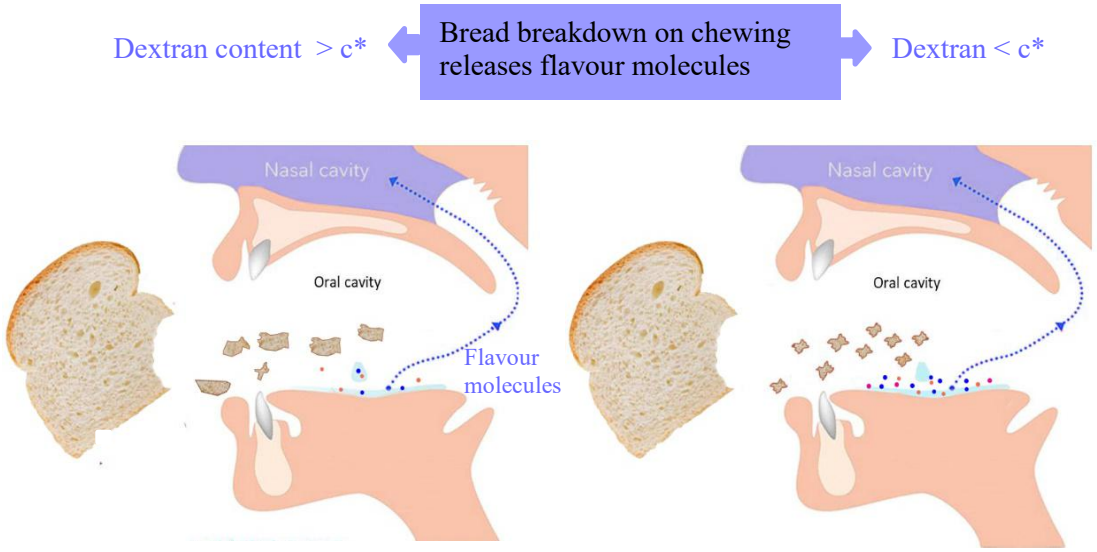

Figure 18. Flavour release and perception in breads containing dextran (% of bread weight) above and below critical overlap concentration c*.

7 CONCLUSION AND FUTURE PROSPECTS

These three studies together, which as a coherent whole constitute the subject of the dissertation, are complementary to understand the functionality of dextran synthesized by lactic acid bacteria fermentation in non-conventional grains (faba bean, wholegrain millet and sorghum) and to exploit it to its fullest in the bread matrix. The tailored bioprocessing method developed in this work showed great potential in improving the nutritional, textural, and sensory properties of composite bread containing 50-70% of wheat flour.

In faba bean matrix, the two strains employed, *W. confusa* E3403 and *L. pseudomesenteroides* DSM 20193, are both promising dextran producers and promote thickening. Sourdough containing dextran formed by *L. pseudomesenteroides* DSM 20193 was characterized by high acidity, which decreased bread dough strength and resulted in lower bread specific volume and harder crumb. In contrast, sourdough fermented by *W. confusa* E3403 presented mild acidification, resulting in increased loaf volume and softer crumb. Therefore, the level of acidification in the matrix must be controlled to obtain positive results.

In millet matrix, *W. confusa* A16 was able to synthesize sufficient dextran *in situ* and low amount of acids were formed. The synthesized dextran effectively compensated the quality deficiencies induced by gluten network "dilution" and counteracted the negative effects of acidification, leading to superior bread quality. The techno-functionality of the synthesized dextran was linked to its structure properties (e.g. high molecular weight and linear structure with low branching). The produced dextran also acted as anti-staling agent, which showed high water binding and retention capacity, resulting in retarded starch retrogradation and crumb firming rate. It is worth noting that the sourdough bioprocessing showed potential advances in terms of increased nutritional quality such as *in vitro* starch and protein digestibility and potential health-promoting functionality such as phenolic compounds bioavailability and antioxidant activity.

In sorghum, the dextran produced by *W. confusa* A16 increased the perceived elasticity, moist mouthfeel, cohesiveness, and softness of the bread. The dextran-enriched sorghum sourdough bread was also perceived as less intense in sour flavour, bitter taste, and aftertaste than the control sourdough bread, leading to hypothesize about the flavour masking ability of dextran. Through adding pure dextran isolated from *W. confusa* A16 in model wheat breads containing taste compounds and following the perceptual changes by magnitude estimation tests, the flavour masking effect was confirmed. The flavour suppressing occurred at dextran concentration above c* and was not affected below this critical point. The intensive texture modifications and consequently altered flavour release kinetics, were suggested to be the main mechanism accounting for the flavour masking phenomenon.

In summary, the *in situ* production of dextran is a promising strategy to formulate high-protein or wholegrain alternative baked goods, creating added-value products with good sensory quality and high consumer acceptance. The method enabled the utilization of

faba bean, millet, and sorghum at a high dosage level (30-50% flour basis) in wheat bread formulations. Furthermore, fermentation is a “clean label” approach which circumvents the labelling requirement on the product package and is appreciated by industry and consumers.

For future prospects, the bioprocessing technology here developed might be also applicable to other protein-rich legumes and wholegrain cereals as well as other food matrices. Furthermore, this thesis introduced the possibility, of the synthesis *in situ* dextran as novel means to mask the undesirable flavours of alternative grains baked goods. In addition, increasing trend to utilize side-streams in food production is likely to create new challenges in flavor design of future foods. This thesis contributes to increase the knowledge for the application of tailored polysaccharide structures as a flavour masking solution in the solid food matrix.

REFERENCES

AACC. (1995). Approved Methods of the American Association of Cereal Chemists (AACC), St. Paul, MN, USA. Method 44-15A: Moisture − Air-Oven Methods.

AACC. (2000). Approved Methods of the AACC. Method 54-21: Rheological Behavior of Flour by Farinograph: Constant Flour Weight Procedure.

Adebiyi, J. A., Obadina, A. O., Adebo, O. A., & Kayitesi, E. (2017). Comparison of nutritional quality and sensory acceptability of biscuits obtained from native, fermented, and malted pearl millet (Pennisetum glaucum) flour. *Food Chem. 232*(Supplement C), 210-217.

Ahmed, J., & Thomas, L. (2018). Effect of xanthan and guar gum on the pasting, stickiness and extensional properties of brown wheat flour/β-glucan composite doughs. *LWT-Food Sci Technol. 87*, 443-449.

Ahmed, R., Siddiqui, K., Arman, M., & Ahmed, N. (2012). Characterization of high molecular weight dextran produced by *Weissella cibaria* CMGDEX3. *Carbohydr Polym. 90*, 441–446.

Akeson, W. R., & Stahmann, M. A. (1964). A pepsin pancreatin digest index of protein quality evaluation. *Nutr J. 83*(3), 257-261.

Amatayakul, T., Halmos, A. L., Sherkat, F., & Shah, N. P. (2006). Physical characteristics of yoghurts made using exopolysaccharide-producing starter cultures and varying casein to whey protein ratios. *Int Dairy J. 16*(1), 40-51.

Amigo, J. M., del Olmo, A., Engelsen, M. M., Lundkvist, H., & Engelsen, S. B. (2019). Staling of white wheat bread crumb and effect of maltogenic α-amylases. Part 2: Monitoring the staling process by using near infrared spectroscopy and chemometrics. *Food Chem. 297*, 124946.

Anderson, J. W., Baird, P., Davis, R. H., Jr, Ferreri, S., Knudtson, M., Koraym, A., Waters, V., & Williams, C. L. (2009). Health benefits of dietary fiber. *Nutr Rev. 67*(4), 188-205.

Angioloni, A., & Collar, C. (2012). High legume-wheat matrices: An alternative to promote bread nutritional value meeting dough viscoelastic restrictions. *Eur Food Res Technol*. *234*, 273–284.

Annor, G. A., Marcone, M., Corredig, M., Bertoft, E., & Seetharaman, K. (2015). Effects of the amount and type of fatty acids present in millets on their *in vitro* starch digestibility and expected glycemic index (eGI). *J Cereal Sci. 64*, 76-81.

Annor, G. A., Tyl, C., Marcone, M., Ragaee, S., & Marti, A. (2017). Why do millets have slower starch and protein digestibility than other cereals? *Trends Food Sci Tech. 66*, 73-83.

Aprodu, I., & Banu, I. (2015). Rheological, thermo-mechanical, and baking properties of wheat-millet flour blends. *Food Sci Technol Int. 21*(5), 342-353.

Arancibia, C., Costell, E., & Bayarri, S. (2013). Sweetness and consistency in semisolid dairy matrices. *J Texture Stud*. *44*, 346-356.

Arendt, E. K., Ryan, L. A. M., & Dal Bello, F. (2007). Impact of sourdough on the texture of bread. *Food Microbiol. 24*(2), 165-174.

Arese, P., & De Flora, A. (1990). Pathophysiology of hemolysis in glucose-6-phosphate dehydrogenase deficiency. *Seminars in hematology. 27*(1), 1-40.

Baines, Z. V., & Morris, E. R. (1987). Flavour/taste perception in thickened systems: The effect of guar gum above and below c*. *Food Hydrocoll. 1*(3), 197-205.

Basedow, A. M., & Ebert, K. H. (1979). Production, characterization, and solution properties of dextran fractions of narrow molecular weight distributions. *J Polym Sci. Polymer Symposia. 66*(1), 101-115.

Belder, A. N. de. (2000). Dextran. In *Ullmann's Encyclopedia of Industrial Chemistry*. Wiley-VCH.

Belz, M. C. E., Axel, C., Arendt, E. K., Lynch, K. M., Brosnan, B., Sheehan, E. M., Coffey, A., & Zannini, E. (2019). Improvement of taste and shelf life of yeasted low-salt bread containing functional sourdoughs using *Lactobacillus amylovorus* DSM 19280 and *Weisella cibaria* MG1. *Int J Food Microbiol. 302*, 69-79.

Biliaderis, C. G., Arvanitoyannis, I., Izydorczyk, M. S., & Prokopowich, D. J. (1997). Effect of hydrocolloids on gelatinization and structure formation in concentrated waxy maize and wheat starch gels. *Starch - Stärke. 49*(7-8), 278-283.

Bjorck, I., & Elmstahl, H. L. (2003). The glycaemic index: Importance of dietary fibre and other food properties. *Proc Nutr Soc. 62*(1), 201-206.

Bozonnet, S., Dols-Laffargue, M., Fabre, E., Pizzut, S., Remaud-Simeon, M., Monsan, P., & Willemot, R.-M. (2002). Molecular characterization of DSR-E, an α-1,2 Linkage-synthesizing dextransucrase with two catalytic domains. *J Bacteriol Res*. *184*(20), 5753-5761.

Braudo, E. E., Plashchina, I. G., Kobak, V. V., Golovnya, R. V., Zhuravleva, I. L., & Krikunova, N. I. (2000). Interactions of flavor compounds with pectic substances. *Food Nahrung. 44*(3), 173-177.

Cagno, R. D., Angelis, M. D., Limitone, A., Minervini, F., Carnevali, P., Corsetti, A., Gänzle, M. G., Ciati, R., & Gobbetti, M. (2006). Glucan and fructan production by sourdough *Weissella cibaria* and *Lactobacillus plantarum*. *J Agric Food Chem. 54*(26), 9873-81.

Călinoiu, L. F., & Vodnar, D. C. (2018). Whole grains and phenolic acids: A review on bioactivity, functionality, health benefits and bioavailability. *Nutrients. 10*(11), 1615.

CAT, Technical Centre for Agricultural and Rural Cooperation. (1988). Bread without wheat. Spore 18. CTA, Wageningen, The Netherlands.

Cencic, A., & Chingwaru, W. (2010). The role of functional foods, nutraceuticals, and food supplements in intestinal health. *Nutrients. 2*(6), 611-625.

Chapot-Chartier, M.-P., Monnet, V., & De Vuyst, L. (2011). Cell-wall and exopolysaccharides of lactic acid bacteria. *The 10th LAB symposium - Thirty Years of Research on Lactic Acid Bacteria. ed.* 37-59.

Chen, W. Z., & Hoseney, R. C. (1995). Development of an objective method for dough stickiness. *LWT-Food Sci Technol. 28*(5), 467-473.

Clarke, C. I., Schober, T. J., Dockery, P., O'Sullivan, K., & Arendt, E. K. (2004). Wheat sourdough fermentation: Effects of time and acidification on fundamental rheological properties. *Cereal Chem. 81*(3), 409–417.

Coda, R., Kianjam, M., Pontonio, E., Verni, M., Di Cagno, R., Katina, K., Rizzello, C. G., Gobbetti, M. (2017a). Sourdough-type propagation of faba bean flour: Dynamics of microbial consortia and biochemical implications. *Int J Food Microbiol*. *248*, 10-21.

Coda, R., Melama, L., Rizzello, C. G., Curiel, J. A., Sibakov, J., Holopainen, U., Pulkkinen, M., & Sozer, N. (2015). Effect of air classification and fermentation by *Lactobacillus plantarum* VTT E-133328 on faba bean (Vicia faba L.) flour nutritional properties. *Int J Food Microbiol. 193*(Supplement C), 34-42.

Coda, R., Varis, J., Verni, M., Rizzello, C. G., & Katina, K. (2017b). Improvement of the protein quality of wheat bread through faba bean sourdough addition. *LWT-Food Sci Technol. 82*, 296-302.

Compaoré-Sérémé, D. (2016). Evaluation de la flore microbiologique associée à la technologie du massa: isolement et caractérisation préliminaire des bactéries lactiques. Master thesis, Mémoire de Master II. Université Ouaga 1 Prof Joseph KI-ZERBO, Burkina Faso.

Cook, S. L., Bull, S. P., Methven, L., Parker, J. K., & Khutoryanskiy, V. V. (2017). Mucoadhesion: A food perspective. *Food Hydrocoll. 72*, 281-296.

Cook, D. J., Hollowood, T. A., Linforth, R. S. T., & Taylor, A. J. (2002). Perception of taste intensity in solutions of random-coil polysaccharides above and below c*. *Food Qual Prefer. 13*(7), 473-480.

Corsetti, A. (2013). Technology of sourdough fermentation and sourdough applications. In Gobbetti, M., & Gänzle, M. (Eds.), *Handbook on Sourdough Biotechnology*. 85-103. Boston, MA: Springer US.

Costell, E., Peyrolón, M., & Duran, L. (2000). Note. Influence of texture and type of hydrocolloid on perception of basic tastes in carrageenan and gellan gels. *Food Sci Technol Int. 6*, 495-499.

Crépon, K., Marget, P., Peyronnet, C., Carrouée, B., Arese, P., & Duc, G. (2010). Nutritional value of faba bean (Vicia faba L.) seeds for feed and food. *Field Crops Res. 115*(3), 329-339.

Cuendet, M., Hostettmann, K., Potterat, O., & Dyatmiko, W. (1997). Iridoid glucosides with free radical scavenging properties from fagraea blumei. *Medicinal and biological Chemistry, HELVETICA. 80*(4), 1144-1152.

De Angelis, M., Siragusa, S., Berloco, M., Caputo, L., Settanni, L., Alfonsi, G., Amerio, M., Grandi, A., Ragni, A., & Gobbetti, M. (2006). Selection of potential probiotic *lactobacilli* from pig feces to be used as additives in pelleted feeding. *Res Microbiol. 157*(8), 792-801.

De Vuyst, L., & Degeest, B. (1999). Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiol Rev. 23*(2), 153-177.

Dertli, E., Mayer, M. J., & Narbad, A. (2015). Impact of the exopolysaccharide layer on biofilms, adhesion and resistance to stress in *Lactobacillus johnsonii* FI9785. *BMC Microbiol. 15*, 8.

Dias-Martins, A. M., Pessanha, K. L. F., Pacheco, S., Rodrigues, J. A. S., & Carvalho, C. W. P. (2018). Potential use of pearl millet (Pennisetum glaucum (L.) R. Br.) in Brazil: Food security, processing, health benefits and nutritional products. *Food Res Int. 109*, 175-186.

Di Cagno, R., De Angelis, M., Limitone, A., Minervini, F., Carnevali, P., Corsetti, A., Gaenzle, M., Ciati, R., & Gobbetti, M. (2006). Glucan and fructan production by sourdough *Weissella cibaria* and *Lactobacillus plantarum*. *J Agric Food Chem*. *54*(26), 9873-81

Dobraszczyk, B. J. (1997). The rheological basis of dough stickiness. *J Texture Stud. 28*(2), 139-162.

Dobraszczyk, B. J., & Morgenstern, M. P. (2003). Rheology and the breadmaking process. *J Cereal Sci. 38*(3), 229-245.

Dobraszczyk, B. J., & Salmanowicz, B. P. (2008). Comparison of predictions of baking volume using large deformation rheological properties. *J Cereal Sci. 47*(2), 292-301.

DTU. (2008). Danmarks Tekniske Universitet, Fodevareinstituttet Wholegrain. Definition and scientific background for recommendations of whole grain intake.

Duboc, P., & Mollet, B. (2001). Applications of exopolysaccharides in the dairy industry. *Int Dairy J. 11*(9), 759-768.

Duc, G., Marget, P., Esnault, R., Le Guen, J., & Bastianelli, D. (1999). Genetic variability for feeding value of faba bean seeds (Vicia faba): Comparative chemical composition of isogenics involving zero-tannin and zero-vicine genes. *J Agric Sci. 133*(2), 185-196.

EFSA. (2010). European Food Safety Authority, Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the substantiation of a health claim related to whole grain.

El Hag, M. E., El Tinay, A. H., & Yousif, N. E. (2002). Effect of fermentation and dehulling on starch, total polyphenols, phytic acid content and *in vitro* protein digestibility of pearl millet. *Food Chem. 77*(2), 193-196.

Erten, H. (1998). Metabolism of fructose as an electron acceptor by *Leuconostoc mesenteroides*. *Process Biochem. 33*(7), 735-739.

Fábián, T. K., Beck, A., Fejérdy, P., Hermann, P., & Fábián, G. (2015). Molecular mechanisms of taste recognition: considerations about the role of saliva. *Int J Mol Sci. 16*(3), 5945-5974.

Fadda, C., Sanguinetti, A. M., Caro, A. D., Collar, C., & Piga, A. (2014). Bread staling: Updating the view. *Compr Rev Food Sci Food Saf. 13*(4), 473-492.

Fallico, V., McSweeney, P. L. H., Horne, J., Pediliggieri, C., Hannon, J. A., Carpino, S., & Licitra, G. (2005). Evaluation of bitterness in ragusano cheese. *J Dairy Sci. 88*(4), 1288-1300.

FAO. (1995). Sorghum and millets in human nutrition. Rome: Lanham, MD: Food and Agriculture Organization of the United Nations; UNIPUB [distributor].

FAOSTAT. (2017). FAO (Food and Agricultural Organization) Statistical Data. Available online: http://www.fao.org/faostat/en/#data/QC. Accessed on Jul 2019.

Feron, G., & Salles, C. (2018). Food oral processing in humans: Links between physiological parameters, release of flavour stimuli and flavour perception of food. *Int J Food Stud*. *7*.

Ferrero, C. (2017). Hydrocolloids in wheat breadmaking: A concise review. *Food Hydrocoll. 68*(Supplement C), 15-22.

Ferruzzi, M. G., Jonnalagadda, S. S., Liu, S., Marquart, L., McKeown, N., Reicks, M., Riccardi, G., Seal, C., Slavin, J., Thielecke, F., van der Kamp, J., & Webb, D. (2014). Developing a

standard definition of whole-grain foods for dietary recommendations: Summary report of a multidisciplinary expert roundtable discussion. *Adv Nutr (Bethesda, Md.). 5*(2), 164-176.

Ferry, A. L., Hort, J., Mitchell, J., Cook, D., Lagarrigue, S., & Valles Pamies, B. (2006). Viscosity and flavour perception: Why is starch different from hydrocolloids? *Food Hydrocoll. 20*, 855-862.

Foster, K. D., Grigor, J. M., Cheong, J. N., Yoo, M. J., Bronlund, J. E., & Morgenstern, M. P. (2011). The role of oral processing in dynamic sensory perception. *J Food Sci. 76*(2), R49-61.

Gallardo-Escamilla, F. J., Kelly, A. L., & Delahunty, C. M. (2007). Mouthfeel and flavour of fermented whey with added hydrocolloids. *Int Dairy J. 17*(4), 308-315.

Galle, S., & Arendt, E. K. (2014). Exopolysaccharides from sourdough lactic acid bacteria. *Crit Rev Food Sci Nutr. 54*(7), 891-901.

Galle, S., Schwab, C., Arendt, E., & Gänzle, M. (2010). Exopolysaccharide-forming *Weissella* strains as starter cultures for sorghum and wheat sourdoughs. *J Agric Food Chem*. *58*(9), 5834–5841.

Galle, S., Schwab, C., Bello, F. D., Coffey, A., Gänzle, M., & Arendt, E. (2012a). Comparison of the impact of dextran and reuteran on the quality of wheat sourdough bread. *J Cereal Sci. 56*(3), 531-537.

Galle, S., Schwab, C., Bello, F. D., Coffey, A., Gänzle, M., & Arendt, E. (2012b). Influence of *in-situ* synthesized exopolysaccharides on the quality of gluten-free sorghum sourdough bread. *Int J Food Microbiol. 155*(3), 105-112.

Germaine, K. A., Samman, S., Fryirs, C. G., Griffiths, P. J., Johnson, S. K., & Quail, K. J. (2008). Comparison of *in vitro* starch digestibility methods for predicting the glycaemic index of grain foods. *J Sci Food Agric*. *88*(4), 652-658.

Gerwig, G. J., Kamerling, J. P., & Vliegenthart, J. F. G. (1979). Determination of the absolute configuration of monosaccharides in complex carbohydrates by capillary g.l.c. *Carbohydr Res. 77*(1), 1-7.

Gobbetti, M., Rizzello, C. G., Di Cagno, R., & De Angelis, M. (2014). How the sourdough may affect the functional features of leavened baked goods. *Food Microbiol. 37*, 30-40.

Gobbetti, M., Simonetti, M. S., Corsetti, A., Santinelli, F., Rossi, J., & Damiani, P. (1995). Volatile compound and organic acid productions by mixed wheat sour dough starters: Influence of fermentation parameters and dynamics during baking. *Food Microbiol. 12*, 497-507.

Goesaert, H., Brijs, K., Veraverbeke, W. S., Courtin, C. M., Gebruers, K., & Delcour, J. A. (2005). Wheat flour constituents: How they impact bread quality, and how to impact their functionality. *Trends Food Sci Tech. 16*(1), 12-30.

Goesaert, H., Leman, P., Bijttebier, A., & Delcour, J. A. (2009). Antifirming effects of starch degrading enzymes in bread crumb. *J Agric Food Chem. 57*(6), 2346-2355.

Goyal, D., Sharma, S., & Mahmood, A. (2013). Inhibition of dextransucrase activity in S*treptococcus mutans* by plant phenolics. *Indian J Biochem Biophys*. *50*(1), 48-53.

Gray, J. A., & Bemiller, J. N. (2003). Bread staling: Molecular basis and control. *Compr Rev Food Sci Food Saf. 2*(1), 1-21.

Guarda, A., Rosell, C. M., Benedito, C., & Galotto, M. J. (2004). Different hydrocolloids as bread improvers and antistaling agents. *Food Hydrocoll. 18*(2), 241-247.

Guichard, E. (2007). Interactions between flavor compounds and food ingredients and their influence on flavor perception. *Food Rev Int*. *18*(1), 49-70.

Guichard, E., Issanchou, S., Descourvieres, A., & Etievant, P. (1991). Pectin concentration, molecular weight and degree of esterification: Influence on volatile composition and sensory characteristics of strawberry jam. *J Food Sci. 56*(6), 1621-1627.

Hammes, W. P., & Gänzle, M. G. (1998). Sourdough breads and related products. In B. J. B. Wood (Ed.), *Microbiology of Fermented Foods*. 199-216. Boston, MA: Springer US.

Hansen, Å. S. (1989). Flavour of sourdough rye bread crumb. *Lebensm Wiss Technol. 22*, 141-144.

Hansen, Å. S., & Hansen, B. (1996). Flavour of sourdough wheat bread crumb. *Z Lebensm Unters Forsch. 202*(3), 244-249.

Hansen, Å. S., & Schieberle, P. (2005). Generation of aroma compounds during sourdough fermentation: Applied and fundamental aspects. *Trends Food Sci Tech. 16*(1), 85-94.
Healthgrain Forum. (2017). Founded association under Finnish law. Perspective: A definition for whole-grain food products—recommendations from the Healthgrain Forum. *Adv Nutr. 8*(4), 525–531.
Hehre, E. J. (1941). Production from sucrose of a-sero-logically reactive polysaccharide by a sterile bacterial extract. *Science*. *93*(2410), 237-238.
Hehre, E. J., & Sugg, J. Y. (1942). Serologically reactive polysaccharides produced through the action of bacteria enzymes. I. Dextran of *leuconostoc mesenteroides* from sucrose. *J Exp Med. 75*(3), 339–353.
Heiniö, R.-L. (2014). Sensory attributes of bakery products. In *Bakery Products Science and Technology*. 2nd Ed, 391-407. Wiley-Blackwell.
Heiniö, R.-L., Liukkonen, K. H., Myllymäki, O., Pihlava, J.-M., Adlercreutz, H., Heinonen, S.-M., & Poutanen, K. (2008). Quantities of phenolic compounds and their impacts on the perceived flavour attributes of rye grain. *J Cereal Sci. 47*, 566-575.
Heiniö, R.-L., Noort, M., Katina, K., Alam, S. A., Sozer, N., de Kock, H. L., Hersleth, M., & Poutanen, K. (2015). Sensory characteristics of wholegrain and bran-rich cereal foods – A review. *Trends Food Sci Tech. 47*.
Hestrin, S., Avineri-Shapiro, S., & Aschner, M. (1943). The enzymic production of levan. *The Biochem J. 37*(4), 450-456.
Hollowood, T. A., Linforth, R. S., & Taylor, A. J. (2002). The effect of viscosity on the perception of flavour. *Chem Senses. 27*(7), 583-591.
Hucker, G. J., & Pederson, C. S. (1930). Studies on the Coccaceae. XVI. The genus *Leuconostoc. N Y Agr Exp Sta Tech Bull. 167*, 1
Innova Database. (2019). Available online: www.innovamarketinsights.com. Accessed on Nov 2019.
Innova consumer survey. (2018). Available online: www.innovamarketinsights.com. Accessed on Nov 2019.
Ioan, C. E., Aberle, T., & Burchard, W. (2000). Structure properties of dextran. 2. Dilute solution. *Macromolecules. 33*(15), 5730-5739.
Ito, K., Ito, S., Shimamura, T., Weyand, S., Kawarasaki, Y., Misaka, T., Abe, K., Kobayashi, T., Cameron A. D., & Iwata, S. (2011). Crystal structure of glucansucrase from the dental caries pathogen *Streptococcus mutans*. *J Mol Biol. 408*(2), 177-186.
Iwaya, H., Lee, J.-S., Yamagishi, S., Shinoki, A., Lang, W., Thawornkuno, C., Kang, H. K., Kumagai, Y., Suzuki, S., Kitamura, S., Hara, H., Okuyama, M., Mori, H., Kimura, A., & Ishizuka, S. (2012). The delay in the development of experimental colitis from isomaltosyloligosaccharides in rats is dependent on the degree of polymerization. *PLoS One. 7*(11), e50658.
Jeanes, A. (1966). In *Encyclopedia of Polymer Science and Technology*. *4*, 805-824. John Wiley and Sons, New York.
Jezierny, D., Mosenthin, R., & Bauer, E. (2010). The use of grain legumes as a protein source in pig nutrition: A review. *Anim Feed Sci Technol. 157*(3-4), 111-128.
Jiang, Z.-q., Pulkkinen, M., Wang, Y., Lampi, A.-M., Stoddard, F., Salovaara, H., Piironen, V., & Sontag-Strohm, T. (2016). Faba bean flavour and technological property improvement by thermal pre-treatments. *LWT-Food Sci Technol. 68,* 295-305.
Juteau, A., Tournier, C., & Guichard, E. (2004). Influence of type and amount of gelling agent on flavour perception: Physicochemical effect or interaction between senses? *Flavour Fragr J. 19*, 483-490.
Kaditzky, S., & Vogel, R. F. (2008). Optimization of exopolysaccharide yields in sourdoughs fermented by *lactobacilli*. *Eur Food Res Technol. 228*(2), 291.
Kang, J., Price, W. E., Ashton, J., Tapsell, L. C., & Johnson, S. (2016). Identification and characterization of phenolic compounds in hydromethanolic extracts of sorghum wholegrains by LC-ESI-MS(n). *Food Chem. 211*, 215-226.
Katina, K., Heiniö, R-L., Autio, K., & Poutanen, K. (2006). Optimization of sourdough process for improved sensory profile and texture of wheat bread. *LWT-Food Sci Technol. 39*(10), 1189-1202.

Katina, K., Maina, N. H., Juvonen, R., Flander, L., Johansson, L., Virkki, L., Tenkanen, M., & Laitila, A. (2009). *In situ* production and analysis of *Weissella confusa* dextran in wheat sourdough. *Food Microbiol. 26*(7), 734-743.

Katina, K., Poutanen, K., & Autio, K. (2004). Influence and interactions of processing conditions and starter culture on formation of acids, volatile compounds, and amino acids in wheat sourdoughs. *Cereal Chem*. *81*(5), 598 - 610.

Kawamura, Y., & Yonezawa, D. (1982). Wheat flour proteases and their action on gluten proteins in dilute acetic acid. *Agric Biol Chem. 46*(3), 767-773.

Kenne, L., & Lindberg, B. (1983). 5-Bacterial polysaccharides. In G. O. Aspinall (Ed.), *The Polysaccharides*. 287-363. Academic Press.

Kieffer, R., Wieser, H., Henderson, M. H., & Graveland, A. (1998). Correlations of the breadmaking performance of wheat flour with rheological measurements on a micro-scale. *J Cereal Sci. 27*(1), 53-60.

Kim, D., Robyt, J. F., Lee , S. Y., Lee, J. H., & Kim, Y. M. (2003). Dextran molecular size and degree of branching as a function of sucrose concentration, pH, and temperature of reaction of *Leuconostoc mesenteroides* B-512FMCM dextransucrase. *Carbohydr Res. 338*(11), 1183-1189.

Kirkwood, J. G., & Riseman, J. (1948). The intrinsic viscosities and diffusion constants of flexible macromolecules in solution. *Chem Phys. 16*(6), 565-573.

Kobue-Lekalake, R. I. (2009). Sensory perception of bitterness and astringency in sorghum. Doctoral thesis. University of Pretoria, Pretoria, South Africa.

Koepsell, H. J., Tsuchiya, H. M., Hellman, N. N., Kasenko, A., Hofman, C. A., Sharpe, E. S., & Jackson, R. W. (1953). Enzymatic synthesis of dextran, acceptor specificity and chain initiation. *J Biol Chem*. *200*, 793–801.

Koliandris, A., Lee, A., Ferry, A.-L., Hill, S., & Mitchell, J. (2008). Relationship between structure of hydrocolloid gels and solutions and flavour release. *Food Hydrocoll. 22*(4), 623-630.

Komlenić, D. K., Ugarčić-Hardi, Ž., Jukić, M., Planinić, M., Bucić-Kojić, A., & Strelec, I. (2010). Wheat dough rheology and bread quality effected by *Lactobacillus brevis* preferment, dry sourdough and lactic acid addition. *Int J Food Sci Tech. 45*(7), 1417-1425.

Korakli, M., & Vogel, R. F. (2006). Structure/function relationship of homopolysaccharide producing glycansucrases and therapeutic potential of their synthesised glycans. *Appl Microbiol Biot. 71*(6), 790-803.

Kothari, D., Das, D., Patel, S., & Goyal, A. (2014). Dextran and food application. In *Polysaccharides.* 735-752. Springer, Cham.

Lacaze, G., Wick, M., & Cappelle, S. (2007). Emerging fermentation technologies: Development of novel sourdoughs. *Food Microbiol. 24*(2), 155-160.

Lawless, H. T., & Heymann, H. (2010). *Sensory Evaluation of Food: Principles and Practices*. Springer New York.

Leemhuis, H., Pijning, T., Dobruchowska, J. M., van Leeuwen, S. S., Kralj, S., Dijkstra, B. W., & Dijkhuizen, L. (2013). Glucansucrases: Three-dimensional structures, reactions, mechanism, α-glucan analysis and their implications in biotechnology and food applications. *J Biotechnol. 163*(2), 250-272.

Lima, V. N., Oliveira-Tintino, C. D., Santos, E. S., Morais, L. P., Tintino, S. R., Freitas, T. S., Geraldo, Y. S., Pereira, R., Cruz, R. P., Menezes, I. R., & Coutinho, H. D. (2016). Antimicrobial and enhancement of the antibiotic activity by phenolic compounds: Gallic acid, caffeic acid and pyrogallol. *Microb Pathog. 99*, 56-61.

Looijesteijn, P. J., Trapet, L., de Vries, E., Abee, T., & Hugenholtz, J. (2001). Physiological function of exopolysaccharides produced by *Lactococcus lactis*. *Int J Food Microbiol. 64*(1), 71-80.

Lotong, V., Chambers, E., & Chambers, D. (2007). Determination of the sensory attributes of sourdough bread. *J Sens Stud. 15*, 309-326.

Lubbers, S., Decourcelle, N., Martinez, D., Guichard, E., & Tromelin, A. (2007). Effect of thickeners on aroma compound behavior in a model dairy gel. *J Agric Food Chem*. *55*(12), 4835-4841.

Lucas, P. W., Prinz, J. F., Agrawal, K. R., & Bruce, I. C. (2002). Food physics and oral physiology. *Food Qual Prefer. 13*(4), 203-213.

Lucy, M., Owuoche, J., & Cheserek, M. (2017). Development of sorghum-wheat composite bread and evaluation of nutritional, physical and sensory acceptability. *Afr J Food Sci Tech. 8*.

Lundgren, B., Pangborn, R. M., Daget, N., Yoshida, M., Laing, D. G., McBride, R. L., Griffiths, N., Hyvönen, L., Sauvageot, F., Paulus, K., & Barylko-Pikielna, N. (1986). An interlaboratory study of firmness, aroma, and taste of pectin gels. *LWT-Food Sci Technol. 19*, 66-76.

Lynch, K. M., Coffey, A., & Arendt, E. K. (2018). Exopolysaccharide producing lactic acid bacteria: Their techno-functional role and potential application in gluten-free bread products. *Food Res Int. 110*, 52-61.

Madigan, M. T., Martinko, J. M., & Brock, T. D. (2006). *Brock biology of microorganisms*. Upper Saddle River, NJ: Pearson Prentice Hall.

Maina, N. H., Pitkänen, L., Heikkinen, S., Tuomainen, P., Virkki, L., & Tenkanen, M. (2014). Challenges in analysis of high-molar mass dextrans: Comparison of HPSEC, AsFlFFF and DOSY NMR spectroscopy. *Carbohydr Polym. 99*, 199-207.

Maina, N. H., Tenkanen, M., Maaheimo, H., Juvonen, R., & Virkki, L. (2008). NMR spectroscopic analysis of exopolysaccharides produced by *Leuconostoc citreum* and *Weissella confusa*. *Carbohydr Res. 343*(9), 1446-1455.

Makokha, A. O., Oniang'o, R. K., Njoroge, S. M., & Kamar, O. K. (2002). Effect of traditional fermentation and malting on phytic acid and mineral availability from sorghum (Sorghum Bicolor) and finger millet (Eleusine Coracana) grain varieties grown in Kenya. *Food Nutr Bull*. *23*(3 suppl), 241–245.

Mälkki, Y., Heiniö, R. L., & Autio, K. (1993). Influence of oat gum, guar gum and carboxymethyl cellulose on the perception of sweetness and flavour. *Food Hydrocoll*. *6*(6), 525-532.

Mariera, L., Owuoche, J. O., & Cheserek, M. (2017). Development of sorghum-wheat composite bread and evaluation of nutritional, physical and sensory acceptability. *Afr J Food Sci Tech. 08*(07).

Masuelli, M. A. (2013). Dextrans in aqueous solution. Experimental review on intrinsic viscosity measurements and temperature effect. *J Polym Bio Phys Chem*. *1*(1), 13-21.

Meignen, B., Onno, B., Gélinas, P., Infantes, M., Guilois, S., & Cahagnier, B. (2001). Optimization of sourdough fermentation with *Lactobacillus brevis* and baker's yeast. *Food Microbiol. 18*(3), 239-245.

Mert, I. D., Campanella, O. H., Sumnu, G., & Şahin, S. (2014). Gluten-free sourdough bread prepared with chestnut and rice flour. *Eur Food Res Technol*. *242*(10).

Mezger, Thomas G. (2006). *The Rheology Handbook*. Vincents Network, 2nd Ed. Hannover.

Mishra, A., & Jha, B. (2013). Microbial exopolysaccharides. In *The prokaryotes: Applied bacteriology and biotechnology*. 179-192. Springer-Verlag Berlin Heidelberg.

Monchois, V., Willemot, R.-M., & Monsan, P. (1999). Glucansucrases: Mechanism of action and structure–function relationships. *FEMS Microbiol Rev. 23*(2), 131-151.

Monsan, P., Bozonnet, S., Albenne, C., Joucla, G., Willemot, R.-M., & Remaud-Siméon, M. (2001). Homopolysaccharides from lactic acid bacteria. *Int Dairy J. 11*(9), 675-685.

Mooser, G., & Iwaoka, K. R. (1989). Sucrose 6-alpha-D-glucosyltransferase from *Streptococcus sobrinus*: Characterization of a glucosyl-enzyme complex. *Biochem J. 28*(2), 443-449.

Moskowitz, H. R., & Arabie, P. (1970). Taste intensity as a function of stimulus concentration and solvent viscosity. *J Texture Stud*. *1*(4), 502-510.

Moulis, C., André, I., & Remaud-Simeon, M. (2016). GH13 amylosucrases and GH70 branching sucrases, atypical enzymes in their respective families. *Cell Mol Life Sci. 73*(14), 2661-2679.

Moulis, C., Joucla, G., Harrison, D., Fabre, E., Potocki-Veronese, G., Monsan, P., & Remaud-Simeon, M. (2006). Understanding the polymerization mechanism of glycoside-hydrolase family 70 glucansucrases. *J Biol Chem. 281*(42), 31254-31267.

Multari, S., Stewart, D., & Russell, W. (2015). Potential of fava bean as future protein supply to partially replace meat intake in the human diet. *Compr Rev Food Sci Food Saf. 14*.

Naser, S. M., Thompson, F. L., Hoste, B., Gevers, D., Dawyndt, P., Vancanneyt, M., & Swings, J. (2005). Application of multilocus sequence analysis (MLSA) for rapid identification of *Enterococcus* species based on rpoA and pheS genes. *Microbiology. 151*(7), 2141-2150.

Nash, D., Lanning, S. P., Fox, P., Martin, J. M., Blake, N. K., Souza, E., Graybosch, R. A., Giroux, M. J., & Talbert, L. E. (2006). Relationship of dough extensibility to dough strength in a spring wheat cross. *Cereal Chem. 83*(3), 255-258.

Neta, E., Johanningsmeier, S., Drake, M. A., & McFeeters, R. F. (2007). A chemical basis for sour taste perception of acid solutions and fresh-pack dill pickles. *J Food Sci. 72*, S352-359.

Netsopa, S., Niamsanit, S., Sakloetsakun, D., & Milintawisamai, N. (2018). Characterization and rheological behavior of dextran from *Weissella confusa* R003. *Int J of Polym Sci. 10*.

Noorfarahzilah, M, Lee, J. S, Sharifudin, M. S, Mohd Fadzelly, A. B, & Hasmadi, M. (2014). Applications of composite flour in development of food products. *Int Food Res J*, *21*(6), 2061-2074.

Nwanekezi, E. C. (2013). Composite flours for baked products and possible challenges – A review. *Niger Food J. 31*, 8-17.

Ohimain, E. I. (2014). The prospects and challenges of composite flour for bread production in Nigeria. *GJHSS-H: Interdisciplinary. 14*, 42-52.

Olano-Martin, E., Mountzouris, K. C., Gibson, G. R., & Rastall, R. A. (2000). *In vitro* fermentability of dextran, oligodextran and maltodextrin by human gut bacteria. *Br J Nutr. 83*(3), 247-255.

Omoba, O. S., Taylor, J. R. N., & de Kock, H. L. (2015). Sensory and nutritive profiles of biscuits from whole grain sorghum and pearl millet plus soya flour with and without sourdough fermentation. *Int J Food Sci Tech. 50*(12), 2554-2561.

Ostman, E., Nilsson, M., Elmståhl, H., Molin, G., & Björck, I. M. E. (2003). On the effect of lactic acid on blood glucose and insulin responses to cereal products: Mechanistic studies in healthy subjects and *in vitro*. *J Cereal Sci. 36*, 339-346.

Overbosch, P., Afterof, W. G. M., & Haring, P. G. M. (1991). Flavor release in the mouth. *Food Rev Int*. *7*(2), 137-184.

Padmanabhan, P. A., Kim, D.-S., Pak, D., & Sim, S. J. (2003). Rheology and gelation of water-insoluble dextran from *Leuconostoc mesenteroides* NRRL B-523. *Carbohydr Polym. 53*(4), 459-468.

Pangborn, R. M., Trabue, I. M., & Szcaesniak, A. S. (1973). *J Texture Stud*. *4*(2), 224-241.

Pangborn, R. M., & Szczesniak, A. S. (1974). Effect of hydrocolloids and viscosity on flavor and odor intensities of aromatic flavor compounds. *J Texture Stud*. *4*(4), 467-482.

Passerini, D., Vuillemin, M., Ufarté, L., Morel, S., Loux, V., Fontagné-Faucher, C., Monsan, P., Remaud-Siméon, M., & Moulis, C. (2015). Inventory of the GH70 enzymes encoded by *Leuconostoc citreum* NRRL B-1299–identification of three novel α-transglucosylases. *FEBS J. 282*(11), 2115-2130.

Pasteur, L. (1861). Sur la fermentation visquese et la fermentation butyrique (On the viscous fermentation and the butyrous fermentation). *Bull Soc Chim Paris. 11*, 30-31.

Pétel, C., Onno, B., & Prost, C. (2017). Sourdough volatile compounds and their contribution to bread: A review. *Trends Food Sci Technol. 59*, 105-123.

Plou, F. J., Martín, M. T., de Segura, A. G., Alcalde, M., & Ballesteros, A. (2002). Glucosyltransferases acting on starch or sucrose for the synthesis of oligosaccharides. *Can J Chem. 80*(6), 743-752.

Previtali, M. A., Mastromatteo, M., De Vita, P., Ficco, D. B. M., Conte, A., & Del Nobile, M. A. (2014), Effect of the lentil flour and hydrocolloids on baking characteristics of wholemeal durum wheat bread. *Int J Food Sci Technol*. *49*, 2382-2390.

Purama, R. K., Goswami, P., Khan, A. T., & Goyal, A. (2009). Structural analysis and properties of dextran produced by *Leuconostoc mesenteroides* NRRL B-640. *Carbohydr Polym. 76*(1), 30-35.

Ramachandra, G., Virupaksha, T. K., & Shadaksharaswamy, M. (1977). Relation between tannin levels and *in vitro* protein digestibility in finger millet (Eleusine coracana Gaertn.). *J Agric Food Chem. 25*(5), 1101-1104.

Rangaraju, V. (2014). Development of bread from fermented pearl millet flour. *J Food Process Technol. 05*.

Reitsma, S., Slaaf, D. W., Vink, H., van Zandvoort, M. A., & oude Egbrink, M. G. (2007). The endothelial glycocalyx: Composition, functions, and visualization. *Pflugers Arch*. *454*(3), 345–359.

Rieder, A., Holtekjølen, A. K., Sahlstrøm, S., & Moldestad, A. (2012). Effect of barley and oat flour types and sourdoughs on dough rheology and bread quality of composite wheat bread. *J Cereal Sci. 55*(1), 44-52.

Rizzello, C. G., Lorusso, A., Montemurro, M., & Gobbetti, M. (2016). Use of sourdough made with quinoa (Chenopodium quinoa) flour and autochthonous selected lactic acid bacteria for enhancing the nutritional, textural and sensory features of white bread. *Food Microbiol. 56*, 1-13.

Robichaud, J. L., & Noble, A. C. (1990). Astringency and bitterness of selected phenolics in wine. ⎡ *J Sci Food Agric*. *53*(3), 343-353.

Robyt, J. F., & Eklund, S. H. (1983). Relative, quantitative effects of acceptors in the reaction of *Leuconostoc mesenteroides* B-512F dextransucrase. *Carbohydr Res*. *121*(C), 279-286.

Robyt, J. F., & Taniguchi, H. (1976). The mechanism of dextransucrase action. Biosynthesis of branch linkages by acceptor reactions with dextran. *Arch Biochem Biophys. 174*(1), 129-135.

Rogošić, M., Mencer, H. J., & Gomzi, Z. (1996). Polydispersity index and molecular weight distributions of polymers. *Eur Polym J. 32*(11), 1337-1344.

Roper, S. D., & Chaudhari, N. (2017). Taste buds: Cells, signals and synapses. *Nat Rev Neurosci. 18*(8), 485-497.

Rosell, C. M., Rojas, J. A., & Benedito de Barber, C. (2001). Influence of hydrocolloids on dough rheology and bread quality. *Food Hydrocoll. 15*(1), 75-81.

Ross, A. S., McMaster, G. J., Tomlinson, J. D., & Cheetham, N. W. H. (1992). Effect of dextrans of differing molecular weights on the rheology of wheat flour doughs and the quality characteristics of pan and arabic breads. *J Sci Food Agric. 60*(1), 91-98.

Ruas-Madiedo, P., & de los Reyes-Gavilán, C. G. (2005). Invited review: Methods for the screening, isolation, and characterization of exopolysaccharides produced by lactic acid bacteria. *J Dairy Sci. 88*(3), 843-856.

Ruas-Madiedo, P., Hugenholtz, J., & Zoon, P. (2002). An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. *Int Dairy J. 12*(2), 163-171.

Rühmkorf, C., Rübsam, H., Becker, T., Bork, C., Voiges, K., Mischnick, P., Brandt, M. J., & Vogel, R. F. (2012). Effect of structurally different microbial homoexopolysaccharides on the quality of gluten-free bread. *Eur Food Res Technol. 235*(1), 139-146.

Rutschmann, M. A., & Solms, J. (1990). Formation of inclusion complexes of starch with different organic compounds. IV, ligand binding and variability in helical conformations of V amylose complexes. *AGRIS*. *23*, 84-87.

Sabatie, J., Choplin, L., Moan, M., Doublier, J. L., Paul, F., & Monsan, P. (1988). The effect of synthesis temperature on the structure of dextran NRRL B 512F. *Carbohydr Polym*. *9*, 87-101.

Saha, D., & Bhattacharya, S. (2010). Hydrocolloids as thickening and gelling agents in food: A critical review. *J Food Sci Technol. 47*(6), 587-597.

Salazar-López, N., Aguilar, G., Rouzaud-Sández, O., & Sánchez, M. (2018). Technologies applied to sorghum (Sorghum bicolor L. Moench): Changes in phenolic compounds and antioxidant capacity. *J Food Sci Technol*. *38*, 369-382.

Salovaara, H., & Valjakka, T. (1987). The effect of fermentation temperature, flour type, and starter on the properties of sour wheat bread. *Int J Food Sci Tech*. *22*, 591-597.

Salunkhe, D. K., & Deshpande, S. S. (1991). Flatus-causing raffinose oligosaccharides. In *Foods of Plant Origin: Production, Technology, and Human Nutrition*. 239-241. Springer US.

Samavati, V., Emam-Djomeh, Z., Mehdinia, A., & Mohammadifar, M. A. (2012). Effect of composition on release of aroma compounds. *IJCCE. 31*(3), 85-96.

Sawadogo-Lingani, H., Diawara, B., Traore, A. S., & Jakobsen, M. (2008). Technological properties of *Lactobacillus fermentum* involved in the processing of dolo and pito, West African sorghum beers, for the selection of starter cultures. *J Appl Microbiol. 104*(3), 873-882.

Sawadogo-Lingani, H., Lei, V., Diawara, B., Nielsen, D. S., Møller, P. L., Traoré, A. S., & Jakobsen, M. (2007). The biodiversity of predominant lactic acid bacteria in dolo and pito wort for the production of sorghum beer. *J Appl Microbiol. 103*(4), 765-777.

Scheibler, C. (1874). Investigation on the nature of the gelatinous excretion (so-called frog's spawn) which is observed in production of beet-sugar juices. *Z Dtsch Zucker-Ind*. *24*, 309-335.

Schmid, J., Fariña, J., Rehm, B., & Sieber, V. (2016). Editorial: Microbial Exopolysaccharides: From Genes to Applications. *Front Microbial. 7*, 308-308.

Sekwati-Monang, B., Valcheva, R., & Ganzle, M. G. (2012). Microbial ecology of sorghum sourdoughs: Effect of substrate supply and phenolic compounds on composition of fermentation microbiota. *Int J Food Microbiol. 159*(3), 240-246.

Selvamuthukumaran, M., & Pathak, Y. V. (2019). In *Flavor Development for Functional Foods and Nutraceuticals*. 1st Ed. CRC Press.

Shahidi, F., & Chandrasekara, A. (2013). Millet grain phenolics and their role in disease risk reduction and health promotion: A review. *J Funct Foods. 5*(2), 570-581.

Shi, Q., Hou, Y., Juvonen, M., Tuomainen, P., Kajala, I., Shukla, S., Goyal, A., Maaheimo, H., & Tenkanen, M. (2016). Optimization of isomaltooligosaccharide size distribution by acceptor reaction of *Weissella confusa* dextransucrase and characterization of novel α-(1→2)-branched isomaltooligosaccharides. *J Agric Food Chem. 64*(16), 3276-3286.

Shobana, S., Sreerama, Y. N., & Malleshi, N. G. (2009). Composition and enzyme inhibitory properties of finger millet (Eleusine coracana L.) seed coat phenolics: Mode of inhibition of α-glucosidase and pancreatic amylase. *Food Chem. 115*(4), 1268-1273.

Shukla, R., & Goyal, A. (2013). Novel dextran from *Pediococcus pentosaceus* CRAG3 isolated from fermented cucumber with anti-cancer properties. *Int J Biol Macromol. 62*, 352-357.

Shumoy, H., Gabaza, M., Vandevelde, J., & Raes, K. (2017). Soluble and bound phenolic contents and antioxidant capacity of tef injera as affected by traditional fermentation. *J Food Compos Anal. 58*, 52-59.

Siepmann, F., Ripari, V., Waszczynskyj, N., & Spier, M. (2017). Overview of sourdough technology: From production to marketing. *Food Bioprocess Tech.* 1-29.

Singh, U., & Singh, B. B. (1992). Tropical grain legumes as important human foods. *Econ Bot. 46*(3), 310-321.

Singh, M., Upadhyaya, H. D., & Bisht, I. S. (2013). *Genetic and Genomic Resources of Grain Legume Improvement*. 1-136. Oxford: Elsevier.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in Enzymology*. *299*, 152-178. Academic Press.

SNF. (2007). Swedish Nutrition Foundation, Health claims in the labelling and marketing of food products. *Scand J Food Nutr. 51*(1), 107–126.

Spence, C. (2015). Multisensory Flavor Perception. *Cell. 161*(1), 24-35.

Stepto, R. F. T. (2010). Dispersity in polymer science (IUPAC Recommendations 2009). *Polym Int*. *59*, 23-24.

Svensson, L., Sekwati-Monang, B., Lutz, D. L., Schieber, A., & Ganzle, M. G. (2010). Phenolic acids and flavonoids in nonfermented and fermented red sorghum (Sorghum bicolor (L.) Moench). *J Agric Food Chem. 58*(16), 9214-9220.

Taylor, J. R. N., Belton, P. S., Beta, T., & Duodu, K. G. (2014). Increasing the utilisation of sorghum, millets and pseudocereals: Developments in the science of their phenolic phytochemicals, biofortification and protein functionality. *J Cereal Sci. 59*(3), 257-275.

Thomson, D. M. H. (1984). Flavour perception. *Nutr Bull*. *9*(2), 69-82.

Tingirikari, J. M., Kothari, D., & Goyal, A. (2014). Superior prebiotic and physicochemical properties of novel dextran from *Weissella cibaria* JAG8 for potential food applications. *Food Funct. 5*(9), 2324-2330.

Tournier, C., Sulmont-Rossé, C., & Guichard, E. (2007). Flavour perception: Aroma, taste and texture interactions. *Food. 1*(2), 246-257.

Troszyńska, A., Narolewska, O., Robredo, S., Estrella, I., Hernández, T., Lamparski, G., & Amarowicz, R. (2010). The effect of polysaccharides on the astringency induced by phenolic compounds. *Food Qual Prefer. 21*(5), 463-469.

Udachan, I. (2018). Enhancement of nutritional and functional characteristics of noodles by fortification with protein and fiber: A review. *J Pharmacogn Phytochem. 7*(1).

Ullrich, M. (2009). Biosynthesis and chemical composition of exopolysaccharides produced by lactic acid bacteria. In *Bacterial Polysaccharides−Current Innovations and Future Trends*. 1st Ed. Caister Academic Press.

U.S. Food and Drug Administration, Code of Food Regulations, 21 CFR 186.1275, 21 CFR 582.127537 Select Committee on GRAS Substances, Life Science Research Office (FASEB). (1975). Evaluation of the health aspects of dextrans as food ingredients. National Technical Information Service (NTIS). Document PB-234 889/4.

U.S. Wheat Associates & Lesaffre. (2017). Sandwich bread in words. A glossary of sensory terms to describe sandwich bread.

Valjakka, T., Katina, K., Kerojoki, H. (2003). Sourdough bread in Finland and Eastern Europe. In *Handbook of Dough Fermentations*. Boca Raton: CRC Press.

Vandamme, T. H. F., Lennoury, A., Charrueau, C., & Chaumeil, J. (2002). The use of polysaccharides to target drugs to colon. *Carbohydr Polym*. 48, 219–231.

van Gunst, A., & Roodenburg, A. (2019). Consumer distrust about E-numbers: A qualitative study among food experts. *Foods* (Basel, Switzerland). *8*(5), 178.

van Hijum, S. A. F. T., Kralj, S., Ozimek, L. K., Dijkhuizen, L., & van Geel-Schutten, I. G. H. (2006). Structure-function relationships of glucansucrase and fructansucrase enzymes from lactic acid bacteria. *Microbiol Mol Biol Rev. 70*(1), 157-176.

Van Tieghem, P. (1878). On sugar-mill gum. *Ann Sci Nat Bot Biol Veg.* 7, 180–203.

Varis, J. (2017). Natiivin ja raskitetun härkäpavun vaikutukset vehnäleivän rakenteeseen ja aistinvaraisiin ominaisuuksiin. Master's thesis. University of Helsinki, Finland.

Vettori, M. H. P. B., Franchetti, S. M. M., & Contiero, J. (2012). Structural characterization of a new dextran with a low degree of branching produced by *Leuconostoc mesenteroides* FT045B dextransucrase. *Carbohydr Polym. 88*(4), 1440-1444.

Voilley, A., & Souchon, I. (2006). Flavour retention and release from the food matrix: An overview. In *Flavour in Food*. 117-132. Woodhead Publishing.

Voon, F. C., Lucas, P. W., Chew, K. L., & Luke, D. A. (1986). A simulation approach to understanding the masticatory process. *J Theor Biol*. *119*(3), 251-262.

Vuillemin, M., Claverie, M., Brison, Y., Séverac, E., Bondy, P., Morel, S., Monsan, P., Moulis, C., & Remaud-Siméon, M. (2016). Characterization of the first α-(1→3) branching sucrases of the GH70 family. *J Biol Chem*. *291*(14), 7687-7702.

Vujicic-Zagar, A., Pijning, T., Kralj, S., Lopez, C. A., Eeuwema, W., Dijkhuizen, L., & Dijkstra, B. W. (2010). Crystal structure of a 117 kDa glucansucrase fragment provides insight into evolution and product specificity of GH70 enzymes. *Proc Natl Acad Sci USA*. *107*(50), 21406-21411.

Wang, S., & Copeland, L. (2013). Effect of acid hydrolysis on starch structure and functionality: A review. *Crit Rev Food Sci Nutr. 55*.

Wieser, H. (2007). Chemistry of gluten proteins. *Food Microbiol. 24*(2), 115-119.

Wisselink, H. W., Weusthuis, R. A., Eggink, G., Hugenholtz, J., & Grobben, G. J. (2002). Mannitol production by lactic acid bacteria: A review. *Int Dairy J. 12*(2), 151-161.

Wolter, A., Hager, A.-S., Zannini, E., & Arendt, E. K. (2014). Influence of sourdough on *in vitro* starch digestibility and predicted glycemic indices of gluten-free breads. *Food Funct*. *5*(3), 564-572.

Wolter, A., Hager, A.-S., Zannini, E., Czerny, M., & Arendt, E. K. (2014). Influence of dextran-producing *Weissella cibaria* on baking properties and sensory profile of gluten-free and wheat breads. *Int J Food Microbio*l. *172*, 83-91.

Xu, Y., Coda, R., Holopainen-Mantila, U., Laitila, A., Katina, K., & Tenkanen, M. (2019). Impact of *in situ* produced exopolysaccharides on rheology and texture of fava bean protein concentrate. *Int Food Res J. 115*, 191-199.

Xu, Y., Pitkänen, L., Maina, N., Coda, R., Katina, K., & Tenkanen, M. (2018). Interactions between fava bean protein and dextrans produced by *Leuconostoc pseudomesenteroides* DSM 20193 and *Weissella cibaria* Sj 1b. *Carbohydr Polym. 190*, 315-323.

Xu, Y., Wang, Y., Coda, R., Sade, E., Tuomainen, P., Tenkanen, M., & Katina, K. (2017). *In situ* synthesis of exopolysaccharides by *Leuconostoc* spp. and *Weissella* spp. and their rheological impacts in fava bean flour. *Int J Food Microbiol. 248*, 63-71.

Yang, Y., Peng, Q., Guo, Y., Han, Y., Xiao, H., & Zhou, Z. (2015). Isolation and characterization of dextran produced by *Leuconostoc citreum* NM105 from manchurian sauerkraut. *Carbohydr Polym. 133*, 365-372.

Ye, G., Li, G., Wang, C., Ling, B., Yang, R., & Huang, S. (2018). Extraction and characterization of dextran from *Leuconostoc pseudomesenteroides* YB-2 isolated from mango juice. *Carbohydr Polym. 207*.

Yven, C., Guichard, E., Giboreau, A., & Roberts, D. D. (1998). Assessment of interactions between hydrocolloids and flavor compounds by sensory, headspace, and binding methodologies. *J Agric Food Chem. 46*(4), 1510-1514.

Zannini, E., Waters, D. M., & Arendt, E. K. (2014). The application of dextran compared to other hydrocolloids as a novel food ingredient to compensate for low protein in biscuit and wholemeal wheat flour. *Eur Food Res and Technol. 238*(5), 763-771.

Zaroug, M., Orhan, I. E., Senol, F. S., & Yagi, S. (2014). Comparative antioxidant activity appraisal of traditional Sudanese kisra prepared from two sorghum cultivars. *Food Chem. 156*, 110-116.

Zarour, K., Llamas-Arriba, M., Prieto, A., Ruas-Madiedo, P., Dueñas, M., de Palencia, P., Aznar, R., Kihal, M., & López, P. (2017). Rheology and bioactivity of high molecular weight dextrans synthesised by lactic acid bacteria. *Carbohydr Polym. 174*, 646-657.

Zhang , Y., Guo, L., Li, D., Jin, Z., & Xu, X. (2019). Roles of dextran, weak acidification and their combination in the quality of wheat bread. *Food Chem. 286*, 197-203.

Zhang, Y., Guo, L., Xu, D., Li, D., Yang, N., Chen, F., Jin, Z., & Xu, X. (2018). Effects of dextran with different molecular weights on the quality of wheat sourdough breads. *Food Chem. 256*, 373-379.

Zhao, C. J., Schieber, A., & Gänzle, M. G. (2016). Formation of taste-active amino acids, amino acid derivatives and peptides in food fermentations – A review. *Int Food Res J. 89*, 39-47.