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UNIVERSITY COLLEGE CORK

School of Food and Nutritional Sciences



THE INVESTIGATION OF TECHNOLOGICAL, NUTRITIONAL AND SENSORIAL CHARACTERISTICS OF WHEAT BREAD, INFLUENCED BY DIFFERENT STRAINS OF *SACCHAROMYCES CEREVISIAE*

Thesis presented by

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Diploma Food Chemist

Under the supervision of

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To obtain the degree of

Doctor of Philosophy - PhD in Food Science and Technology

Head of School

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April 2017

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Declaration

I hereby declare that this thesis is my own work and effort, and that it has not been submitted for another degree, neither at the National University Ireland, Cork nor elsewhere. Where other sources of information have been used, they have been acknowledged.

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Mareile Heitmann

Abbreviations

A	Aconitase
a_w	Water activity
Ac-CoA-S	Acetyl-CoA synthase
AcD	Acetaldehyde dehydrogenase
AD	Alcohol dehydrogenase
APA	Aroma profile analysis
ATP	Adenosine triphosphate
AUC	Area under the curve
AWCD	Average well colour development
cfu	Colony forming units
CS	Citrate synthase
DAD	Diode array detector
DNS	3,5-Dinitro salicylic acid reagent
EFSA	European Food Safety Authority
F	Fumarase
FAO	Food and Agriculture Organization of the United Nations
G	Glucosidase
GABA	γ-aminobutyric acid
GAD	Glutamic acid decarboxylase
GC-MS	Gas chromatography-mass spectrometry
GDL	Glucono-delta-lactone
GI	Glycaemic index
pGI	Predicted glycaemic index
Glu	Glucose
GP	Glycerol-3-phosphatase
GPD	Glycerol-3-phosphate dehydrogenase
GRAS	Generally Regarded as Safe
GS	Glutamate synthase
HI	Hydrolysis index
Hm	Maximum height
HPLC	High performance liquid chromatography
I	Invertase
ID	Isocitrate dehydrogenase
IL	Isocitrate lyase
αKD	α-ketoglutarate dehydrogenase
L*	Lightness
M.	*Meyerozyma*
Mal	Maltose
MalT	Maltotriose

MD	Malate dehydrogenase
MS	Malate synthase
opt	Optimised
orig	Original
P.	*Penicillium*
p	Probability
PCA	Principle component analysis
PD	Pyruvate dehydrogenase
PDc	Pyruvate decarboxylase
pred	Predicted
QPS	Qualified Presumption of Safety
r	Correlation coefficient
RAS	Rapid available sugars
RH	Relative humidity
RID	Refractive index detector
RS	Resistant starch
RSM	Response surface methodology
RSR	Reducing sugars released
S.	*Saccharomyces*
SAS	Slowly available sugars
S-CoA-S	Succinyl-CoA synthase
SD	Succinate dehydrogenase
SSL	Sodium stearoyl lactate
T1	The time the dough needs to achieve Hm
TAC	Total available carbohydrates
TCA	Tricaboxylic acid
TD	Thermal desorption
TPA	Texture profile analysis
TS	Total starch
TTA	Total titratable acid
vit.	Vitamin
V_{lost}	The carbon dioxide volume released by the dough
$V_{retention}$	Carbon dioxide volume kept in the dough
V_{total}	Total carbon dioxide volume reached
WHO	World Health Organisation

Abstract

Yeast induced fermentation of dough is an important process in bread making and one of the oldest biochemical processes in the world. Surprisingly, our knowledge about the dough fermentation step in correlation to product quality parameters is scarce and still not completely understood. A literature review revealed that yeast fermentation can be used to improve technological, nutritional and sensorial quality characteristics of wheat breads. However less attention was drawn on the dough fermentation step using Baker's yeast, whereas yeast selection is an established part of the production process in beer and wine making. Initially, this thesis investigates the suitability of various *Saccharomyces cerevisiae* strains originating from the beverage industry in dough leavening to enhance bread quality characteristics compared to commercial Baker's yeast. The results revealed that various yeast strains showed large differences in technological bread quality parameters. Especially *S. cerevisiae* T-58 and s-23 showed the features desired for fermenting wheat flour such as adequate gas production and strong dough formation resulting in an increase of specific volume with a simultaneously reduction in hardness. In the case of bread, starch being the main carbohydrate source, glycaemic control is of great interest. Therefore the *in vitro* starch digestibility was analysed in comparison to Baker's yeast bread (100) for the predicted glycaemic index (pGI). pGI values were significantly lower for *S. cerevisiae* s-23 (71.6), wb-06 (63.0) and Blanc (77.9). PCA confirmed that the breads were quite different in terms of their technological properties, chemical composition and the resulting pGI. Yeast fermentation, next to the baking process, also plays a key role in the formation of aroma compounds. The impact of yeast strains on sensory characteristics, flavour and aroma profile was investigated by gas chromatography mass spectrometry after thermal desorption (GC-MS TD) and descriptive sensory analysis. The production of different aroma active compounds as well as the impact on technological parameters such as specific volume and number of cells significantly impacted the flavour profile and consumer acceptance. Due to their specific metabolite production, another important aspect was to adapt the process parameters (fermentation time and temperature). For that reason, response surface methodology (RSM) was used as a model design. A change in fermentation parameters showed further improvement of the breads mainly in terms of prolonged shelf life, lowered pGI and higher consumer acceptance. These differences, between the optimised and original procedures, were confirmed by PCA. The increasing fundamental knowledge about dough fermentation generates new opportunities for their use in the baking industry. Furthermore yeast can be used to replace or reduce the amount of expensive additives and dough improvers. This study also opens alternatives to better satisfy the high demand of consumers for an increasing variety of bread products by only changing the yeast culture.

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Best Friends live forever in the memories we keep!

Alan Lucid (1989 – 2017)

1. Chapter Introduction

1.1. Context and aims

Bread is one of the most important staple food products for the human diet. Yeast fermentation in leavening of dough – next to beer making – is one of the oldest biochemical processes in the world (Linko, Javanainen, & Linko, 1997). In 2001 a food consumption survey in Ireland showed that 99% of the participants had a mean intake of 139 g bread per day, with white breads and rolls covering 78 g/day. This amounts to an average of 50.7 kg bread, respectively 28.47 kg white bread and rolls per year (IUNA, 2001). However, changing eating patterns and a large variety of substitute products such as fast food and breakfast cereals have led to a decrease in bread consumption (Prättälä, Helasoja, & Mykkanen, 2001; Siega-Riz, Popkin, & Carson, 2000). Surprisingly, our knowledge is scarce concerning the dough fermentation process in correlation to product quality parameters and is not completely understood (Mondal & Datta, 2008). Only limited effort has been put in to the investigation of the technological performance of *Saccharomyces cerevisiae* referred as "Baker's yeast" in bread. The key role yeast strains play in a bread system are underestimated, and in contrast to brewer's and wine making yeast, less attention has been drawn to the dough fermentation step using Baker's yeast (Dequin, 2001). In Ancient times in Egypt and the Middle East, brewing and baking were closely linked and originally only one single strain was used for both processes. Until the nineteenth century, bakeries used yeast left over from the breweries for dough leavening. These days, microbial cultures are available which are genetically improved to better suit the need of these fermentation processes (Amendola & Rees, 2003). A literature review, as part of this study (Chapter 2) gives an overview of the impact metabolites produced during fermentation by *S. cerevisiae* have on bread quality characteristics. The comparison of strains used for brewing and baking revealed that brewer's yeast mainly focuses on alcohol production in a long fermentation process, and Baker's yeast concentrates on carbon dioxide production to ensure a uniform dough leavening in a relatively short fermentation process. By connecting the results of brewer's and Baker's yeast it could be possible and highly profitable to better understand the fundamental fermentation process. Therefore, the suitability of *S. cerevisiae* strains originating from the brewing industry were investigated in Chapter 3. The results can be used as a tool in dough leavening to enhance bread quality characteristics. The yeast strains used in this study are summarised in Table 1-1.

Table 1-1 Summary about important parameters for *Saccharomyces cerevisiae* strains used in this doctoral dissertation

S. cerevisiae	Application[1]	Temperature optimum [°C][1]	Fermentation time[1]	Flocculation [1]	% based on flour
Baker's yeast	Baked goods	-	Hours	Low	2
s-23	Lager	12-15 Lower temperature tolerance	Up to 14 days	High	4
T-58	Ale	15-20 Higher temperature tolerance	2-3 days	Powdery	2
us-05	Ale	15-22 Higher temperature tolerance	2-3 days	Medium	6
wb-06	Wheat beer	18-24	2-3 days Long storage	Low	2
Blanc	White wine	18-30	5-24 days	Low	½

[1] according to the specification sheet

As an important carbohydrate source, bread also plays big a role in human nutrition. Carbohydrates in general account for 45-70% of the total energy intake (Lafiandra, Riccardi, & Shewry, 2014). The FAO/WHO recommends a dietary carbohydrate intake of 50-75% of the total energy intake (Mann et al., 2007). Therefore, carbohydrates are important for the energy metabolism and glucose homeostasis in humans (Lau, Soong, Zhou, & Henry, 2015). The quantity and quality of consumed carbohydrates might be one aspect which has an influence on type 2 diabetes. The field of clinical nutritional studies is looking for methods to decrease the risk if certain non-communicable diseases, such as type 2 diabetes and cardiovascular diseases. Therefore, the impact carbohydrates have on the postprandial glycaemic response during digestion is measured as the glycaemic index (GI) (Jenkins et al., 2002).

The GI is defined as:

"The GI is the incremental area under the curve (AUC) of the blood glucose concentration occurring upon ingestion of a carbohydrate-containing food relative to a reference food (glucose or white wheat bread)"

Carbohydrate containing food products can furthermore be categorised based on their GI. There are three categories reaching from products with a low GI (GI< 55; nuts, dairy products, legumes, pasta), over intermediate GI foods (GI between 55 and 70; certain breads, muesli) and products with a high GI (GI > 70; white wheat bread, whole meal

barley flour) (Atkinson, Foster-Powell, & Brand-Miller, 2008). Factors affecting digestibility and therefore also the glycaemic response of bread can come both from the technological aspects of bread production as well as the used raw materials (Fardet, Leenhardt, Lioger, Scalbert, & Rémésy, 2006). Besides components of the food product, the technological processing of the breads also has an influence on the GI. The more a food product is processed, the higher the digestibility of the starch. The glycaemic response depends on indigenous factors of the food matrix (starch susceptibility, and protein and lipid content) as well as on the macroscopic structure of the food (botanical integrity of ingredients and physical texture). The rate of *in vitro* starch hydrolysis during a multi-enzyme dialysis system corresponded well with the postprandial blood glucose response (Singh, Dartois, & Kaur, 2010). Nowadays, the lowering of the GI in baked products is undertaken by the inclusion of whole kernels or intact grains. Therefore, Fardet et al., (2006) recommended a reduction in yeast quantity to increase the density of the end-product for the production of low-GI breads. Since consumers prefer a soft and flexible crumb which corresponds to a low hardness (Hager et al., 2012), the focus should concentrate on changing the chemical characteristics rather than the physical characteristics of wheat bread. This could be achieved by the application of different strains of *S. cerevisiae*. Therefore, the influence of different strains from the species *S. cerevisiae* on starch digestibility and glycaemic index was shown in Chapter 4 using a multi-enzymatic- *in vitro* -system to obtain predicted GIs.

However the application of different strains from the species *S. cerevisiae* can influence all quality characteristics of bread which are highly important for consumer acceptance. Not only the technological and nutritional parameters of the end product, but also aroma and flavour profile are affected by yeast addition. The aroma fraction of bread consists of about 600 volatile compounds reported by Schieberle & Grosch, (1991). Although a lot of these flavour and aroma compounds are formed during the baking process, also fermentation and yeast metabolism play a key role in the unique bread flavour (Hui, 2006). Essential contributors to the flavour of fermented foods are alcohols, aldehydes, esters, ketones and acids originating from the yeast metabolism (McKinnon, Gélinas, & Simard, 1996; Suomalainen & Lehtonen, 1978; Whiting, 1976). The main pathway responsible for aroma production by yeast is the Ehrlich pathway (Hazelwood, Daran, van Maris, Pronk, & Dickinson, 2008). In the beverage industry, it is quite common to use yeast as an

important parameter to alter the flavour and aroma profile of the end product (Pires, Teixeira, Branyik, & Vicente, 2014; Swiegers, Francis, Herderich, & Pretorius, 2006; Wondra & Berovič, 2001). However, in bread making only recently flavour and aroma profiles became more recognised as a quality parameter (Birch, Petersen, Arneborg, & Hansen, 2013; Birch, Petersen, & Hansen, 2014; Cho & Peterson, 2010; Pico, Bernal, & Gómez, 2015). Therefore, a commercial interest in the field of bread fermentation to alter flavour and aroma characteristics has arisen in recent years. In Chapter 5 the impact of yeast strains on aroma and flavour profile was investigated. Furthermore, a trained panel was used for descriptive sensory analysis to predict sensory acceptance.

Due to the specific metabolism and optimum conditions each starter cultures shows, fermentation time and temperature are important factors. In general, most often a fermentation time of 55 min is used with a temperature range between 33-54°C for the production of pan bread (Pyler & Gorton, 2010). Since the lack of knowledge about the impact of fermentation parameters in combination with the application of starter cultures next to *S. cerevisiae* Baker's yeast, response surface methodology (RSM) was used to investigate the optimal fermentation times and temperatures for every *S. cerevisiae* strain individually (Chapter 6) to enhance bread quality.

The aim of this study was to identify the possibility to use different *S. cerevisiae* strains originating from the brewing and wine making industry in a wheat bread system. The increasing fundamental knowledge about dough fermentation generates new opportunities for their use in the bakery technology. Using adapted fermentation parameters further allows to create breads with improved characteristics in case of technological, nutritional and sensorial characteristics to satisfy consumer needs. This doctoral dissertation opens opportunities to satisfy the high consumer demand for an increasing variety of bread by only changing the starter culture

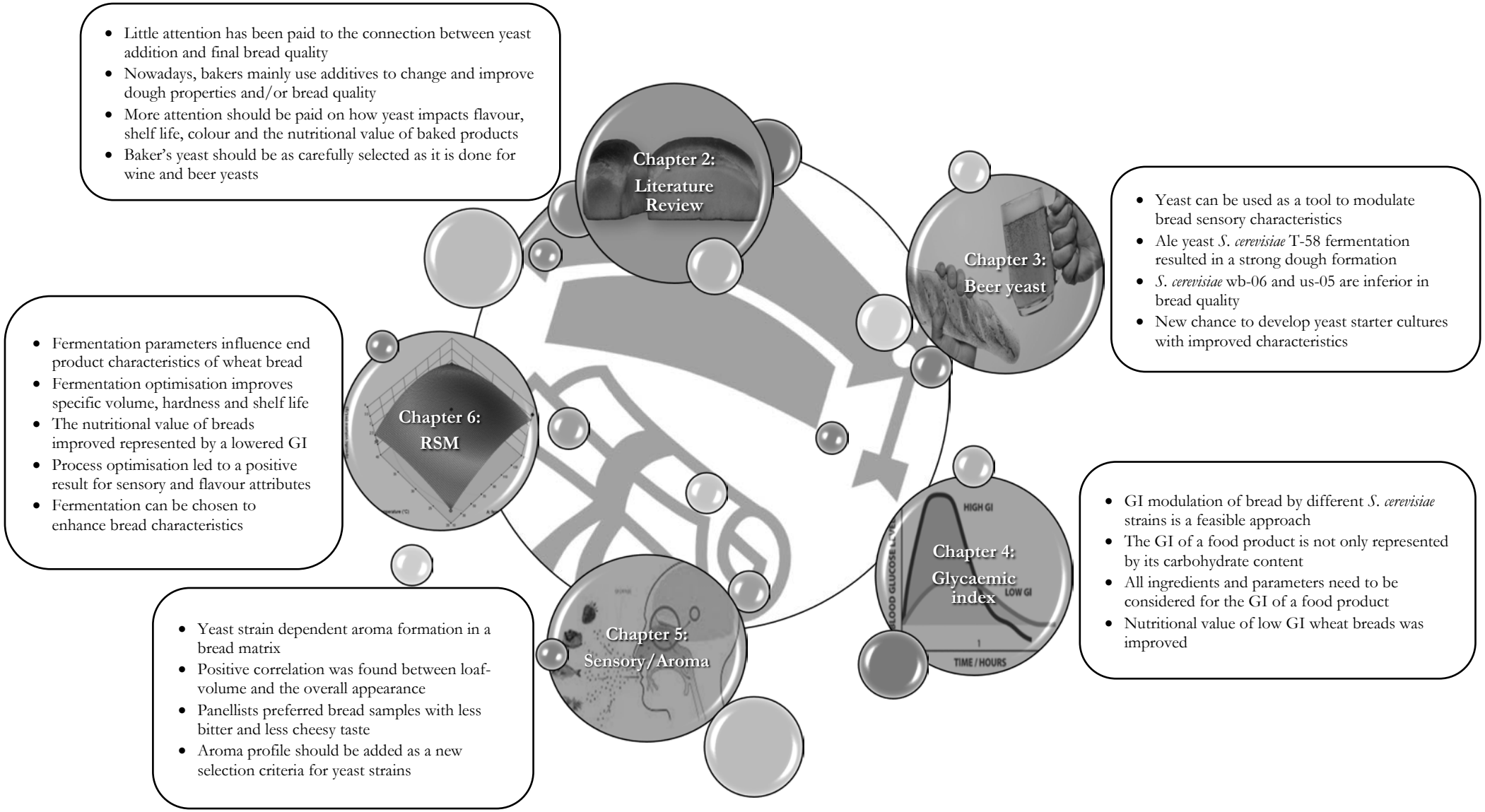


Figure 1-1 Schematic overview of the chapters in this doctoral dissertation

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2. Chapter Impact of *Saccharomyces cerevisiae* metabolites produced during fermentation on bread quality parameters: a review

Mareile Heitmann, Emanuele Zannini, Elke K. Arendt

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2.1. Abstract

Although bread making with the use of Baker's yeast has a long tradition in human history, little attention has been paid to the connection between yeast addition and the final bread quality. Nowadays, bakers mainly use different flour additives such as enzymes (amylases, hemicellulases, and proteases) to change and improve dough properties and/or bread quality. Another strategy is the use of modified industrial Baker's yeast. To date, there is no yeast strain used in the baking industry, which is genetically modified, despite some studies demonstrating that the application of recombinant DNA technology is a possibility for improved strains suitable for baking. However, due to the fact that the majority of consumers in Europe highly reject the use of genetically modified microorganisms in the production of food, other strategies to improve bread quality must be investigated. Such a strategy would be a reconsideration of the selection of yeast strains used for the baking process. Next to the common criteria, the requirement for adequate gas production, more attention should be paid on how yeast impacts flavour, shelf life, colour and the nutritional value of baked products, in a similar way to which yeast strains are selected in the wine and brewing industries.

2.2. Introduction

Bread making is one of the oldest biochemistry processes in the world. *Saccharomyces cerevisiae* also known as Baker's yeast is one of the main ingredients for bread making. The term "cerevisiae" (meaning beer) signifies closely linked relationship between beer and bread making, originating centuries ago in Egypt and the Middle East. Historically, the same strain of yeast was used for both processes. In the nineteenth century, yeasts' left over from the brewing industry were shared with the bakers for bread production. Today, thousands of different genetically improved microbial cultures are used for different applications, like baking, brewing and wine making. Although bread making has a long tradition throughout human history, little attention has been focused on the connection between yeast addition and the final bread product quality (Dequin, 2001; Mondal & Datta, 2008). To date, bakers mainly use different flour additives, such as enzymes (amylases, hemicellulases, and proteases) to change and improve dough properties and/or bread quality. Another strategy is the use of modified industrial Baker's yeast. During fermentation yeast produces mainly carbon dioxide and ethanol, but the role of yeast goes much deeper than just gas production (Randez-Gil, Sanz, & Prieto, 1999b), concerning the production of other secondary metabolites, which have an impact on the final product quality. Yeast affects the volume, structure, flavour, colour and shelf life of each fermented product (Fleet, 2007). The characteristic volume and aerated cell structure of bread are mainly influenced by the addition of yeast its metabolism and carbon dioxide production during fermentation. Due to the production of secondary metabolites through different metabolic pathways, yeast influences the flavour (by producing precursors such as esters, aldehydes and ketones), colour (carbohydrates, amino acids) and shelf-life (acids, glycerol) of baked products. All these metabolic products demonstrate the important role of yeast in bread making. Nevertheless, the most important characteristic, which is usually considered during strain selection is the ability to ferment sugars anaerobically with adequate gas production (Reed & Nagodawithana, 1991). In our opinion, the production of other metabolites by the yeast is underestimated when considering the selection of yeast strains. The purpose of this study is therefore to describe the impact of yeast in view of final bread quality parameters. This article reviews critically published literature on studies related to yeast and bread quality and identifies potential future investigations for applied yeast research with particular reference to the production of wheat bread. The

main intention of this study is to better understand the complex dough fermentation reactions performed by yeast and their impact on product quality. This may enable the adaption of new yeast strains including those currently used in other applications, such as the brewing and wine industries, which can specifically enhance bread properties and so the final bread quality. The present review further examines the metabolites produced by yeast during dough fermentation and their impact on bread quality parameters. This knowledge could help to create new procedures and criteria for yeast strain selection for application in bread making.

2.3. Yeast in bread making

Yeast is an ubiquitous, unicellular, asexual eukaryote belonging to the kingdom Fungi, which is able to ferment sugars (added or produced by enzymatic hydrolysis) into alcohol and carbon dioxide (Cauvain & Young, 2007b) and therefore is known as the leavening agent in baked goods. Their shape is typically spherical, oval or cylindrical with an average diameter of around 8 μm. The cells contain a double layered cell wall through which the cell is able to absorb nutrients and release metabolites. The main yeast strains related to bread making are from the species *S. cerevisiae* (Cauvain & Young, 2007b; Fleet, 2007). Fresh Baker's yeast comprises of 30-33% of dry materials, 40.6-58.0% of proteins, 35.0-45.0% of carbohydrates, 5.0-7.5% of minerals, 4.0-6.0% of lipids and several vitamins (vit.) (Bekatorou, Psarianos, & Koutinas, 2006). The European yeast industry produces 1 million tonnes of yeast annually of which around 30% is exported globally (http://www.cofalec.com/business-and-economy/). The annual growth rate of the global market is expected to be 8.8% from 2013 to 2018 (http://www.marketsandmarkets.com/Market-Reports/yeast-industry-268.html).

Typically, an aerobic fed-batch process with molasses as a nutrient source is used for the commercial production of yeast (Attfield, 1997). The process consist of growing, separating, washing and processing to remove extracellular and intracellular water by filtration or pressing (Randez-Gil et al., 1999b). To decrease damage to the yeast cells additives like emulsifiers and/or antioxidants are added during production. Growth conditions including temperature (25-30°C), moisture, and nutrients (starch, sugar) must be optimised. When yeast is grown outside these optimal parameters, a complex stress response occurs (Attfield, 1997). Stresses can cause direct and/or indirect cell damage

influencing the membrane permeability, inhibiting enzymes activity and result in the formation of reactive oxygen species. Cell responses include a decrease in intracellular pH through glycerol, formation of several antioxidant defences such as glutathione and increased membrane permeability for intracellular components. Stress response is an important factor for survival and growth in industrial applications (Attfield, 1997). Intensive biochemical, microbiological and technical knowledge has led to commercial Baker's yeast preparations which contain one or more strains from the species *S. cerevisiae*. Through the addition of sourdough, other species can be incorporated in the bread making process like *Pichia* and *Candida* (De Vuyst & Neysens, 2005). In general, a yeast used for the bakery industry should fulfil specific requirements with respect to the application and processing characteristics, such as adequate gas production to ensure a uniform dough leavening, tolerance to a wide range of pH, temperature and salt/sugar concentrations, as well as formation of desirable aroma compounds (Linko et al., 1997). Specialised brewers and/or distillers yeast could be incorporated in the bread making process, but because they are not adapted for the bread making process, it is common knowledge that they are unsuitable due to their different metabolism and tolerances (Cauvain & Young, 2007b). Nevertheless, from the safety point of view European Union regulations allow these yeasts for use in dough fermentation and bread production. In fact, the safety of food is a major concern of consumers. Therefore, regulations and safety assessment guidelines are available in the European Union. The Qualified Presumption of Safety (QPS) list summarises a wide variety of biological agents including bacteria, yeasts, fungi and viruses that may be used in the food and feed chain (EFSA, 2012). In the United States, food and substances used in food are regulated by the U.S. Food and Drug Administration and are summarised in the Generally Regarded as Safe (GRAS) status.

Interesting yeast species that could be used as an alternative to *Saccharomyces* include *Debaromyces*, *Kluyveromyces* and *Schizosaccharomyces*. Heitmann et al., (2015) recently studied the impact of different beer yeasts in comparison to Baker's yeast on wheat bread quality. This study showed that various beer yeasts are suitable for bread making and the resulting wheat bread showed both superior and inferior characteristics in comparison to the Baker's yeast control bread. Nowadays, yeast is produced in a huge variety of different forms throughout the world. However, these "domestic" yeasts are different from "wild

strains" due to genetic modification and adaption, which allows them to grow in inappropriate situations (Ali, Shehzad, Khan, Shabbir, & Amjid, 2012). The main formats in which yeast is available include fresh, compressed, active dry and instant active dry yeast. The difference between these formats is related to their physical appearance due to differences in moisture content. Reduction in moisture content is used to prolong the shelf life of the strain but such preservation methods have an impact on yeast performance factors such as metabolic activity, acid- and osmo-tolerance as well as temperature stability (Cauvain & Young, 2007b). Product shelf life ranges from 3 weeks (fresh and compressed yeast) to 1 (dry yeast) or 2 (instant dry yeast) years (Hui, 2006). Fresh and compressed yeasts are most commonly used in industry, since they are considered to be the most reliable. The format of the yeast also has an influence on the fermentation intensity. Fresh yeast produces the most carbon dioxide during fermentation resulting in superior dough-rising capacity. Considering the fermentation speed, the yeast acts in the following order: compressed yeast > instant active dry yeast > active dry yeast (Hui, 2006). Although fresh yeast is slightly dehydrated it doesn't need hydration time like dry yeast. The biggest problem is the shorter shelf life of fresh yeast in comparison to processed yeast. Instant yeast is available since the 1960s and is characterised by its very low moisture content and its fine particle size. In comparison to dried yeast, instant yeast can be directly added to the flour and its main use is for bread and pizza premixes (Cauvain & Young, 2007b). Some studies have already investigated the impact of these different formats on product quality parameters. Codina and Voica, (2010) studied the impact of different yeast (*S. cerevisiae*) forms on carbon dioxide retention using a rheofermentometer. They found that active dry yeast had the highest fermentation rate followed by compressed yeast and active instant dry yeast (Codina & Voica, 2010). Rollini et al., (2007) analysed four commercial compressed Baker's yeast (*S. cerevisiae*) strains which were originally used in different applications (pastries, bread, frozen doughs and Panettone) and tested them in complex dough formulations. They found that the different strains were indeed suitable for different applications in contrast to what was indicated by the producer. However, all their Baker's yeasts belonged to the species *S. cerevisiae* and they showed variations regarding their growth efficiency and gassing power.

To the authors' knowledge, there is no yeast strain used in the baking industry which is genetically modified. However Randez-Gil et al., (1999) showed that recombinant DNA technology is a possibility of constructing new strains with improved suitability for the baking industry.

2.4. Yeast vs. chemical leavening agents

Besides yeast, it is a common practice to produce leavened products using chemical leavening systems which produce carbon dioxide either through chemical decomposition using heat or through an acid-base reaction. For bakery products, particularly pastries, the two major gas producing chemicals used are sodium bicarbonate (baking soda) and ammonium bicarbonate (Amendola & Rees, 2003). Baking soda is a powerful leavening agent which starts as soon as it comes into contact with an acidic environment like batter or dough (Amendola & Rees, 2003). The disadvantages of these chemical leavening agents are the creation of off-flavours as well as an over browning. An advantage is the short production time; no fermentation step has to be included in the production, since some gas is already released at room temperature with the majority released during baking. In comparison to yeast the release of gas is much faster and the gas cells are therefore much bigger. A few products naturally containing acids can be used for leavening like lemon juice or sour milk. Other chemical leavening agents include salts of phosphoric acid such as aluminium phosphate, mono-calcium phosphate, sodium acid pyrophosphate and di-calcium phosphate. Mono-potassium tartrate (cream of tartar) and glucono-delta-lactone (GDL) can be also used. The common baking powder consists of a mixture of different acids, mostly in combination with baking soda and starch as a carrier to separate the acids and bases to prevent premature reactions (Hui, 2006). Due to the neutralising effect of the different ingredients, no off-flavour is left and the pH is not influenced (Amendola & Rees, 2003). Similar to yeast, chemical leavening agents affect the structure, colour, flavour and pH of the final product. Each leavening agent creates a slightly different texture, so when choosing the appropriate leavening agent, the reaction rate and desired effects in the finished products must be known (Hui, 2006). Plessas et al., (2005) produced leavened bread by using kefir grains instead of yeast or chemical leavening. The produced bread showed a smaller specific volume but better ability to retain moisture after production, with a firmer texture. Another advantage was

the lower acidity when using kefir grains with a positive effect on the mould-free shelf life. This study highlights that other substrates should be considered as leavening agents for baked goods beside Baker's yeast and chemical leavening agents.

2.5. Main Metabolic pathways

Yeasts are facultative anaerobes which means that they can grow with or without oxygen. In general, yeasts convert sugars into carbon dioxide, energy, biomass and ethanol in the presence of oxygen. In the absence of oxygen they use alcoholic fermentation to convert sugar into ethanol, carbon dioxide and glycerol. The dominant fermentation products, which have the greatest impact on bread quality are carbon dioxide and ethanol (Pronk, Steensma, & van Dijken, 1996; Trevelyan & Harrison, 1952). They are formed as soon as the yeast has been added to the dough/batter. *S. cerevisiae* also produces a range of other secondary metabolites as glycerol, organic acids, flavour compounds and precursors. The production of these compounds is linked to several different metabolic pathways, like glycolysis, alcoholic fermentation, the tricarboxylic acid cycle (TCA) and the glyoxylate cycle, which are summarised in Figure 2-1. The primary carbon metabolism is performed by glycolysis. During glycolysis the yeast produces energy by consuming low molecular weight sugars available in the dough (sucrose, maltose, glucose and fructose). Hexoses such as glucose and fructose, are the preferentially utilised sugars which enter the glycolytic metabolic pathway. However, glucose is preferred over fructose, since they are transported with the same carrier into the cell which has a greater binding specificity for glucose (Verstrepen et al., 2004). When glucose and fructose are consumed, the yeast starts to deplete maltose but without hydrolysing it to glucose, as Baker's yeast lacks the necessary enzyme. In beer production the most fermentable sugars are maltose, maltotriose and glucose. Again, glucose is the preferred sugar, but to obtain appropriate substantial alcohol content, the complete fermentation of maltose and maltotriose is also required. Consequently, the majority of brewing yeasts are able to ferment maltose and maltotriose after glucose. However, some yeast cells are not able to take up maltotriose for their metabolism which can lead to difficulties in beer brewing leading to lower ethanol yields or atypical beer flavours (Alves-Jr, Herberts, Hollatz, Miletti, & Stambuk, 2007). To utilise maltose the yeast requires an active transport system across the plasma membrane. Subsequently, the maltose is hydrolysed by glucosidase enzymes (G) into two

glucose molecules (Alves-Jr et al., 2007). The repression of the synthesis of glucosidase enzymes is a major concern in limiting the dough fermentation rate (Needleman, 1991) which is also the reason for a lag phase in the carbon dioxide production. Osinga et al., (1988) suggested a means of avoiding this lag phase by replacing the promoters of the maltose permease and maltase with constitutive promoters to increase the metabolic conversion of maltose. Other higher sugars like sucrose need to be degraded by invertase (I) before the yeast can use them for metabolism.

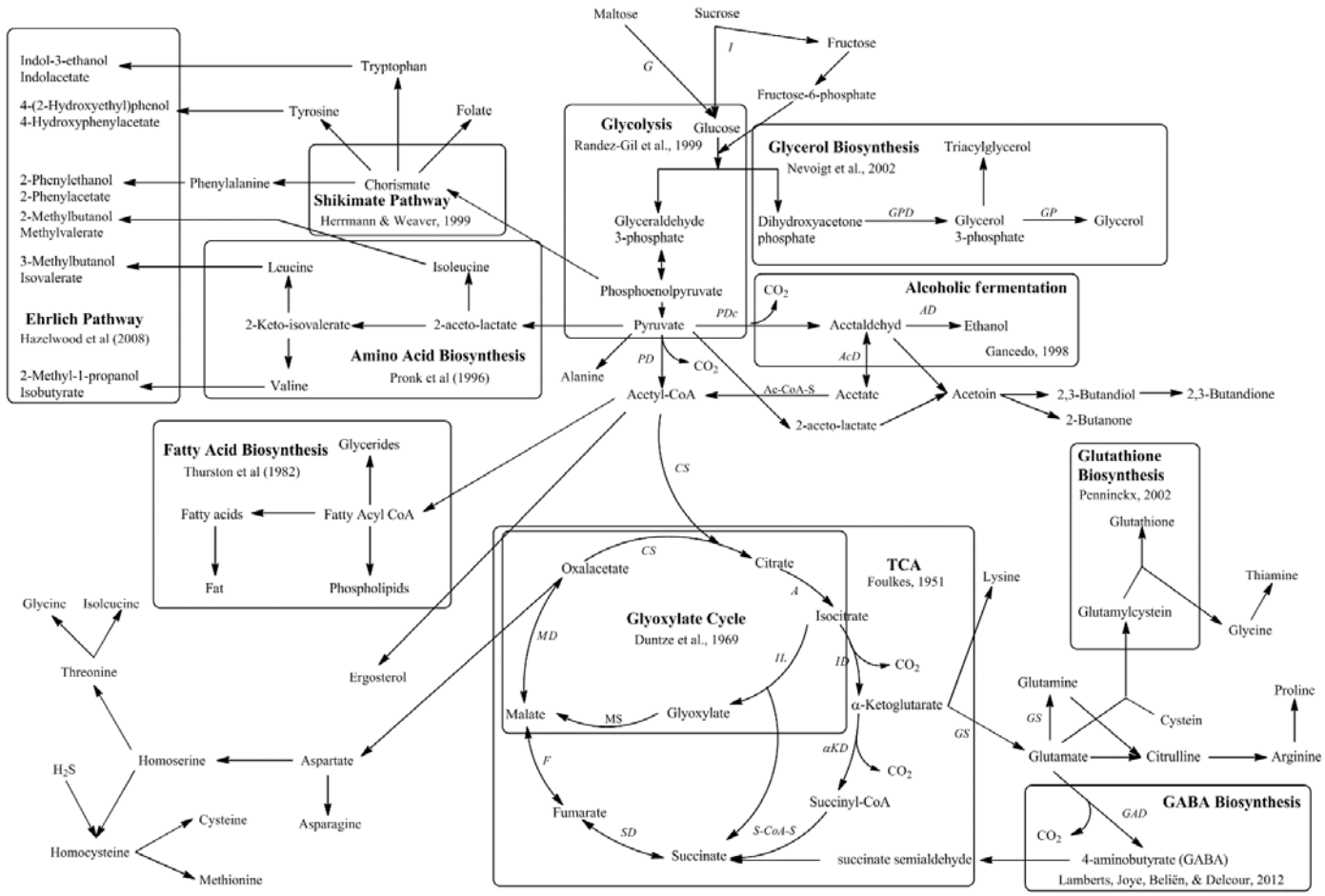


Figure 2-1 Schematic representation of the most important metabolic pathways, following the carbohydrate dissimilation, their enzymes (Abbreviation) and references in *Saccharomyces cerevisiae* influencing bread quality parameters

Therefore, the yeast harbours two different invertase enzymes. One invertase is located in the cytoplasm of the yeast cell and therefore it requires sucrose uptake. The second invertase is located between the plasma membrane and cell wall. The hexoses formed by this enzyme are taken up by hexose transport systems, and made available for yeast metabolism (Pronk et al., 1996). Codina and Voica, (2010) showed that after mixing no sucrose was left in the dough samples due to the presence of yeast invertase, which degraded the sucrose to glucose and fructose for yeast fermentation. Maltose concentration increases during dough fermentation due to activity of amylases found in wheat flour. A common pathway which is involved in all sugar-metabolising microorganisms is the lower part of the Embden-Meyerhof pathway and the formation of pyruvate (Koshland & Westheimer, 1950; Pronk et al., 1996). Pyruvate has a central position in many metabolic pathways as it can be seen in Figure 2-1. The production of pyruvate and, therefore glycolysis, plays a key role in the fermentation metabolism of yeast. The definition of glycolysis is well known as a sequence of ten enzyme-catalysed reactions, which converts sugars like glucose to pyruvate coupled with the production of ATP as an energy source. Di-hydroxy acetone phosphate as an intermediate in glycolysis and a precursor of glycerol, a compound which plays an important role in the cytosolic redox balance during anaerobic growth (Ansell, Granath, Hohmann, Thevelein, & Adler, 1997; Bakker et al., 2001; Nevoigt et al., 2002; Nevoigt & Stahl, 1997; van Dijken & Scheffers, 1986). Di-hydroxy acetone phosphate is reduced to glycerol-3-phosphate by glycerol-3-phosphate dehydrogenase (GPD) and finally dephosphorylated to glycerol by glycerol-3-phosphatase (GP) (Nevoigt et al., 2002; Sigler & Hofer, 1991). In addition, during growth of yeast, pyruvate is transformed into many different compounds, such as carbon dioxide, ethanol and other organic metabolites, which have an influence on bread quality (Pronk et al., 1996). Since yeast favours an alcoholic fermentation metabolism over respiration ("Crabtree effect") (De Deken, 1966), in the presence of high sugar concentrations the main metabolic pathway which must be considered is the alcoholic fermentation, starting from pyruvate (Fiaux et al., 2003; Gancedo, 1998). This "Crabtree effect" can cause several problems, such as an incomplete fermentation, production of off-flavours, undesirable by-products and loss of biomass yield (Verstrepen et al., 2004). During alcoholic fermentation ethanol is produced, via pyruvate decarboxylase (PDc),

after conversion of pyruvate into acetaldehyde and carbon dioxide. Further, alcohol dehydrogenase (AD) reduces acetaldehyde to ethanol, by oxidation of NADH.

Another important metabolite is acetyl-CoA, which can be formed in two different pathways; either from pyruvate (glycolysis) or acetaldehyde (alcoholic fermentation). In the latter pathway, acetaldehyde is oxidised to acetate by acetaldehyde dehydrogenase (AcD). Acetate is converted to acetyl-CoA by acetyl-CoA synthase (Ac-CoA-S). With lowering sugar concentrations the yeast switches their metabolism from alcoholic fermentation to respiration which utilises the tricarboxylic acid cycle (TCA), known as "diauxic shift" (DeRisi, Iyer, & Brown, 1997; Foulkes, 1951; Galdieri, Mehrotra, Yu, & Vancura, 2010; Gasch & Werner-Washburne, 2002). The production of acetyl-CoA from pyruvate is performed by pyruvate dehydrogenase (PD). Acetyl-CoA can be used for the production of fatty acids and fat. Thurston et al., (1982) showed that fatty acids are mainly produced during the first four hours of fermentation in beer with a four-fold increase over this time. Another fate of acetyl-CoA is its funnelling into the TCA cycle within the mitochondria, with the ultimate production of secondary metabolites and additional carbon dioxide. By definition, the TCA cycle is known as a series of chemical reactions used for carbon dioxide and ATP generation through oxidation of acetate. In this cycle, pyruvate is oxidised to carbon dioxide and water with the concomitant production of ATP. Most of the carbon dioxide involved in dough fermentation comes from alcoholic fermentation due to the "Crabtree effect" of yeast. The primary role of the TCA cycle is production of additional ATP. The expression of genes involved in the TCA cycle is down regulated during the first 30 min of dough fermentation, however, because of the presence of glucose, these enzymes still have a low level activity which explains the production and excretion of organic acids such as citrate, malate and succinate. Several research groups showed that the such organic acids are produced in the TCA cycle (Arikawa, Kobayashi, et al., 1999; Arikawa, Kuroyanagi, et al., 1999; Whiting, 1976). Another enzyme in the TCA cycle is aconitase (A), which converts citrate into isocitrate (Gangloff, Marguet, & Lauquin, 1990). Aconitase is located in the mitochondria but also in the cytosol as part of the glyoxylate cycle (Duntze, Neumann, Gancedo, Atzpodien, & Holzer, 1969; Regev-Rudzki, Karniely, Ben-Haim, & Pines, 2005). The enzyme isocitrate dehydrogenase (ID) oxidases isocitrate to α-ketoglutarate, with the production of carbon dioxide, which also represents the starting point of glutamate metabolism. Via the

reductive pathway of the TCA cycle, beginning from oxaloacetate, malate and fumarate can be produced (Arikawa, Kobayashi, et al., 1999). Further oxidation to succinyl-coenzyme A is catalysed by α-ketoglutarate dehydrogenase (αKD) with the production of carbon dioxide. Beside the TCA cycle, the formation of succinate and malate by the enzymes isocitrate lyase (IL) and malate synthase (MS) can take place in the glyoxylate cycle which occurs in the cytosol (Arikawa, Kuroyanagi, et al., 1999; Fernandez, Moreno, & Rodicio, 1992). In addition to the production of glycerol, ethanol and organic acids, the yeast is able to produce free amino acids using the Ehrlich Neubauer-Fromherz pathway, which is linked to the shikimate pathway (Herrmann & Weaver, 1999; Maga & Pomeranz, 1974). Amino acid biosynthesis is controlled by about 30 enzymes involving different pathways (Pronk et al., 1996). Coming from the amino acids flavour formation takes place. It can start with a deamination of free amino acids like valine, leucine, phenylalanine or tryptophan followed by a decarboxylation, which can produce aldehydes. These aldehydes can be reduced to higher alcohols (isobutyl alcohol, isoamyl alcohol, phenylethanol) or transformed into acids by oxidation (Maga & Pomeranz, 1974). In general, the biosynthesis of higher alcohols commences with a transamination reaction of amino acid such as valine, leucine and phenylalanine and is catalysed by aminotransferases. The produced α-keto acid is further converted by decarboxylation into fusel alcohols, and finally reduced to higher alcohols via the Ehrlich pathway (Hazelwood et al., 2008; Procopio, Qian, & Becker, 2011). In addition, the corresponding organic acids can be produced, like phenylacetate, hydroxyphenylacetate, or isobutyrate (Hazelwood et al., 2008).

2.6. Loaf volume/Cell structure

Achieving a desired loaf volume by yeast fermentation is only possible by providing a favourable environment for yeast growth and for formation of gluten matrix that enables maximum gas retention (Sahlström, Park, & Shelton, 2004). The gas bubbles, which are incorporated in the dough after mixing, grow during fermentation until the liquid dough phase is saturated with carbon dioxide. This growth leads to expanding of the dough and thinning of the dough matrix between the gas cells. If over-fermentation occurs the dough is not capable of retaining the additional gas produced by the yeast and the gas bubbles fracture, which leads to a lower bread volume. The gas holding capacity is an important

characteristic for determining the bread quality and suitability of yeast for baking. The more gas is entrapped in the dough, the smaller the gas cells and the higher their distribution after proofing. These gas cells can resist more strength before they rupture, which leads to lower extensibility and a higher specific volume (Dobraszczyk, 2003; Sroan, Bean, & MacRitchie, 2009; Verheyen, Jekle, & Becker, 2014). During the baking process, the ethanol produced evaporates with some of the water, which helps to develop the aerated structure of the cell crumb. It is well known that dough mixing time can be reduced by adding instant active dry yeast, due to an effect on the gluten network development (Pyler & Gorton, 2008b). In dried yeast some non-viable cells are present which release glutathione as a stress response (Penninckx, 2002; Reed & Nagodawithana, 1991; Verheyen et al., 2015). Rheological dough properties are influenced by oxidising and reducing agents, which have an effect on the glutenin subunits that are linked by disulphide bonds and can affect their degree of polymerization (Delcour & Hoseney, 2010). The release of glutathione has a strong reducing effect and therefore increases the rate of thiol-disulphide interchange reactions which leads to a modification of the viscoelastic gluten network (Verheyen et al., 2015). As a result, gluten proteins with reduced size and lower molecular weight are present (Delcour & Hoseney, 2010). For the reason, that rheological dough properties are strongly influenced by thiol-disulphide exchange reactions; by removing thiol groups the dough gets stronger (Frater & Hird, 1963). Strong and weak flours differ in their amount of protein-bond glutathione. Li et al., (2004) measured 10 different flours varying in their amount of protein-bond glutathione; Only 5 flours resulted in bread doughs showing a strong performance. They reported that flours with a significantly higher amount of protein-bond glutathione result in a strengthening effect on the dough and therefore a stronger gluten-network development and better bread characteristics. Moreover, yeast is able to produce glycerol and pyruvic acid in the early stage of fermentation (Whiting, 1976). Glycerol has a positive effect on the texture of bread, especially during freezing. Corsetti et al., (2000) reported that the addition of glycerol reduces the firming of baked products during storage.

2.7. Flavour and aroma

Aroma and flavour are important quality parameters of bread. These are mainly affected by ingredients and secondary fermentation products produced by yeast and generated

under baking conditions (Birch, Petersen, & Hansen, 2013; Frasse, Lambert, Richard-Molard, & Chiron, 1993; Gassenmeier & Schieberle, 1995; Maeda et al., 2009; Schieberle & Grosch, 1991). The most influential compounds are volatile metabolites like alcohols, aldehydes and ketones and non-volatile compounds like acids, esters, sugars, polyphenols, free fatty acids and lipids (Hui, 2006). Non-volatile compounds act mainly as precursors for reactions that form new flavour compounds (Hui, 2006). Sugars remaining from the fermentation have an effect on aroma due to their high reactivity in Maillard reactions (Nilsson, Öste, & Jägerstad, 1987). The Maillard reaction is a complex mechanism, between reducing sugars like maltose, glucose and fructose and amino acids like leucine and phenylalanine, peptides and/or proteins during baking, influencing the colour, flavour and nutritional properties of baked products (O'Brien, Morrisey, & Ames, 1989). Dough fermentation with yeast results in a decrease of the concentration in free amino acid content. An increased amount could influence the aroma through Maillard reactions and the Ehrlich pathway. Some volatile compounds are lost during baking, while others form complexes with various dough constituents, thus affecting the flavour profile of the final product. Not all components which contribute to the overall flavour and aroma of yeast leavened breads have been identified thus far. The total number of contributing components is enormous and their specific interactions in flavour and aroma formation are still not fully understood (Reed & Nagodawithana, 1991). A few authors have reviewed flavour formation in bread (Cho & Peterson, 2010; Maga & Pomeranz, 1974; Pyler & Gorton, 2008a; Rothe, 1988). Most of the compounds responsible for aroma formation in bread crumb made from yeast fermented dough result from yeast metabolism (Frasse et al., 1993; Schieberle & Grosch, 1991), whereas the aroma compounds of the crust are products of Maillard reactions (Purlis, 2010). The most significant compounds reported in the literature are alcohols and aldehydes such as 2,3-butanedione and 3-hydroxy-2-butanone and esters which are produced by yeast cells using the Ehrlich Pathway to degrade amino acids (Hazelwood et al., 2008). Nowadays in the baking industry the trend is to use a short bread making process in terms of fermentation, whereby the development of aroma and flavour is very limited (Cauvain & Young, 2007b; Maeda et al., 2009). The application of different bacterial starter cultures, such as wine or beer yeast could compensate for these short fermentation process and produce flavour and aroma during such short fermentations (McKinnon et al., 1996;

Suomalainen & Lehtonen, 1978). Research on alcoholic beverage fermentation and production reveals that the choice of starter cultures is an important factor, related to the formation of aroma and flavour in the final product (Procopio et al., 2011; Suárez-Lepe & Morata, 2012). Several studies have dealt with the effects of yeast on aroma development during the production of wine and beer (Molina, Swiegers, Varela, Pretorius, & Agosin, 2007; Saerens, Verbelen, Vanbeneden, Thevelein, & Delvaux, 2008). In these industries, yeast identification and strain characterisation is essential, due to the wide variety of different flavour and aroma profiles yeast can impart (Dashko et al., 2015; Furdíková, Makyšová, Ďurčanská, Špánik, & Malík, 2014; Huang et al., 2010; Pires et al., 2014; Vararu, Moreno-Garcia, Zamfir, Cotea, & Moreno, 2016). In the recent years the aroma of bread gain more focus and recognition as an important bread quality parameter (Birch, Petersen, Arneborg, et al., 2013; Birch et al., 2014; Birch, Petersen, & Hansen, 2013).

Birch et al., (2013a) studied the influence of seven commercial compressed Baker's yeasts on the formation of bread aroma using dynamic headspace extraction. They found significant differences in the aroma profile of the bread crumb by varying fermentation time, between the breads. Furthermore, they stated that the choice of Baker's yeast is a very important decision for the bakers with respect to fermentation activity and aroma formation potential. Another study by the same group showed that with increasing yeast concentration, the main flavour components like 2-methyl-1-propanol, 2-phenylethanol, phenylacetaldehyde, 2,3-butanedione, ethyl acetate, ethyl 3-methylbutanoate, ethyl hexanoate, ethyl octanoate and phenyl-ethyl acetate increase concomitantly (Birch, Petersen, & Hansen, 2013). On the other hand, an increase in fermentation temperature caused an increase in lipid oxidation products, which are often described as off-flavours. However, their formation is independent of yeast concentration. It was suggested that short fermentation time at low temperatures and high yeast concentrations could be used to develop a bread with a high concentration of aroma compounds and less off-flavour. Thurston et al., (1982) suggested a relationship between yeast's fatty acids and the aromatic profile of fermented foods. Such fatty acids have been shown to contribute to the production of fatty acid ethyl esters especially in beer. They are connected to the yeast cell wall and can be released when yeast cells dies. Fatty acid esters are secondary metabolites produced by yeast and many bacteria, which play a key role in the flavour of

alcoholic beverages. Ethyl esters of short and medium fatty acids are important flavour compounds characterised by their strong fruity flavour. Beside their application in food and beverage production, they are also used by the cosmetic and pharmaceutical industries.

2.8. Colour

Another important attribute for consumer's acceptability is colour. Surface colour is of considerable importance in the baking industry (Pathare, Opara, & Al-Said, 2013; Purlis, 2010; Zanoni, Peri, & Bruno, 1995) as it is the first parameter assessed by consumers. Colour formation depends on physico-chemical characteristics such as moisture, pH, sugar concentration, amino acid content and the process conditions used during production, like baking temperature, fermentation time and temperature and starter culture (Zanoni et al., 1995). Colour formation results due to chemical, biochemical, microbial and physical changes, which arise during production (Pathare et al., 2013). Colour formation on the crust develops mainly throughout baking due to chemical changes via the Maillard reactions (Purlis, 2010). Maillard reaction occurs between proteins and carbohydrates at temperatures higher than 50°C at a pH range of 4-7. Another important reaction is caramelisation (Kroh, 1994; Zanoni et al., 1995), which is the direct degradation of sugars and starch occurring in high-sugar foods at higher temperatures, >120°C or 9<pH<3 (Kroh, 1994; Zanoni et al., 1995). Both reactions appear concurrent and depend on the type of sugar and amino acids present as well as the pH and water activity of the product (Zanoni et al., 1995). The residual reducing sugars remaining after fermentation strongly influence the crust and crumb colour (Finot, 1990; O'Brien et al., 1989). Due to increased mobility of reactants, the reaction rate increases exponentially with higher moisture content, up to a maximum at 30% moisture (Wolfrom & Rooney, 1953; Wolfrom, Schuetz, & Cavalieri, 1948). Both the initial pH of the product and the buffering capacity of the system influence the rate and direction of the reaction. The rate of browning is low at acidic pH values and intensifies with increasing pH to a maximum at a pH of ~10 (Ashoor & Zent, 1984; Wolfrom, Kolb, & Langer, 1946). In general, the rate of Maillard reaction is higher if excess reducing sugars are present rather than excess amino compounds (O'Brien et al., 1989). Crust colour can be also controlled by using different starter cultures. Alpha amylase activity is the main

reaction to be considered in relation to crust colour formation, due to the production of increasing amounts of maltose and dextrins, which participate both in the Maillard and caramelisation reactions. Use of different starter cultures can have an influence on colour formation due to differences in sugar metabolism (Goesaert et al., 2005; Heitmann et al., 2015; Ormrod, Lalor, & Sharpe, 1991).

2.9. Shelf life

Shelf life is a parameter relating to the loss of perceived freshness. This can be correlated to several different factors which are summarised in two different categories, staling and microbial spoilage. These parameters will be discussed further in the next two paragraphs.

2.9.1. Staling/Hardness/Firmness

Modifications in crumb structure due to changes other than spoilage organisms, such as chemical and physical changes of the crust (soft, leathery) and crumb (hard, dry, and crumbly) is referred to as staling (Kulp & Ponte, 1981). Bread staling is mainly associated with the firming of the crumb, which is an important factor in terms of consumer acceptability (Pateras, 2007). Although bread staling is not yet completely understood, the baking industry uses different anti-staling agents to inhibit staling. These include enzymes, alcohol, lipids, emulsifiers and sweeteners (Hui, 2006; Pateras, 2007). In particular, alpha-amylase is well known to retard crumb firming (Giménez et al., 2007; Hui, 2006; Pateras, 2007). Lipases, lipoxygenases, endoxylanase, arabinosidase and protease are also known to prevent bread staling due to a crumb softening effect. Heitmann et al., (2015) examined the effect of different yeast strains on bread hardness during storage. By using different starter cultures they were able to produce a significant change in crumb hardness, explained by the negative correlation of $r = -0.90$ ($p < 0.05$) between crumb hardness and specific volume. It is known that breads produced with a bulk fermentation step have a longer shelf life, due to larger amounts of alcohol produced during fermentation. Some studies examining the effect of ethanol on bread staling, showed that the crumb modulus of bread, treated with ethanol, increases during storage at a slower rate than the control bread using a differential scanning calorimeter and crumb compressibility measurement (Fearn & Russell, 1982; Russell & Chorleywood, 1983). Russell and Chorleywood, (1983)

showed, that bread treated with ethanol firms at a slower rate than control bread. Increasing sugar and salt levels are also known to slow the staling of baked products (Cairns, Miles, & Morris, 1991; Taylor, Maga, & Pomeranz, 2009). I'Anson et al., (1990) reported a decreasing effect of ribose> sucrose> glucose on the retrogradation of wheat starch, but the full mechanism of action is not fully understand. It is suggested that sugars are able to increase the glass transition temperature and concurrently decrease the diffusion of polymers to a crystal nucleus (I'Anson et al., 1990). On the other hand Levine & Slade, (1990) stated that sugars increase the glass transition temperature of the amylose matrix and therefore the re-crystallization of amylopectin is repressed. Glycerol has been reported to influence moisture distribution and staling of bread during storage. Yeast leavened breads show a higher water content than unleavened breads which results in more carbon dioxide and therefore a coarser bread crumb (Mondal & Datta, 2008).

2.9.2. Microbial spoilage

Microbial spoilage is another important factor when considering bread shelf life due to post-processing contamination (Pateras, 2007). Microbial spoilage is commonly caused by microorganisms belonging to the species *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus*, *Mucor*, *Endomyces* and *Cladosporium* (Legan, 1993). Aside from the economic losses caused by these microorganisms, consumers are concerned about the potential mycotoxins produced by these microorganisms. Mycotoxins can cause severel health problems in humans (Legan, 1993; Pateras, 2007). The parameters determining the microbial shelf life are water activity, pH and storage conditions. The most common method for preventing mould growth is the application of chemical preservatives such as propionic acid and its salts or potassium sorbate. However, the current trend is towards production without the use of additives. One solution is the incorporation of sourdough as a natural bio-preservative to increase the mould-free shelf life of baked products. Lactobacilli produce weak organic acids, other low molecular weight compounds, peptides, cyclic dipeptides and proteins, which are known for their antifungal activity (Axel et al., 2015; Axel, Zannini, Arendt, Waters, & Czerny, 2014; Magnusson & Schnürer, 2001; Niku-Paavola, Laitila, Mattila-Sandholm, & Haikara, 1999; Okkers, Dicks, Silvester, Joubert, & Odendaal, 1999; Röcken & Vorsey, 1995; Stiles, 1996). Physical methods of prolonging the shelf life are modified atmosphere packaging, pasteurisation or irradiation of packed

bread (Legan, 1993; Pateras, 2007). Some spoilage organisms such as spoilage yeast cause off-odours. Post-processing contamination is likely due to physical contact with contaminated equipment. The two main types of yeast associated in spoilage are filamentous yeast ("chalky moulds") and fermentative yeast (Berni & Scaramuzza, 2013; Pateras, 2007). *S. cerevisiae* is the most common fermentative yeast spoilage organism, characterised by an alcoholic or estery off-odour. Filamentous yeast like *Pichia burtonii* form white colonies on the surface of bread. This growth can be easily referred to as mould (Legan, 1993; Pateras, 2007). Berthels et al., (2004) identified yeast strains with a discrepancy in their consumption preference for glucose and fructose, and found the ability of such strains to reduce residual fructose levels and increase ethanol yield to be helpful in partially solving the spoilage problem. They suggested use of such strains as a criteria for selection of new yeast strains for wine production. Heitmann et al., (2015) demonstrated the effect of different *S. cerevisiae* strains, originating from the brewing industry, on the shelf life of white wheat bread. They showed both inferior and superior behaviour in terms of mold-free shelf life of the breads, which ranged between 3 and 5 days. They also demonstrated different abilities to propagate mould on breads baked with the different *S. cerevisiae* strains.

It is well known that some fungi and bacteria are able to produce secondary metabolites with antifungal properties. Nowadays most of the attention has been given to antifungal lactic acid bacteria present in sourdough bread and little attention has been given to yeast as possible producers of antifungal compounds. Yeasts, however, are promising candidates as fungicides. Coda et al., (2013) screened 146 different yeast strains (genera including *Candida, Metschnikovia*, *Debaromyces*, *Pichia* and *Kazachstania*) focusing on antifungal activity against *Penicillium roqueforti*. They found six *Meyerozyma guilliermondii* with noticeable *in vitro* activity. Their work showed the possibility of extending shelf-life of baked goods using *M. guilliermondii* LCF1353 as a mixed starter while maintaining optimal taste and structure at the same time. In another study the same group demonstrated, similarly, the potential of *Wickerhamomyces anomalus* as a mixed starter to extend the shelf-life of baked goods (Coda et al., 2011). Mo & Sung, (2014) investigated *Pichia anomala* SKM-T, which is known for its antagonistic properties against some spoilage moulds like *Penicilium paneum* KACC44834, and found it to be suitable as a leavening agent for the production of white pan bread. The bread containing this strain exhibited less *P. paneum*

spoilage colonies on the surface than bread baked with *S. cerevisiae*, due to a production of the flavour compounds 2-phenylethyl acetate, 2-phenylethyl alcohol, 2-decenal and nonanal, which enabled a shelf life extension. The production of antifungal compounds is considered in the selection of yeast strains for wine production. However, it is not a characteristic considered when choosing Baker's yeast.

2.10. Nutrition

Since ancient times, cereals have been a staple food in the human diet. They are considered as an important source of energy and supply macronutrients including complex carbohydrates, fibre, protein as well as micronutrients such as calcium, phosphorus, iron, sodium, magnesium and potassium. Cereal grains can be considered a source of vitamins, especially B vitamins like thiamine (vit. B1), riboflavin (vit. B2) and niacin (vit. B3) (Cauvain & Young, 2007b). Yeast represent a nutritional source of carbohydrates, fats, vitamins, especially B vitamins, minerals and amino acids, in particular, lysine (Rincón & Benítez, 2001). Studies on the effects of cereal fermentation on nutritional quality are scarce. Due to bread being a staple food it represents an important means to supplementing human nutrition. During fermentation yeast can have an effect on the levels of vitamins, phenolic compounds, phytates and folates, which is discussed more detail below.

During the production of yeasted bread, a 48% loss of thiamine (vit. B1) and pyridoxine (vit. B6) were observed (Batifoulier, Verny, Chanliaud, Rémésy, & Demigné, 2005). However, a longer fermentation increased the levels again. Compared to thiamine (vit. B1) and pyridoxine (vit. B6), folate (vit. B9) showed good stability during bread making and an increased amount could be found in comparison to the flour (Osseyi, Wehling, & Albrecht, 2001). The content of thiamine (vit. B1) has also been reported to decrease in the wheat and rye baking process, (Martinez-Villaluenga et al., 2009) but to increase with a longer fermentation time (Batifoulier et al., 2005). The fermentation step can therefore have an effect on the overall formation or retention of vitamins during baking. A short baking process was also presented to reduce the content of thiamine (vit. B1) in whole-wheat, but a prolonged yeast or sourdough fermentation maintained its levels (Batifoulier et al., 2005). Batifoulier et al., (2005) also found that the thiamine content was increased when fermentation time was prolonged and that the increase was significantly higher in

white bread with yeast compared to sourdough, despite comparable vitamin production by the microorganisms (0.25 mg/g dry matter). Long fermentations could support a net synthesis of thiamine by yeast, while fermentation with lactic acid production in sourdough bread could origin in a decrease of thiamine (Khetarpaul & Chauhan, 1989). In contrast to these findings, (Rucker, Suttie, & McCormick, 2006) reported a 35% loss of thiamine during bread making. A small amount of the riboflavin (B2) in bread derives from yeast. As a result, bread often contains more riboflavin than the original flour. Sourdough fermentation does not lead to any enrichment of riboflavin (Batifoulier et al., 2005). Whole-wheat bread making with yeast (from kneading to final bread) undergoing a long fermentation process, resulted in a 30% enrichment in riboflavin. The use of yeast and sourdough during fermentation did not show a synergistic effect on B vitamin levels (Batifoulier et al., 2005), but a longer fermentation time could increase the level of pyridoxine (Batifoulier et al., 2005). Yeast fermentation has been shown to result in an increase of folate in the baking process of wheat and rye breads (Kariluoto et al., 2006). Kariluoto et al., (2006) investigated the ability of yeasts and lactic acid bacteria to have an influence on the folate content in a rye sourdough and showed that the effects of sourdough bacteria are negligible. Proofing does not influence the total folate content but changes in vitamin distribution were observed. Folate losses during baking were about 25% (Kariluoto et al., 2004). However, the synthesis of folate by yeast results in an increase of the content over three-fold in bread. Another important advantage of yeast fermentation is the reduction of phytates (phytic acid) by phytase activity which results in an increase of the bioavailability of magnesium and phosphorus. However, phytase activity depends on the substrate flour, proofing temperature and time as well as dough pH and the amount of yeast (Pozrl et al., 2009). Commercial Baker's yeast has been shown to express phytase activity (Tu, Sandberg, Carlsson, & Andlid, 2000). A wide variation in phytase activity was identified in sourdough starters containing both yeast and lactic acid bacteria (Chaoui, Faid, & Belhcen, 2003; Reale et al., 2004). Another potential suggestion was the use of high-phytase yeast strains to act as phytase carriers in the gastrointestinal tract. The reduction of phytic acid has repeatedly been reported in yeast and sourdough processes. Although yeast fermentation reduces the unfavourable effects of phytic acid, sourdough bread seems to be a better source of available minerals, especially magnesium, iron and zinc (De Angelis et al., 2003; Turk, Carlsson, & Sandberg, 1999). Therefore, it

should be possible to control the phytase activity by modifying the process conditions or by selecting specific microbial starters. Losses have been observed for tocopherol (vit. E) during sourdough preparation and dough making (Wennermark & Jägerstad, 1992). Katina et al., (2007) observed reduction in tocopherol (vit. E) and tocotrienol (vit. E) content. This may have been due to oxygen sensitivity. Fermentation has been shown to increase the antioxidant activity in the methanol extracted fraction of rye sourdough, concurrent with increased levels of easily extractable phenolic compounds (Katina et al., 2007). A reduction in kneading time combined with a longer fermentation time could be able to retain carotenoids and vitamin E contents. Yeast fermentation of rye bran also showed an increase of free ferulic acid (Katina et al., 2007). Rye breads baked with sourdough also showed an increase in the antioxidant capacity in comparison to white wheat bread. The highest values were reported for breads using wholemeal flour (Martinez-Villaluenga et al., 2009; Michalska et al., 2007). Recently, it was shown that a yeast fermentation using wheat bran together with cell wall hydrolytic enzymes increased the bioaccessibility of phenolic compounds in breads as well as the metabolite 3-phenylpropionic (Anson et al., 2009). An increase in free ferulic acid was observed as a result of dough mixing and proofing (Poutanen, Flander, & Katina, 2009). However, the amount of released ferulic acid was about 1% of the total amount of ferulic acid originate from wholemeal rye. An increase of the levels of total phenolic compounds and free phenolic acids could be found by sourdough and yeast fermentation of wholemeal rye (Katina et al., 2007). In contrast, Boskov Hansen et al., (2002) did not observe a significant change in the content of phenolic acids during dough proofing. Baking showed a slightly increase of the concentration of phenolic compounds in the crust, probably through Maillard reaction (Gélinas & McKinnon, 2006). However, this effect was not detected in wholemeal bread (Boskov Hansen et al., 2002; Dewettinck et al., 2008; Gélinas & McKinnon, 2006). One other study used different yeast strains for the production of selenium enriched baked products. Stabnikova et al., (2008) used a yeast, which biomass was enriched with organic forms of selenium, to increase the amount of selenium in bread. The non-protein monocarboxylic acid, γ–aminobutyric acid (GABA), plays an important role in the animal and human nervous system as a neurotransmitter. An increased intake of GABA can be related to different health benefits, such as lowering of blood pressure, prevention of diabetes, inhibition of leukaemia cell proliferation and

cancer cell apoptosis. Collar et al., (1992) and Benedito De Barber et al., (1989) suggested, however, that GABA is rapidly consumed by yeast at the beginning of a fermentation, due to the high demand of nitrogen for cell growth, or takes part in the Maillard reaction. More recently Lamberts et al., (2012) showed the important role of yeast in the GABA dynamics during bread making. During dough mixing the level of GABA is increasing, but during fermentation yeast consumes it as a nitrogen source. However, the authors were able to produce GABA enriched bread through the addition of exogenous glutamic acid decarboxylase (GAD) in the recipe. Hudec et al., (2015) screened different microorganisms from 10 different food applications as well as seven pure bacterial strains for GABA. They showed a small production of GABA from *S. cerevisiae* Baker's yeast and wine yeast of 0.8 and 1.3%, respectively. The highest GABA production of 90.0% could be detected by using *Lactobacillus delbrueckii* subsp. *bulgaricus*. Using strains from the genera *Lactobacillus*, via sourdough production, could be a good alternative to increase the GABA content in bread. Rizzello et al., (2008) previously reported a GABA concentration of 258.7 mg/kg in a wholemeal wheat sourdough by the addition of an adjunct culture using lactic acid bacteria.

2.11.Conclusion

The baking industry is currently selecting their yeast strains based on their ability to ferment sugars anaerobically with adequate gas production. However, other important quality parameters for consumer acceptance of bread including colour, texture and flavour, are not considered when selecting yeast strains. At the moment the production of additional metabolites by yeast plays an underestimated role in the selection of strains. Wine yeasts have been traditionally selected by their fermentative power, suitable fermentation kinetics, in additional to their low acetic acid production and resistance to sulphur dioxide (Suárez-Lepe & Morata, 2012). Recently, new selection criteria have been sought to improve the technological properties and sensorial features of wines, since the metabolic uniqueness and physiological properties of yeast could, through the production of metabolites, improve the sensorial properties of wine. Included in these criteria are the ability to enhance wine colour, the absence of β-glucosidase activity, the facilitation of colloidal stabilisation in red wines, the appropriate enhancement of aroma via the production of volatile compounds and the provision of structure and body (Suárez-Lepe & Morata, 2012). Similarly detailed selection criteria are commonplace in the production of beer. The brewing industry, in general, separates yeast strains into ale and lager yeasts. In addition, they also use more specific selection criteria, such as the fermentation behaviour (top or bottom fermentation), fermentation performance (fermentation rate and degree of attenuation), the ability to ferment meliobiose, temperature tolerance, ability to flocculate (powdery or flocculant yeast), oxygen requirements and the ability to form or remove fermentation metabolites (aroma compound formation) (Bokulich & Bamforth, 2013; Kunze, 2014). Therefore, specific selection of Baker's yeast should be as carefully considered as it is done for wine and beer yeasts, particularly in terms of flavour, colour and shelf life. The wine industry has also recognised the potential of non-Saccharomyces yeast strains, which haven't yet been studied in the process of bread making (Suárez-Lepe & Morata, 2012). Randez-Gil et al., (1999) previously suggested to use recombinant DNA technology for the creation of new yeast strains expressing enzymes to allow elimination of the extensive use of baking additives. Choosing the perfect starter culture for bread/baked product manufacturing should not solely be determined by the gas production capacity during fermentation. Other characteristics like enzyme activity are an important parameters to predict the final bread quality, due to their

impact on shelf life (microbial and staling) as well as colour and flavour formation. Consumer acceptance will not allow the use of genetically engineered yeasts. More targeted yeast selection, based on broader criteria, offers a good way to obtain yeast strains from the species *S. cerevisiae* (even other genera and species) with novel technological properties, within the limitation of current Food Legislation. Such strains should enable improvements in the technological and/or sensorial qualities of baked products.

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3. Chapter Impact of different beer yeasts on wheat dough and bread quality parameters

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3.1. Abstract

In order to investigate the impact of different yeast strains from the species *Saccharomyces cerevisiae* on the dough and bread quality parameters, wheat flour was fermented using different beer yeasts. The results show that beer yeast strains could be included in the baking process since *S. cerevisiae* T-58 and *S. cerevisiae* s-23 provided adequate gas production and dough formation with superior structural properties like extensibility and stickiness to *S. cerevisiae* Baker's yeast. The resulting breads show the highest specific volume with the highest slice area and the highest number of cells and the lowest hardness over time. The different yeasts had also an impact on the crust colour due to their abilities to ferment different sugars and on shelf life due to the production of a range of different metabolic by-products. According to this study it was possible to produce higher quality bread by using yeast coming from the brewing industry, instead of bread containing standard Baker's yeast.

3.2. Introduction

One of the oldest biochemical processes in the whole world is the preparation of bread and beer through yeast fermentation (Linko et al., 1997). Surprisingly, the fermentation process and its correlation between product quality parameters are still not completely understood (Mondal & Datta, 2008) and next to brewer's and wine making yeast less attention was demanded to Baker's yeast (Dequin, 2001). Most of the baking processes are linked to a fermentation step mainly dominated by the yeast strain *Saccharomyces cerevisiae*, regularly mentioned as Baker's yeast (Fleet, 2007). A Baker's yeast with excellent processing characteristics should ensure a uniform dough leavening, be a good flavour producer and tolerate a wide range of temperatures, pH, as well as sugar and salt concentration (Linko et al., 1997). Therefore, fermentation as a step in bread making, has a large impact on the improvement of shelf life, texture, taste and flavour of the final product (Fleet, 2007). Yeasts can also have an impact on the production, quality, sensory and safety of each bakery product (Fleet, 2007). The main ingredients for baking are flour and water, which influences the overall texture and the crumb, as well as salt which strengthens the gluten network and yeast as a leavening agent for a good dough development. Additionally, sugar (for starting the fermentation), fat (for a better machinability), sodium steaoryl lactylate (emulsifier) and ascorbic acid (for strengthen the dough) are added. Freshly baked bread is generally characterised by a crispy crust, soft crumb, a pleasant mouth feel and an intensive flavour (Giannou, Kessoglou, & Tzia, 2003). The review of published literature showed that most of the studies detailed experimental aspects (temperature, volume expansion and moisture content), analytical aspects (energy requirement and rheological properties) (Ktenioudaki, Butler, & Gallagher, 2010; Salvador, Sanz, & Fiszman, 2006), along with the development of new baking technologies (new materials and ingredients as well as un-proofed, cooled or frozen doughs) (Decock & Cappelle, 2005) and new techniques (different dough mixing procedures) (Giannou et al., 2003) in the bread making process (Mondal & Datta, 2008). Besides the baking process, the research effort has focused almost exclusively on yeast activity during the dough fermentation and the aroma profile developed in the resulting bread (Connelly & McIntier, 2008). However, limited effort has been put in the investigation of the technological performance of *S. cerevisiae* in baking applications by underestimating the key roles that yeast strains play in bread system. Therefore, in the

present study, systematic baking trials and dough analysis were carried out, based on a standard recipe and procedure with 4 different beer yeasts in comparison to Baker's yeast used as a control. Originally one single strain of yeast was used for both processes brewing and baking. Long ago in Egypt and the Middle East, both processes, brewing and baking, were closely linked. That remained until the nineteenth century where yeast left over from the breweries was used for bread making. Nowadays, genetically improved microbial cultures are available for commercial use to better suit the need of the operator (Amendola & Rees, 2003). Beer yeast strains feature optimized metabolism suitable for beer making in terms of flavour compounds and alcohol production differently to Baker's yeast which concentrates on a fast fermentation and uniform dough leavening due to carbon dioxide production. Connecting the results of brewer's yeast with Baker's yeast could be highly profitable, to better understand the fundamental fermentation process. This study could open unexplored scenarios on yeast application through a tailored modulation of dough characteristics, bread quality parameters and sensory profiles and help to develop a new generation of yeast strains with enhanced technological characteristics.

3.3. Experimental

3.3.1. Materials

The suppliers of the ingredients used were Unifoods ingredients for bakers' flour (12.7% moisture, 0.8% ash, without any further additives); sugar from Siucra, Ireland; salt from Glacia British Salt Limited, UK; Sodium steaoryl lactylate (SSL) from Danisco, Denmark; commercially available sunflower oil and ascorbic acid from Storefast Solutions, UK. Instant active dry Baker's yeast was obtained from Puratos, Belgium; Dry yeast s-23 (Lager yeast), T-58, us-05 (Ale yeast) and wb-06 (wheat beer yeast) were supplied by Fermentis Division of S. I. Lesaffre, France. All the used yeasts belonged to the species *S. cerevisiae*.

3.3.2. Cell Count

To determine the cell viability (cfu/g) of the yeast powders, 1 g freeze dried yeast was suspended in 10 mL distilled water. From this stock solution, serial dilutions were prepared with ringer solution and spread on malt extract agar (Merck, Germany) plates and incubated aerobically for 2 days at 25°C. Plates with 30 to 300 colonies were selected for yeast cell counts.

3.3.3. Bread-making

Wheat breads were prepared using 2% salt, 1.5% sugar, 3% fat, 0.5% SSL, 0.1% ascorbic acid, 63% water (based on Farinograph 500BU based on Farinograph 500BU) and 2% yeast, based on flour. The amount of yeast was adapted according to the number of cell count of Baker's yeast in order to standardize the inoculum size. Yeast was dissolved in water (25°C) and activated for 10 min. The yeast/water mixture was added to the premixed dry ingredients and the fat. Mixing was performed for 1 min at speed 1 with a spiral mixer Pietroberto SF (Food Equipment Service, Northern Ireland). The dough was scraped down from the bowl, and a further mixing step at speed 2 was carried out for 7 min. Bulk fermentation for the wheat dough was performed for 15 min in a proofer (KOMA SunRiser, Roermond, The Netherlands) set at 30°C with a relative humidity (RH) of 85%. The doughs were scaled to 400 g into 9 baking tins of 15x9.5x9.7 cm and

placed again in the proofer for 60 min (30°C, 85% RH). Baking was carried out for 30 min at 230°C top and bottom temperature in a deck oven (MIWE condo, Arnstein, Germany), previously steamed with 0.3 L of water and subsequently with 0.7 L of water. After baking the bread loaves were directly removed from the tins and cooled down at room temperature for 120 min. Finally the loaves were analysed and stored in plastic bags at room temperature.

3.3.4. Rheofermentometer Analysis

The rheofermentometer (Chopin, France) measures the dough development according to the production and retaining of carbon dioxide during fermentation. Wheat dough was fermented with four different beer yeasts to determine its gaseous release and dough development characteristics. For the measurement, three hundred grams of dough were prepared as described for bread making. The experimental dough was placed into the fermentation chamber and fermented at 30°C over 180 min. A cylindrical weight of 1500 g was attached to the fermentation chamber. The fermentation performance of the dough is expressed using several parameters such as; the dough development curve (maximum height of the dough sample - Hm), the time the dough needs to achieve this height (T1) and the dough volume reached through carbon dioxide production throughout the whole fermentation process (V_{total}).

3.3.5. Extensibility

Dough extensibility and resistance to extension was measured by a TA-XT2i texture analyser (Stable Micro Systems, Surrey, UK) equipped with a Kieffer Dough and Gluten Extensibility Rig with a 5 kg load cell (Verheyen et al., 2014). Dough was mixed according to bread making procedure. All doughs were measured 5 times after 60 min of proofing at 30°C and RH of 85%. The measurement was performed under the following settings: pre-test speed of 2 mm/s, test speed of 3.3 mm/s, post-test speed of 10.0 mm/s and a force of 5 g. The following values calculated by the TA-XT2i software were chosen to describe the behaviour of the dough: extensibility (distance to break [mm]) and resistance to extension (maximum force [N]).

3.3.6. Dough stickiness

Dough stickiness was measured using a TA-XT2i texture analyser (Stable Micro Systems, Surrey, UK) equipped with a 1" spherical probe (plastic 13097) and a 5 kg load cell. Dough was prepared according to the bread making procedure and the measurement was done before and after 1 h proofing. The settings used for this measurement were: pre-test speed of 0.4 mm/s, test speed of 0.5 mm/s, post-test speed of 10.0 mm/s, return distance 50 mm, contact time 0.1 s and a force of 40 g.

3.3.7. Total available carbohydrates

The total available carbohydrate level from freeze-dried breadcrumb samples was determined spectrophotometrically by using an enzyme kit (K-TSTA) supplied by Megazyme, Ireland. After hydrolysis of starch by thermostable a-amylase maltodextrins are formed which were degraded by amyloglucosidase to D-glucose. The further oxidation of D-glucose to D-gluconate released hydrogen peroxide which was quantitatively measured using peroxidase and formation of quinoneimine dye (λ = 510 nm).

3.3.8. Sugar

Sugar levels of flour, dough and bread crumb were analysed for sucrose, maltose, glucose and fructose. Concentrations were quantified by an Agilent 1260 high performance liquid chromatography system (HPLC) with a Hi-Plex H^+ column (Agilent, Cork, Ireland) coupled to a refractive index detector (RID). The sugars were extracted with distilled water for 20 min under shaking and clarified with Carrez I and II. The HPLC analysis was performed at 25°C column temperature with water (HPLC-grade) at a flow rate of 0.6 mL/min.

3.3.9. Loaf Characteristics

Loaf-specific volume was analysed after cooling using a Volscan Profiler (Stable Micro Systems, UK). Crumb texture was determined on the baking day as well as after 2 and 5 days of storage using TA-XT2i texture analyser (Stable Micro Systems, Surrey, UK). In

details, five bread slices (20 mm thickness) from each loaf were used to evaluate the crumb texture. The system was equipped with a 25 kg load cell and a 35 mm cylindrical probe. The measurement was performed under the following settings: test speed of 5 mm/s, post-test speed of 10.0 mm/s, a force of 0.05 N and 5 s waiting time in between the first and second compression. Hardness as a value calculated by the TA-XT2i software was chosen to describe crumb texture.

3.3.10. Crumb Grain

Image analysis of the structure of bread slices was carried out 2 h after baking by a C-cell Bread Imaging system (Calibre Control International Ltd., UK). The analysis was achieved on 3 central slices (20 mm) of each loaf. Image analysis parameters investigated were slice area and number of cells.

3.3.11. Crust Colour

The colour values of bread samples were measured using the CIE L* a* b* colour system, where L* is lightness, a* is redness, and b* is yellowness. The instrument used was a Colorimeter CR-400 (Konica Minolta, Osaka, Japan). The colorimetric parameters L*, a* and b* were referred to CIE standard illuminant D65. The bread crust of each bread sample was measured 30 times.

3.3.12. Shelf Life

The shelf life of the breads against environmental moulds were determined adapting the method described by Dal Bello et al., (2007). Each loaf was sliced in a sterile manner to obtain slices of 20 mm thickness. Each slice was microbiologically challenged by exposing them to the environmental air for 5 min on each side and then packed in a plastic bag and heat sealed. On each side of the bag a filter tip of a transfer pipette was inserted to guarantee similar aerobic conditions. Bags were stored at room temperature and examined for mould growth in the time of a 28 day storage period. Mould growth was evaluated on the basis of the fungal outgrowth appeared as percentage of the total surface area.

3.3.13. Statistical Analysis

Minitab 16 software was used to carry out statistical analysis on the results. Exploratory data analysis was followed by a multiple comparison procedure of variance (one way ANOVA, Tuckey's test) to describe significant differences at a level of significance from 5% ($p<0.05$) between samples made from different yeast. Between some of the parameters a simple regression was performed, to find significant correlations. All analysis was performed in triplicates and the results are shown as average with confidence interval.

3.4. Results and Discussion

3.4.1. Cell Count

The cell viability of the freeze dried yeasts from the various suppliers was obtained using the cell count method to be able to standardise the inoculum level of yeast for the baking trials. The freeze dried powder of ale yeast *S. cerevisiae* T-58 as well as *S. cerevisiae* wb-06 had the same total cell count as Baker's yeast. The total cell count from *S. cerevisiae* s-23 contained just half the amount of viable cells (4.80E+08 cfu/g) and *S. cerevisiae* us-05 (3.87E+08 cfu/g) just one third. The yeast addition level for every yeast was based on the *S. cerevisiae* Baker's yeast cell count of 1.13E+09 cfu/g. Considering the amount of cells from *S. cerevisiae* Baker's yeast the breads were all prepared with 1.13E+09 cfu/g yeast. The lower addition of *S. cerevisiae* s-23 and *S. cerevisiae* us-05 to the breads therefore containes more dead yeast cells. Christoph Verheyen & Jekle, (2016) showed a linear correlations (r=0.8486) between the amount of glutathione and the concentration of dead cells. Already small amounts of glutathione can result in a softer dough with a loss of specific volume. However, no effect can be seen for *S. cerevisiae* s-23 regarding dough and bread properties. Only *S. cerevisiae* us-05 with the highest amount of non-viable yeast cells showes inferior results in comparison to *S. cerevisiae* Baker's yeast (see below).

3.4.2. Rheofermentometer Analysis

The rheofermentometer evaluates the gas holding capacity and dough development height, which gives an indication about yeast fermentation rate and yeast activity. In Table 3-1, the effect of different yeast with the same inoculum size to gas production and dough development is presented. *S. cerevisiae* Baker's yeast, used as a control, was compared to the different beer yeasts based on the dough development height (Hm), and the time to reach this value (T1), as well as total gas production. Surprisingly all yeasts except *S cerevisiae* us-05 (40.5 ± 4.9 mm) showed no significantly differences in their dough development height. The total volume of carbon dioxide produced by the yeast (V_{total}) during the fermentation was also recorded. The production of carbon dioxide is directly linked to the yeast fermentation rate and is also expressed as yeast activity. As seen in Table 3-1 the highest total gas production (V_{total}) was measured for *S. cerevisiae* ale yeast T-

58 (2081 ± 54 mL). The total gas production of *S. cerevisiae* Baker's yeast and *S cerevisiae* s-23 was comparable (1700 ± 22 mL). The results showed that, the highest yeast activity was obtained for *S. cerevisiae* T-58. *S. cerevisiae* Baker's yeast and *S. cerevisiae* s-23 showed less activity due to the lower total gas production during fermentation and *S. cerevisiae* us-05 and *S. cerevisiae* wb-06 produced even less total gas volume of 1217 ± 139 and 1163 ± 31 mL, respectively. This indicates that yeast *S. cerevisiae* us-05 forms less carbon dioxide. The results also revealed that this yeast has a slower fermentation rate of sugars. Beer yeasts are usually applied in a long fermentation process with colder temperatures (13 - 20°C), where a slower fermentation rate than Baker's yeast is advantages (White & Zainasheff, 2010). The time to achieve the maximum dough rise (T1) was longer for yeast *S. cerevisiae* us-05, *S. cerevisiae* s-23 and *S. cerevisiae* Baker's yeast. T1 is related to yeast speed and activity. Yeast *S. cerevisiae* T-58 had the shortest T1 requiring less time to reach the same values than Baker's yeast. Beer yeast undergoes a much faster fermentation at higher temperatures. Ale strains (T-58) are growing much faster at 32°C and lager strains (s-23) at 27°C, which is the reason why T-58 needed less time to reach the maximum dough rise at the fermentation temperature of 30°C than s-23 (White & Zainasheff, 2010).

Table 3-1 Dough Characteristics for dough development, gas production, extensibility, resistance to extension and stickiness after 60 min proofing at 30°C and a relative humidity of 85%[#]

S. cerevisiae	Dough development curve		gaseous release curve			Weakening coefficient [%]	Resistance to extension [N]	Extensibility [mm]	Stickiness [N]	
	Hm[*] [mm]	T1[*] [min]	V_{total}[*] [mL]	$V_{retention}$[*] [mL]	V_{lost}[*] [mL]				0 min	60 min
Baker's yeast	69.4 ± 5.8^{a}	174 ± 10^{a}	1700 ± 22^{b}	1408 ± 21^{a}	291 ± 11^{b}	83 ± 1^{bc}	0.214 ± 0.021^{b}	22.158 ± 3.494^{b}	36.283 ± 2.212^{a}	40.236 ± 2.383^{d}
s-23	63.3 ± 4.8^{a}	101 ± 5^{c}	1706 ± 78^{b}	1370 ± 16^{a}	336 ± 54^{b}	80 ± 2^{c}	0.166 ± 0.017^{c}	23.985 ± 3.017^{ab}	36.910 ± 2.461^{a}	45.075 ± 3.294^{bc}
T-58	71.2 ± 6.2^{a}	129 ± 14^{b}	2081 ± 54^{a}	1534 ± 155^{a}	546 ± 112^{a}	74 ± 6^{c}	0.212 ± 0.018^{b}	17.439 ± 1.096^{b}	39.479± 1.847^{a}	42.545± 1.569^{cd}
us-05	40.5 ± 4.9^{b}	115 ± 11b^{c}	1217 ± 139^{c}	1114 ± 98^{b}	103 ± 27^{c}	92 ± 2^{ab}	0.076 ± 0.004^{d}	33.156 ± 7.509^{a}	40.487 ± 3.199^{a}	61.626 ± 1.908^{a}
wb 06	62.2 ± 2.8^{a}	172 ± 9^{a}	1163 ± 31^{c}	1117 ± 65^{b}	45 ± 3^{c}	96 ± 2^{a}	0.264 ± 0.013^{a}	25.606 ± 2.093^{ab}	39.876± 2.965^{a}	47.373± 1.586^{b}

[#] Shown results are average values and confidence interval of three independent fermentations, each sample measured 5 times. Values in one column followed by the same upper case are not significantly different ($p<0.05$)

[*] Hm: maximum height of the dough; T1: the time the dough needs to achieve Hm; V_{total}: carbon dioxide volume reached; $V_{retention}$: carbon dioxide volume kept in the dough; V_{lost}: the carbon dioxide volume released by the dough

3.4.3. Dough Extensibility

The Kieffer dough and gluten extensibility rig measures the uniaxial extension of a dough sample after resting time, which gives an indication of dough quality. To measure the effect of different yeasts on the extensibility, the dough was measured after 60 min of proofing to evaluate changes during the bread making process. As seen in Table 3-1 the results showed significant differences. The highest extensibility was found for *S. cerevisiae* us-05 (33.1 mm) compared to *S. cerevisiae* Baker's yeast (22.1 mm). The lowest extensibility was revealed for *S. cerevisiae* T-58 (17.4 mm). For the resistance of extension the lowest value was found for *S. cerevisiae* us-05 (0.07 N) and much higher values for *S. cerevisiae* Baker's yeast, *S. cerevisiae* T-58 and *S. cerevisiae* wb-06 with 0.21 N, 0.21 N and 0.26 N, respectively. Strong doughs have a high resistance to extension and a low extensibility (Bordes, Branlard, Oury, Charmet, & Balfourier, 2008). The doughs produced with *S. cerevisiae* Baker's yeast and *S. cerevisiae* T-58, characterised by the highest resistance to extension and the lowest extensibility show properties which are correlated to strong doughs. A decreased resistance to extension and an increased extensibility was revealed for *S. cerevisiae* us-05 with the lowest total gas production, which are associated with the properties of weak doughs. These findings confirm the rheofermentometer results, since strong doughs entrap more gas, produced from the yeast, than weak doughs (Dobraszczyk, 2003). The relationship between carbon dioxide production and extension properties of dough, leads in the end to a higher dough development height and a higher specific volume. It is easier to stretch doughs with less force, since they have entrapped more gas cells. At the beginning of the fermentation process, the dough contains a lot of small air bubbles and as a result of increasing carbon dioxide concentration throughout fermentation the small bubbles grow and merge into bigger gas filled cells (Verheyen et al., 2014). The growing induces a biaxial elongation of the gluten-starch matrix (Sroan et al., 2009). Over the process of expansion, the gluten-starch matrix is stretched until it ruptures, which causes thinning of the entrapped gas cells. Since small gas bubbles have a thicker cell wall and less internal pressure than bigger gas cells it is easier to stretch them (Dobraszczyk, 2003). When more gas is entrapped in the dough the gas cells are smaller and the distribution is higher after proofing. Those gas cells can resist more strain before they rupture, which leads to a lower extensibility and a higher specific volume

(Dobraszczyk, 2003; Sroan et al., 2009; Verheyen et al., 2014). Another reason could be because of the amount of dead yeast cells which produces the reducing agent glutathione. A high amount of glutathione results in a modification of the viscoelastic gluten network, which has a weakening effect on the dough and influences the bread quality, as can be seen for *S. cerevisiae* us-05, which showed the lowest cell viability (Verheyen et al., 2014).

3.4.4. Dough stickiness

Dough stickiness is an important characteristic as a dough quality parameter. Dough stickiness is caused through the interactive balance between the two forces adhesion and cohesion (Hoseney & Smewing, 1999). Adhesion represents the interaction between a material (dough) and a surface (probe), whereas cohesion describes the interactions inside the material. Summarised, dough stickiness is a result of surface and rheological properties (Adhikari, Howes, Bhandari, & Truong, 2001). If either the adhesive force or the cohesive force is low, the material will appear with a non-sticky character (Hoseney & Smewing, 1999). In the baking industry stickiness is a problem since it causes difficulties during dough handling (low dough mixing tolerance, reduced dough strength) by interruptions, waste and contaminations as well as a decreased bread making quality such as bread volume (Adhikari et al., 2001). The results obtained from the TA-XT2i texture analyser are depicted in Table 3-1 for the doughs prepared with the different yeasts. Stickiness from the different doughs was compared before and after 60 min of proofing. Before proofing the results for stickiness are not significantly different. During proofing stickiness increased and showed significant changes. The highest stickiness was found for *S. cerevisiae* us-05 of 61.626 N and the lowest stickiness for *S. cerevisiae* Baker's yeast of 40.236 N. The increase of stickiness is linked to several factors influencing the dough including protein composition most likely available amino acids (Dhaliwal, Mares, Marshall, & Skerritt, 1988). Water as an increase in the relative humidity during proofing is influencing the dough stickiness as well, since more water can be absorbed from the surface (Adhikari et al., 2001). Low molecular weight sugars which are produced by the yeast in time of fermentation can have also an effect on dough stickiness (Adhikari et al., 2001).

3.4.5. Total Starch

The total starch content is measured by using an enzymatic kit, which is based on the use of α-amylase and amyloglucosidase, in a wide range of food and cereal products. As shown in Table 3-2, the results differed significantly between the bread samples. The lowest value in comparison to *S. cerevisiae* Baker's yeast (69.9%) was found for *S. cerevisiae* us-05 and *S. cerevisiae* s-23 of 60.2% and 60.9%, respectively. The lower content of total starch for the doughs prepared with beer yeast is based on the fact that beer yeast has higher enzyme activities in comparison to Baker's yeast, which degrade starch into more fermentable sugars (White & Zainasheff, 2010).

Table 3-2 Carbohydrate levels in dough and bread made with different yeast after 60 min proofing at 30°C and a relative humidity of 85% and after baking[#]

Concentration [mmol/kg] / *S. cerevisiae*	Dough				Bread				
	Maltose	**Glucose**	**Fructose**	**Sum**	**Maltose**	**Glucose**	**Fructose**	**Sum**	**Total starch**
Baker's yeast	63.7 ± 2.4^{a}	33.0 ± 3.7^{bc}	42.4 ± 5.2^{c}	139.1 ± 11.3^{b}	93.0 ± 13.4^{a}	28.6 ± 1.7^{b}	57.5 ± 14.7^{ab}	182.6 ± 59.0^{ab}	69.9 ± 3.2^{a}
s-23	49.6 ± 5.0^{b}	45.1 ± 8.7^{b}	83.9 ± 2.1^{ab}	188.4 ± 17.5^{ab}	60.6 ± 11.5^{ab}	34.8 ± 3.5^{ab}	87.1 ± 14.5^{ab}	182.5 ± 29.5^{ab}	60.9 ± 2.2^{b}
T-58	39.0 ± 0.9^{bc}	47.0 ± 3.1^{bc}	38.4 ± 1.5^{c}	124.4 ± 5.5^{b}	61.9 ± 14.4^{ab}	14.5 ± 3.1^{b}	43.4 ± 3.6^{b}	121.3 ± 17.9^{b}	65.3 ± 1.9^{ab}
us-05	33.2 ± 4.8^{c}	73.7 ± 11.3^{a}	98.6 ± 11.0^{a}	226.2 ± 45.3^{a}	81.5 ± 11.0^{a}	50.7 ± 7.0^{a}	113.2 ± 6.2^{a}	245.4 ± 24.1^{a}	60.2 ± 4.2^{b}
wb 06	42.8 ± 4.4^{bc}	26.4 ± 0.3^{c}	63.3 ± 6.6^{bc}	126.1 ± 1.8^{b}	43.4 ± 8.8^{b}	23.5 ± 9.6^{b}	50.6 ± 7.1^{b}	117.5 ± 7.7^{b}	64.9 ± 1.3^{ab}
	Sucrose	**Glucose**	**Fructose**	**Sum**					**Total starch**
Wheat flour	48.5 ± 8.8	1.3 ± 0.5	1.7 ± 0.3	51.4 ± 7.0					72.4 ± 3.4

[#] Shown results are average values and confidence interval. Values in one column followed by the same upper case letter are not significantly different ($p<0.05$)

3.4.6. Sugar

Maltose, sucrose, glucose and fructose are the most important sugars for the yeast fermentation. The HPLC-RID analysis showed significantly differences in the amount of sugars in dough and bread fermented with the different yeasts (Table 3-2). In general, yeast strains have a higher ability to ferment glucose than fructose (Hopkins & Roberts, 1936). As a result in this study, the amount of fructose increased steadily during fermentation. Accordingly to the increased amount of fructose, sucrose was equally converted to glucose and fructose during mixing and early stages of proofing. Codina and Voica, (2010) pointed out how compressed yeast, instant dry yeast and active dry yeast of *S. cerevisiae* Baker's yeasts under different fermentation times influence the concentration of sugars throughout fermentation. They found that in the beginning the yeast mainly ferments glucose and only in later stages of fermentation it also uses maltose and fructose as a carbohydrate source for their metabolism. The highest amount of maltose was found in the dough sample fermented with Baker's yeast. Due to the concentration of glucose and fructose being high enough, amylase, which is naturally present in flour, generates maltose out of starch (Codina & Voica, 2010). The results for the different beer yeasts indicate less maltose in the dough and in the resulting bread, due to the fact that beer yeast uses maltase enzymes to hydrolyse maltose into two glucose units (White & Zainasheff, 2010). Maltose degradation is repressed, when the glucose concentration is higher than the maltose concentration, as can be seen for *S. cerevisiae* T-58, *S. cerevisiae* us-05 and *S. cerevisiae* wb-06. The sum of low molecular weight sugars, (Table 3-2), which are not fermented by the yeast have an effect on dough stickiness (Adhikari et al., 2001). The highest amount of available sugars after 60 min of proofing was found for *S. cerevisiae* us-05 (226.2 mmol/kg) compared to *S. cerevisiae* Baker's yeast (139.1 mmol/kg). The lowest amount of sugars in comparison to the control was revealed for *S. cerevisiae* T-58 of 124.4 mmol/kg. The results for stickiness showed no significant differences between all the different doughs before fermentation as long as the amount of available sugars is identical between all the doughs. After fermentation the stickiness increases with the amount of available low molecular sugars since they have a high hygroscopicity and solubility in the different doughs, due to the ability of the different yeasts to ferment and generate different sugars.

3.4.7. Loaf Characteristics

The results from the dough analysis (rheofermentometer and extensibility) already suggested that all yeasts are more or less suitable for fermenting wheat flour and therefore for the production of bread. However, the bread quality parameters of the resulting end products vary significantly and are presented in Table 3-3.

Table 3-3 Bread crust and crumb characteristics from bread made with different yeasts[#]

S. cerevisiae	Specific volume [mL/g]	Slice area [mm²]	Number of cells	L* Lightness
Baker's yeast	3.66 ± 0.5^{b}	7,946 ± 395^{ab}	4,814 ± 442^{ab}	40.77 ± 1.12^{b}
s-23	4.20 ± 0.1^{ab}	8,715 ± 231^{a}	5,260 ± 187^{a}	43.60 ± 1.72^{a}
T-58	4.45 ± 0.2^{a}	8,937 ± 760^{a}	5,514 ± 642^{a}	44.52 ± 1.46^{a}
us-05	2.89 ± 0.1^{c}	6,193 ± 250^{c}	4,075 ± 246^{b}	35.79 ± 0.60^{c}
wb-06	3.52 ± 0.1^{bc}	6,241 ± 110^{b}	5,256 ± 161^{a}	44.86 ± 1.01^{a}

[#] Values given as mean ± confidence interval (α =0.05). Values in one column followed by the same upper case are not significantly different ($p<0.05$)

The quality evaluation showed differences in specific volume, crumb structure and crumb characteristics. One of the most important characteristics for consumer's acceptability is the overall texture and the loaf-specific volume. A high ratio of volume per weight is desired by the consumers (Hager et al., 2012). According to the results for gas retention and dough development height from the rheofermentometer during proofing, the highest specific volume of 4.45 ± 0.2 mL/g was obtained by *S. cerevisiae* T-58 and the smallest specific volume of 2.89 ± 0.1 mL/g by *S. cerevisiae* us-05. Two positive correlations could be found between the dough development height and the specific volume of $r=0.99$ ($p<0.05$) and the carbon dioxide volume which is retained in the dough and the specific volume of $r=0.94$ ($p<0.05$). Since the loaf volume depends on the gluten-starch matrix, a good balance between viscosity and elasticity is important for bread making properties (Goesaert et al., 2005). Strong doughs can be stretched up to a certain loaf volume since they can entrap more gas than weak doughs (*S. cerevisiae* Baker's yeast, *S. cerevisiae* s-23 and *S. cerevisiae* T-58) (Sim, Noor Aziah, & Cheng, 2013). When the dough is too strong and rigid the rising can be hindered (Goesaert et al., 2005). On the other side, dough which is too elastic (*S. cerevisiae* us-05, *S. cerevisiae* wb-06), is difficult to process and decrease the

quality of the finished product (Goesaert et al., 2005). The texture of the crumb was determined using the TA-XT2i texture analyser and the results are shown in Figure 3-1.

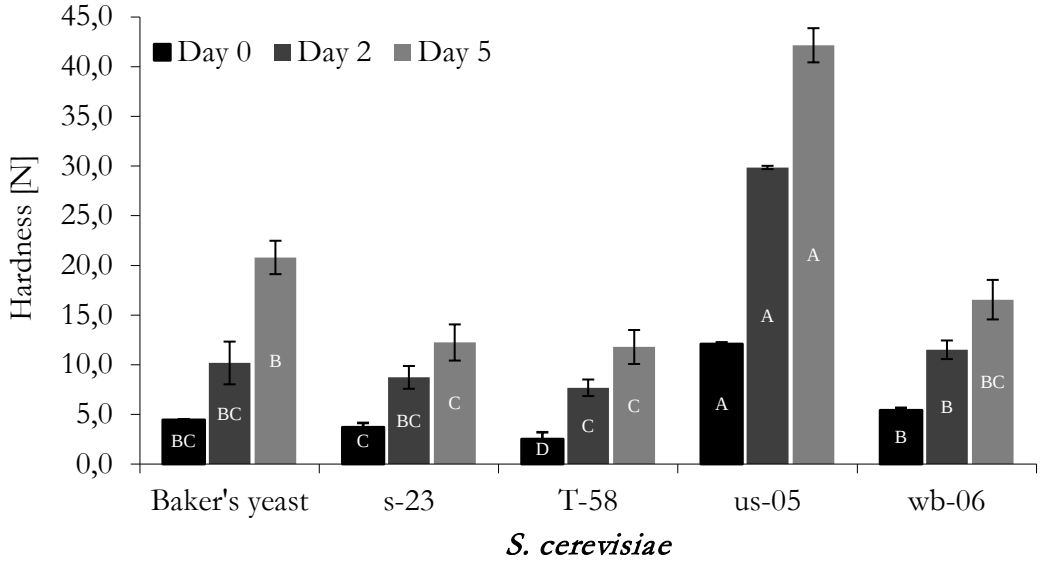


Figure 3-1 Crumb hardness for wheat breads baked with different beer yeasts and Baker's yeast as a control. Mean values ± confidence interval ($\alpha = 0.05$)

Hardness as a change in crumb structure over time indicates a decreasing consumer acceptability due to the staling process which occurs as a consequence of chemical and physical changes over storage (Cauvain & Young, 2007a). At time zero, *S. cerevisiae* T-58 and *S. cerevisiae* s-23 had the softest crumb of 2.53 ± 0.8 N and 3.72 ± 0.4 N, respectively. *S. cerevisiae* wb-06 had a crumb hardness of 5.01 ± 0.75 N followed by *S. cerevisiae* Baker's yeast and *S. cerevisiae* us-05 with the highest hardness of 5.25 ± 1.4 N and 11.35 ± 1.3 N, respectively. Low values for crumb hardness are desired, since consumers relate high hardness values to a stale bread product. Moreover the results showed an increase of hardness over time. The hardness for *S. cerevisiae* us-05 was on all days significantly different to all the other results. Comparing the hardness of *S. cerevisiae* us-05 to *S. cerevisiae* Baker's yeast, *S. cerevisiae* s-23, *S. cerevisiae* T-58 and *S. cerevisiae* wb-06 lower values on day 2 and day 5 were shown (Figure 3-1). Regarding the overall texture, the bread made using *S. cerevisiae* T-58 was the most favourable, compared to *S. cerevisiae* Baker's yeast. It shows the highest specific volume and the lowest hardness over time. The bread made with *S. cerevisiae* us-05 with the lowest specific volume and the highest hardness over time was the

least favourable. A negative correlation could be determined of r=-0.90 ($p<0.05$) which explains the increasing hardness values in comparison to small specific volumes.

3.4.8. Crumb Grain

Next to the physical texture, the visual appearance of the crumb is an important quality parameter. A C-cell which uses digital image analysis software to describe the crumb grain was applied to characterise the visual appearance and the results are shown in Table 3-3. Comparing the results, it is visible that crumb structure and cell characteristics are clearly different. According to the slice area, *S. cerevisiae* T-58 and *S. cerevisiae* s-23 had the highest area of 8,937 mm^2 and 8,715 mm^2, respectively. In contrast the other yeasts, *S. cerevisiae* Baker's yeast, S. cerevisiae wb-06 and *S. cerevisiae* us-05 showed lower slice areas. Concerning the number of cells, *S. cerevisiae* T-58 had the highest amount of cells (5514 ± 642) and *S. cerevisiae* us-05 the lowest amount of (4075 ± 246) cells per slice. Those results are directly linked to the results from the gas production, due to the fact that gas cells are smaller and have a higher distribution, when more gas is entrapped in the dough (Dobraszczyk, 2003; Sroan et al., 2009; Verheyen et al., 2014). *S. cerevisiae* T-58 which produces the most gas throughout fermentation is correlated to more cells per slice and a bigger slice area in the end product (Sroan et al., 2009).

3.4.9. Crust Colour

The external colour of bread is formed by the Maillard reaction and caramelisation. This complex series of reactions between reducing sugars and amino acids is responsible for colour and flavour. The results showed in Table 3-3 indicate significantly differences in crust colour among the yeast bread samples. The lightness (L*) of the bread crust vary widely with values from 35.79 (*S. cerevisiae* us-05) to 44.86 (*S. cerevisiae* wb-06). The breads are ordered respectively from the darkest to the whitest in the following way: *S. cerevisiae* us-05, *S. cerevisiae* Baker's yeast, *S. cerevisiae* s-23, *S. cerevisiae* T-58 and *S. cerevisiae* wb-06 (Table 3-3). A difference in L* values were expected due to the Maillard reactions and caramelisation process, which are influenced by water, reducing sugars and amino acids (Gallagher, Gormley, & Arendt, 2003). The establishing of Maillard products is influenced by the amount of reducing sugars and free amino acids in the dough which will form

pigments by performing the reaction. The more Maillard products the darker will be the colour and the more intensive the flavour in the end product. A darker colour may refer to the ability of the yeast to produce protease, thus releasing amino acids (Ormrod et al., 1991), and the amount of reducing sugars, which are not fermented during proofing (Goesaert et al., 2005). According to the relationship between crust colour and the amount of reducing sugars it can be seen that *S. cerevisiae* us-05 with the darkest crust colour have the most unfermented sugars ($r=-0.92$, $p<0.05$).

3.4.10. Shelf Life

The two factors influencing the shelf-life of baked products are microbial spoilage (mould-, bacterial-, and yeast-spoilage) and chemical or physical changes referred as staling (Cauvain & Young, 2007a). Fresh baked products are mould free due to the thermal inactivation during baking (Cauvain & Young, 2007a). The contamination with mould spores occurs during cooling, slicing and packaging of bread, which are present in the air (Cauvain & Young, 2007a). Throughout this study, the shelf life was judged by the evaluation of mould free shelf life and the length of time before first mould appeared after the slices were exposed to the bakery air for 5 min. As seen in Figure 3-2, the first appearance of mould was between 3 to 5 days after production, depending on the yeast. *S. cerevisiae* Baker's yeast showed the first mould after 4 days of storage as did *S. cerevisiae* s-23 and *S. cerevisiae* us-05. According to the first appearance of mould *S. cerevisiae* T-58 is superior in shelf life while the first mould was detected after 5 days of storage and *S. cerevisiae* wb-06 is inferior in shelf life concerning the first mould appeared after 3 days. Comparing the development of mould after the first appearance the results allow to highlight three different categories: *S. cerevisiae* Baker's yeast, *S. cerevisiae* s-23 and *S. cerevisiae* T-58 had a lower appearance of mould with a weak resistance to propagation because the curves grew quickly.

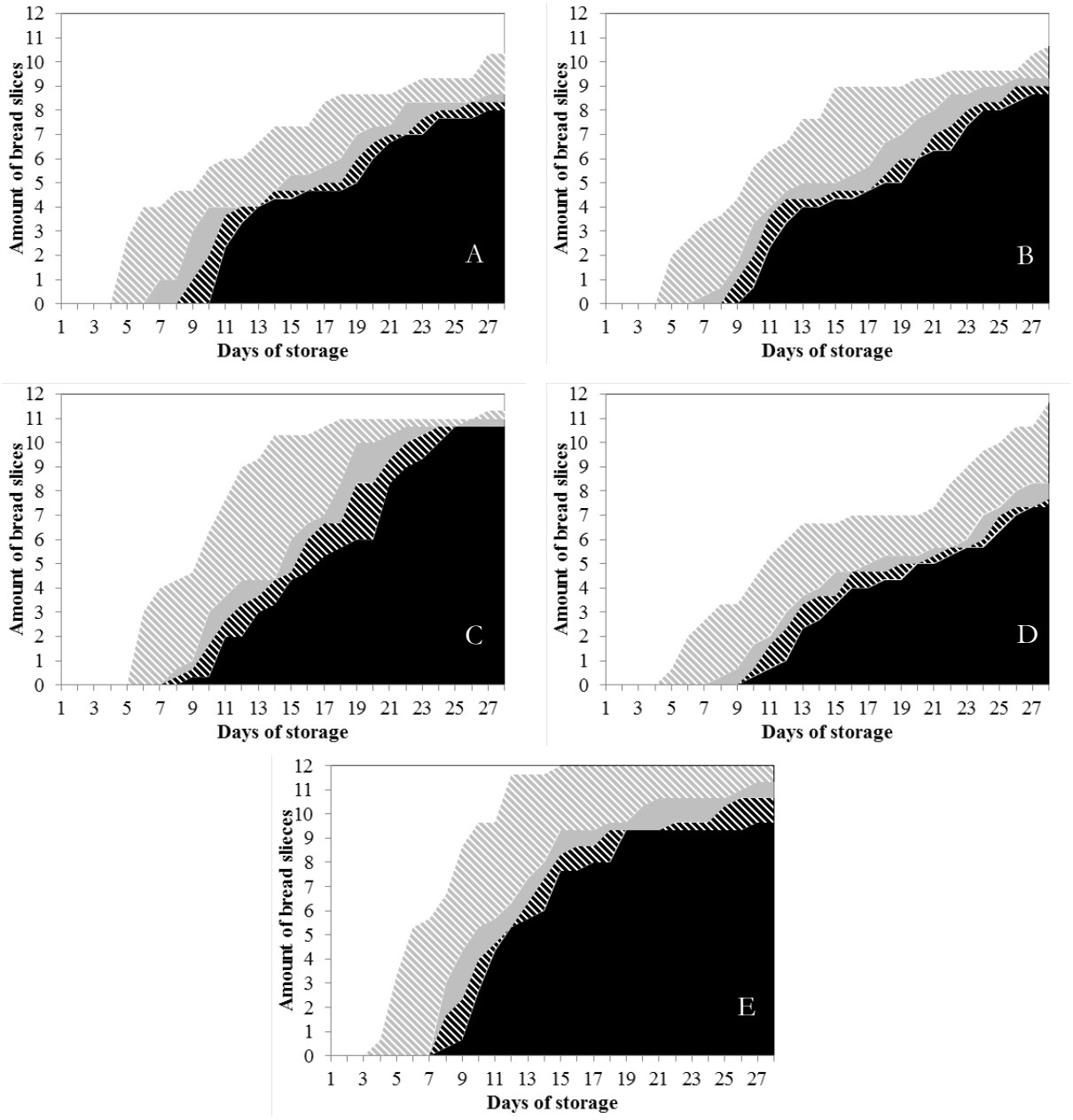


Figure 3-2 Shelf life of bread baked with different yeasts (A. Baker's yeast, B. s-23, C. T-58, D. us-05, E. wb-06) against environmental moulds during a 28-day storage period. Bread spoilage is indicated as percentage of the total surface area of each of the 12 slices where fungal outgrowth occurred: Mould free slices (white area), <10% mouldy (grey diagonally striped area), 10–24% mouldy (grey area), 25-49% mouldy (black diagonally striped area) and >50% mouldy (black area). Mean values are shown (n = 3)

Compared to Baker's yeast, *S. cerevisiae* us-05 showed higher antifungal performance than all the other yeasts due to the fact that it had a lower antifungal performance in time. *S. cerevisiae* wb-06 showed the highest appearance of mould but with a high resistance to propagation since colonies appeared quicker in the time of storage. The longest shelf life as well as overall resistance to the propagation of mould was shown for *S. cerevisiae* us-05. These results could refer to the ability of the different yeast to produce other metabolic by-products of the main metabolic pathway like organic acids (Whiting, 1976). These compounds are mainly attributed to the partial finished tricarboxylic acid cycle for the period of yeast growth (Pronk et al., 1996). The most common produced secondary metabolites are organic acids like succinate, acetate and citrate (Pronk et al., 1996).

3.5. Conclusion

This study showed differences in dough and bread quality parameters using different strains of *Saccharomyces cerevisiae*. Although the strains are all from the same species *S. cerevisiae*, they showed strain specific performances. Ale yeast *S. cerevisiae* T-58 and lager yeast *S. cerevisiae* s-23 are superior to Baker's yeast. *S. cerevisiae* T-58 and *S. cerevisiae* s-23 have the features desired for fermenting wheat flour like adequate gas production and strong dough formation. Two positive correlations could be found between the dough development height and the specific volume of $r=0.99$ ($p<0.05$) and the CO_2 volume which is retained in the dough and the specific volume of $r=0.94$ ($p<0.05$). Concerning their baking quality, they showed also higher specific volumes and less staling over time. A relationship between the loaf volume and the hardness could be found of $r=-0.90$ ($p<0.05$). Further, a negative correlation could be found between the sum of reducing sugars and the L* colour value of $r=-0.92$ ($p<0.05$). Wheat beer yeast *S. cerevisiae* wb-06 and ale beer *S. cerevisiae* us-05 are inferior in comparison to Baker's yeast as a control showing lower quality parameters of dough and bread. In conclusion, yeast strains provide a significant impact on quality parameters of dough and bread and they can be used as a tool to modulate bread characteristics. Additionally, it is possible to formulate a tailored yeast starter culture, merging the different yeast strains in a specific ratio to optimise the quality of the final wheat bread.

3.6. Acknowledgement

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4. Chapter Modulation of *in vitro* predicted Glycaemic Index of White Wheat Bread by different strains of *Saccharomyces cerevisiae* originating from various beverage applications.

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4.1. Abstract

A low glycaemic index (GI) in bakery products can be associated with a decrease of cardiovascular disease and a protective role against the development and management of non-insulin-dependent diabetes. In this study, the impact of different *Saccharomyces cerevisiae* strains applied to the bread making process on the pGI of bread was investigated. The pGI was measured using an *in vitro* enzymatic model system in relation to the metabolic patterns of the different yeast strains and the compositional analysis of the breads. Although total and resistant starch contents of the breads were similar, a significant reduction in pGI was obtained for breads fermented with *S. cerevisiae* s-23, wb-06 and Blanc. Differences in the amount of protein and fat were observed. However, their proportion when related to carbohydrate content was not high enough to effectively alter the pGI of the breads. Considering the fermentation process, *S. cerevisiae* wb-06, Blanc and to some extent s-23 were characterised by slower fermentation rates. The resulting breads were reduced in pGI with lower specific volumes as well as firmer crumb structures. Breads high in pGI were either characterised by an increased glucose content (*S. cerevisiae* us-05) or high specific bread volumes and soft crumbs (*S. cerevisiae* Baker's yeast and T-58) indicating a relationship between complete starch gelatinisation during baking and starch digestibility. Conclusively, the pGI of white wheat bread can be significantly decreased by using different strains from the species *S. cerevisiae*.

4.2. Introduction

Glycaemic index (GI) is defined as the relative rate of glucose entering the bloodstream compared to the effect of a reference carbohydrate source (Jenkins et al., 1981) and have been investigated intensively in bread (Björck, Liljeberg, & Östman, 2000; Borczak, Sikora, Sikora, & Van Haesendonck, 2011; Dewettinck et al., 2008; Jenkins et al., 1986).

These investigations have determined how different types of flour and variations in the baking process can alter the GI. Clinical studies have also investigated the effect of GI on blood glucose levels and their relationship to human health (Åkerberg, Liljeberg, & Björck, 1998; Björck et al., 2000; Liljeberg, Lönner, & Björck, 1995). Bread-making which usually involves a fermentation step is one of the oldest known cereal processes. The standard yeast strain for bread baking is almost exclusively *Saccharomyces cerevisiae* "Baker's yeast". Other strains of this species are used for the production of beer and wine mastering a wide variety of desired technological and sensorial performances. Bread is an important carbohydrate source and plays therefore a big role in human nutrition. Bread carbohydrates in general account for 45-70% of the total energy intake. To characterise the effect that carbohydrate containing foods have on the blood glucose level, the GI was introduced. There are several factors influencing the glycaemic response of carbohydrate containing foods, such as matrix structure, particle size, amount and degree of damaged starch, starch structure and the addition of several other ingredients (Björck & Liljeberg Elmståhl, 2003; Fardet et al., 2006). The main factors influencing enzymatic starch digestibility are its physical encapsulation by either fibre and/or protein, the proportion of damaged granules, its crystallinity, the native structure and the degree of gelatinisation and retrogradation. The main method used to reduce the GI of bread so far has been the inclusion of whole kernels into the bread (Fardet et al., 2006; Singh et al., 2010). Those kernels have a network of insoluble fibre that surrounds the starch fraction. The fibre network then acts as a physical barrier towards the starch degrading α-amylase and limits starch gelatinisation during baking which influences the resulting GI (Fardet et al., 2006; Singh et al., 2010). Starch gelatinisation is further influenced by the surrounding gluten matrix and the baking process. Besides starch gelatinisation, another major influence on the GI is the presence of organic acids. Several studies have shown that sourdough bread, characterised by the production of organic acid through lactic acid bacteria fermentation, can reduce the postprandial glucose response of wheat bread (De Angelis et al., 2007,

2009). The lower pH of the sourdough bread inhibits the hydrolytic salivary amylases *in vivo* (Liljeberg et al., 1995). Contrastingly, during dough fermentation, a reduced pH causes the activation of flour enzymes, which result in a reduction of the total starch content. Further promoted by the addition of acid is the formation of resistant starch. Resistant starch can not be digested but nevertheless accounts for a part of the total starch content of food products (Haralampu, 2000). Technological parameters also can have an effect on the GI of a food product. Burton & Lightowler, (2006)found a significant reduction in GI by lowering the loaf volume with a greater satiety index, due to the limited swelling and gelatinisation of the starch granules and the higher density of the bread. Following the same approach, Fardet et al., (2006) suggested a reduction in yeast quantity, which results in high density products and will lead to products with a reduced GI. Many consumers prefer a soft and flexible crumb which corresponds to a low hardness. Accordingly the focus should better concentrate on changing the chemical composition rather than the physical characteristics of wheat bread. Heitmann, Zannini, & Arendt, (2015) recently investigated the impact of different *S. cerevisiae* strains on wheat bread characteristics, which showed differences in their technological parameters like specific volume and hardness as well as in their sugar composition. These characteristics can influence the GI of the resulting breads. However, the glycaemic response of those breads was not further investigated. The objective of this study was to assess whether the quality and chemical composition of white wheat bread can be changed by using different *S. cerevisiae* for bread dough fermentation and whether these changes have an impact on the GI.

4.3. Materials and Methods

4.3.1. Materials

The suppliers of the ingredients used were Voigtmühle Illertissen, Germany for Baker's flour (13.6% moisture; 12.2% protein; 0.59% ash); salt from Glacia British Salt Limited, UK; Palm fat from Vandemoortele, Izegem, Belgium. Instant active dry Baker's yeast was obtained from Puratos, Belgium; Dry yeast s-23, T-58, us-05 and wb-06 were supplied by Fermentis Division of S. I. Lesaffre, France. Dry yeast Blanc was supplied from Vinoferm, Brouwland, Beverlo, Belgium. All the used yeasts belonged to the species *S. cerevisiae*. All chemicals, enzymes and dialysis tubing were from Sigma-Aldrich, Arklow, Ireland.

4.3.2. Bread Preparation

The breads baked using different *S. cerevisiae* strains were prepared using 2.2% salt, 1% palm fat, 62% water and 2% Baker's yeast, based on flour. The amount of the yeasts coming from the brewing and wine making industry were adapted, according to the cell count, to standardise the inoculum size in comparison to *S.cerevisiae* Baker's yeast (1.13 E+09 cfu/g) (Heitmann et al., 2015). The different yeasts were activated by dissolving in water (25°C) for 10 min. The suspension was continuously stirred for one minute. All dry ingredients were premixed in a spiral mixer (Mac Pan, Thiene, Italy). The fat and yeast/water mixture were added after premixing. The first mixing step of the dough was at speed 1 for two minutes followed by a second mixing step at speed 2 for 5 min. After a resting period of 5 min, the dough was divided into 500 g pieces and placed in baking tins (15 cm x 9.5 cm x 9.7 cm), greased prior to use. The tins were then placed in the proofer (KOMA sunriser, Roermond, the Netherlands) at 75% relative humidity and 35°C for 85 min. The pre-heated deck oven (MIWE Condo, Arnstein, Germany) was steamed with 0.4 L of water 30 sec before placing the loaves in the oven. The breads were baked for 35 min at 220 °C top and bottom temperature. Before analysis the breads were cooled for 2 h.

4.3.3. *In vitro* Starch Digestibility and Reducing Sugars Released

The *in vitro* starch digestibility of white wheat bread was evaluated using the method previously described by Brennan & Tudorica, 2008 and Hager, Czerny, Bez, Zannini, & Arendt, 2013. The bread samples were prepared by processing an aliquot of the breadcrumbs in a mixer (Major Titanium, Kenwood) with glass blender attachment. In a first step, 4 g of breadcrumbs were mixed with 20 mL of sodium potassium phosphate buffer (0.2 M, pH 6.9). The pH was adjusted to 1.5 with 8 M HCl. Subsequently 5 mL of pepsin solution (EC 3.4.23.1, 526 U/mg solid, 115 U/mL) was added and the sample was incubated at 37°C for 30 min. After incubation, the pH was readjusted to 6.9 with 6 N NaOH and 1 mL α-amylase solution (EC 3.2.1.1, 15 U/mg solid, 110 U/mL) was added. The sample was adjusted to 50 mL with sodium potassium phosphate buffer and transferred into a dialysis tube (25 mm width, length 40 cm, 14 kDa) containing glass beads which were placed in a beaker containing 450 mL sodium potassium phosphate buffer and incubated in a water bath for 4 h at 37°C. During incubation, the tubes were inverted several times every 15 min. Every 30 min an aliquot of 1 mL dialysate was taken and replaced by the same amount of fresh buffer. The content of reducing sugars released (RSR) during dialysis was measured spectrophotometrically (λ=546 nm). For that purpose, 100 µL dialysate was mixed with 100 µL 3,5-dinitrosalicylic acid reagent (DNS) (2 M sodium hydroxide, 3,5-dinitrosalicylic acid, potassium sodium tartrate tetrahydrate in distilled water). The sample tubes were heated for 10 min in a boiling water bath and immediately cooled on ice. Before reading absorbance the samples were diluted with 1 mL of distilled water. The amounts of RSR [%], were calculated as maltose equivalents (in g) and expressed as percentage of the total available carbohydrates (TAC) in the bread samples (4 g). The amount of RSR (g/100g TAC) was plotted against the digestion time (min). Using the trapezoidal method described by Wolever & Jenkins, (1986), the area under the hydrolysis curve (AUC) was calculated for the first 210 min of dialysis. The hydrolysis index (HI) was calculated from the AUC of the analysed bread samples (1).

$$HI = \frac{AUC(sample)}{AUC(control)} * 100 \qquad (1)$$

In order to calculate the predicted GI of the samples the following equation (2) was applied (Brennan & Tudorica, 2008):

$$pGI = 0.862HI + 8.189 \quad (2)$$

For the *in vitro* starch digestibility and the calculation of the glycaemic index bread fermented with *S. cerevisiae* was used as a reference.

4.3.4. Yeast metabolism characterisation

The individual yeast-metabolic properties were determined using the YT MicroPlate™ from Biolog, USA, which determines the yeast growth based on the reduction of tetrazolium as a response to the metabolism of different sugars (Praphailong, Van Gestel, Fleet, & Heard, 1997). The test is designed to be able to characterise and identify a wide range of yeasts according to their metabolic pattern. The higher the absorption values and more intense the colour change of the reduced tetrazolium, the more positive is the reaction and metabolism of the yeast. The yeast strains were cultured on Sabouraud agar. Individual cells were removed from the surface by using sterile swabs (Biolog, USA) and suspended in 12 mL of sterile water. Colonies were gradually added to increase the turbidity until 46%. From this yeast solution 100 µL were added to each of the 96 wells of the YT MicroPlate™. The YT MicroPlate™ was incubated at 28°C for 72 hours. The YT MicroPlate™ were read with the Micro plate reader (Multiskan FC, Thermo Fischer Scientific) at a wavelength of 600 nm. Results are calculated and expressed as average well colour development (AWCD). For the judgment of fructose metabolism a test based on the YT MicroPlate™ was performed. Therefore, a microplate was prepared using 100 µL of Sabouraud broth as a substrate, where glucose was replaced with fructose, in each well. The sample preparation and incubation conditions were the same as described above. Growth was read as turbidity after 72 hours with the Micro plate reader at a wavelength of 595 nm.

Additionally carbon dioxide production was measured by a Rheofermentometer F3 (Chopin, France) according to Heitmann et al., (2015), for the further evaluation of yeast metabolism characteristics.

4.3.5. Loaf characteristics

Loaf specific volume was analysed using the Vol-Scan Profiler (Stable Micro Systems, Surrey, UK) Crumb texture was measured two hours after baking using the TA-XT2i Texture Analyser (Stable Micro Systems, Surrey, UK). Four bread slices were cut from three loaves with a thickness of 25 mm (the end pieces were discarded). The Texture Profile Analysis (TPA) measurements were done with a 35 mm cylindrical probe and a 25 kg load cell. To compress the central area of a bread slice to 40% of its original height the following test parameters were used. Test speed was set to 5 mm/s, the post-test speed was 10.0 mm/s, the distance was 10 mm, trigger force was 0.05 N and the waiting period between the first and second compression was 5 s. To characterise the crumb texture the TPA software calculated the hardness of the samples. A C-cell Imaging system (Calibre Control International Ltd., UK) was used to characterise the structure of bread slices. The following parameters were used to describe the crumb grain characteristics: number of cells and area of cells.

4.3.6. Compositional Analysis

For the compositional analysis of bread samples, freeze-dried breadcrumbs were used. Total and resistant starch content were determined using Megazyme (International, Bray, Ireland) enzymatic kit K-TSTA 09/14 and K-RSTAR 09/14 respectively. The analysis for the TS was necessary for the calculations of the pGI after the *in vitro* digestion. The analyses of protein and fat content of the bread crumbs were based on the AACCI – 46-12.01 (protein factor 5.7) and AACCI – 30-1 0.01 method, respectively. The levels of citric acid, succinic acid and acetic acid were measured using an Agilent1260 HPLC system with Diode-Array Detection (DAD) and a HiPlex H^+ Column (65°C) (Agilent, Cork, Ireland). The samples were eluted with 0.005 M H2SO4 at a flow rate of 0.5 mL/min. The levels of fructose, maltose and glucose were analysed with the same system coupled to a refractive index detector (RID) (35°C) with a HiPlex H^+ column (30°C) (Agilent, Cork, Ireland). Elution of the samples was carried out with water at a flow rate of 0.5 mL/min. Sample preparation for both HPLC analysis was carried out by extracting freeze-dried samples with water while shaking them for 20 min. The samples were

clarified with 7% perchloric acid overnight. The samples were then centrifuged (3500 rpm, 10 min) and filtrated (0.450 μm) prior to injection.

4.3.7. Statistical Analysis

Results are presented as average ± confidence interval of three individual measurements. Minitab 17 was used for the evaluation of significant difference. Therefore a one-way analysis of variances (ANOVA, Tuckey's test) was performed. The chosen level of significance was 5% ($P < 0.05$). Additionally a simple regression analysis was performed to find significant correlations between the compositions as a result from the yeast metabolism and the pGI. Furthermore, the data was investigated by multivariate data analysis (Principle component analysis (PCA)) with R software version 3.3.1. to describe the differences among the samples.

4.4. Results

In this study six different *S. cerevisiae* strains originating from the baking, beer and wine industry were used for the production of wheat bread. The pGI was measured and correlated to the change in composition as a result from the metabolic activity of the various yeasts. The differences in yeast characteristics and parameters which can have an influence on the GI of fermented wheat bread are summarised in Table 4-1.

Table 4-1 Summary about important *S. cerevisiae* characteristics

S. cerevisiae	Application	Temperature optimum [°C][1]	Fermentation time[1]	Flocculation[1]	Dosage [%] based on flour	Metabolic patterns				
						Sugar metabolism[2]			CO_2 production[3]	Acid production[4]
						MalT	Mal	Glu		
Baker's yeast	Baked goods	25-30	Hours	Low	2	++	+	+	+++	++
s-23	Lager	12-15 (27 faster) Lower temperature tolerance	Up to 14 days	High	4	++	+++	+++	++	++
T-58	Ale	15-20 (32 faster) Higher temperature tolerance	2-3 days	Powdery	2	++	++	++	+++	++
us-05	Ale	15-22 Higher temperature tolerance	2-3 days	Medium	6	+++	+	+++	++	+++
wb-06	Wheat beer	18-24	2-3 days	Low	2	+	++	++	+	+
Blanc	White wine	18-30	5-24 days	Low	½	++	++	++	+	+

[1] According to specification sheet, [2] According to YT MicroPlateTM, [3] According to Rheofermentometer analysis, [4] According to HPLC-DAD analysis

MalT: Maltotriose; Mal: Maltose; Glu: Glucose

+++ high; ++ moderate; + low

4.4.1. *In vitro* Starch Digestibility and Glycaemic Index (pGI)

The GI of the breads produced with six different yeasts were analysed using an *in vitro* model system and the results are depicted in Figure 4-1. The breads made with yeast originating from different beverage applications were compared to the bread fermented with *S. cerevisiae* Baker's yeast (pGI 100) as the reference product. The pGI values of the bread samples ranged between 63.02 for the bread fermented with *S. cerevisiae* wb-06 and 103.63 for the bread made with *S. cerevisiae* us-05. In comparison to *S. cerevisiae* Baker's yeast, *S cerevisiae* wb-06 showed the highest reduction of -36.98% followed by *S. cerevisiae* s-23 with -28.42% and *S. cerevisiae* Blanc with -22.91%. The lowest decrease of -2.30% in pGI was determined for breads fermented with *S. cerevisiae* T-58. Only for *S. cerevisiae* us-05 an increase of pGI equal to +3.63% was observed.

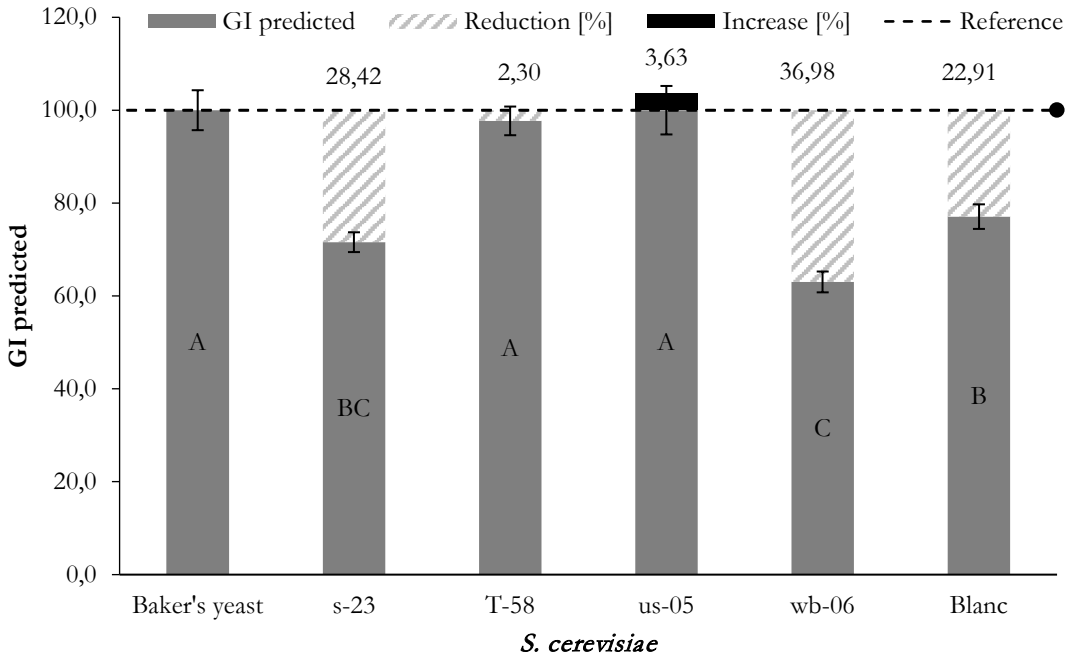


Figure 4-1 Reduction and increase of the predicted Glycaemic index with confidence interval ($p<0.05$) in comparison to Baker's yeast bread as a reference product

4.4.2. Yeast metabolism identification

Yeast fermentation is an important technological process in bread making, influencing the carbohydrate fractions, which are important for the evaluation of the GI. Therefore, it is of advantage to know the differences in the metabolic pathways of the various yeasts and their ability to ferment certain sugars like maltotriose, maltose and glucose. The evaluation of the growth absorption values by YT MicroPlate™ showed varying metabolic patterns for the different *S. cerevisiae* strains as it can be seen in Table 4-2. The most relevant sugars for dough fermentation are maltose, glucose, fructose and sucrose (Henry & Saini, 1989). The metabolism for these sugars was significant different among the yeast strains. In general, the slowest yeast-cell growth could be seen for *S. cerevisiae* Baker's yeast on maltose as a substrate with an absorption value of 0.26 after 72 hours of incubation. All the yeasts originating from the beverage industry showed higher values between 0.46 and 0.64. The uptake of glucose (0.47) and galactose (0.54) was moderate for *S. cerevisiae* Baker's yeast. In terms of the utilisation of maltotriose, the fastest growth was visible for *S. cerevisiae* us-05 (0.72), whereas *S. cerevisiae* wb-06 hardly grew on this trisaccharide (0.19). Sucrose, as a substrate provided a high survival rate for all the yeast strains, which was between 0.61 for *S. cerevisiae* wb-06 and 0.78 for *S. cerevisiae* us-05. The absorption values for dextrin as a polysaccharide revealed a quite poor growth for all the yeasts except *S. cerevisiae* T-58 of 0.47. Raffinose as a minor compound in wheat flour was a moderate nutrient source for the different *S. cerevisiae* strains. All *S. cerevisiae* strains demonstrated excellent survival on fructose.

Table 4-2 Characterisation of metabolic patterns for bread making relevant sugars using the micro plate reader after 72h of incubation[#]

	average well colour development (AWCD)							Turbidity
S. cerevisiae	Dextrin	Maltotriose	Maltose	Glucose	Sucrose	Raffinose	Galactose	Fructose
Baker's yeast	0.14 ± 0.05^{B}	0.58 ± 0.07^{BC}	0.26 ± 0.07^{C}	0.47 ± 0.03^{D}	0.63 ± 0.17^{AB}	0.43 ± 0.01^{B}	0.54 ± 0.02^{D}	0.62 ± 0.10^{A}
s-23	0.13 ± 0.00^{B}	0.64 ± 0.03^{AB}	0.64 ± 0.01^{A}	0.73 ± 0.04^{A}	0.68 ± 0.06^{AB}	0.53 ± 0.04^{A}	0.78 ± 0.03^{A}	0.64 ± 0.10^{A}
T-58	0.47 ± 0.08^{A}	0.51 ± 0.00^{C}	0.46 ± 0.00^{B}	0.57 ± 0.01^{C}	0.61 ± 0.02^{AB}	0.25 ± 0.01^{D}	0.65 ± 0.00^{BC}	0.69 ± 0.05^{A}
us-05	0.23 ± 0.11^{AB}	0.72 ± 0.00^{A}	0.37 ± 0.05^{BC}	0.72 ± 0.04^{A}	0.78 ± 0.01^{A}	0.39 ± 0.03^{BC}	0.59 ± 0.04^{CD}	0.63 ± 0.05^{A}
wb-06	0.22 ± 0.22^{AB}	0.19 ± 0.04^{D}	0.46 ± 0.04^{B}	0.56 ± 0.01^{C}	0.61 ± 0.01^{B}	0.34 ± 0.02^{C}	0.70 ± 0.05^{AB}	0.71 ± 0.04^{A}
Blanc	0.36 ± 0.10^{AB}	0.55 ± 0.02^{BC}	0.48 ± 0.00^{B}	0.64 ± 0.00^{B}	0.65 ± 0.00^{AB}	0.25 ± 0.02^{D}	0.74 ± 0.00^{A}	0.66 ± 0.06^{A}

[#] Results are presented as average values with confidence interval of three independent measurements. Values in one column followed by the same upper case capital letter are not significantly different ($p<0.05$)

4.4.3. Loaf characteristics

The impact of the different yeast strains on bread quality characteristics were evaluated. One of the most important parameter is the loaf-specific volume. *S. cerevisiae* Blanc produced the bread with the lowest volume of 2.17 mL/g and *S. cerevisiae* T-58 had the highest volume of 3.55 mL/g. Another important quality characteristic of bread is hardness. The results are shown in Table 4-3. The hardness of the breads ranged from 5.73 N for bread produced with *S. cerevisiae* T-58 and 14.72 N for bread which was fermented with *S. cerevisiae* us-05. These large differences can be attributed to the different metabolites produced by the various yeast strains such as the amount of CO_3 (specific volume), variations in sugar composition, ethanol and glycerol (moisture distribution). Next to physical texture also the crumb grain characteristics are an important attribute. Digital image analysis using C-cell was performed to describe crumb grain structure (Table 4-3). Regarding the number of cells, *S. cerevisiae* Baker's yeast showed the highest (5353), whereas *S. cerevisiae* s-23 had the lowest number (4269). Higher area of cells, as found in *S. cerevisiae* Baker's yeast, s-23 and T-58, indicate a more open structure. The smallest area of cells was found for *S. cerevisiae* wb-06 and Blanc, indicating a firmer structure.

4.4.4. Compositional Analysis

Compositional analysis was carried out on the breads produced with different *S. cerevisiae* strains and the outcome of the final composition is summarised in Table 4-3. Fermentation with the various *S. cerevisiae* strains did not significantly change the amount of total and resistant starch. The protein content showed the highest value for bread fermented with *S. cerevisiae* us-05 (8.46%) and the lowest quantity for bread made with *S. cerevisiae* Blanc (7.16%) propably due to the activation of proteases. *S. cerevisiae* Blanc resulted in the lowest fat content of 1.28%. Whereas the highest concentration was obtained by applying *S. cerevisiae* us-05 (1.87%).

Table 4-3 Chemical composition based on dry matter (total starch, resistant starch, protein, fat) and technological bread parameters (specific volume, hardness)[#]

S. cerevisiae	Total starch [%]	Resistant starch [%]	Protein [%]	Fat [%]	Specific volume [mL/g]	Hardness [N]	Number of cells	Area of cells [%]
Baker's yeast	46.17 ± 4.35 ^{A}	0.71 ± 0.10 ^{A}	7.69 ± 0.12 ^{BC}	1.48 ± 0.02 ^{B}	3.52 ± 0.15 ^{A}	6.28 ± 0.75 ^{B}	5353 ± 388 ^{A}	51.7 ± 0.5 ^{A}
s-23	45.81 ± 1.20 ^{A}	0.58 ± 0.10 ^{A}	8.11 ± 0.13 ^{AB}	1.55 ± 0.10 ^{AB}	2.92 ± 0.02 ^{B}	8.86 ± 1.30 ^{B}	4269 ± 100 ^{D}	52.2 ± 0.4 ^{A}
T-58	41.25 ± 1.81 ^{A}	0.55 ± 0.07 ^{A}	7.66 ± 0.05 ^{BC}	1.48 ± 0.02 ^{B}	3.55 ± 0.14 ^{A}	5.73 ± 0.67 ^{B}	5161 ± 111 ^{AB}	52.2 ± 0.2 ^{A}
us-05	41.63 ± 0.60 ^{A}	0.59 ± 0.09 ^{A}	8.46 ± 0.20 ^{A}	1.87 ± 0.03 ^{A}	2.51 ± 0.17 ^{C}	14.72 ± 3.43 ^{A}	4505 ± 79 ^{CD}	50.2 ± 0.8 ^{B}
wb-06	43.43 ± 1.45 ^{A}	0.58 ± 0.13 ^{A}	7.51 ± 0.27 ^{C}	1.41 ± 0.15 ^{B}	2.36 ± 0.03 ^{C}	14.72 ± 0.03 ^{A}	4862 ± 155 ^{BC}	48.9 ± 0.2 ^{C}
Blanc	43.26 ± 0.97 ^{A}	0.47 ± 0.18 ^{A}	7.16 ± 0.26 ^{C}	1.28 ± 0.12 ^{B}	2.17 ± 0.13 ^{C}	14.49 ±0.84 ^{A}	4989 ± 180 ^{AB}	48.7 ± 0.7 ^{C}

[#] Results are presented as average values with confidence interval of three independent measurements. Values in one column followed by the same upper case capital letter are not significantly different ($p<0.05$) compared to Baker's yeast

The results for the carbohydrate and acid HPLC-analysis are presented in Table 4-4. The three main sugars maltose, glucose and fructose fermented by yeast were investigated. A significant lower amount of maltose was measured in the breads fermented with *S. cerevisiae* T-58 and *S. cerevisiae* Baker's yeast. Glucose evaluation showed minimum concentration of 0.36 mmol/kg for the bread made with *S. cerevisiae* Baker's yeast and a maximum content of 18.14 mmol/kg for the bread produced with *S. cerevisiae* us-05. 8.83 mmol/kg of fructose was left in the bread fermented with *S. cerevisiae* s-23 and 30.0 mmol/kg in the bread baked with *S. cerevisiae* Blanc. The total amount of sugars were highest for breads fermented with *S. cerevisiae* us-05 and lowest for breads made with *S. cerevisiae* Baker's yeast.

The citric acid, succinic acid and acetic acid content differed significantly among the breads. Fermentation with *S. cerevisiae* Blanc and *S. cerevisiae* wb-06 resulted in the lowest concentration of citric acid of 109.51 and 109.09 mmol/kg, respectively in comparison to *S. cerevisiae* us-05 with the highest amount of 116.75 mmol/kg. Similar findings were found for the concentration of succinic acid. *S. cerevisiae* Blanc (150.86 mmol/kg) resulted in a minor succinic acid production. Whereas, the most was formed by *S. cerevisiae* us-05 (215.98 mmol/kg). *S. cerevisiae* us-05 revealed also the highest content of 176.04 mmol/kg acetic acid. In contrast, *S. cerevisiae* wb-06 produced only 167.91 mmol/kg. Therefore the overall acid content was found to be the highest in breads fermented with *S. cerevisiae* us-05 and the lowest occurred in *S. cerevisiae* Blanc.

Table 4-4 Carbohydrates (maltose, glucose, and fructose) and organic acids (citric acid, succinic acid, acetic acid) analysed in bread crumb samples[#]

S. cerevisiae	Sugars [mmol/kg]			Acids [mmol/kg]		
	Maltose	**Glucose**	**Fructose**	**Citric acid**	**Succinic acid**	**Acetic acid**
Baker's yeast	55.99 ± 2.36 ^{B}	0.36 ± 0.18 ^{B}	16.85 ± 0.76 ^{B}	112.97 ± 0.33 ^{AB}	161.20 ± 3.15 ^{B}	175.35 ± 0.53 ^{AB}
s-23	67.47 ± 1.55 ^{A}	1.37 ± 0.34 ^{B}	8.83 ± 1.27 ^{C}	110.70 ± 1.83 ^{AB}	185.31 ± 13.23 ^{C}	171.63 ± 4.07 ^{AB}
T-58	54.22 ± 1.29 ^{B}	0.39 ± 0.19 ^{B}	10.94 ± 0.95 ^{BC}	111.17 ± 1.48 ^{AB}	168.74 ± 11.86 ^{BC}	172.89 ± 2.76 ^{AB}
us-05	63.04 ± 6.35 ^{AB}	18.14 ± 3.88 ^{A}	25.76 ± 4.15 ^{A}	116.75 ± 4.82 ^{A}	215.98 ± 7.29 ^{A}	176.04 ± 3.38 ^{A}
wb-06	72.45 ± 2.49 ^{A}	2.71 ± 0.51 ^{B}	26.89 ± 1.93 ^{A}	109.51 ± 0.88 ^{B}	173.47 ± 3.07 ^{BC}	167.91 ± 1.78 ^{B}
Blanc	67.10 ± 2.22 ^{A}	4.43 ± 0.63 ^{B}	30.00 ± 1.96 ^{A}	109.09 ± 0.53 ^{B}	150.86 ± 2.57 ^{C}	169.08 ± 1.51 ^{AB}

[#] Results are presented as average values with confidence interval of three independent measurements. Values in one column followed by the same upper case capital letter are not significantly different ($p<0.05$)

4.4.5. Impact of chemical composition and technological characteristics on the pGI

PCA analysis was performed for technological characteristics as well as for compositional parameters of the breads baked with different strains of *S. cerevisiae* (Figure 4-2a). The first PC models primarily a measure of the acids, fat, protein, glucose and pGI. Whereas the second PC represents principally the technological properties (hardness and specific volume) as well as maltose, fructose and starch. Using hierarchical classification, it was possible to differentiate the used *S. cerevisiae* strains into 3 groups (Figure 4-2b): (A) *S. cerevisiae* Baker's yeast, T-58 and s-23, whereas the latter one is separated by a sub-group; (B) *S. cerevisiae* wb-06 and Blanc and (C) *S. cerevisiae* us-05. Indeed, breads of group A were characterised by high specific volume, low hardness which resulted in a high pGI. Contrastingly, group B showed opposed technological features but shared lower sugar content which overall decreased the pGI of the breads. The appearance of *S. cerevisiae* s-23 in the sub-group of A displays its lower specific volume resulting in higher bread density. The application of *S. cerevisiae* us-05 is set apart mainly due to its high glucose level represented by high glycaemic response. Statistical analysis showed correlations between specific volume and pGI and hardness and pGI of $r= 0.903$, $p \leq 0.04$ and $r= -0.873$, $p \leq 0.05$, respectively. However, it has to be mentioned that *S. cerevisiae* us-05 had to be excluded for these tests.

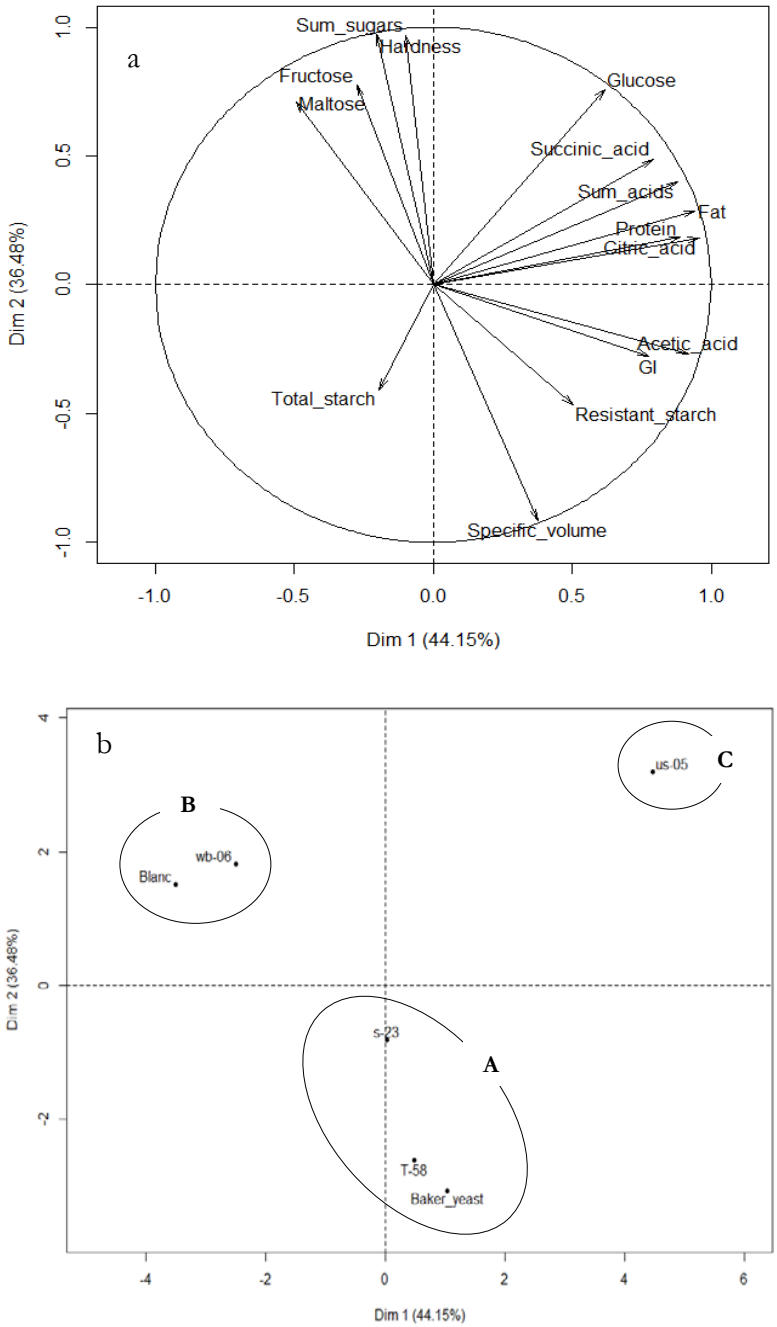


Figure 4-2 Principal component analysis of technological and compositional parameters: (a) distribution of analysed parameters, (b) hierarchical classification of breads fermented with different *S. cerevisiae* strains

4.5. Discussion

The GI refers to a measurement of glucose entering the blood stream and is related to the rate of carbohydrate-containing food absorption and digestion in the human body. A low GI in bakery products can reduce the risk of coronary heart disease and showed beneficial effects in the management of diabetes. In this study, the impact of the different yeast strains applied to the bread making process on the pGI of bread was investigated. The pGI was measured using an *in vitro* enzymatic model system in relation to the metabolic patterns of the different *S. cerevisiae* strains used and the compositional analysis of the breads.

Apart from the bread fermented with *S. cerevisiae* us-05, all other breads showed lower pGI values than the reference bread (*S. cerevisiae* Baker's yeast). However, a pGI reduction was only significant for breads baked with *S. cerevisiae* wb-06, s-23 and Blanc.

The modification of the digestibility of a bread product directly influences the GI. The absorption of carbohydrates can be altered by changes in formulation and processing of cereal products (Fardet et al., 2006). Therefore, it is not surprising that starch characteristics have a significant impact on the GI. Other factors influencing the GI are the food product matrix, determined by protein and lipid content or physical texture (Fardet et al., 2006). Berti, Riso, Monti, & Porrini, (2004) suggested a high gluten content has a GI lowering effect. The authors explained this result by the fact that gluten surrounds the starch granules, which makes them less accessible to amylases (Fardet et al., 1998). Compositional analysis of the baked breads in this study revealed that their total and resistant starch contents did not differ significantly. Regarding protein and lipids, lowering of a food products' GI was only seen at a protein to carbohydrate ratio of 3:5 and only large amounts of fat in the ratio of 1:1 (Arvidsson-Lenner et al., 2004). Although differences in the amount of protein and fat were observed, their proportion when related to carbohydrate content was not high enough to effectively alter the GI of the breads. Therefore, total and resistant starch as well as protein and fat content can be neglected for having an impact on the pGI change of the breads in our study.

Processing can also have an influencing effect on the GI (Ross, Brand, Thorburn, & Truswell, 1987). It has been shown by Fardet et al., 2006 and Burton & Lightowler, 2006 that fermentation conditions and metabolites can impact on enzyme activity as well as the

individual components found in bread. One example how processing impacts on the GI is that during baking starch is gelatinised which makes the starch granules more accessible to enzymatic degradation and therefore increases the GI of a food product (Berti et al., 2004; Brand, Nicholson, Thorburn, & Truswell, 1985; Ross et al., 1987). Considering the fermentation process, inefficient dough rising results in denser products. This can lead to a lower core temperature during baking, which limits gelatinisation and swelling of the starch granules and consequently causes a decrease in starch digestibility (Burton & Lightowler, 2006). *S. cerevisiae* wb-06, Blanc and to some extent s-23 are characterised by slower fermentation rates (Table 4-1), which were obtained by scoring the metabolic patterns (YT MicroPlateTM) and the gas production (rheofermentometer). Their breads were reduced in GI and had lower specific volumes as well as firmer crumb structures as confirmed by C-Cell measurements (Table 4-3). Hence, starch gelatinisation in doughs fermented with these yeasts was probably more restricted. Nevertheless, a small and dense bread can give a high pGI product. This evidences that there was another main contributor increasing the rate of glycaemic response for the dense bread fermented with *S. cerevisiae* us-05.

In addition to starch gelatinisation, a second major influencing factor is the presence of organic acids. Several studies have shown that the use of sourdough in bread production and the thereby generated organic acids can reduce the postprandial glucose response of wheat bread (Borczak et al., 2011; De Angelis et al., 2007; Lappi et al., 2010; Scazzina, Del Rio, Pellegrini, & Brighenti, 2009; Siragusa et al., 2009). One main mechanism how the addition of sourdough is reducing the GI is explained as follows: the addition of sourdough reduces the pH, which promotes the activity of flour enzymes which results in a reduction of the starch content (Liljeberg et al., 1995; Wolter, Hager, Zannini, & Arendt, 2014). Although *S. cerevisiae* us-05 produced the most acids (Table 4-4), a significant pH decrease was not measured in the resulting bread crumbs (data not shown). However, this could have given improved starch degradation during the dough fermentation stage. In the beginning of the bread making process, yeast first ferments free available sugars like sucrose, raffinose, glucose and fructose (Randez-Gil, Sanz, & Prieto, 1999a). As dough fermentation continues, flour amylases provide further growth substrate by the release of dextrins, maltotriose, maltose and glucose due to the starch degradation (Randez-Gil et al., 1999a). Fructan hydrolysis also occurs due to the activity

of yeast's invertase (Nilsson et al., 1987). Since these sugars cause different glycaemic responses, their uptake and metabolism by yeast highly impact the GI. A high concentration of glucose (GI 100) and maltose (105) therefore suggest a higher GI of the product. The total amount of sugars were highest for breads fermented with *S. cerevisiae* us-05. In particular, although this strain revealed a very good proliferation on glucose as substrate (Table 4-2), which would support a fast glucose depletion, residual glucose was extraordinary high in these breads (Table 4-4). It is possible that glucose was accumulated during the fermentation process. A possible explanation for this effect could be the high growth rate of *S. cerevisiae* us-05 on maltotriose (Table 4-2) liberating even more glucose during the fermentation which the strain could not all utilize. To conclude, this high glucose content is probably the main reason for the increased glycaemic response of the dense breads fermented with *S. cerevisiae* us-05. Contrastingly, high levels of fructose (GI of 15) refer to a lower GI of a product (Atkinson et al., 2008). Fermentation with *S. cerevisiae* wb-06 and Blanc resulted in higher residual fructose amounts than fermentation with the *S. cerevisiae* Baker's yeast and T-58 (Table 4-4). This contribution further supports the low pGI measured in these denser breads. In the breads fermented with *S. cerevisiae* Baker's yeast and T-58, the amount of sugars was low and notably very little glucose was detected which would have favoured a pGI reduction of these products. Lower fructose levels also indicated fructose consumption. Nevertheless, all possible factors have to be considered when judging a product's GI. Concluding for these breads and their increased glycaemic response, it was probably their highest carbon dioxide production (Table 4-1) which let the dough rise best during fermentation and was then followed by a complete starch gelatinisation during baking resulting in soft breads with high specific volumes, but also in a better starch digestibility.

This study showed that the pGI of white wheat bread can be significantly decreased by using different strains from the species *S. cerevisiae*. PCA confirmed that those breads were quite distant in terms of their technological properties, chemical composition and the resulting pGI. This opens opportunities for an increasing variety of bread by only changing the starter culture, without necessary adaptations to the bread-making process and/or recipe, which is of interest to the baking industry.

4.6. Acknowledgment

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5. Chapter Correlation of flavour profile to sensory analysis of bread produced with different *Saccharomyces cerevisiae* originating from the baking and beverage industry

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5.1. Abstract

Aroma is an important quality parameter for wheat bread and most of the aroma compounds in yeast-fermented bread result from the fermentative action of yeast. In this study, the impact of various strains of *Saccharomyces cerevisiae*, originating from the beverage industry, were investigated on the aroma profile of wheat bread. Seven volatiles were analysed by gas chromatography-mass spectrometry after thermal desorption (GC-MS TD) from the bread crumb. The results showed yeast strain dependent production of aroma compounds. Descriptive sensory analysis resulted in an overall taste acceptance by the panellists for breads baked with *S. cerevisiae* Baker's yeast, T-58 and Blanc. The panel acceptance can be explained by the production of sensory active compounds such as 3-methyl-1-butanol and 2,3-butandiol. Furthermore the panellists preferred bread samples with a less bitter ($r=0.934$, $p<0.01$) and less cheesy taste ($r=-0.865$, $p<0.03$). Also the visual aspects play an important role, shown by correlation between the specific-volume and the overall appearance ($r=0.928$, $p<0.01$). Aroma profile analysis offers a tool for the selection of new yeast strains, increasing the bread variety on the market. Consequently, aroma production as a yeast quality characteristic should be taken into account for the selection of new strains involved in bread making.

5.2. Introduction

Although a large amount of aroma compounds are formed during the baking process, fermentation plays a key role in the development of the unique bread flavour (Hui, 2006). As a result of the yeast metabolism, a wide range of aroma-active volatiles have been identified (Birch, Petersen, Arneborg, et al., 2013). Overall, the main groups responsible for bread crumb aroma are alcohols, aldehydes, esters, ketones and acids. From the beer and wine industry it is common knowledge that the choice of yeast strain is an important parameter to alter flavour perception of the end product (Pires et al., 2014; Swiegers et al., 2006; Wondra & Berovič, 2001). Only recently, flavour and aroma profiles are considered as quality parameters during bread making (Birch, Petersen, Arneborg, et al., 2013; Birch et al., 2014; Cho & Peterson, 2010; Pico et al., 2015). This has led to an increasing commercial interest within the field of bread fermentation to change the aroma characteristics of bread. Styger, Jacobson, & Bauer, (2011) explained that differences in the genes of *Saccharomyces cerevisiae* strains play a major role in the change of the aroma profiles. The main genes responsible for aroma formation by yeast have been recently investigated by Hazelwood, Daran, van Maris, Pronk, & Dickinson, (2008) and belong to the Ehrlich pathway. Another study showed variation in the aroma profile of bread with the application of seven commercial Baker's yeasts (Birch, Petersen, Arneborg, et al., 2013). These modifications might be due to changes in the gene-regulating mechanisms and pathways of aroma compounds. The formation of 1-propanol, 2-methyl-1-propanol and 3-methylbutanal from valine and leucine via the Ehrlich pathway was higher in two Belgian Baker's yeasts when compared to yeasts produced in other European countries (Birch, Petersen, Arneborg, et al., 2013). Several carboxylases have been investigated to be important for the catabolism of these branched-chain amino acids as well as the aromatic amino acid phenylalanine (Dickinson, Salgado, & Hewlins, 2003). Hence, the activity of these carboxylases in commercial Baker's yeasts is strain dependent. Furthermore yeast associated extracellular enzymes like proteases, lipases and amylases can have an influence on the aroma profile. A minor role of enzymes during bread making is the production of flavour precursors. Residual sugars mainly glucose and fructose originating from amylase activity are able to participate in the Maillard reaction during baking (Maga & Pomeranz, 1974). Proteases increase amino acids and peptide

concentrations which are participating as precursors for aroma production in the Ehrlich pathway as well as Maillard reaction. Lipase activity is responsible for the production of short chain fatty acids and therefore induces changes in lipid composition, which also contribute to flavour changes (Martínez-Anaya, 1996). A higher yeast quantity can increase the concentration of the aroma compounds 3-methyl-1-butanol, 2-phenylethanol and 3-hydroxy-2-butanone as reported by Birch, Petersen, & Hansen, (2013). 3-methylbutanal, 3-methyl-1-butanol, phenylacetaldehyde and 2,3-butandione were predicted to be the most important aroma-active volatiles. An extensive review of the available literature showed that yeast strains indeed influence the aroma profile of fermented products such as beverages (Huang et al., 2010; Pires et al., 2014; Suárez-Lepe & Morata, 2012). However, only little attention has focused on the impact of yeast strains on bread aroma. This study investigated how different yeasts originating from the beer and wine industry alter the aroma profile in comparison to Baker's yeast. In addition, a descriptive sensory analysis was performed using a trained panel. The present findings further add knowledge to improve the understanding of aroma formation during dough fermentation.

5.3. Experimental

5.3.1. Materials

The suppliers of the ingredients were Voigtmühle Illertissen, Germany for Baker's flour; Glacia British Salt Limited, UK for salt and Vandemoortele, Izegem, Belgium for palm fat. Instant active dry Baker's yeast was obtained from Puratos, Belgium; Dry yeast s-23, T-58, us-05 and wb-06 were supplied by Fermentis Division of S. I. Lesaffre, France. Dry yeast Blanc was supplied from Vinoferm, Brouwland, Beverlo, Belgium. All the yeasts applied in this study belonged to the species *S. cerevisiae*. All chemicals were supplied by Sigma-Aldrich, Arklow, Ireland.

5.3.2. Bread making

Bread making was performed as previously described by Heitmann et al., (2015) with some modifications. Wheat breads were prepared using 2.2% salt, 1% palm fat, 62% water and different amounts of yeast (Table 5-1), based on flour weight. The amount of yeast was adapted to the amount of Baker's yeast (1.13 E+09 cfu/g) analysed by the total cell count (Heitmann et al., 2015). Yeast was dissolved in water (25 °C) and activated for 10 min. The yeast/water suspension was added to the premixed dry ingredients and the fat. Mixing was performed for 2 min at low speed with a spiral mixer (mac. pan, Thiene, Italy). A further mixing at higher speed was carried out for 5 min. The doughs were scaled to 500 g, moulded and placed into baking tins which were proofed (KOMA sunriser, Roermond, the Netherlands) for 85 min (35 °C, 75% relative humidity). Baking was carried out for 35 min at 230 °C top and bottom temperature in a deck oven (MIWE condo, Arnstein, Germany), previously steamed with 0.35 L of water. After baking the bread loaves were directly removed from the tins and cooled down at room temperature for 120 min. Finally, the bread crumb and crust was separated and stored frozen for further analysis.

5.3.3. Technological Bread and Dough Characteristics

Loaf-specific volume and bake loss were analysed after cooling for 2 hours with a Vol-scan Profiler (Stable Micro System, UK) (Heitmann et al., 2015). A C-cell bread imaging

system (Calibre Control International Ltd., UK) was used for the evaluation of the structure of bread crumb. The analysis was also performed with three central slices of three loafs. Parameters investigated by the C-cell were total number of cells and number of cells/mm^2 (Heitmann et al., 2015). Rheofermentometer F3 (Chopin, France) measurements were used for the investigation of the carbon dioxide production (V_{total}) and retention during fermentation ($V_{retention}$) (Heitmann et al., 2015).

5.3.4. Extraction of Volatile Aroma Compounds by Thermal Desorption (TD) and Quantification using GC-MS

For the extraction of volatile aroma compounds samples were prepared by weighing 0.1g into a clean glass thermal desorption (TD) tube to concentrate the volatile aroma compounds in a gas stream prior to injection (Perkin Elmer Turbomatrix 650). Subsequently, the aroma compounds were absorbed at 90°C for 10 min. Quantification of the aroma-active volatiles was made using a gas chromatography mass spectrometer (GC-MS, Agilent 5977B MSD) with a Rxi 624-Sil 20m column and helium as carrier gas. The details for the temperature profile are start temperature: 35°C (4 min) with an increase of 15°C/min to 220°C (hold 1 minute). The total run time was 17.3 min. For the detected compounds a database search was conducted. The aroma compounds detected and analysed in this study by GC-MS TD were ethanol, acetic acid, 3-methyl-1-butanol, isobutyric acid, 2,3-butanediol, 1-hexanol and 2-phenylethanol.

5.3.5. Sensory Analysis

Descriptive sensory analysis was performed using a trained panel consisting of 15 panellists (10 male, 5 female, aged 25-34 years) using the aroma profile analysis (APA). During a period of six month prior to participation in the sensory, weekly sessions were held to train the panellists to be able to orthonasal recognise 120 selected odorants at different odorant concentrations. Odour qualities and quantities were determined by smelling reference solutions. The performance was assessed via standard procedures for each panellist. All training and sensory analyses were performed in a sensory panel room at 21 ± 1°C. Based on reference aroma solutions with certain concentrations a "flavour language" was developed to define the specific smell of a compound corresponding to a

certain aroma attribute. For descriptive aroma profile analysis, each wheat bread sample was cut in slices (thickness: 2 cm) and presented to the sensory panel. The sensory panel had to sniff the crumbs and describe the odour intensity they perceived on a scale from 0 (not detectable) to 10 (high intensity). For descriptive taste profile analysis, the panellists tasted the bread crumb and scored the intensities of the taste attributes on the same scale. In order to evaluate the aroma, taste and overall liking, the panel evaluated the liking of each sample on a 0 (dislike very much) to 10 (like very much) scale. Arithmetic means of each sensory score was calculated.

5.3.6. Data Analysis

Results are shown as average ± confidence interval of at least triplicate measurements. Minitab 16 software was used to carry out statistical analysis. Exploratory data analysis was followed by a multiple comparison procedure of variance (one way ANOVA) followed by a post-hoc Tukey test to describe significant differences at a level of significance of 5% ($p<0.05$). In addition, Pearson correlation was performed to find linear dependencies between all the various parameters ($p<0.01$).

5.4. Results and Discussion

5.4.1. Loaf Characteristics

Standard bread quality parameters were analysed (Table 5-1). *S. cerevisiae* us-05, wb-06 and Blanc showed the least gas production during 3 hours of fermentation indicating a slow fermentation rate. Accordingly, their bread specific volume was significantly lower ($p<0.05$). A positive correlation between gas production and specific volume was found to underline this finding ($r=0.92$ $p<0.001$). Since the amount of viable cells was for all yeasts the same, this fact can be mainly explained due to the applications commonly used for the various yeasts. The used yeasts are normally applied in the production of beer and wine where fermentation in general takes place several days as well as the different substrate used as an energy source. However, the positive results for *S. cerevisiae* T58 and s-23 might be explained by their higher temperature tolerance. They are therefore better adapted to the bread fermentation process.

Table 5-1 Technological bread characteristics and dough fermentation parameters[#]

S. cerevisiae	Dosage [%] based on flour	Specific volume [mL/g]	Bake loss [%]	Number of cells	Number of Cells/mm²	V_{total} [mL]	$V_{retention}$ [mL]
Baker's yeast	2	3.52 ± 0.15 ^{A}	10.52 ± 0.21 ^{A}	5353 ± 388 ^{A}	0.57 ± 0.04 ^{BC}	1372.0 ± 37.2 ^{A}	1229.0 ± 25.5 ^{A}
s-23	4	2.92 ± 0.02 ^{B}	11.08 ± 0.26 ^{A}	4269 ± 101 ^{D}	0.54 ± 0.01 ^{C}	1030.5 ± 148.0 ^{BC}	961.5 ± 104.9 ^{BC}
T-58	2	3.55 ± 0.14 ^{A}	10.45 ± 0.40 ^{AB}	5161 ± 112 ^{AB}	0.54 ± 0.02 ^{C}	1321.0 ± 96.0 ^{AB}	1172.0 ± 58.8 ^{AB}
us-05	6	2.51 ± 0.17 ^{C}	10.28 ± 0.40 ^{AB}	4504 ± 79 ^{CD}	0.64 ± 0.05 ^{B}	822.5 ± 61.7 ^{C}	789.0 ± 49.0 ^{C}
wb-06	2	2.36 ± 0.03 ^{C}	9.16 ± 0.58 ^{C}	4862 ± 155 ^{BC}	0.72 ± 0.01 ^{A}	402.5 ± 8.8 ^{D}	400.0 ± 9.8 ^{D}
Blanc	½	2.17 ± 0.13 ^{C}	9.39 ± 0.36 ^{BC}	4989 ± 180 ^{AB}	0.74 ± 0.01 ^{A}	371.0 ± 184.2 ^{D}	369.0 ± 184.2 ^{D}

[#] Values in one column followed by the same superscript letter are not significantly different ($p<0.05$).

5.4.2. Analyses of Volatile Aroma Compounds in Bread Crumb

In addition to ethanol, which is the highest amount of volatile aroma compound produced during bread fermentation, many other key aroma components have been identified in the crumb of wheat bread such as 3-methyl-1-butanol, 2-phenylethanol and 2,3-buntanedione (Gassenmeier & Schieberle, 1995; Schieberle & Grosch, 1991). Also various acids like acetic acid, butyric acid, valeric acid and isobutyric acid contribute to the overall aroma (Frasse et al., 1993). Non-volatile compounds, which have an influence on the flavour of wheat bread are lactic acid (originating from sourdough) and salt (Calvel, Wirtz, & MacGuire, 2001). The corresponding pathways responsible for the aroma formation by *S. cerevisiae* are presented in Figure 5-1. The resulting concentrations are summarised in Table 5-2. Ethanol concentration showed significant differences in bread crumbs. The highest amount was found in breads baked with *S. cerevisiae* T-58 probably due to its higher temperature tolerance. *S. cerevisiae* Blanc was expected to show the highest alcohol production, since this yeast, is normally used for wine production and has a high alcohol tolerance.

3-methyl-1-butanol was only detectable in bread samples baked with *S. cerevisiae* Baker's yeast, s-23 and T-58. Birch et al., (2014) and Frasse et al., (1993) stated that 2- and 3-methyl-1-butanol are the most important aroma and sensorial compounds in yeast fermented bread crumb although they are not significantly influenced by yeast concentration. Nevertheless, they show an increase when the fermentation time is prolonged (Frasse et al., 1993; Gassenmeier & Schieberle, 1995; Schieberle & Grosch, 1991). As well as in alcoholic beverages 2- and 3-methyl-butanol next to n-propanol, iso-butanol and ethanol are the most significant alcoholic aroma compounds which contribute to a warm mouth feel and are mainly produced via the Ehrlich pathway (Brányik, Vicente, Dostálek, & Teixeira, 2008). Therefore, the final concentration of alcohols is evaluated by the utilisation of amino acids and sugar uptake rate. The amino acid composition of the fermentation substrate, the fermentation stage and yeast strain consequently have an influence on the biosynthetic pathways.

Table 5-2 Concentration and organoleptic descriptions of volatile aroma compounds in bread-crumb samples as determined by GC-MS TD

Compound	organoleptic description	Concentration [µg/kg]					
		S. cerevisiae					
		Baker's yeast	**s-23**	**T-58**	**us-05**	**wb-06**	**Blanc**
ethanol	alcoholic, sweet	3900	4400	5500	2400	2100	3200
acetic acid	vinegar, pungent, sour	50	130	n.d.*	110	110	93
3-methyl-1-butanol	alcoholic, fermented, fruity	86	70	64	n.d.*	n.d.*	n.d.*
isobutyric acid	acidic, sour, cheesy	20	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*
2,3-butanediol	fruity, creamy, buttery	n.d.*	38	62	11	12	n.d.*
1-hexanol	green, fruity	n.d.*	n.d.*	n.d.*	23	n.d.*	n.d.*
2-phenylethanol	bready, flowery, sweet	15	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*

* not detected

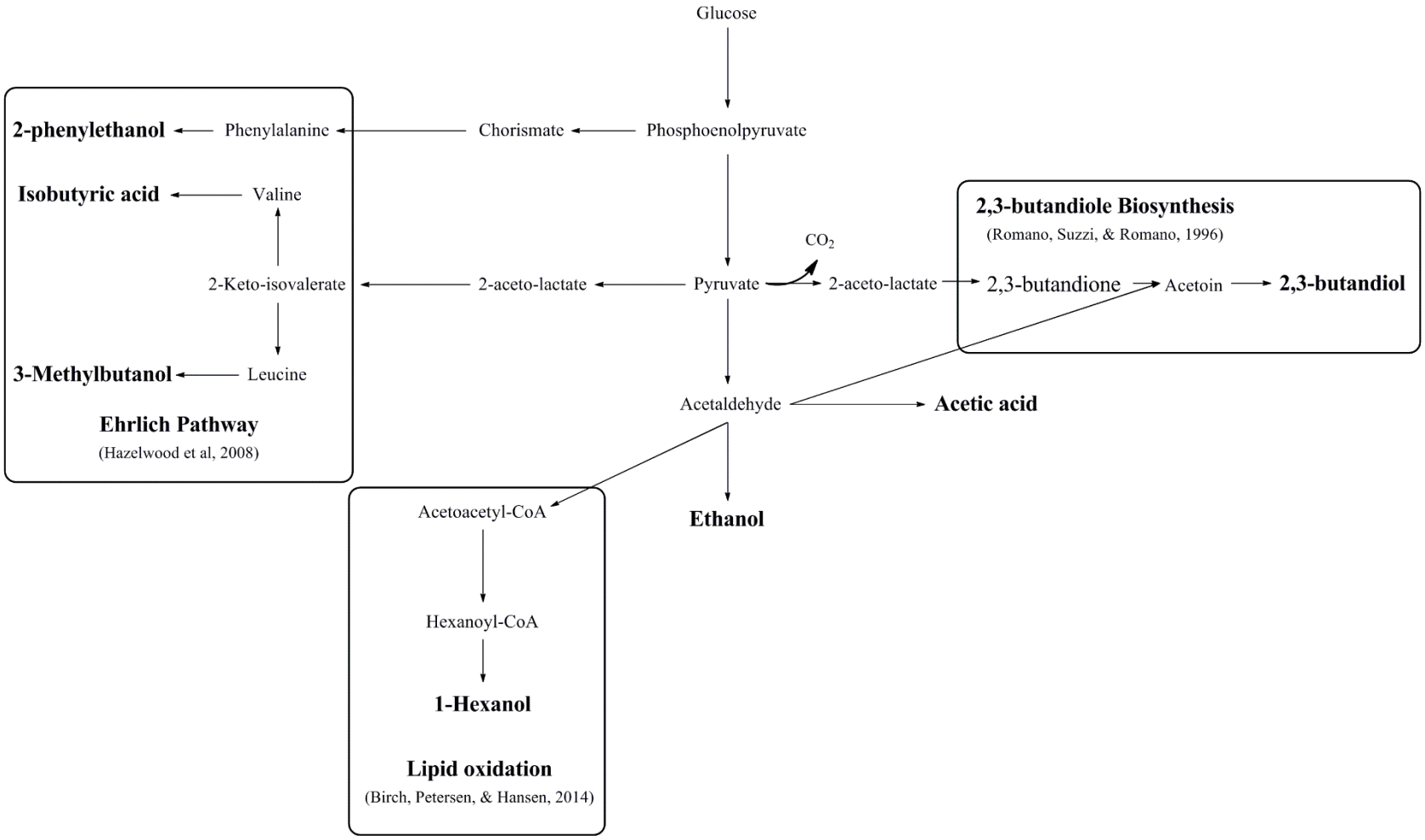


Figure 5-1 Yeast metabolism pathways of relevant aroma compounds analysed during this study

Since the production of the aroma compounds analysed in this study is related to the Ehrlich pathway, it is noteworthy to mention the BAP2 gene. This gene controls the expression of branched chain amino acid permeases, which are responsible for the uptake of leucine, isoleucine and valine (Grauslund, Didion, Kielland-Brandt, & Andersen, 1995). Kodama, Omura, Miyajima, & Ashikari, (2011) showed a constitutive expression of the BAP2 gene by using the brewing strain BH-225 at higher temperatures. The consequent higher utilisation of valine, leucine and isoleucine promoted the production of 3-methyl-1-butanol. The temperature dependency was confirmed by Abe & Minegishi, (2008), who showed a reduced gene expression at lower temperatures. However, it was demonstrated that for the uptake of all branched chain amino acids in *S. cerevisiae* the main regulatory signal for the transcriptional induction of BAP2 is the presence of micromolar amounts of leucine (Didion, Grauslund, Kielland-Brandt, & Andersen, 1996). Since in this study the used flour was always the same and therefore the amino acid composition did not change, it is suggested that *S. cerevisiae* us-05, wb-o6 and Blanc are lacking the BAP2 gene. As known from the suppliers specification sheet *S. cerevisiae* s-23 and T-58 are able to perform a faster fermentation at higher temperatures, which promotes the production of 3-methyl-butanol. 2-phenylethanol is another sensorial important volatile formed in yeast fermented bread crumb which is characterised by a flowery aroma. Birch et al., 2014 and Frasse et al., (1993) mentioned that it is one of the aroma compounds produced with the highest concentration in bread crumb. This fact could not be confirmed by this study, since 2-phenylethanol was only produced by *S. cerevisiae* Baker's yeast in a small degree (15 µg/kg). The formation of 2-phenylethanol in *S. cerevisiae* is through the Ehrlich pathway using the amino acid phenylalanine. Brewer's yeast assimilates phenylalanine slower than other amino acids such as leucine and lysine (Reed & Nagodawithana, 1991). Hence, it is not surprising that there was no detectable production of 2-phenylethanol when applying beer yeast strains in a bread system. Birch, Petersen, Arneborg, et al., (2013) explained this by different carboxylases present in *S. cerevisiae*. Dickinson et al., (2003) found that carboxylases are important for the utilisation of branched-chain amino acids (leucine, valine) and aromatic amino acids (phenylalanine) in the Ehrlich pathway. Therefore, the aroma profile of fermented products is yeast strain dependent. Isobutyric acid is produced by *S. cerevisiae* via the Ehrlich pathway by the utilisation of valine. Russell & Stewart, (1987) stated that in wort fermentation the

availability of high leucine levels stimulate the formation of isobutyric and isovaleric acid. Isobutyric acid was also only produced by *S. cerevisiae* Baker's yeast (20µg/kg). A production of 2,3-butanediol was only found in breads fermented with the beer yeasts *S. cerevisiae* s-23, T-58, us-05 and wb-06. The responsible pathway for the production of 2,3-butanediol by yeast is the oxidative decarboxylation of 2-acetolactate to 2,3-butanedione, which is further enzymatically reduced to 2,3-butanediol (Romano & Suzzi, 1996; Wainwright, 1973), see also Figure 5-1. Maiorella, Blanch, & Wilke, (1983) showed that at higher 2,3-butanediol concentrations, more energy is needed for the active transport mechanism, since it is only slightly lipid-soluble. The active transport mechanism removes the internally produced 2,3-butanediol through the lipid membrane, which could result in an increased ethanol production to provide the necessary amount of ATP. Therefore, *S. cerevisiae* T-58 with the highest ethanol concentration of 5500 µg/kg shows also the highest production of 2,3-butanediol of 62 µg/kg ($r=0.987$ $p<0.01$). Since in most of the beers a high concentrations of 2,3-butandione is undesirable, the removal of 2,3-butandione is considered as the rate-limiting step during beer maturation. In beer production, a maximum 2,3-butandione production occurs around seventy-two hours after production, so „aging" is a major step in beer making (Godtfredsen & Otresen, 1982). In brewing, there are various strategies to remove 2,3-butandione, the most common practice is to use higher temperatures to enhance the decarboxylation to acetoin and 2,3-butanediol. Therefore, since dough fermentation is performed at higher temperatures than beer fermentation, the production of 2,3-butanediol is favoured. 2,3-butanediole is also the most prominent diol produced during wine fermentation. However, in wine it has only little sensory significance since it has minor effect on the odour of the wine and only a slightly bittersweet taste.

Hexanol, is a metabolite originating from lipid oxidation and was only found in bread crumb baked with *S. cerevisiae* us-05. Frasse et al., (1992) stated that a higher yeast activity results in a reduced lipoxygenase activity. These enzymes need oxygen to convert fatty acids into the corresponding aldehydes (propanal, pentanal, hexanal etc.) and alcohols (1-propanol, 1-pentanol, 1-hexanol etc.), therefore a lower yeast concentration or activity and therefore a slower consumption of oxygen may induce an increase in lipid oxidation products. The only yeast showing a production of 1-hexanol is *S. cerevisiae* us-05. This is in accordance with the relatively low carbon dioxide production during fermentation

(Table 5-1). *S. cerevisiae* Blanc showed generally no flavour production besides acetic acid and ethanol during baking. The production of 1-hexanol depends on the presence and concentration of six-carbon precursors (glucose, fructose and amino acids) in grapes during wine fermentation (Killian & Ough, 1979). Wine yeast is highly adapted to the fermentation of grapes and less well to grain or flour fermentation. This is reflected as well in the inferior loaf characteristics of the corresponding breads.

5.4.3. Descriptive Sensory Evaluation

Eight sensory attributes were collected for the smell and taste of the bread-crumb samples. The dominating characteristics were roasted, salty, buttery, yeasty, bitter and cheesy. The sensory analysis showed no significant differences for the smell among the panellists (Figure 5-2A). In terms of taste only significant differences for cheesy and bitter were detectable by the panellists (Figure 5-2B). The panellist revealed the highest perception of a cheesy aroma for breads fermented with *S. cerevisiae* s-23 and us-05 and a lower perception for breads produced with all of the remaining *S. cerevisiae* strains. The panellist observed a bitter taste in breads fermented with *S. cerevisiae* us-05. The lowest bitter perception was associated to *S. cerevisiae* Baker's yeast. The highest panel acceptance in terms of overall taste were found for breads baked with *S. cerevisiae* Baker's yeast and T-58 (Figure 5-2B). In both breads a high concentration of 3-methyl-1-butanol was detected. Recently, a positive correlation between the aroma of wheat bread and the concentration of 3-methyl-1-butanol and 2-phenylethanol has been shown by Salim-ur-Rehman, Paterson, & Piggott, (2006). Bread fermented with *S. cerevisiae* Blanc showed also a high taste acceptance, although it resulted in the lowest production of aroma compounds. Only ethanol and acetic acid could be detected by GC-MS TD analysis. Some aroma compounds like hexanal (Martínez-Anaya, 1996), butyric acid (Quílez, Ruiz, & Romero, 2006) and higher alcohols are originated from lipid oxidation. 1-hexanol and 1-octen-3-ol were found to have a negative correlation on consumer preference due to their unpleasant aroma (Paraskevopoulou, Chrysanthou, & Koutidou, 2012). The sensory analysis of the bread revealed *S. cerevisiae* us-05 as the least preferred one by the panellists. This might be linked to the production of 1-hexanol during bread making as well as a significant higher roasted smell and taste.

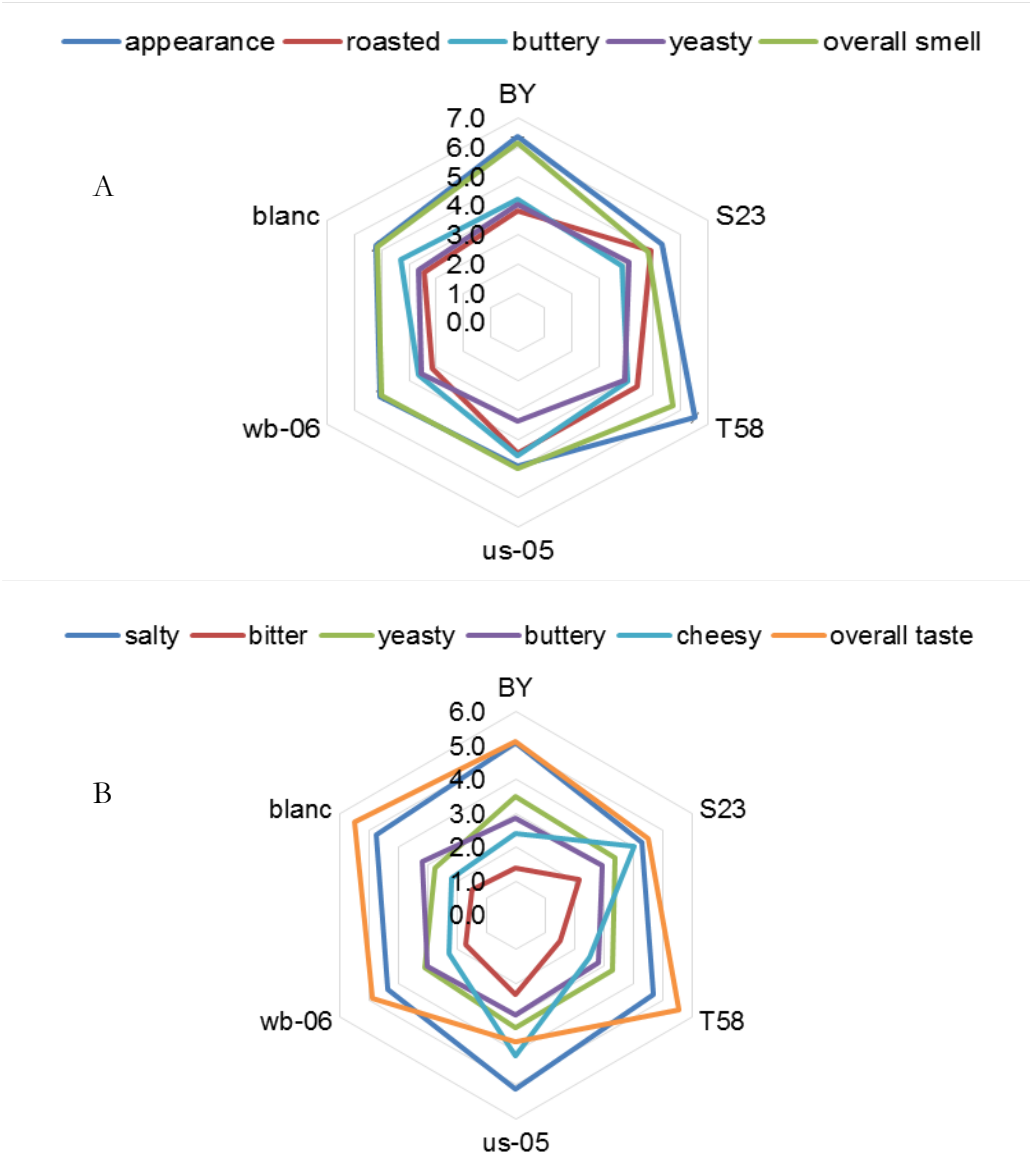


Figure 5-2 Sensory analysis of bread crumbs for the smell (A) and taste (B) of breads baked with different *S. cerevisiae* strains on a scale from 0 (not detectable) over 10 (high intensity)

5.4.4. Correlation between Aroma Compounds, Technological Bread Characteristics and Sensory Acceptance.

Pearson correlation analysis revealed several linear dependencies among the various parameters analysed. The technological bread parameter, number of cells/mm^2 showed a negative relationship to the overall appearance ($r=-0.752$ $p<0.09$). Panellists associate a

low number of cells with a dense and dry product which decreases the acceptance. As expected, a positive correlation was found between the specific volume and the overall appearance ($r=0.928$, $p<0.01$) as judged by the trained panel. A high specific volume is also related to a soft crumb.

Due to their alcoholic organoleptic characteristics, another positive correlation was found between the yeasty smell and the ethanol concentration in the bread samples ($r=0.816$, $p<0.05$) as well as the yeasty taste and the 3-methyl-1-butanol concentration ($r=0.985$, $p<0.1$). Since 3-methyl-1-butanol is one of the most important aroma compounds within yeast fermented bread crumbs, it is not surprising that *S. cerevisiae* Baker's yeast, s-23 and T-58, reached the high acceptance from the panel. These were the only yeasts showing the production of 3-methyl-1-butanol. In general, it is known that high levels of aroma compounds like alcohol, ketones and esters (Birch, Petersen, & Hansen, 2013; Plessas et al., 2005) in combination with low amounts of acids and aldehydes are more accepted from the consumers in sensorial analysis of wheat bread-crumb (Quílez et al., 2006). Therefore, the panellists preferred bread samples with a less bitter ($r=0.934$, $p<0.01$) and less cheesy taste ($r=-0.865$, $p<0.03$). These taste attributes are associated with off-flavours. Furthermore, the panellists observed a more roasted smell for breads having a higher bake loss ($r=0.927$, $p<0.01$), due to higher water evaporation and higher bake loss more heat transfer appears leading to more Maillard products (Eichnerl & Karel, 1971).

5.5. Conclusion

The influence of various strains of *S. cerevisiae* on the production of volatile aroma compounds in bread crumb by GC-MS TD in combination with a descriptive sensory analysis was investigated. The results revealed strain dependent aroma formation in a bread matrix and significant differences among the sensory acceptance. Several correlations between aroma profiles, sensory and technological loaf characteristics were found. Therefore, not only flour composition and quality have to be considered for the bread making process. The choice of yeast strain is also a very important parameter concerning the impact on aroma profile, sensory acceptance and technological loaf characteristics. Aroma profile therefore should be added as a new selection criteria for yeast strain development and could be of industrial interest. Since European regulations and consumer acceptance prohibit the use of genetically modified microorganisms, yeast strain selection offers an alternative approach to improve wheat bread quality.

5.6. Acknowledgements

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6. Chapter Response surface methodology as a quality optimisation tool for wheat bread produced with different *Saccharomyces cerevisiae* strains

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6.1. Abstract

The present study used response surface methodology as a tool to optimise proofing conditions (time and temperature) for the application of different *Saccharomyces cerevisiae* strains originating from the beverage industry in a wheat bread system. In general beer and wine are fermented at lower temperatures for a longer time than bread fermentation resulting in mainly produced ethanol and less carbon dioxide. Differently in bread making, the main focus lays in the production of carbon dioxide and not ethanol. Likewise, the fermentation process needs to be adapted when other strains different to Baker's yeast are applied. Individual optimisations were carried out for five *S. cerevisiae* strains in comparison to *S. cerevisiae* Baker's yeast. The application of different fermentation parameters showed a significant effect on a wide range of technological, sensorial and nutritional product parameters. The main influence resulted in a prolonged shelf life and a reduced glycaemic index with a higher sensory preference, which was confirmed by PCA. Overall, it was shown that optimisation of the fermentation conditions is essential, when yeasts from different origins are used.

6.2. Introduction

Studies on the impact of different yeast strains on bread quality parameters are rare, even though bread leavening with yeast is one of the oldest biotechnological processes in the world. The fermentation process is well known as the leavening step in bread making and makes a contribution to the crumb structure formation. Literature states a great variety in terms of how long and at which temperature a bread should be proofed. The most used fermentation time was 55 min. An early study by Freilich, (1949) investigated proofing times between 0-150 min, where a window between 45-60 min showed to be the optimum. With a longer fermentation time the specific volume still increased but the crumb structure was unacceptable open (Siffring & Bruinsma, 1993). Typical fermentation temperatures are reported to be 33-54°C, but most bakeries use 41-43°C for the fermentation of pan bread (Pyler & Gorton, 2010). Kamman, (1970) reported that temperatures around 46-48°C were commonly used in the industry. However, these temperatures can also result in the inhibition of yeast. Siffring & Bruinsma, (1993) speculated that the higher the final proofing temperature the faster the thermal death of yeast cells occurs during baking, due to the shorter period of time to overcome temperature differences. A longer time with a cooler proofing temperature could therefore result in additional gas production and expansion in the early stages of baking, which is known as oven spring. A further hypothesis is that with higher proofing temperatures enzymes like proteases and amylases become more active which could result in weaker doughs with less oven spring (Siffring & Bruinsma, 1993). Nowadays, proofing takes place in general for 55-65 min at 30-35 °C (Delcour & Hoseney, 2010). Flander, Salmenkallio-Marttila, Suortti, & Autio, (2007) showed the significant effect of final fermentation temperature and time on specific volume and hardness of oat breads. Clarke, Schober, Angst, & Arendt, (2003) also showed a significant influence between yeast addition and fermentation time on specific volume for wheat sourdough breads. More precisely, the specific volume increased with a longer fermentation time until a maximum was reached before the structure collapsed as a consequence of over proofing. Therefore, fermentation temperature and time showed to have a high impact on bread quality parameters (Flander et al., 2007). Previously, the application of starter cultures different to Baker's yeast impacted greatly bread quality, like texture and flavour (Heitmann et al., 2015). This opens opportunities to satisfy the high demand of the

consumers for increasing the variety of bread by only changing the starter culture. Fermentation parameters in terms of temperature and time are even more important factors when applying different starter cultures due to their specific metabolism. Due to the lack of knowledge about the impact of fermentation conditions when using different starter cultures, response surface methodology (RSM) was applied as a tool to optimise the process conditions of white wheat bread. The increasing fundamental knowledge about dough fermentation generates new opportunities for their use in the baking industry. Using adapted fermentation parameters further allows to create breads with improved characteristics in case of specific volume, crumb structure and flavour.

6.3. Materials and Method

6.3.1. Materials

The ingredients used in this study were wheat flour (without any additives) (Voigtmühle Illertissen, Germany), salt (Glacia British Salt Limited, UK), palm fat (Vandemoortele, Belgium). The suppliers for the different yeasts were Puratos, Belgium for Baker's yeast; Fermentis Division of S. I. Lesaffre, France for yeast s-23 (Lager beer), T-58 (Ale beer), us-05 (Ale beer) and wb-06 (wheat beer); Vinoferm, Belgium for white wine yeast Blanc. All yeasts were from the species *S. cerevisiae*. All other chemicals were purchased from Sigma Aldrich (Sigma, Arklow, Ireland).

6.3.2. Bread-making

The wheat dough recipe (based on flour weight) was 62% water, 2.2% salt, 1% fat and 2% yeast. To standardise the inoculation level of all yeasts, the amount of yeast was adapted to the amount of Baker's yeast analysed by total cell count (Heitmann et al., 2015). Yeast was activated for 10 min in water (25 °C). The dough was prepared by blending the yeast/water mixture with the dry ingredients and mixed for 2 min at low speed in a spiral mixer Pietroberto SF (Food Equipment Service, Northern Ireland). A further mixing at higher speed was carried out for 5 min. A bulk fermentation step was performed for 5 min at room temperature. 500g of dough was scaled into baking tins of 15x9.5x9.7 cm and placed in the proofer (KOMA sunriser, Roermond, the Netherlands). The original fermentation time was 85 min at 35°C and 75% relative humidity. The breads were baked 35 min at 220 °C top and bottom temperature in a deck oven (MIWE condo, Arnstein, Germany), previously steamed with 0.4 L of water. After baking, the bread loaves were cooled down at room temperature for 120 min prior to analysis.

6.3.3. Experimental design/Process Optimisation

To evaluate the effect of the independent variables (fermentation time and temperature) on the dependent variables (specific volume, crumb hardness, slice area and number of cells) response surface methodology (RSM) was applied. A two-dimensional central composite design was established featuring variations in fermentation time and

temperature for the use of different yeast cultures. The used fermentation temperature ranged from 20-40 °C, according to proofer regulations. The applied fermentation times were between 10 and 180 minutes. In total 13 trials were carried out for each yeast strain (three central points, six axial points and four factorial points). Each dependent variable was analysed using the appropriate model with least square regression in order to identify significant effects of the fermentation conditions on the responses ($p < 0.05$). To judge the adequacy of model fit, the significance of the lack-of fit error term, R^2, coefficient of variation, and model significance were used. Where contradiction between these requirements were found, the best overall solution was considered. For the optimisation of fermentation time and temperature desirability a multiple response method was applied. In terms of responses the following parameters were used: specific volume (maximise), crumb hardness (minimise), slice area (maximise) and number of cells (maximise).

6.3.4. Rheofermentometer analysis

Carbon dioxide production during fermentation was measured using a rheofermentometer (Chopin, France). Dough samples (300 g) were prepared as described above. The samples were placed in the fermentation chamber and a cylindrical weight (1500 g) was set on top of the dough. The samples were first fermented under standard conditions of 30°C for 180 minutes. Following the RSM analysis, the temperature of the fermentation was adjusted for each microorganism individually.

6.3.5. Physicochemical analysis of bread

Specific volume was analysed after cooling using a Vol-scan Profiler (Stable Micro Systems, UK). Crumb texture was determined on the baking day. Four bread slices (25 mm thickness) from each loaf were used to evaluate the physical crumb texture. Texture profile analysis (TPA) was performed by a TA-XT2i texture analyser (Stable Micro Systems, Surrey, UK) (Heitmann et al., 2015). Image analysis of the crumb structure of bread slices was carried out using a C-cell Bread Imaging system (Calibre Control International Ltd., UK). The parameters slice area and number of cells were chosen for the characterisation on 4 central slices (25 mm) for each loaf. Shelf life against

environmental moulds was determined using the method described by (Dal Bello et al., 2007). The breads where cut in a sterile manner into slices of 20 mm thickness. The slices were than exposed to the environmental air for 5 min on each side. Afterwards the slices were packed in a plastic bag and heat sealed. In each side of the plastic bag a filter tip was inserted to ensure similar aerobic conditions. The bags were incubated at room temperature and inspected for mould growth over a 14 day period. Total titratable acids (TTA) and pH were measured as described in Arbeitsgemeinschaft Getreideforschung, (1994). Water activity measurements of the bread crumb were determined using a Hygrolab C1 water activity meter (rotronic, UK).

6.3.6. Compositional analysis of bread

The compositional analysis was performed using freeze-dried bread crumbs. Megazyme enzymatic Kits K-TSTA 09/14 and K-RSTAR 09/14 (International, Bray, Ireland) were used for the determination of total and resistant starch, respectively. Protein and fat content analysis was based on the AACCI – 46-12.01 (protein factor 5.7) and AACCI – 30-10.01, respectively. Organic acids and simple carbohydrates were measured using an Agilent 1260 HPLC system. Sample extraction was performed with water while shaking for 20 min. After clarification with 7% perchloric acid overnight, the samples were centrifuged (3500 rpm, 10 min) and filtered prior to injection. Organic acids were detected after separation using a HiPlex H^+ column (Agilent, Cork, Ireland) at 65°C using 0.005 M H_2SO4 with Diode-Array Detector (DAD). For the detection of the simple carbohydrates a Refractive Index Detector (RID) (35°C) in combination with a HiPlex H^+ column (30°C) and water was used. Both elution methods were carried out at a flow rate of 0.5 mL/min.

6.3.7. Predictive glycaemic index

For the nutritional value of the wheat breads the in vitro starch digestibility was investigated using the method described by (Brennan & Tudorica, 2008; Hager et al., 2013). Bread crumbs were prepared by processing an aliquot in a mixer (Major Titanium, Kenwood) with glass blender attachment. The bread crumbs were mixed with sodium potassium phosphate buffer (0.2 M, pH 6.9). Afterwards the pH was adjusted to 1.5 using

8 M HCl and 5 mL of pepsin solution (EC 3.4.23.1, 526 U/mg solid, 115 U/mL) was added. After incubation at 37°C for 30 min, the pH was once more adjusted to 6.9 using 6 N NaOH and 1 mL α-amylase solution (EC 3.2.1.1, 15 U/mg solid, 110 U/mL) was added. The sample volume was filled up to 50 mL with sodium phosphate buffer and moved into a dialysis tube (25 mm width, length 40 cm, 14 kDa), with the addition of glass beads. The dialysis tube was placed in a beaker containing 450 mL sodium phosphate buffer and incubated at 37°C for 4 hours. Every 15 min the tubes were inverted several times and every 30 min an aliquot of the dialysate was removed and replaced by the same amount of fresh buffer. The concentration of reducing sugars released (RSR) was investigated using a spectrophotometer at 546 nm using 3,5-dinitrosalicylic acid reagent (DNS) (2 M sodium hydroxide, 3,5-dinitrosalicylic acid, potassium sodium tartrate tetra hydrate in distilled water). The glycaemic index was calculated using the formulation described by Brennan & Tudorica, (2008). For the in vitro starch digestibility and the calculation of the glycaemic index bread fermented with *S. cerevisiae* Baker's yeast was used as a reference.

6.3.8. Flavour profile and sensory analysis

The volatile aroma compounds were concentrated in a gas stream prior to injection after weighing 0.1 g into a glass thermal desorption (TD) tube (Perkin Elmer Turbomatrix 650). Afterwards the aroma compounds were absorbed for 10 min at 90°C. The aroma active volatiles were quantified by gas chromatography mass spectrometry (GC-MS, Agilent 5977B MSD) using an Rxi 624-Sil 20m column with helium as carrier gas. The oven temperature was programmed from 35°C (4 min) to 220°C at a heating rate of 15°C/min and for 1 min. A database search was conducted for the detected compounds.

A trained panel consisting of 15 panellists (5 female, 10 male, 25-34 years) was used for the descriptive sensory analysis by applying the aroma profile analysis (APA). Over a six month period prior to the sensory, weekly sessions were performed for the aroma recognition test. By smelling reference solutions odour qualities and quantities were determined. Afterwards the panellists were able to orthonasal recognise 120 odorants at different concentrations. Training and sensory analysis was performed at 21 ± 1°C in a sensory panel room. A consensus with the sensory panel on terms of "flavour language" based on aroma solutions was developed to define the specific smell of a compound.

APA was performed on each bread sample (slices of 2 cm thickness). The odour and taste intensities was judged on a scale from 0 (not detectable) to 10 (high intensity). The overall taste, aroma and liking was evaluated by the panellists on a scale from 0 (dislike very much) to 10 (like very much). In the end the panellists had to decide whether they preferred the original or optimised procedure.

6.3.9. Statistical Analysis

Minitab 17 software was used to carry out statistical analysis of the results. Exploratory data analysis was followed by a multiple comparison procedure of variance (one way ANOVA, Tuckey's test) to describe significant differences at a level of significance from 5% ($p<0.05$) between samples. All analysis were performed in triplicates and the results are shown as average with confidence interval. Design Expert 7 (Stat-Ease Inc., USA) was used for the experimental response surface methodology. 3 dimensional surface plots were used for the evaluation of the interactive effects of the variables to optimise fermentation parameters for the different yeasts applied. In addition, to describe the differences between the samples, multivariate data analysis (Principle component analysis (PCA)) was performed using R software version 3.3.1.

6.4. Results and Discussion

6.4.1. Optimisation of fermentation parameters

In the present study RSM was used as a tool to evaluate the effect of fermentation time and temperature on technological, sensorial and nutritional bread quality parameters. The optimisation using RSM resulted mainly in an increase of fermentation time (87-124 min) with a decrease of fermentation temperature (20-25°C), in comparison to the original fermentation conditions. Only *S. cerevisiae* T-58 with a shorter fermentation time (70 min) and *S. cerevisiae* us-05 with a higher temperature (40°C) show ambivalent results. The application of the optimised fermentation parameters lead to an improvement of technological characteristics. Specific volume increased when *S. cerevisiae* Baker's yeast, wb-06 and Blanc were used. Breads fermented with *S. cerevisiae* us-05, wb-06 and Blanc were significant softer. No changes were observed for *S. cerevisiae* T-58 and s-23. Freilich, (1948) studied the impact of the fermentation variables time and amount of yeast on bread staling. However they did not find a significant impact by changing these fermentation variables. Gomez, Oliete, Pando, Ronda, & Caballero, (2008) investigated the influence of yeast concentration, fermentation time and temperature on bread staling parameters and specific volume over time on wholemeal and white wheat bread. They showed that the specific volume increased with increasing yeast dose as well as fermentation time and temperature. However, between 30 and 35°C no significant differences could be found for the volume. They explained these findings by the optimum gas production of the yeast and the ability of the dough to retain the produced carbon dioxide. Their observed effect on the firmness was mainly explained by the increase in volume. Another reason could be the improved activity of flour enzymes mainly α-amylase by prolonging the fermentation time, resulting in an reduced recrystallisation of the starch component amylopectin (Siljeström et al., 1988).

6.4.2. Influence of fermentation parameters on gas production during fermentation

In general beer and wine are fermented at lower temperatures for a longer time in comparison to bread fermentation, with a higher production of ethanol and lower carbon dioxide concentrations. The used beer and wine yeasts with the exception of *S. cerevisiae* s-23 show the highest specific volume at higher temperatures in comparison to Baker's yeast. Merritt, (1966) measured the amount of carbon dioxide production by yeast in a wort fermentation. They stated that the temperature optimum for the rate of carbon dioxide production has to be seen in relation to the fermentation time and temperature. For the first 30 minutes the fermentation activity was greatest at 40°C but over a longer fermentation time of 2 hours the best fermentation activity was determined at 35°C. An important parameter for cell growth and metabolic activity of yeast is fermentation temperature. Rezaei et al., (2014) showed that the physiological state of yeast cells affects the production of metabolical by-products. Cells harvested between exponential growth and diauxic shift resulted in a gradualy increase in the production of carbon dioxide and the maximum dough height. Our study confirmed differences in the amount of carbon dioxide production (*S. cerevisiae* Baker's yeast, T-58, us-05 and Blanc) measured with a rheofermentometer (Table 6-1). This explains the differences in volume due to the correlation between those two parameters ($r=0.873$, $p<0.001$).

Table 6-1 Optimisation settings; original, predicted and optimised values for the responses at control and optimum fermentation time and temperature[#]

	Settings for Optimisation			*Saccharomyces cerevisiae*																
	Target	Lower limit	Upper limit	Baker's yeast			s-23			T-58			us-05			wb-06			Blanc	
				orig[1]		opt[3]	orig[1]		opt[3]	orig[1]		opt[3]	orig[1]		opt[3]	orig[1]		opt[3]	orig[1]		opt[3]
Fermentation time [min]	**minimise**	**10**	**150**	85		124	85		93	85		70	85		87	85		106	85		112
Fermentation temperature [°C]	**in range**	**20**	**40**	30		20	30		20	30		23	30		40	30		25	30		36
				orig[1]	pred[2]	opt[3]	orig[1]	pred[2]	opt[3]	orig[1]	pred[2]	opt[3]	orig[1]	pred[2]	opt[3]	orig[1]	pred[2]	opt[3]	orig[1]	pred[2]	opt[3]
Specific volume [mL/g]	**maximise**	2.0	5.0	3.52	3.90	3.93*	2.92	2.84	2.80	3.55	3.72	3.70	2.51	2.71	2.75	2.36	2.37	2.48*	2.17	1.79	2.53*
Crumb hardness [N]	**minimise**	4.0	20.0	6.3	8.4	7.1	8.9	7.5	9.3	5.7	4.1	6.1	14.7	11.60	9.5*	15.33	11.0	13.0*	14.5	19.6	13.3*
Slice Area [mm²]	**maximise**	5500	10000	9383	7827	8460*	7861	7553	7778	9499	9587	8868	7090	6796	7434	6795	6367	6936	6766	5429	6975
Number of cells	**maximise**	4500	6000	5353	5523	5758	4269	4656	4436	5161	4854	5254	4504	4636	3950*	4862	4889	4630	4989	4822	5415*
V_{total}				1372		1248*	1030		1002	1321		1654*	822		1250*	402		408	371		806*
$V_{retention}$				1229		1247	961		994	1172		1196	789		1082*	400		406	369		786*
	Desirability				0.549			0.734			0.743			0.758			0.632			0.693	

[#] significant differences ($p>0.05$) between original and optimised procedure are presented by *

[1] results using the original procedure, [2] results predicted by the RSM tool, [3] results using the optimised procedure

6.4.3. Influence of fermentation parameters on crumb grain characteristics

Crumb grain characteristics such as slice area and number of cells also give an indication about the gas production of the yeast. The rise of the dough is influenced by the growth of the gas cells during fermentation and the oven spring resulting in biaxial extension (Kokelaar, van Vliet, & Prins, 1996). Regarding rheology, number of cells are influenced by strain hardening (the stress increases more than proportionally to the strain) resulting in different stresses in the dough system, which have an impact on disproportionation and coalescence of the gas cells. By applying different temperatures during fermentation the biaxial extension of dough is influenced (Kokelaar et al., 1996). Lower strain hardening and less stress are the consequence of higher temperatures, resulting in a higher possibility of disproportionation. Therefore, the number of cells are decreasing with the application of higher temperatures during fermentation. The same study further showed a stronger strain rate thinning at higher temperatures, which could also explain the loss in gas cells by applying a high temperature with a long fermentation time (Kokelaar et al., 1996). Larger cells are desired since small cells are associated with a low specific volume. However, these cells must be still in the range, not produce holes and result in a good specific volume (Clarke et al., 2003). For the optimisation of the fermentation parameters not only the used microorganism needs to be taken into account but also the changes in dough rheology at different times and temperatures due to the effect on the gluten network stability and gas retention.

6.4.4. Influence of fermentation parameters on shelf life

Shelf life is one of the most important bread characteristics and can be positively influenced by process optimisation. In Table 6-2 the results for the shelf life trials in combination with the influencing factors (organic acid concentrations, a_w, pH and TTA) are presented. A shelf life increase from 1-3 days was observed depending on the yeast when fermentation conditions were optimised. After the application of the optimised fermentation conditions the concentrations of citric acid, and succinic acid were significantly lowered. The concentrations of acetic acid were higher except for *S. cerevisiae* s-23 and *S. cerevisiae* wb-06. During exponential growth, yeast cells are known for a higher production of high concentrations of acetic acid and low concentrations of succinic acid (Rezaei et al., 2014). Responsible for the change in acid concentrations especially a decrease in citric and succinic acid is the repression of the TCA cycle. The decrease of TCA cycle metabolites using the optimised fermentation conditions also shows the higher fermentative activity with less respiration. However, these variations in the acid content resulted in a decrease in pH and increase in TTA values. Gould, (1996) stated that the best prevention of spoilage is the concurrent presence of weak organic acids and a reduction of pH. Even though Jayaram et al., (2013) stated succinic acid as the main pH-determining factor, for the breads analysed in this study acetic acid seems to have a higher influence on the resulting pH. The prolonged shelf life can therefore be explained by the decrease in pH ($r=-0.703$, $p<0.011$) with a simulatneously increase of acetic acid as well as a drop in water activity for some of the breads. Furthermore glycerol is an important metabolite influencing the final pH. Less glycerol production because of lower temperatures → less osmotic pressure → less buffering capacity.

Table 6-2 Shelf life, organic acids, a_w, pH and TTA characteristics from breads baked at original and optimised fermentation conditions using different yeasts[#]

			Shelf life [days]	Acids [mmol/kg]				a_w	pH	TTA
				Acetic acid	Succinic acid	Citric acid	Sum			
S. cerevisiae	**BY**	**orig[1]**	4	175.3 ± 0.5	161.2 ± 3.2	113.0 ± 0.3	449.5 ± 1.4	0.958 ± 0.002	5.90 ± 0.05	2.61 ± 0.09
		opt[3]	5*	256.6 ± 3.7*	27.5 ± 0.6*	11.0 ± 0.1*	302.2 ± 1.1*	0.965 ± 0.004*	5.56 ± 0.06*	2.77 ± 0.09
	s-23	**orig[1]**	5	171.6 ± 4.1	185.3 ± 13.2	110.7 ± 1.8	467.6 ± 6.4	0.958 ± 0.005	5.86 ± 0.08	3.04 ± 0.02
		opt[3]	7*	72.6 ± 2.5*	62.2 ± 2.6*	13.0 ± 0.8*	177.5 ± 2.0*	0.955 ± 0.008	5.55 ± 0.06*	3.23 ± 0.13*
	T-58	**orig[1]**	5	172.9 ± 2.8	168.7 ± 11.9	111.2 ± 1.5	452.8 ± 5.4	0.963 ± 0.004	5.92 ± 0.08	2.65 ± 0.07
		opt[3]	6*	304.1 ± 3.3*	37.0 ± 0.3*	11.4 ± 0.0*	360.5 ± 0.9*	0.938 ± 0.007*	5.49 ± 0.03*	2.83 ± 0.09*
	us-05	**orig[1]**	4	176.0 ± 3.4	216.0 ± 7.3	116.8 ± 4.8	508.8 ± 5.2	0.956 ± 0.007	5.94 ± 0.08	3.23 ± 0.14
		opt[3]	6*	351.9 ± 1.9*	40.9 ± 3.5*	13.9 ± 0.1*	416.8 ± 1.4*	0.953 ± 0.007	5.59 ± 0.05*	3.55 ± 0.10*
	wb-06	**orig[1]**	4	167.9 ± 1.8	173.5 ± 3.1	109.5 ± 0.9	450.9 ± 1.9	0.963 ± 0.010	6.06 ± 0.05	2.49 ± 0.15
		opt[3]	5*	49.7 ± 2.6*	15.2 ± 0.3*	12.9 ± 0.1*	96.2 ± 0.8*	0.950 ± 0.007	5.55 ± 0.02*	2.71 ± 0.06*
	blanc	**orig[1]**	4	169.1 ± 1.5	150.9 ± 2.6	109.1 ± 0.5	429.1 ± 1.5	0.964 ± 0.009	5.90 ± 0.04	2.65 ± 0.09
		opt[3]	7*	276.2 ± 3.5*	31.5 ± 0.6*	10.0 ± 0.0*	324.3 ± 1.0*	0.959 ± 0.014	5.74 ± 0.07*	2.95 ± 0.06*

[#] significant differences ($p>0.05$) between original and optimised procedure are presented by *

[1] results using the original procedure, [3] results using the optimised procedure

6.4.5. Influence of fermentation parameters on Glycaemic Index

The process optimisation improved in most cases the nutritional value of the breads (except breads made with *S. cerevisiae* s-23 and wb-06) resulting in a lowered GI (Table 6-3). A low GI in bakery products is favourable, since it showed beneficial effects in the management of diabetes and can reduce the risk of coronary heart disease (Brand-Miller, McMillan-Price, Steinbeck, & Caterson, 2009). The GI is influenced by a range of different parameters such as carbohydrate and organic acid content but also by protein and fat concentrations (Fardet et al., 2006). The optimisation of the fermentation parameters resulted in significant changes in the carbohydrate composition as well as the protein concentration. The amount of resistant starch and fat was only influenced in breads fermented with *S. cerevisiae* s-23 and us-05, respectively. In general, altering the GI of a food product also means influencing its digestibility. The presence of organic acids (mainly acetic, propionic and lactic acid) in bread can limit the accessibility of enzymes to the food product. The acids promote interactions between starch and gluten during starch gelatinisation and thereby reduce the rate of starch digestion due to slowing down the gastric emptying (Fardet et al., 2006; Ostman, Nilsson, Elmstahl, Molin, & Bjorck, 2002). Organic acids are also known to reinforce interactions between starch and protein resulting in limited enzyme accessibility. This effect is stronger if the acid is already present during thermal treatment and starch gelatinisation (Fardet et al., 2006). Therefore the results show an increase in GI with a simultaneously decrease in acetic acid concentration for breads baked with *S. cerevisiae* s-23 and *S. cerevisiae* wb-06. Besides the composition, the technological processing also has an influence on the GI. In general, it is known, that the more a food product is processed the higher is its digestibility (Berti et al., 2004). Some researcher have been able to show, that shorter kneading times and / or longer fermentation times results in a more compact structure of the bread. This decreased density can have a reducing effect on the gastric emptying rate (Fardet et al., 2006). Therefore, it might be possible, that extended periods of proofing, in particular with increased temperatures could lead to the starch being more susceptible to gelatinisation during baking and possibly an increased digestibility (Berti et al., 2004; Brand et al., 1985; Ross et al., 1987). However, bread fermented with *S. cerevisiae* us-05 showed the highest reduction in pGI of 34.4%. This bread was fermented at 40°C

Table 6-3 Compositional and nutritional characteristics from breads baked at original and optimised fermentation conditions using different yeasts[#]

S. cerevisiae		GI	Total Starch [%]	Resistant starch [%]	Protein [%]	Fat [%]	Sugars [mmol/kg]			
							Maltose	Glucose	Fructose	sum
Baker's yeast	**orig**[1]	100.0 ± 0.0	46.2 ± 4.4	0.71 ± 0.10	7.7 ± 0.1	1.5 ± 0.0	56.0 ± 2.3	0.36 ± 0.18	16.9 ± 0.8	73.2 ± 3.7
	opt[3]	78.3 ± 0.1*	38.3 ± 1.0*	0.76 ± 0.28	7.0 ± 0.2*	1.1 ± 0.2	168.4 ± 11.0*	1.52 ± 0.15*	17.0 ± 2.2	186.9 ± 16.1*
s-23	**orig**[1]	71.6 ± 2.1	45.8 ± 1.2	0.58 ± 0.10	8.1 ± 0.1	1.6 ± 0.1	67.5 ± 1.6	1.37 ± 0.34	8.8 ± 1.3	77.7 ± 3.5
	opt[3]	81.3 ± 1.0*	38.8 ± 1.1*	0.82 ± 0.06*	7.1 ± 0.0*	1.3 ± 0.0	127.7 ± 5.1*	1.64 ± 0.21	2.5 ± 0.6*	131.9 ± 7.1*
T-58	**orig**[1]	97.7 ± 3.1	41.3 ± 1.8	0.55 ± 0.07	7.7 ± 0.1	1.5 ± 0.0	54.2 ± 1.3	0.39 ± 0.19	10.9 ± 1.0	65.6 ± 0.9
	opt[3]	82.1 ± 0.2*	38.7 ± 1.1	0.50 ± 0.09	7.3 ± 0.0*	1.4 ± 0.0	94.8 ± 36.7	1.22 ± 0.02*	15.1 ± 1.0*	111.1 ± 45.5
us-05	**orig**[1]	103.6 ± 5.2	41.6 ± 0.6	0.59 ± 0.09	8.5 ± 0.2	1.9 ± 0.0	63.0 ± 6.4	18.14 ± 3.88	25.8 ± 4.2	106.9 ± 16.4
	opt[3]	68.0 ± 0.4*	37.1 ± 2.3*	0.70 ± 0.20	7.4 ± 0.1*	1.3 ± 0.1*	254.2 ± 0.0*	0.83 ± 0.00*	3.6 ± 0.0*	258.7 ± 32.2*
wb-06	**orig**[1]	63.0 ± 2.2	43.4 ± 1.5	0.58 ± 0.13	7.5 ± 0.3	1.4 ± 0.2	72.5 ± 2.5	2.71 ± 0.51	26.9 ± 1.9	102.0 ± 6.0
	opt[3]	92.6 ± 1.4*	31.1 ± 4.8*	0.64 ± 0.10	6.9 ± 0.1*	1.2 ± 0.2	125.4 ± 29.1*	3.93 ± 0.74	15.3 ± 9.5	144.6 ± 41.0
blanc	**orig**[1]	77.1 ± 2.7	43.3 ± 1.0	0.47 ± 0.18	7.2 ± 0.3	1.3 ± 0.1	67.1 ± 2.2	4.43 ± 0.63	30.0 ± 2.0	101.5 ± 5.8
	opt[3]	62.9 ± 1.3*	39.6 ± 2.2	0.84 ± 0.02	6.8 ± 0.0	1.1 ± 0.1	187.0 ± 4.2*	2.36 ± 0.04*	21.0 ± 0.3*	210.3 ± 4.9*

[#] significant differences ($p>0.05$) between original and optimised procedure are presented by *

[1] results using the original procedure, [3] results using the optimised procedure

for 87 min. Manipulating proofing times of bread doughs can consequently show a change in loaf volume and also alter glycaemic response (Burton & Lightowler, 2006). Burton & Lightowler, (2006) found that the longer the proofing time, the higher was the GI of the white wheat breads. They explained this by both the higher specific volume and the higher amount of easily digestible starch. The extended proofing time leads to a more extensive disruption and breakdown of the starch granules and therefore also more simple sugars. Conditions during fermentation can also have an impact on the activation and performance of enzymes, including amylases and proteases (Poutanen et al., 2009) as well as on the metabolism of the applied microorganisms. Conclusively, proofing times and temperature have an influence on the composition and characteristics of white wheat bread and as a result can also have an impact on the GI. In general, physical structure, including both specific volume and hardness of a bread, can influence the glycaemic response of bread. However in this study, the altered proofing conditions mainly resulted in a decreased GI. The glycaemic index was lowered, due to the reduction in total starch content and change in monosaccharide composition as well as an increase in organic acids mainly acetic acid. Overall, it is not possible to determine one main influencing factor on the GI of white wheat bread. Both the microbiological and the technological parameters affect the GI and it is possible to increase and decrease the GI using different microorganism and varying processing procedures. As Englyst, Vinoy, Englyst, & Lang, (2003) concluded in their study, it is not the direct absorption of carbohydrates in the small intestine but rather the combined effect of all food properties on the glucose uptake that influences the GI. The results only give predicted GI values and therefore a limited overall picture. For the complete investigation of the influence of various yeast strains on the GI of baked products an *in vivo* study would be necessary for future investigations.

6.4.6. Influence of fermentation parameters on sensorial characteristics

Most importantly the impact of process optimisation led to a positive result for sensory and flavour attributes of the individual breads. This was not only shown by the sensory evaluation using a trained panel (Figure 6-1) but was also supported by GC-MS analysis (Table 6-4). Aroma formation in bread crumb has been shown to be yeast strain, fermentation time and temperature dependent. Temperature is an important key factor influencing the production of ethanol as shown by Fakruddin, Quayum, Ahmad, &

Choudhury, (2012) on molasses as carbon source. They found a gradually increase between 25 to 30°C with a drastically decrease at higher fermentation temperatures. In addition they showed an effect of pH on the ethanol production. The highest ethanol production was achieved at pH 6.0 (80.42 g/L) in comparison to 5.4 (48.82 g/L).

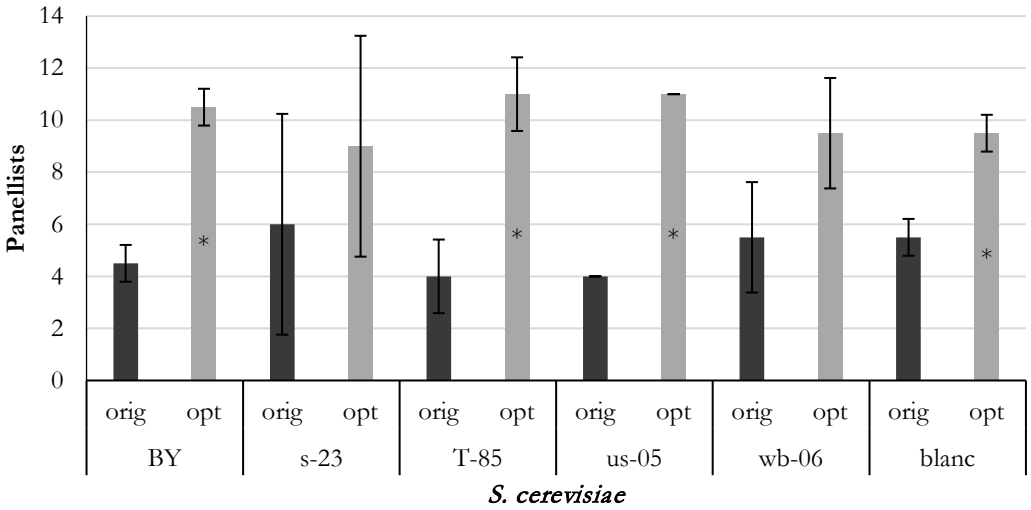


Figure 6-1 Panellists preferences between breads fermented at original and optimised conditions (* indicating significant differences between original and optimised conditions)

Therefore, the ethanol concentration decreased by applying the optimised procedure due to the change in temperature and the lowered pH. The reduction in ethanol concentration by using the optimised procedure might be also explained by the presence of citric acid. Since a high concentration of citric acid is known to shift the metabolism from ethanol to glycerol production (Lawrence, Botting, Antrobus, & Coote, 2004). A production of 2,3-butandiole was only shown by the brewing strains *S. cerevisiae* s-23, T-58, us-05 and wb-06, which is formed by oxidative decarboxylation of 2-acetolactate to 2,3-butandione and further reduced to 2,3-butandiole (Romano & Suzzi, 1996; Wainwright, 1973). The removal of 2,3-butandiol trough the lipid membrane by the active transport mechanism needs energy (Maiorella et al., 1983), which is provided by ethanol production and confirmed by correlation analysis ($r=0.901$, $p<0.006$). The decrease in 2,3-butandiol concentration by applying the optimised process for *S. cerevisiae* T-58 might be a result of the high citric acid production. As described above, citric acid can be responsible for a shift of ethanol to glycerol production, therefore less ATP is present for the

Table 6-4 Volatile aroma compounds and their organoleptic descriptions from breads baked at original and optimised fermentation conditions using different yeasts

		Concentration [µg/kg]											
		S. cerevisiae											
	organoleptic description	**BY**		**s-23**		**T-58**		**us-05**		**wb-06**		**blanc**	
		orig[1]	**opt[3]**	**orig[1]**	**opt[3]**	**orig[1]**	**opt[3]**	**orig[1]**	**opt[3]**	**orig[1]**	**opt[3]**	**orig[1]**	**opt[3]**
ethanol	alcoholic, sweet	3900	3700	4400	4100	5500	3800	2400	2300	2100	1600	3200	3400
acetic acid	pungent, sour	50	110	130	190	n.d.*	170	110	160	110	110	93	57
3-methyl-1-butanol	alcoholic, fermented, fruity	86	50	70	n.d.*	64	53	n.d.*	n.d.*	n.d.*	33	n.d.*	19
isobutyric acid	acidic, sour, cheesy	20	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*
2,3-butanediol	fruity, creamy, buttery	n.d.*	n.d.*	38	64	62	43	11	n.d.*	12	12	n.d.*	n.d.*
1-hexanol	green fruity	n.d.*	10	n.d.*	n.d.*	n.d.*	10	23	18	n.d.*	n.d.*	n.d.*	n.d.*
2-phenylethanol	bready, flowery, sweet	15	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*

* not detected

[1] results using the original procedure, [3] results using the optimised procedure

active transport mechanism. Richard-Molard, Nago, & Drapron, (1979) showed the fermentation time dependency of acetic acid production in bread crumb. Their results revealed an increasing content of acetic acid when the fermentation time was increased from 3 to 10 hours. The optimised fermentation conditions resulted in breads having an increased amount of aroma active acetic acid except for *S. cerevisiae* wb-06 and Blanc. Gassenmeier & Schieberle, (1995) showed the formation of 3-methyl-1-butanol and 2-phenylethanol is influenced by fermentation time and temperature. The optimum fermentation temperature was found to be 35°C with a long fermentation time (3h) in liquid pre-ferments (flour, water, yeast). In this study the amount of 3-methyl-1-butanol was increased for *S. cerevisiae* wb-06 and Blanc by the application of a longer fermentation time and decreased for *S. cerevisiae* T-58 with a shorter fermentation time. However, *S. cerevisiae* Baker's yeast and s-23 both showed a reduction in 3-methyl-1-butanol with longer fermentation times probably due to the colder temperature used. Since the production of 3-methyl-1-butanol and 2-phenylethanol is linked to the Ehrlich pathway, the BAP2 gene needs to be mentioned. BAP2 controls the expression of branched chain amino acid permeases (uptake of leucine, isoleucine and valine) (Grauslund et al., 1995). The brewing strain BH-225 showed a constitutive expression of BAP2 at higher temperatures (Procopio et al., 2011) which was also confirmed by Abe & Minegishi, (2008). Therefore, increased temperatures promote the higher production of 3-methyl-1-butanol. On the other hand 2-phenylethanol was only detectable in breads fermented with *S. cerevisiae* Baker's yeast using the original procedure. The concentration of 1-Hexanol, which is originating from lipid oxidation, is lowered after the application of the optimised procedure for *S. cerevisiae* us-05. In general, it is known that with increasing temperatures the amount of lipid oxidation products is increasing (Birch, Petersen, & Hansen, 2013). The increase of lipid oxidation rate can be explained by an increase in lipase activity. However, the optimum lipase temperature is 37°C. The activity decrease at higher temperature which is the case for *S. cerevisiae* us-05. Nevertheless, *S. cerevisiae* Baker's yeast and T-58 showed a small production of 1-hexanol with decreasing temperatures in the optimised procedure. Isobutyric acid was only produced by *S. cerevisiae* Baker's yeast using the original procedure. The alteration in fermentation parameters showed no change for this aroma compound. The sensory evaluation revealed that in general the panellists preferred breads produced with the optimised fermentation

conditions, except for breads made with *S. cerevisiae* s-23 and wb-06. For these breads the opinions of the panellists were quite divided. These preferences are a result from various combination of circumstances. The panellists preferred breads with less bitter ($r=-0.886$, $p<0.001$) and cheesy ($r=-0.802$, $p<0.002$) but with a more buttery ($r=0.704$, $p<0.011$) taste. Another factor influencing the panellists choice was the pH ($r=-0.896$, $p<0.001$). It was suggested that the sensory characteristics of bread perceived by a trained panel can be related to the ingredients used (Hersleth, Berggren, Westad, & Martens, 2005). However in most of the cases, a change of ingredients, process fermentation and baking conditions will have an influence on the perception of sensory characteristics. In this study, yeast strain fermentation temperature and time are the main factors influencing the bread crumb aroma. In general, a change in fermentation temperature and time was found to decrease ethanol and 3-methylbutanol and an increase of acetic acid. However, contradictory results were found regarding 2,3-butandiol and 1-hexanol concentrations. This observation highlights the complexity of aroma formation of different strains from the species *S. cerevisiae* in combination with fermentation conditions.

6.4.7. Principal component analysis

PCA analysis was performed on all the experimental data to understand the correlation between the composition and nutritional improvement as well as the prolonged shelf life (Figure 6-2). The first PC models primarily describes the compositional analysis and the shelf life with its influencing factors (Figure 6-2a). Whereas the second PC represents the technological properties (hardness, specific volume, number of cells/cm^2, slice area V_{total} and $V_{retention}$). The mono- and disaccharides in combination with the GI are to a greater extent presented in PC 3 and 4. Using hierarchical classification, it was possible to differentiate the used procedures into 2 groups (Figure 6-2b): (A) optimised procedure, (B) original procedure. Group A representing the optimised procedure is well characterised by a prolonged shelf life resulting from the high sum of acids especially acetic acid but as well by a high sum of sugars with a reduction in the rest of the compositional compounds resulting in a low GI. Contrastingly, the original procedure (Group B) is therefore more described by high total starch, protein, fat, pH, glucose, fructose, citric and succinic acid which showed a high GI and a shorter shelf life.

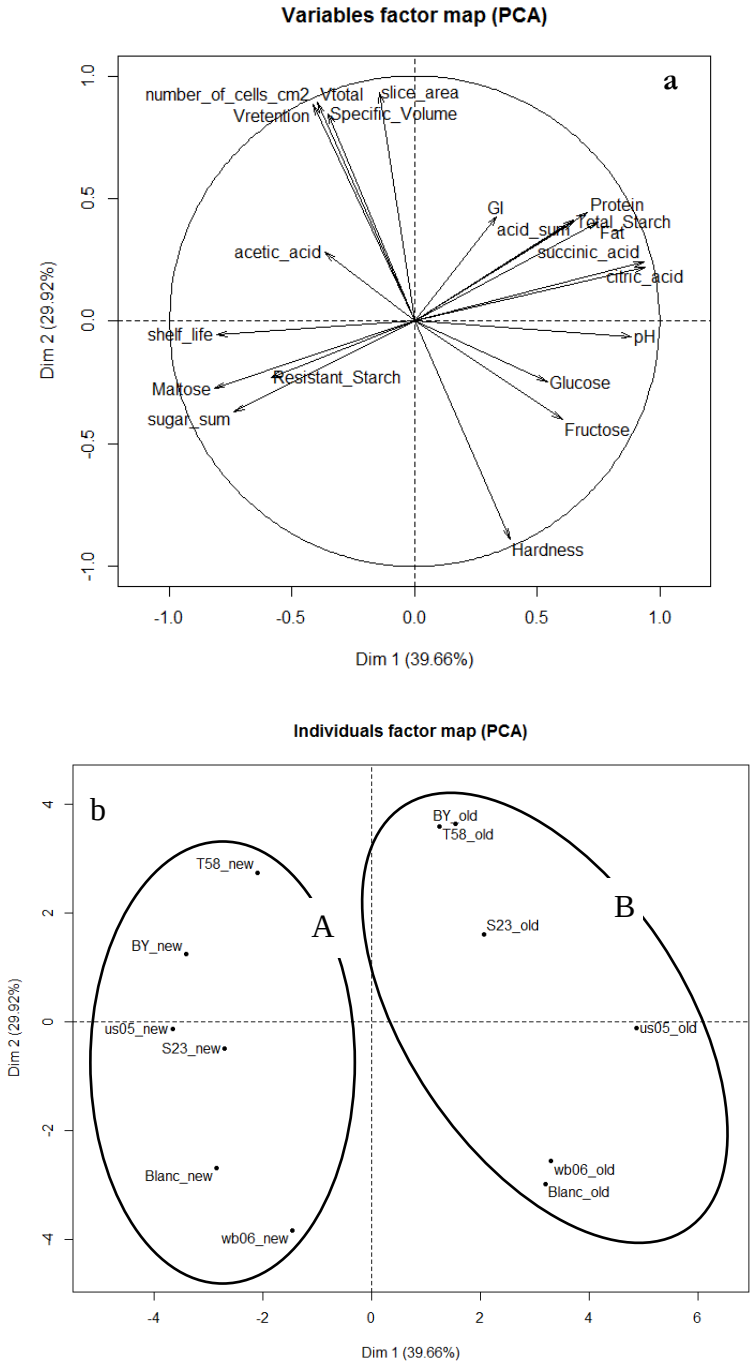


Figure 6-2 Principal component analysis (PCA) of technological and compositional parameters: (a) distribution of analysed parameters, (b) hierarchical classification of breads fermented with different *S. cerevisiae* strains for the original and optimised fermentation procedure

6.5. Conclusion

Conclusively, in this study RSM was used as a tool to optimise the process conditions for yeast originating from different fermentation sources applied to white wheat bread. By adapting the fermentation conditions in terms of time and temperature the optimal process can be chosen to enhance bread characteristics. The resulting breads were compared based on a wide range of technological, sensorial and nutritional product parameters and main effect can be seen in significant differences in terms of shelf life, GI, aroma production and sensory preference. PCA confirmed that the breads prepared with the optimised fermentation conditions were quite distant in their technological and nutritional parameters in comparison to the original procedure. Considering the panellists preference, breads fermented with the optimised procedure were generally preferred in most cases. Overall, it was shown that optimisation of the process conditions is essential when yeasts from different origins are used. A good tool for this type of optimisation is to use RSM.

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7. Chapter General Discussion

7.1. Conclusion

The dough fermentation step using yeast is considered as one of the oldest biochemical processes next to beer making. However our knowledge in terms of yeast impact on bread quality parameters is scarce. A close link between brewing and baking was common in Egypt and the Middle East in ancient times, where for both processes only one single yeast strain was used. Until the nineteenth century, bakeries were still using left over yeast from the breweries for dough leavening. Nowadays, the yeasts are genetically improved for the better suitability of each application (Amendola & Rees, 2003).

A literature review, revealed that yeast strain selection by industry is mainly based on adequate gas production with the ability to ferment sugars anaerobically (Chapter 2). Currently the production of additional metabolites and other important quality characteristics such as colour, texture and flavour are not considered by industry for the choice of suitable yeasts. In the beverage industry much more effort is made in the selection of yeast strains and for each product a different yeast with special characteristics is used. Beer yeasts are commonly divided into ale and lager yeasts, nevertheless more specific criteria are also used such as the fermentation behaviour (top or bottom fermentation), fermentation performance (fermentation rate and degree of attenuation), the ability to ferment meliobiose, temperature tolerance, ability to flocculate (powdery or flocculant yeast), oxygen requirements and the ability to form or remove fermentation metabolites (aroma compound formation) (Bokulich & Bamforth, 2013; Kunze, 2014). Next to beer yeasts, wine yeasts are mainly selected by fermentative power, suitable fermentation kinetics, their low acetic acid production and resistance to sulphur dioxide with the recently addition of the ability to enhance wine colour, the absence of β-glucosidase activity, the appropriate enhancement of aroma via the production of volatile compounds and the provision of structure and body (Suárez-Lepe & Morata, 2012). Taking the beverage industry as a role model, Baker's yeast should be more carefully selected to enhance bread quality and should not only focus on gas production. Much more parameters need to be taken into account such as enzyme activity, colour and flavour formation as well as their impact on shelf life (staling and microbial).

To increase our knowledge about the dough fermentation step, the first part of this study focused on the impact different *S. cerevisiae* strains originating from the brewing industry

have on technological bread and dough characteristics (Chapter 3). To ensure the comparability of the results the cell viability in the freeze dried powders was analysed. The inoculum size of all yeasts was adapted to *S. cerevisiae* Baker's yeast. By only changing the yeast strain used, significant impact on dough extensibility and stickiness were shown. In the resulting breads, the two yeasts *S. cerevisiae* T-58 and s-23 showed the necessary gas production in combination with strong dough formation resulting in superior bread characteristics such as higher specific volume and less staling over time. Moreover a positive influence on the shelf life of bread was achieved by using *S. cerevisiae* us-05, which showed the longest shelf life and the best resistance to propagation of mould. In conclusion, yeasts show a significant impact on dough and bread characteristics and they can be used as a tool to enhance technological end product quality. Furthermore differences in the carbohydrate concentrations for maltose, glucose and fructose were shown.

Bread, as a carbohydrate source, also plays an important role in human nutrition since it accounts for 45-70% of the total energy intake (Lafiandra et al., 2014). Also, the FAO/WHO recommends a dietary carbohydrate intake of 50-75% of the total energy intake (Mann et al., 2007). However, the quantity and quality of consumed carbohydrates might be one aspect which has an influence on type 2 diabetes. Compositional analysis showed no differences in total and resistant starch content. Even so protein and lipid content showed significant differences, only a lowering effect can be expected at a protein carbohydrate ratio of 3:5 and a fat carbohydrate ratio of 1:1 (Arvidsson-Lenner et al., 2004). Therefore, another part of this study focused on the nutritional value of breads in terms of postprandial glycaemic response during digestion measured as the pGI using an *in vitro* multi-enzyme dialysis method (Chapter 4). The GI is defined as the relative rate of glucose entering the bloodstream compared to the effect of a reference carbohydrate source (Jenkins et al., 1981). In general, breads have a high GI (>70 in comparison to glucose (100)), due to the high starch gelatinisation occurring during baking (Fardet et al., 2006). However, the application of the various *S. cerevisiae* strains showed a reduction in pGI for *S. cerevisiae* s-23 (71.6), wb-06 (63.0) and Blanc (77.9) in comparison to *S. cerevisiae* Baker's yeast bread (100). No effect was investigated for *S. cerevisiae* T-58 (97.7) and us-05 (103.6). The fermentation process however, can lead to differences in starch gelatinisation and swelling of starch granules during baking, affected by core temperature.

Therefore inefficient dough rising can result in limited starch gelatinisation and consequently in a decrease of starch digestibility. *S. cerevisiae* wb-06, Blanc and s-23 showed a slower fermentation rate after YT MicroPlate™ analysis resulting in lower specific volume with a firmer crumb structure and lowered pGI. In addition *S. cerevisiae* wb-06 and Blanc showed as well a high residual fructose (GI of 15) content which further contributes to a reduction in pGI. Another major influencing factor to reduce the GI is the presence of organic acids. However *S. cerevisiae* us-05 with the highest amount of acids produced showed an increased glycaemic response. This higher pGI can be explained by the high concentrations of glucose (GI of 100) and maltose (GI of 105) present in the breads. After principle component analysis (PCA) and hierarchical classification a separation into 3 groups characterised by different parameters was possible. Therefore, a low pGI was described by low specific volume, high hardness along with a lower sugar content. In conclusion it was shown that the application of various *S. cerevisiae* strains can significantly decrease the GI of wheat bread and improve nutritional parameters.

However not only the technological and nutritional characteristics are important for consumer's acceptance, also the aroma and flavour profile are a significant factor as a quality parameter. During the baking process a large amount of aroma compounds are formed, nevertheless fermentation also plays a key role in the production of the unique bread flavour (Hui, 2006). The beverage industry shows that the choice of yeast strain is essential to alter flavour perception in the end product (Pires et al., 2014; Swiegers et al., 2006; Wondra & Berovič, 2001). The influence of *S. cerevisiae* strains on the production of volatile aroma compounds on bread crumb was investigated in Chapter 5. Ethanol, acetic acid, 3-methyl-1-butanol, isobutyric acid, 2,3-butandiol, 1-hexanol and 2-phenylethanol were analysed in this study by gas chromatography mass spectrometry after thermal desorption (GC-MS TD). The main pathway responsible for aroma compound formation in yeast cells is the Ehrlich pathway, which is utilising amino acids to their corresponding higher alcohols and organic acids (Hazelwood et al., 2008). Consequently, the amino acid composition of the fermentation substrate, the fermentation stage and the gens present in the yeast strains have an influence on the biosynthetic pathway. The results showed yeast strain dependent aroma formation in wheat bread crumb. In addition, a trained panel was used to predict consumer acceptance by descriptive sensory analysis. Several correlations between aroma profiles, sensory and technological loaf characteristics were

found. Especially 3-methyl-1-butanol, which is one of the most important aroma compound in bread flavour, and ethanol corresponded well with yeasty taste ($r=0.985$, $p<0.1$) and smell ($r=0.816$, $p<0.05$), respectively. However the sensory acceptance is also influenced by technological parameters such as number of cells/mm^2 ($r=-0.752$, $p<0.09$) and specific volume ($r=0.928$, $p<0.01$) in correlation to overall appearance. The highest sensory acceptance therefore, was found for breads fermented with *S. cerevisiae* T-58 and s-23. Aroma profile should be considered as a new selection criteria for yeast strain development and could be of industrial interest. Yeasts should be selected according to a high production of alcohol, ketones and esters with a limited production of acids, and aldehydes (Birch, Petersen, & Hansen, 2013; Plessas et al., 2005). This combination showed in general the best panel acceptance in sensorial analysis of wheat bread-crumbs (Quílez et al., 2006).

Another important factor for the application of different starter cultures in dough leavening are the process conditions. In terms of yeast fermentation the most important parameters are fermentation time and temperature. The various yeasts used in this study are normally used for the production of different end products, consequently the influence of fermentation time and temperature on bread quality characteristics was studied in Chapter 6. Due to the lack of knowledge about the impact of fermentation conditions by using different starter cultures, response surface methodology (RSM) was applied as a tool to optimise the process conditions for yeasts originating from different fermentation sources applied to white wheat bread. The optimisation mainly resulted in an increase of fermentation time (87-124 min) with a decrease of fermentation temperature (20-25°C). Solely, a shorter fermentation time (70 min) and a higher fermentation temperature (40°C) was found for *S. cerevisiae* T-58 and us-05, respectively. However, the application of the optimised procedure only lead to an improvement of specific volume for *S. cerevisiae* Baker's yeast, wb-06 and Blanc. On the contrary, *S. cerevisiae* us-05, wb-06 and Blanc resulted in softer crumbs. The optimisation however, did not negatively impact on the remaining yeasts. In addition the results showed a significant influence of the fermentation conditions on microbial shelf life, pGI, aroma production and sensory preference. An increase from 1-3 days in shelf life was observed after the application of the optimised procedure due to an increase of citric acid in combination with a lowered pH and increased TTA values. Moreover the optimisation of fermentation

conditions resulted in an improved nutritional value represented by a lowered glycaemic index (pGI). A further reduction in pGI was seen for *S. cerevisiae* Baker's yeast (78.3), T-58 (82.1), us-05 (68.0) and Blanc (62.9). Next to technological and nutritional characteristics, most importantly the process optimisation also led to an enhancement of sensory and flavour attributes. The results showed aroma formation is dependent of yeast strain, fermentation time and temperature. The change in fermentation parameters led to a decrease in ethanol and 3-methyl-1-butanol with a simultaneously increase of acetic acid. For 2,3-butandiol and 1-hexanol contradictory results were found. Sensory analysis, however, revealed that in general the panellists preferred breads produced using the optimised procedure. Except breads fermented with *S. cerevisiae* s-23 and wb-06, where the opinions were quite divided among the panellists. The acceptance was influenced by the combination of various circumstances. Overall the panellists preferred breads with less bitter ($r=-0.886$, $p<0.001$) and cheesy ($r=-0.802$, $p<0.002$) but with a more buttery ($r=0.704$, $p<0.011$) taste. All these results highlight the complexity of aroma formation by *S. cerevisiae* together with fermentation conditions. PCA in combination with hierarchical classification confirmed the difference between the breads prepared with different procedures. It was possible to differentiate the used procedures into 2 groups: (A) optimised procedure, (B) original procedure. Therefore, Group A (optimised procedure) is well represented by a prolonged shelf life, a high concentration of citric acid, a high sum of sugars with a reduction in the rest of the compositional compounds and a low pGI. On the contrary Group B (original procedure) is more described by high total starch, protein, fat, glucose, fructose, acetic and succinic acid content which showed a high pGI and a shorter shelf life. Overall, it was shown that optimisation of the process conditions is essential when yeasts from different origins are used and can be used as a tool to enhance bread quality characteristics. A good tool for this type of optimisation is RSM.

This doctoral thesis showed that strains of the species *S. cerevisiae* originating from the beverage industry can be suitable for dough leavening. The application of different *S. cerevisiae* showed a positive influence on technological characteristics such as higher specific volume with a simultaneously reduction in hardness and a prolonged shelf life. Furthermore, the composition of the resulting breads were significantly influenced by yeast metabolism leading to breads with improved nutritional values represented by a

lowered pGI. Besides the improvement of technological and nutritional characteristics also a change in aroma profile and sensory acceptance was shown. However, most essential is the fermentation optimisation for yeast leavened products. Therefore, not only the raw materials have to be considered to enhance bread quality, but yeast choice needs to be taken into account as a new quality parameter due to its impact on aroma profile, sensory acceptance and technological loaf characteristics. Literature also suggests to use recombinant DNA technology to create new yeast strains, which would eliminate the extensive use of baking additives such as enzymes (Randez-Gil et al., 1999a). However, consumer acceptance and the current Food Legislation (EFSA, 2012) will not allow the use of genetically engineered yeast strains. In the wine industry also non-*Saccharomyces* yeasts have shown great potential (Suárez-Lepe & Morata, 2012) and could be of interest for the baking industry. Interesting yeast species as an alternative to *Saccharomyces* could be *Debaromyces*, *Kluyveromyces* and *Schizosaccharomyces*. For that reason, yeast strain selection offers an alternative approach to improve wheat bread quality, to satisfy the high demand of consumers for an increasing variety of bread by only changing the starter culture.

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8. Appendix

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8.3. Publications and Presentations

Peer reviewed first author publications:

Published

Heitmann, M., Zannini, E., Arendt, E.K., Impact of different beer yeasts on wheat dough and bread quality parameters, *Journal of Cereal Science* (2015), doi: 10.1016/j.jcs.2015.02.008.

Heitmann, M., Zannini, E., Arendt, E.K., Impact of *Saccharomyces cerevisiae* metabolites produced during fermentation on bread quality parameters: a review, *Critical Reviews in Food Science and Nutrition* (2016), doi: 10.1080/10408398.2016.1244153 (accepted 29.09.2016)

Heitmann, M., Axel, C., Zannini, E., Arendt, E.K., Modulation of *in vitro* predicted Glycaemic Index of White Wheat Bread by different strains of *Saccharomyces cerevisiae* originating from various beverage applications, *European Food Research and Technology,* (2017), doi: 10.1007/s00217-017-2894-2

Heitmann, M., Zannini, E., Axel, C., Arendt, E.K., Correlation of flavour profile to sensory analysis of bread produced with different *Saccharomyces cerevisiae* originating from the baking and beverage industry, *Cereal Chemistry,* (2017), doi: 10.1094/CCHEM-03-17-0044-R

Second author publications:

Published

Horstmann, S., Belz, M.C.E., **Heitmann, M.**, Zannini, E., Arendt, E.K., Fundamental Study on the impact of gluten-free starches on the quality of gluten-free model breads, *Foods (2016),* doi: 10.3390/foods5020030 (see Chapter 9)

Oral Presentations:

M. Heitmann, E. Zannini, E. K. Arendt 2014: Impact of different yeasts on wheat bread quality, *13th European Young Cereal Scientists and Technologists Workshop,* Munich, Germany, May 2014

M. Heitmann, E. Zannini, E. K. Arendt 2014: Impact of different yeasts on wheat bread quality, *AACCI Annual Meeting,* Providence, Rhode Island, USA, October 5th – 8th 2014

M. Heitmann, E. Zannini, E. K. Arendt 2016: Influence of sourdough type and quantity on gluten quality parameters measured by Gluten Peak Tester following a correlation analysis on bread quality parameters, *15th European Young Cereal Scientists and Technologists Workshop,* Bergamo, Italy, May 2016

Poster presentation:

Belz, M.C.E., Hager, A-S., **Heitmann, M.**, Horstmann, S., Arendt, E.K., 2013: Elasticity – an important quality characteristic to be addressed for gluten-free bread, *3rd International Symposium on Gluten-Free Cereal Products,* Vienna, Austria, 12th – 14th June 2013

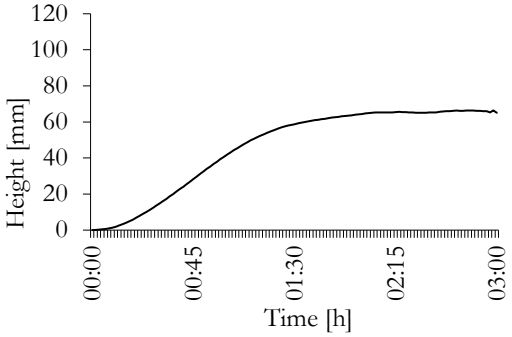
Matullat I., Lösche K., **Heitmann M.**, Arendt E. K., Döring S.: Sensory acceptance and perception of ready-to-bake rolls of German and Irish consumers, *Pangborn conference,* Gothenburg, Sweden, 23rd – 27th August 2015

Horstmann, S., Belz, M.C.E., **Heitmann, M.**, Zannini, E., Arendt, E.K.: Fundamental Study on the impact of gluten-free starches on the quality of gluten-free model breads, *4th International Gluten-free Symposium,* Cork, Ireland, 18th – 19th October 2016

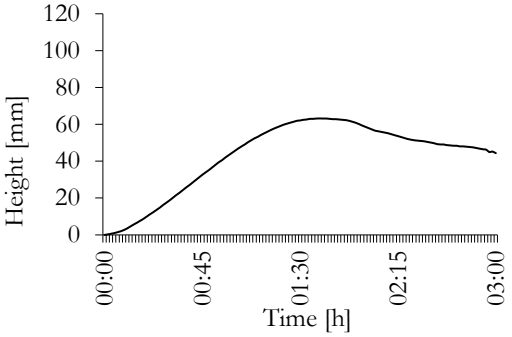
Heitmann, M., Zannini, E., Axel, C., Arendt, E.K., The investigation of technological, nutritional and sensorial characteristics of wheat bread, influenced by different strains of *Saccharomyces cerevisiae*, *ISSY 33*, Cork, Ireland, 26th – 29th June 2017

8.4. Complementary Data

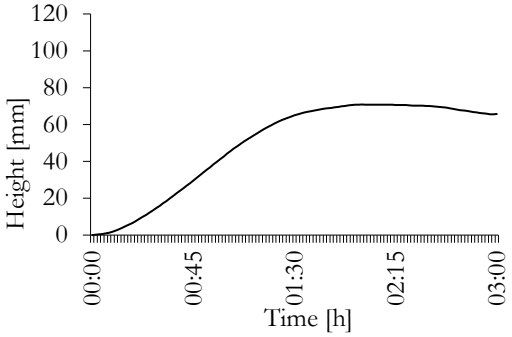
8.4.1. Chapter 3 Impact of different beer yeasts on wheat dough and bread quality parameters



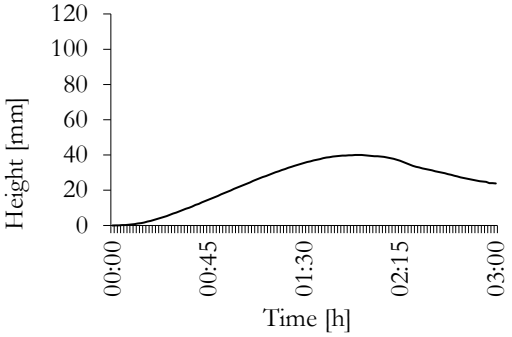
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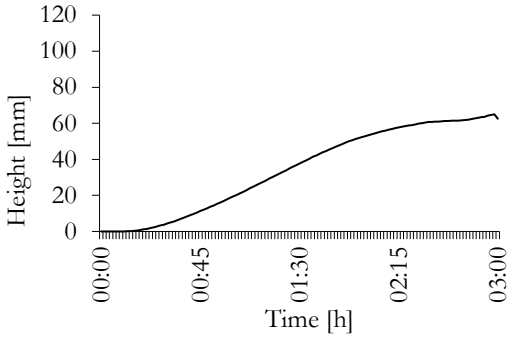
S. cerevisiae S-23



S. cerevisiae T-58



S. cerevisiae us-05



S. cerevisiae wb-06

Figure 8-1 dough development curves of dough fermented with different *S. cerevisiae*

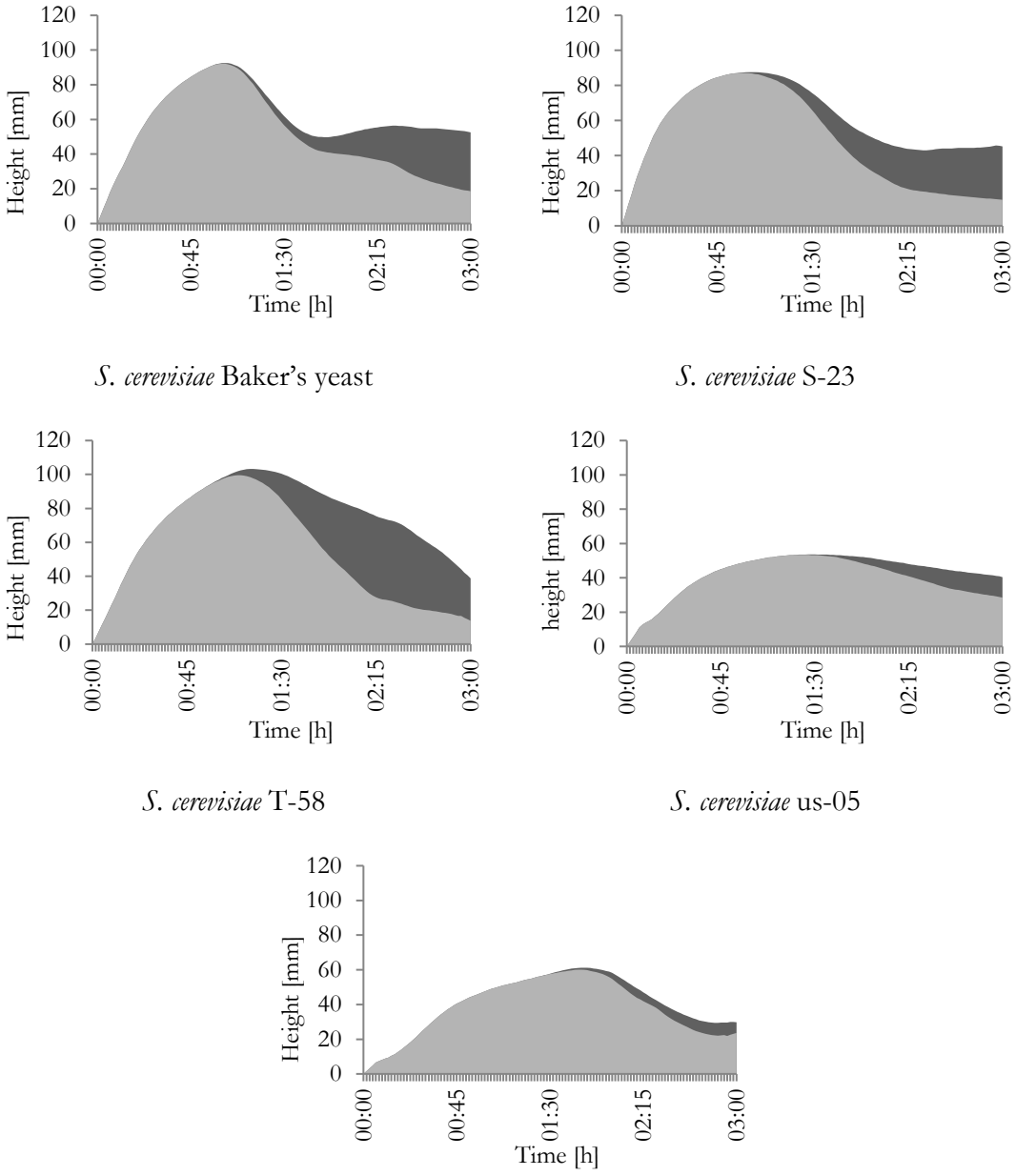
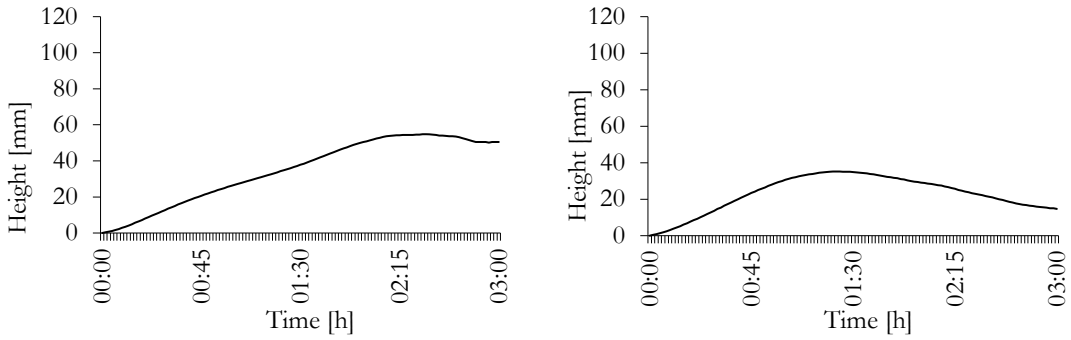


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8.4.2. Chapter 4 Modulation of *in vitro* predicted Glycaemic Index of White Wheat Bread by different strains of *Saccharomyces cerevisiae* originating from various beverage applications.

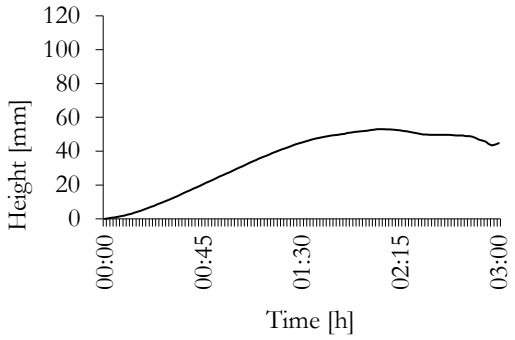
Table 8-1 Flour characteristics

pH	6.70 ± 0.03
TTA	2.67 ± 0.12
Damaged starch	5.94 ± 0.30
Total starch	70.25 ± 2.14
Moisture	13.60 ± 0.04
Ash	0.59 ± 0.11
Fat	2.68 ± 0.12
Protein	12.2 ± 0.47
Glutopeak	
Peak time	55.5 ± 0.72
Peak torque	85.5 ± 0.68
Wet gluten	24.6 ± 1.2
Dry gluten	9.9 ± 0.11
Gluten index	84.3 ± 3.28
Water absorption	62.0 ± 0.12

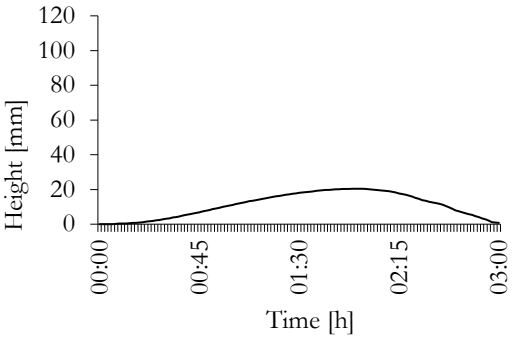


S. cerevisiae Baker's yeast

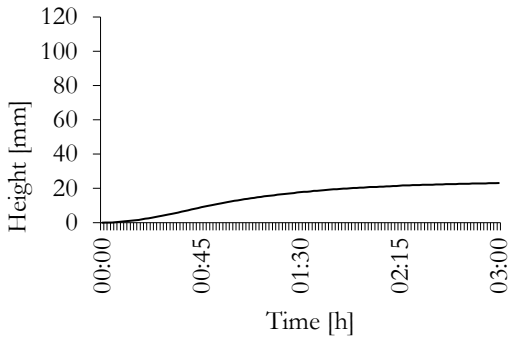
S. cerevisiae S-23



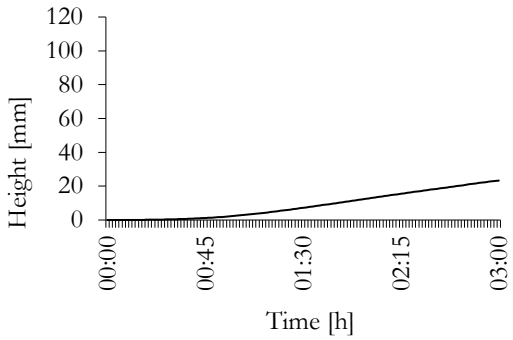
S. cerevisiae T-58



S. cerevisiae us-05



S. cerevisiae wb-06



S. cerevisiae Blanc

Figure 8-3 dough development curves of dough fermented with different *S. cerevisiae* using the original procedure

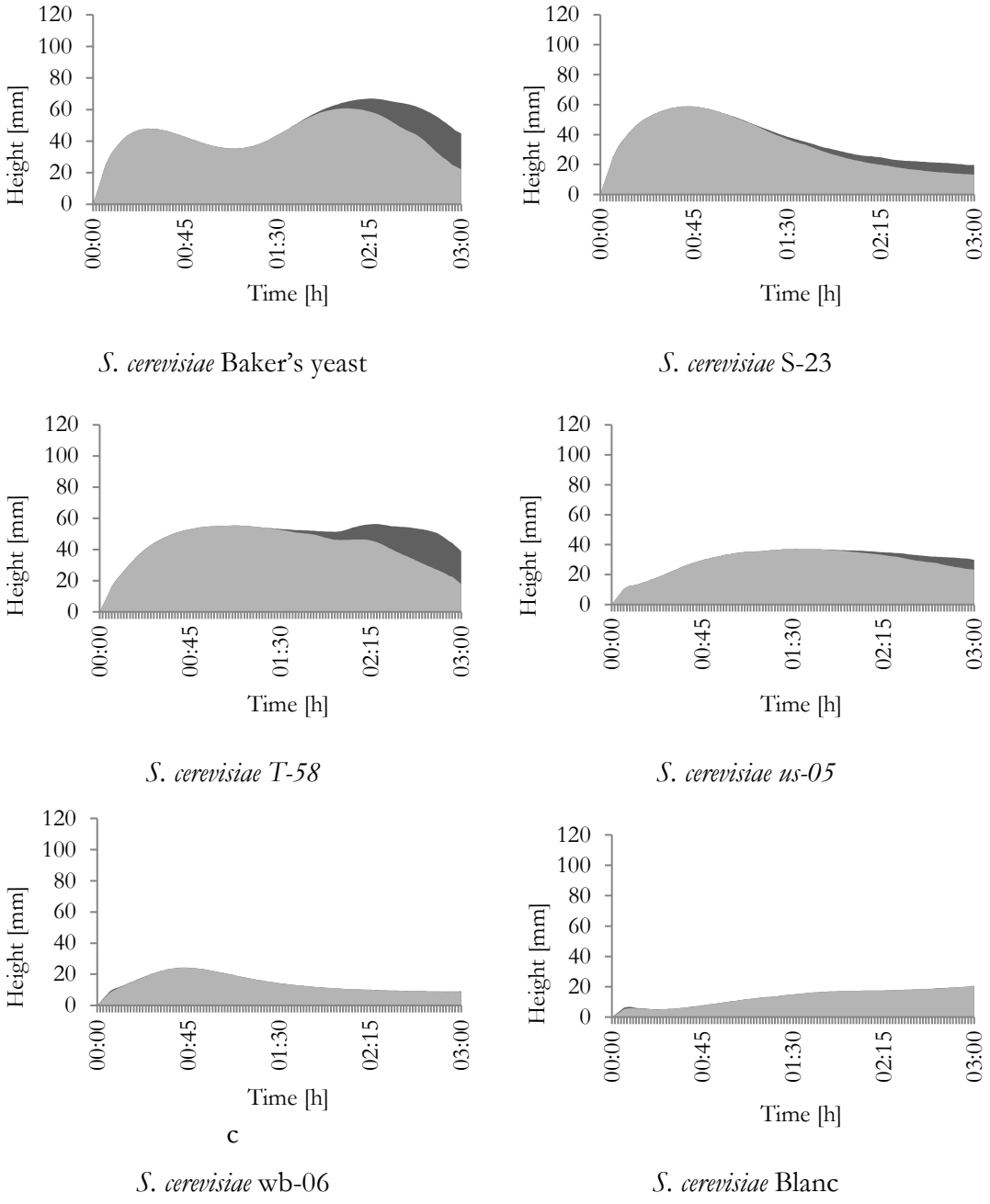
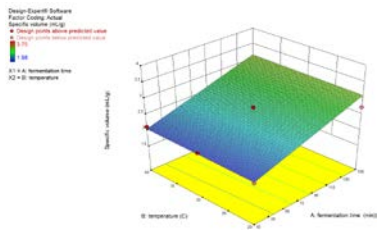
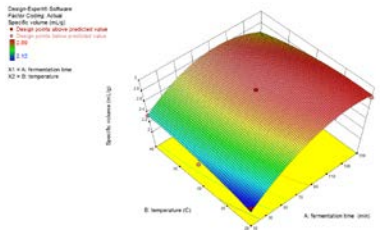


Figure 8-4 gaseous release curves of dough fermented with different *S. cerevisiae* using the original procedure

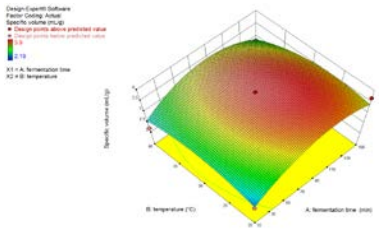
8.4.3. Chapter 6 Influence of fermentation time and temperature on wheat bread quality prepared with different yeasts using response surface methodology



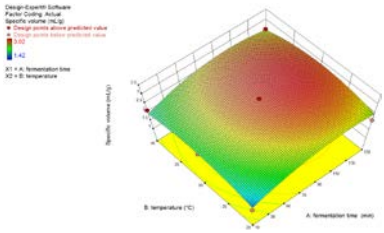
S. cerevisiae Baker's yeast



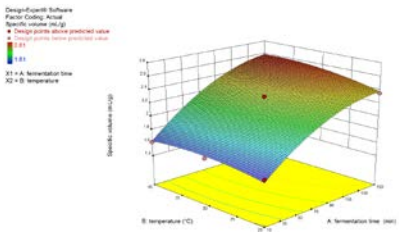
S. cerevisiae S-23



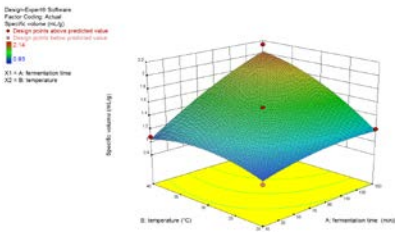
S. cerevisiae T-58



S. cerevisiae us-05

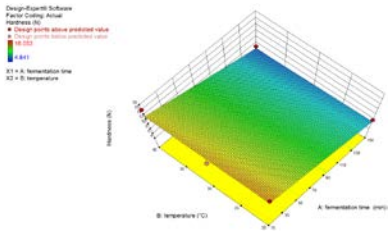


S. cerevisiae wb-06

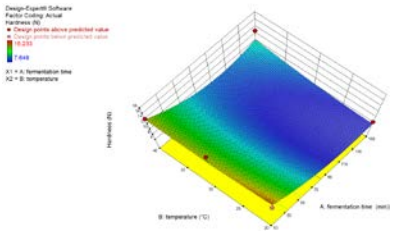


S. cerevisiae Blanc

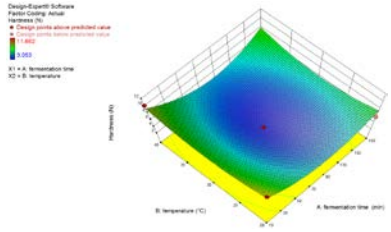
Figure 8-5 Influence of fermentation temperature and time on the specific volume of bread prepared with different *S. cerevisiae* using response surface methodology



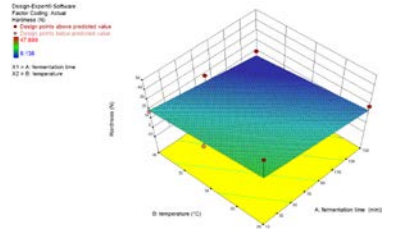
S. cerevisiae Baker's yeast



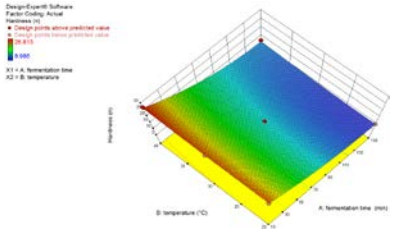
S. cerevisiae S-23



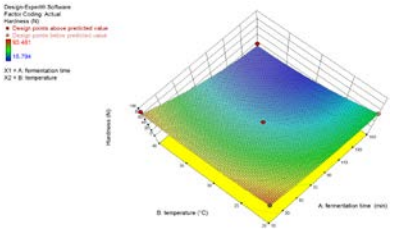
S. cerevisiae T-58



S. cerevisiae us-05

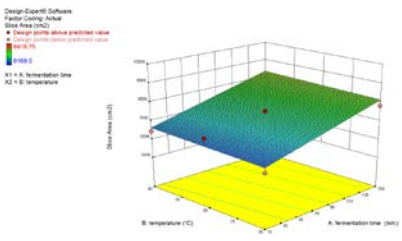


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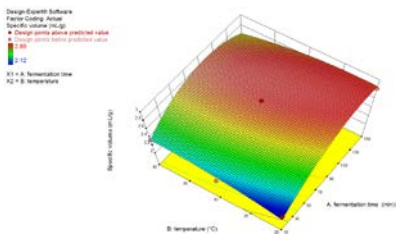


S. cerevisiae Blanc

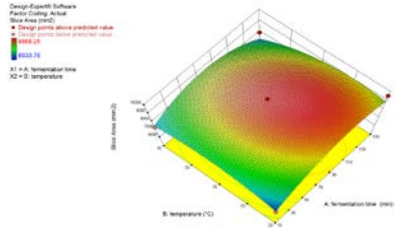
Figure 8-6 Influence of fermentation temperature and time on the hardness of bread prepared with different *S. cerevisiae* using response surface methodology



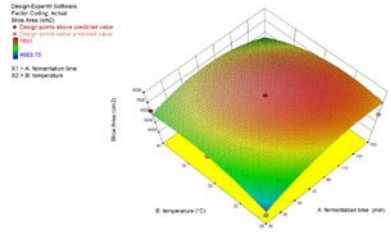
S. cerevisiae Baker's yeast



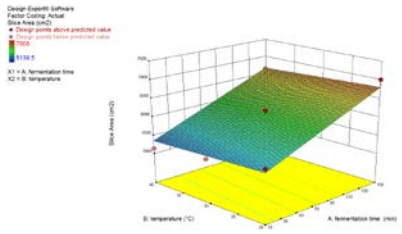
S. cerevisiae S-23



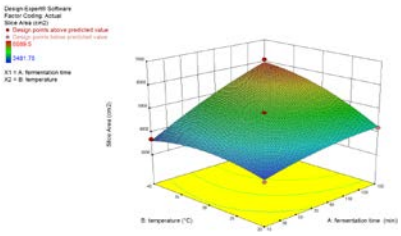
S. cerevisiae T-58



S. cerevisiae us-05

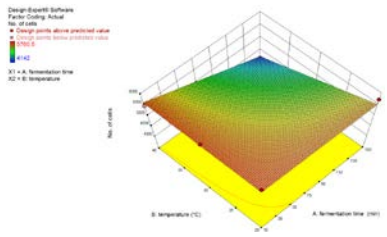


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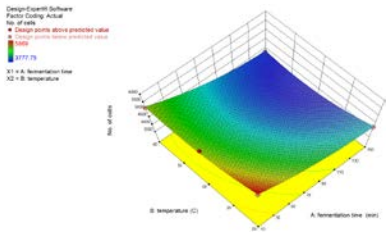


S. cerevisiae Blanc

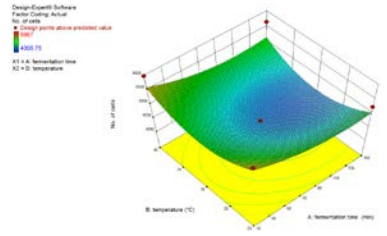
Figure 8-7 Influence of fermentation temperature and time on the slice area of bread prepared with different *S. cerevisiae* using response surface methodology



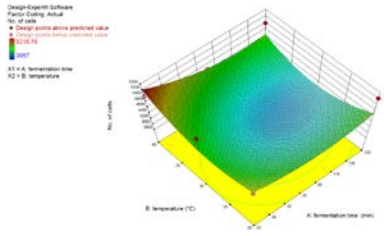
S. cerevisiae Baker's yeast



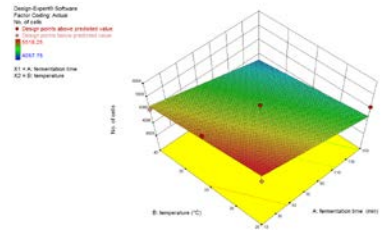
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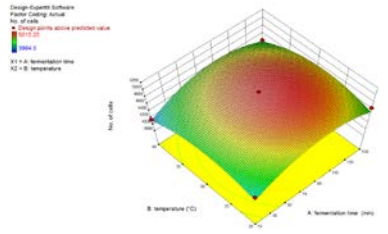
S. cerevisiae T-58



S. cerevisiae us-05



S. cerevisiae wb-06



S. cerevisiae Blanc

Figure 8-8 Influence of fermentation temperature and time on the number of cells of bread prepared with different *S. cerevisiae* using response surface methodology

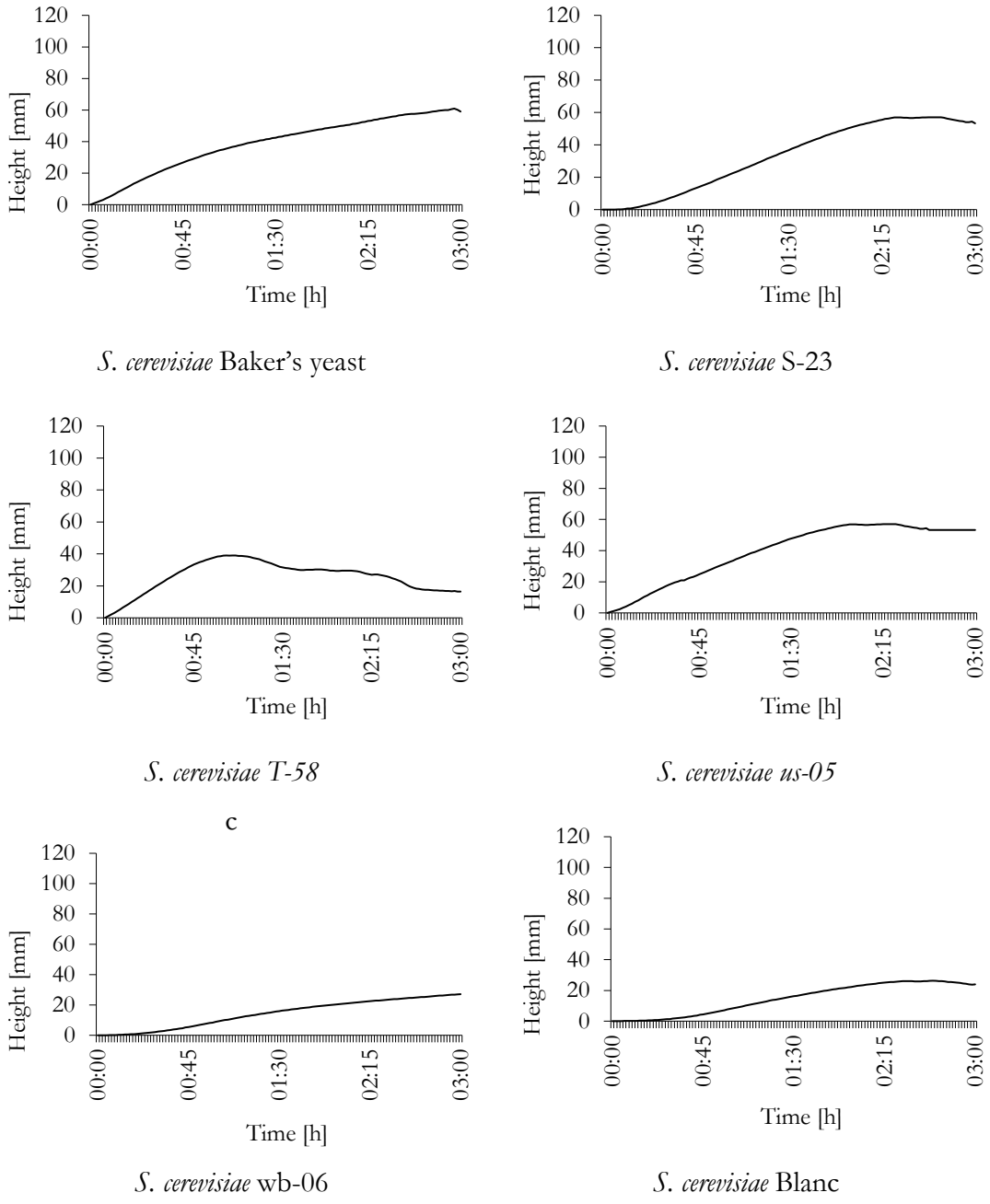
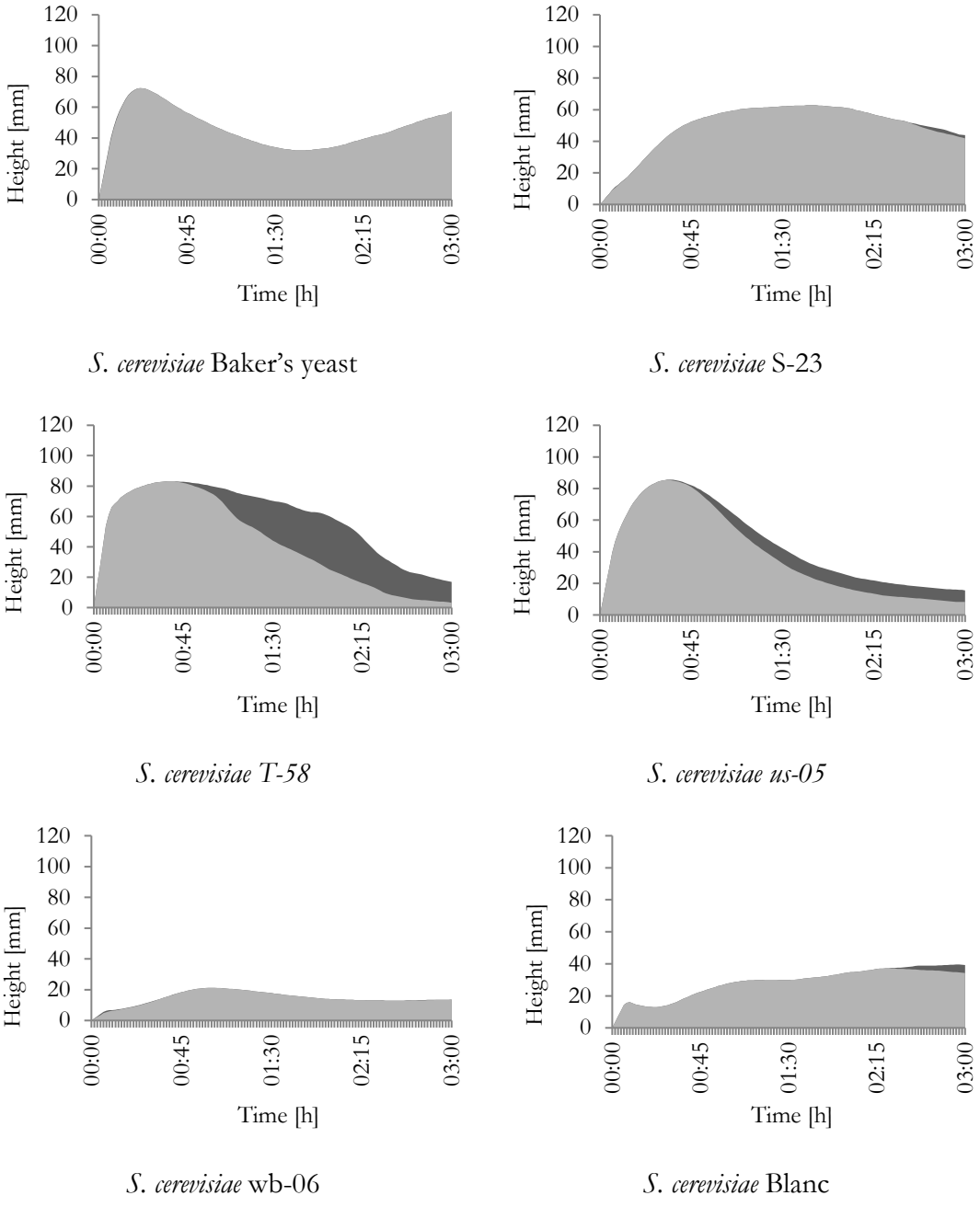


Figure 8-9 dough development curves of dough fermented with different *S. cerevisiae* using the optimise



S. cerevisiae Baker's yeast

S. cerevisiae S-23

S. cerevisiae T-58

S. cerevisiae us-05

S. cerevisiae wb-06

S. cerevisiae Blanc

Figure 8-10 gaseous release curves of dough fermented with different *S. cerevisiae* using the optimised procedure

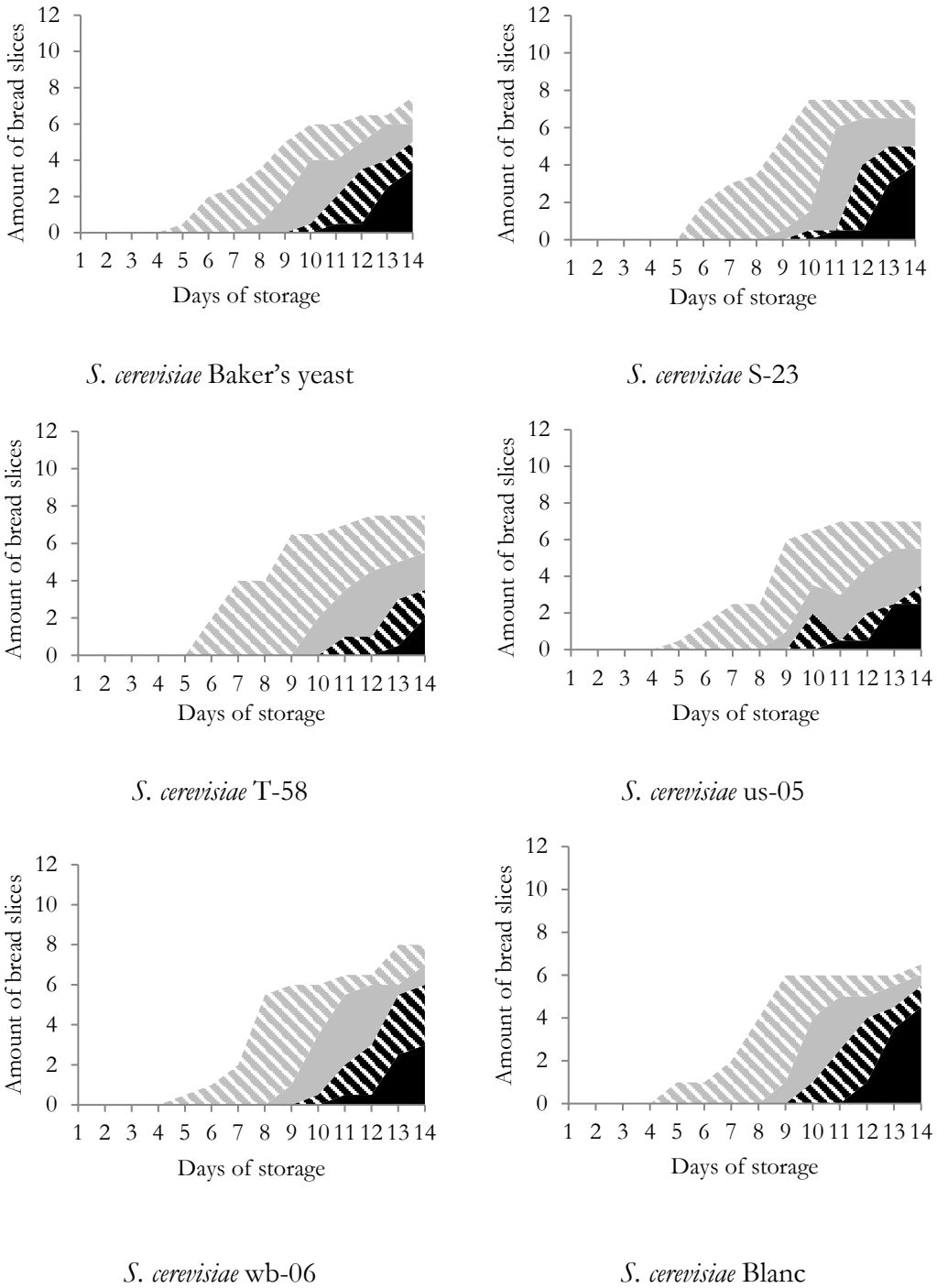
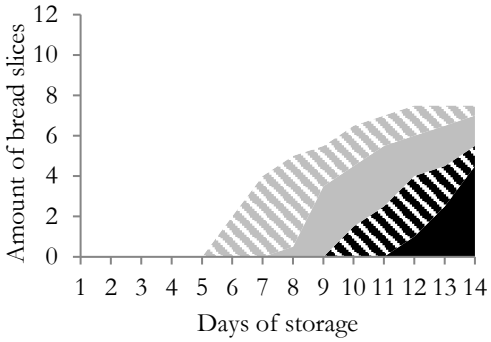
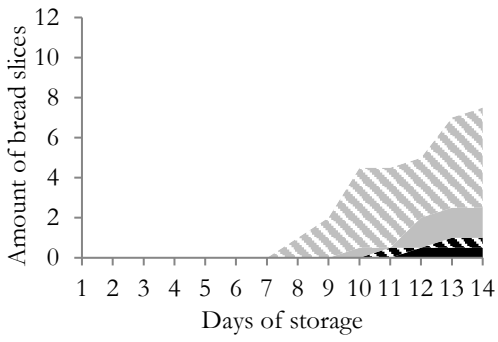


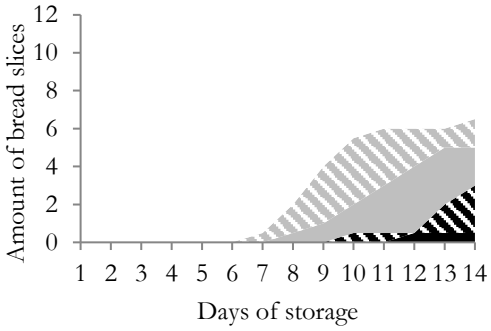
Figure 8-11 Shelf life of bread baked with different yeasts using the original procedure against environmental moulds during a 28-day storage period. Bread spoilage is indicated as percentage of the total surface area of each of the 12 slices where fungal outgrowth occurred: Mould free slices (white area), <10% mouldy (grey diagonally striped area), 10–24% mouldy (grey area), 25-49% mouldy (black diagonally striped area) and >50% mouldy (black area). Mean values are shown (n = 3)



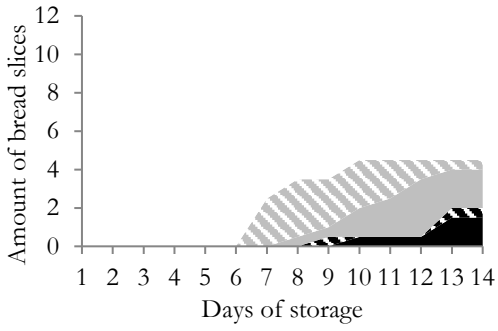
S. cerevisiae Baker's yeast



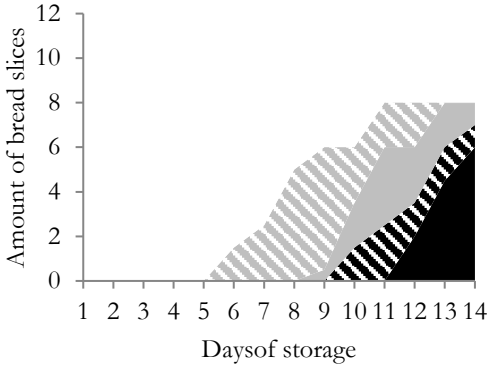
S. cerevisiae S-23



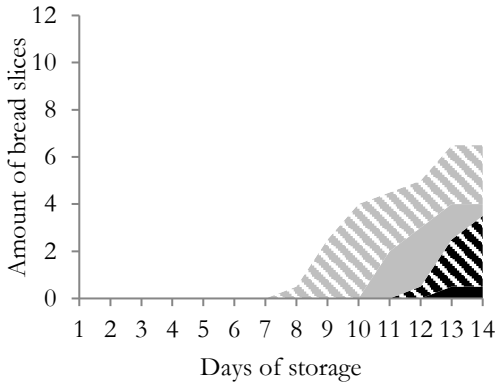
S. cerevisiae T-58



S. cerevisiae us-05



S. cerevisiae wb-06



S. cerevisiae Blanc

Figure 8-12 Shelf life of bread baked with different yeasts using the optimised procedure against environmental moulds during a 28-day storage period. Bread spoilage is indicated as percentage of the total surface area of each of the 12 slices where fungal outgrowth occurred: Mould free slices (white area), <10% mouldy (grey diagonally striped area), 10–24% mouldy (grey area), 25-49% mouldy (black diagonally striped area) and >50% mouldy (black area). Mean values are shown (n = 3)

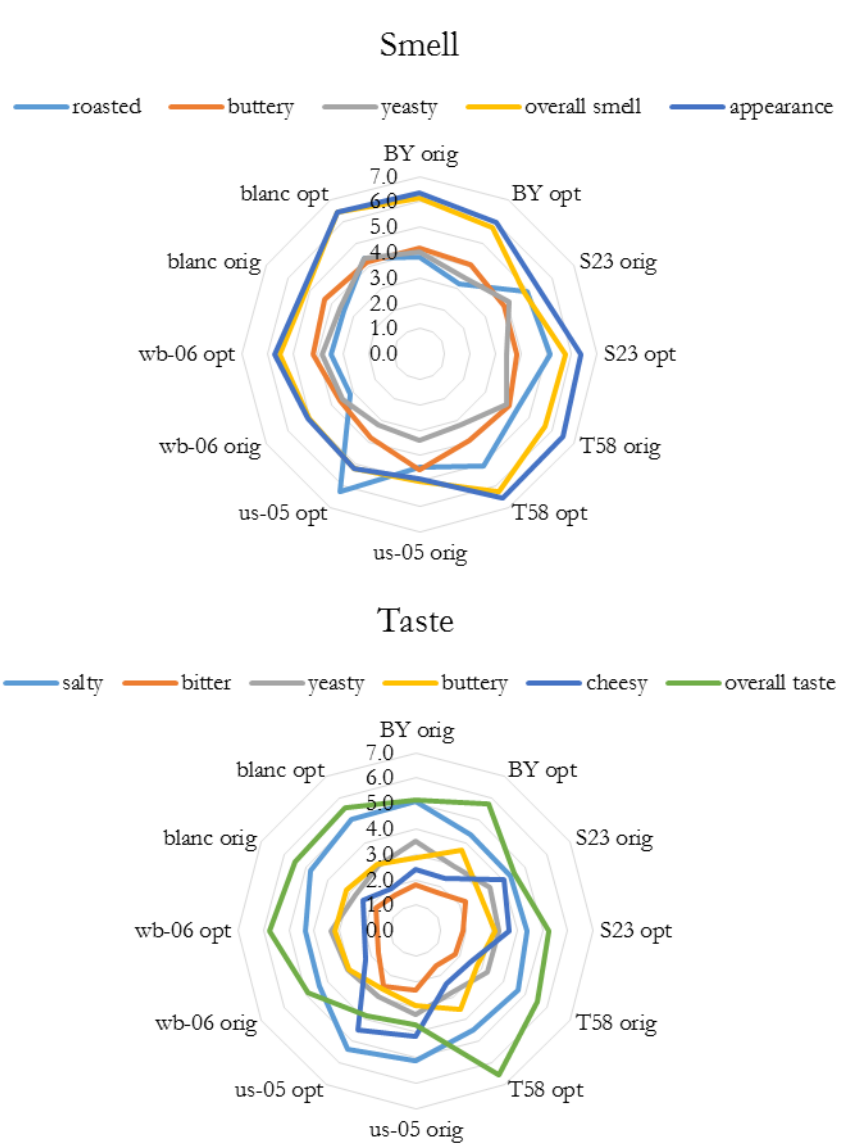


Figure 8-13 Sensory analysis of bread crumbs for the smell (A) and taste (B) of breads baked with the original and optimised procedure on a scale from 0 (not detectable) over 10 (high intensity)

9. Chapter Fundamental Study on the impact of gluten-free starches on the quality of gluten-free model breads

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9.1. Abstract

Starch is widely used as an ingredient and significantly contributes to texture, appearance, and overall acceptability of cereal based foods, playing an important role due to its ability to form a matrix, entrapping air bubbles. A detailed characterisation of five gluten-free starches (corn, wheat, rice, tapioca, potato) was performed in this study. In addition, the influence of these starches, with different compositional and morphological properties, was evaluated on a simple gluten-free model bread system. The morphological characterisation, evaluated using scanning electron microscopy, revealed some similarities among the starches, which could be linked to the baking performance of the breads. Moreover, the lipid content, though representing one of the minor components in starch, was found to have an influence on pasting, bread making and staling. Quality differences in cereal root and tuber starch based breads were observed. However, under the baking conditions used, gluten-free rendered wheat starch performed best, followed by potato starch, in terms of loaf volume and cell structure. Tapioca starch and rice starch based breads were not further analysed, due to an inferior baking performance. This is the first study to evaluate gluten-free starch on a simple model bread system.

9.2. Introduction

Starch is the primary energy storage compound in many plants including cereals, legumes, potatoes and tubers and provides 70-80% of the calories consumed by humans worldwide [1]. Starches are widely used as ingredients in many foods to improve appearance, texture and overall acceptability. They are used as gelling, thickening, adhesion, moisture-retention, stabilizing, film forming and texturizing agents [2]. In gluten-free products, starch may be successfully incorporated into the food formula to improve one or more of these properties depending on the interaction with other ingredients in the formulation and the type of food products. The main starch sources in gluten-free systems are corn, tapioca, potato, rice and gluten-free wheat [3]. Native starch exists in the form of granules. The size, shape and molecular arrangement inside the granules depend on the species, cultivar, variety of plant, as well as the environmental growing conditions. The starch biosynthesis pathway generally results in two types of glucose based polymers being formed, the semi-linear amylose and the highly branched amylopectin. In addition, other components like proteins and lipids are associated with starch [4,5]. Cereal starches are usually considered gelling materials, and in baking they significantly contribute to texture, appearance and overall acceptability of cereal based foods [4,5]. During the bread baking process starch granules gelatinize i.e. they swell and are partially solubilised, but still maintain their granular identity [6]. Starch gelatinisation plays an important role in gluten-free formulations, due to the ability of starch to form a matrix in which air bubbles are entrapped. For this reason, the addition of gel forming starches such as pre-gelatinised starches and air cell stabiliser such as gums have been suggested as a means to provide gas occlusion and stabilizing mechanisms [2]. Moreover, the addition of a certain type of starch in the gluten-free formula could improve the batter consistency during mixing, enhance the softness of the crumb and control starch gelatinization during the baking process [7]. Gluten-free products are important for people who suffer from coeliac disease (CD), an immune mediated enteropathy causing inflammation in the small intestine [8]. It is triggered by the ingestion of prolamins from wheat, rye and barley in genetically susceptible individuals [8]. CD is one of the most widespread food intolerances with a prevalence of 1% [8]. The avoidance of gluten intake is currently the only safe treatment for coeliac disease. This means that patients with CD have to strictly adhere to a gluten-free diet, being unable to enjoy common foods such as bread, pizza, pasta or beer that are commonly

based on gluten-containing grains. Due to the increasing prevalence of CD, there is a growing demand for palatable and nutritious gluten-free products from consumers. Hence, scientists aim to develop gluten-free products, which include a complex and well-matched list of ingredient to guarantee the production of high quality gluten-free breads with improved health and nutritional properties [9]. Recent studies have demonstrated a number of options for gluten replacement, including the use of various combinations of hydrocolloids [10], modifying the interaction between gluten-free proteins and starches [11] and the use of pseudo cereal flours such as quinoa [12]. In addition, the impact of modified starches on gluten-free dough and bread has been investigated by Ziobro et al. [13]. Modification of starch has an important impact on the consistent production of good quality gluten-free products. There are many commercial gluten-free breads on the market using an array of ingredients, without knowing how these ingredients interact and influence final product quality. The present work is novel in its use of a simple gluten-free model formulation (7 ingredients), enabling the examination of the bread making performance of different starches. It aims to deepen the knowledge on how different starches influence baking performance. This is accompanied by a wide range of analyses characterising the gluten-free starches.

9.3. Materials and Methods

9.3.1. Materials

The suppliers for the ingredients used were Cargill, UK for corn starch; Agrana, Austria for potato starch; Roquette, France for gluten-free wheat starch; Tradelink, UK for tapioca starch and Beneo Remy, Belgium for rice starch. Dry yeast was supplied by Puratos, Belgium; sugar from Siúcra Nordzucker, Ireland; salt from Glacia British Salt Limited, UK and hydroxypropyl methylcellulose (HPMC) from J. Rettenmaier, Germany.

9.3.2. Microscopy

Samples of starch were dried in an air-oven for 1h at 103°C. Samples were affixed with double-sided carbon tape to an aluminium stub and coated with a layer of 25 nm of sputtered palladium-gold. Hereupon, samples were examined under high vacuum in a field emission scanning electron microscope (SEM) with a working distance of 8 mm. Secondary electron images were acquired at an accelerating voltage of 5 kV. For processing of the images SEM Control User Interface software, Version 5.21 (JEOL Technics Ltd., Japan) was used.

9.3.3. Particle size

Analysis of the particle size distribution was carried out by laser diffraction using a dry feed cell (Malvern Mastersizer 3000, Instruments Ltd., UK). The sample was dispersed in air using 1.5 bar pressure and measured when an obscuration of 5-7% was achieved, with a refractive index of 1.45.

9.3.4. Chemical characterisation of the starches

Chemical characterisation of the starch samples was performed using laboratory standard methodologies. Moisture determination was performed using the air oven method (AACC Method 44-15 A). Total starch (AACC Method 76.13), damaged starch (AACC Method 76-31.01), amylose / amylopectin content of total starch, α–amylase activity (AACC Method 22-02.01) and β-amylase activity were determined utilizing commercially available assay kits (Megazyme International, Ireland LTD). The protein content was determined

using the Bradford assay with bovine serum albumin as the calibration standard. For the determination of the lipid content, the starch samples were first digested according to the Weibull-Stoll method, to release bound lipids. The lipid content was then determined using AACC Method 30-25.01.

9.3.5. Rapid visco analysis

The pasting properties of the starch samples were determined according to the Newport Scientific Method 6, Version 4, December 1997, using a RVA Super 3 Rapid Visco Analyser (Newport Scientific, Warriewood, Australia) with thermocline control and data collecting software. Starch sample of 3g (on a basis of 14% moisture) were weighed into an RVA aluminium canister, and 25g of distilled water were added, to prepare a starch-water sample of 9.2% (w/w). The temperature profile used was heating to 95 °C (6.3 °C/s), holding at 95 °C for 162 s and cooling to 50 °C (5.1 °C/s) and holding at 50 °C for 120 s.

9.3.6. Bread making procedure

Bread samples were produced based on a simple recipe (100% starch, 80% water, 2% HPMC, 2% salt, 4% sugar, 2% yeast, based on starch weight). For the pre-fermentation, yeast was suspended in warm water (25°C) and regenerated for a period of 10 min. Mixing was carried out with a k-beater (Kenwood, Havant, UK) at low disk speed (level 1 of 3) for 1 min in a Kenwood Major Titanium KM 020 Mixer (Kenwood, Havant, UK). After that the dough was scraped down from the bowl walls and a further mixing of 2 minutes at higher disk speed (level 2 of 3) was carried out. The batter was scaled to 300 g into 9 baking tins of 16,5 cm x 11 cm x 7 cm and placed in a proofer for 45 min at 30 °C and 85% relatively humidity (RH). The breads were baked for 55 min at 220 °C top and bottom heat in a deck oven, previously steamed with 0.7 L of water. After baking, bread loaves were removed from the tins and cooled at room temperature for 2 h. The loaves were subsequently analysed or packaged in plastic bags (polystrol- ethylene vinyl alcohol-polyethylene) for storage. Each starch bread batch was prepared in three replicates.

9.3.7. Bread characteristics

The bread analyses were performed two hours after baking. Four of 8 loaves were immediately used for performing the texture and structural analyses on day 0, the remaining four loaves were used for the texture analysis on storage day 2 (50 hours after baking) and storage day 5 (122 hours after baking), two each day. For the analysis performed on day 2 and 5, the bread samples were packaged in polythene bags (polystyrol –ethylene vinyl alcohol- polyethylene) and stored at room temperature. Specific volume was determined using a Vol-scan profiler (Stable Micro Systems, Godalming, England), equipped with a non-contact measurement device. The bake loss was measured as the difference between the initial weight of the sample (dough in the tin before fermentation) and the final weight of the sample (bread after baking and cooled for two hours). For measuring the moisture of the bread crumb an air oven was used according to AACC Method 44-15A. The texture profile analysis (TPA) of the bread samples was determined using a universal testing machine TA-XT2i (Stable Micro Systems, Godalming, England). This measurement, also called the "two-bite-test", evaluated seven parameters assigning independent numeric values to bread attributes normally estimated with sensory test. For analysing the texture changes over times, the measurement was done on day 0 (the baking day), day 2 and day 5. On the baking day, two breads were sliced transversely by using a slice regulator and bread knife to retain consistent slices of 20 mm thickness. Eight bread slices, taken from the center of the breads, were used to evaluate the texture parameters using a universal testing machine TA-XT2i equipped with a 25 kg loading cell and a 35 mm aluminium cylindrical probe. The settings used were a test speed of 5 mm/s with a trigger force of 25 g to compress the middle of the bread crumb to 50% of its original height. The measurement was repeated after 2 and 5 days of storage using the bread samples packaged in a plastic bag and stored at room temperature. For crumb grain evaluation a C-cell Bread Imaging system (Calibre Control International Ltd., UK) equipped with C-Cell version 2.0 software was used. The area of holes (the total area of holes as a percentage) and the wall thickness (the average thickness of cell walls) were chosen to describe the crumb grain.

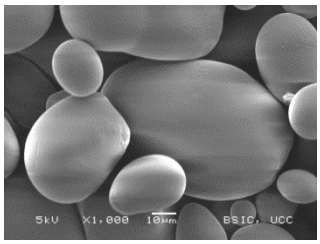
9.3.8. Statistical analysis

All measurements were performed at least in triplicate. The confidence interval was calculated and the results were checked using the Grubbs-test (outlier-test) with a significance level of α=0.05. Pearson correlation coefficient (r) and p-value were used to show correlations and their significance using the Basic Statistics package of the software MINITAB version 15 (MINITAB Ltd., UK). Differences of $p < 0.05$ were considered significant. The correlation coefficient is classified in different levels of correlation: perfect ($|r| = 1.0$), strong ($0.80 \leq |r|$ b 1.0), moderate ($0.50 \leq |r|$ b 0.80), weak ($0.10 \leq |r|$ b 0.50), and very weak (almost none) correlation ($0.10 \leq |r|$).

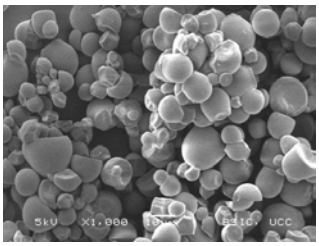
9.4. Results and Discussion

9.4.1. Starch granule morphology

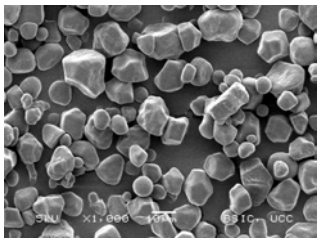
SEM was used to compare the microstructure of the different starches. Different granule sizes (A and B granules), shapes and the mix of different size granules in the starch have an influence on the rheology and the functional and structural properties of starch based foods [14]. The morphological characterisations are depicted in Figure 9-1.



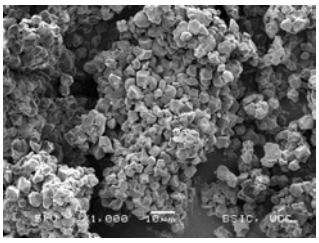
(a)



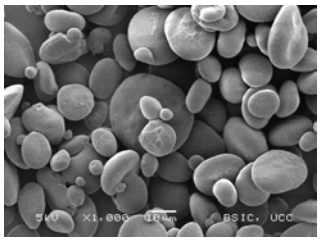
(b)



(c)



(d)



(e)

Figure 9-1 Micrographs of (a) potato starch, (b) tapioca starch, (c) corn starch, (d) rice starch, (e) wheat starch. (Magnification 1000x)

The granular size of the different starches was determined by laser diffraction using a dry feed cell. The results are shown in Figure 9-2.

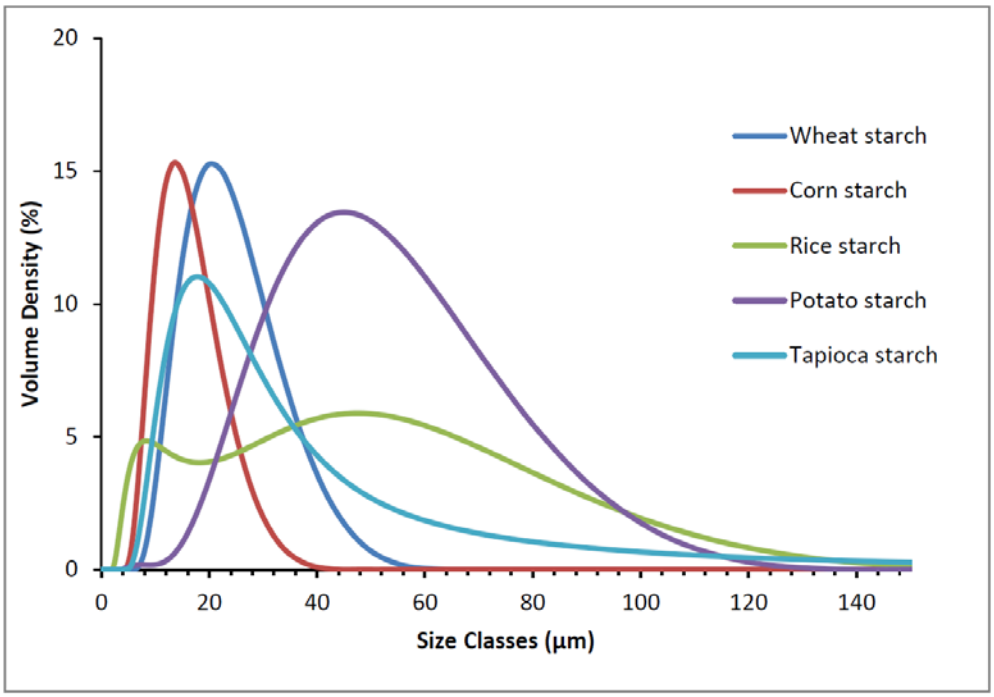


Figure 9-2 Granule size distributions of the different starches

The comparison of the surface area mean diameter (D [3,2]) also known as the Sauter mean showed the following order, starting with the biggest one: potato starch (36.7 µm), gluten-free wheat starch (18.9 µm), tapioca starch (18.1 µm), corn starch (12.9 µm) and rice starch (12.3 µm). However, the size distribution showed that rice starch had high values, similar to potato starch. This is due to the agglomerated state of rice starch, which leads to a detection of big granules (Figure 9-1). The micrographs in Figure 9-1 reveal that the potato starch has big and small granules, referred to as A and B granules, respectively. Gluten-free wheat starch also showed big and small granules. For the tapioca starch granules, agglomerated granules were found.

9.4.1. Chemical characterisation of the starches

The results of the chemical characterisation of potato, tapioca, corn, rice and gluten-free wheat starch are shown in Table 9-1. The cereal starches (wheat, corn and rice starch) contained a lower moisture level compared to the tuber starches (potato and tapioca starch). The total starch contents were, except for tapioca starch and corn starch, significantly different. From the starches analysed, gluten-free wheat starch showed the highest, and rice starch the lowest, total starch content. As expected, for the amylose and amylopectin contents significant differences between the starches were found. The potato starch had the highest amylose content (54.2%), while gluten-free wheat starch had the lowest (25.1%). Properties such as susceptibility to enzymatic hydrolysis and the gelling and pasting behaviour can be related to the amylose content. Furthermore, a higher content of damaged starch was found for the cereal starches. These differences were also detected by Schirmer et al. [14]. The higher content of damaged starch reflects the effect of milling, which is involved in the extraction process of rice and wheat starch. Corn starch undergoes wet milling which is a softer process leading to a lower starch damage content. Instead of milling, a process of rasping and washing (lixiviation) is applied for the extraction of starch from root and tubers [15]. The lipid content of the starches was also analysed. Low but significantly different values were determined. In the cereal starches, values between 0.26% and 0.71% were found, while in potato and tapioca starch no lipids were detectable. In general, the lipid content in starch is low and depends on the source of origin. It has been reported that the cereal starches have higher lipid contents than root and tuber starches [14]. However, even though the lipids belong to the minor components in starch, they have significant influence on the gelatinisation properties and on bread making properties. Lipids in starch occur, mainly due to their structure (linear), in complexes within the helical amylose or within the long branch-chains of amylopectin, or form complexes with amylose during heating [16]. It can be assumed that the difference in the lipid contents is influenced by the source of the starch and its internal structure. This assumption is supported by Lindeboom et al. [17], who analysed the biochemical and physiochemical aspects of starch granule size. 1The presence of lipids in starch is important in bread making due to the positive properties imparted during the baking process and the storage [18]. The effect of lipids on the baking process has been mainly reported for flours, where a higher lipid

Table 9-1 Overview of the different starch compositions. Results are shown as the mean values ± confidence interval of 3 replicates[#]

Starch Sample	Moisture [%]	Total starch (db) [%]	Amylose [%]	Damaged Starch (db) [%]	Protein (db) [%]	Lipids (db) [%]	Beta-Amylase [U/g]
Potato	18.0 ± 0.1a	92.2 ± 2.1a	54.2 ± 0.6a	1.0 ± 0.1a	0.02 ± 0.03a	n.d.	0.18 ± 0.0a
Tapioca	13.7 ± 0.0b	94.2 ± 0.1b	36.0 ± 0.3b	0.7 ± 0.0b	0.03 ± 0.04a,b	n.d.	0.25 ± 0.0a
Corn	12.2 ± 0.1c	92.5 ± 0.9b	28.9 ± 0.2c	1.0 ± 0.1a	0.04 ± 0.05a,b	0.26 ± 0.01 a	0.32 ± 0.0a
Rice	12.5 ± 0.1d	83.4 ± 2.5a	46.4 ± 0.4d	7.4 ± 0.2c	0.04 ± 0.06b	0.71 ± 0.02 b	2.33 ± 0.2b
Wheat	12.8 ± 0.2e	97.4 ± 5.7b	25.1 ± 0.3e	2.5 ± 0.2d	0.10 ± 0.14c	0.50 ± 0.01 c	0.33 ± 0.0 a

[#]Means in the same column with different letters are significantly different (≥3 = One-way ANOVA; ≥2 0 =t-Test, $p < 0.05$). n.d. = not detected

content and interactions with proteins, compared to starches occur [19]. These effects are also important in gluten-free baking. The main lipids in starch are polar lipids. Polar lipids can act as surface active components, which are known to stabilize liquid films at gas-liquid interfaces. This can help to maintain the integrity of gas cells during mixing and baking [18]. Hence, it could help gas retention as well as the expansion of gas cells / bubbles during proofing. During baking the better cell wall stability leads to greater retention of evaporated water and CO2 produced by the fermentation process. This retention leads to a larger loaf volume and a finer crumb texture [19]. This explains the significantly higher loaf volumes of wheat and corn starch compared to potato starch breads. Alpha-amylase is an important enzyme occurring in different starch sources [20]. It is an endogenous amylase, which is able to cleave α-1,4-glycosidic bonds present in the amylose or amylopectin chain. The end products of α-amylase action are oligosaccharides. However, no α-amylase activity was detected. In contrast to the α-amylase, beta-amylase is an exogenous amylase, which cleaves exclusively α-1,4-glycosidic bonds from the non-reducing end of the chain. The beta-amylase acts on the external glucose residues of amylose or amylopectin and only maltose and some glucose units are generated [20]. The significantly highest β-amylase activity was found in rice starch, it was more than 7 times higher than the remaining starches. Amylases have an influence on baked breads, due to the production of maltose and glucose, which leads to improvement in colour as a result of caramelisation and Maillard reactions. Furthermore these monosaccharides are fermented by yeast into alcohol and carbon dioxide, which causes rising of the bread [20].

9.4.2. Pasting properties

The pasting properties of the different starches were determined with the rapid visco analyser (RVA), an instrument that measures the viscosity (cP) of a sample while a specific temperature profile is applied. Figure 9-3 illustrates representative RVA pasting patterns for the different starches.

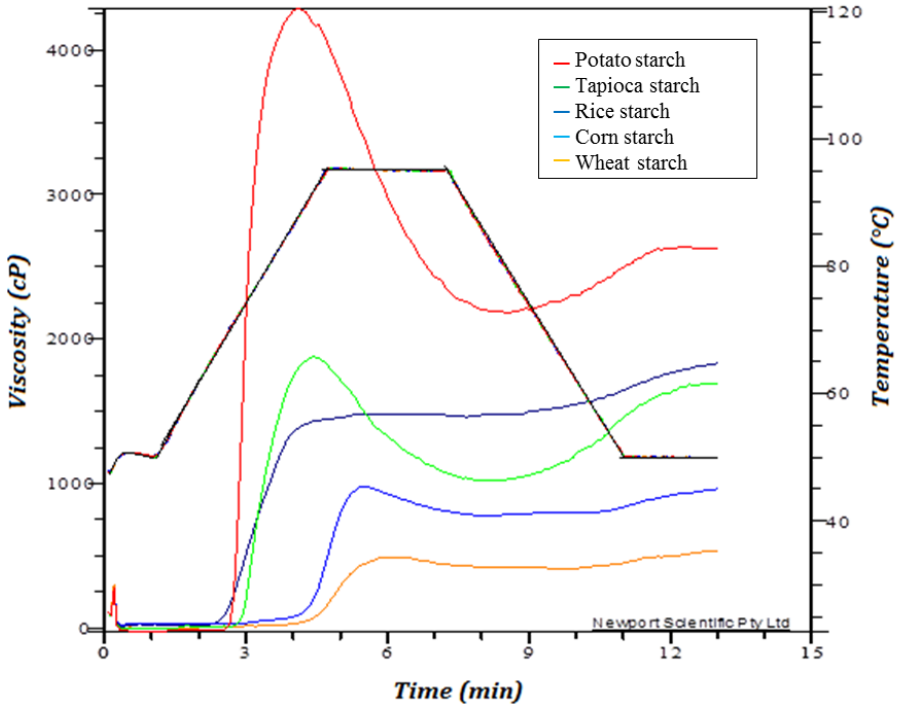


Figure 9-3 Pasting profiles of the different starches: potato starch, tapioca starch, rice starch, corn starch and wheat starch

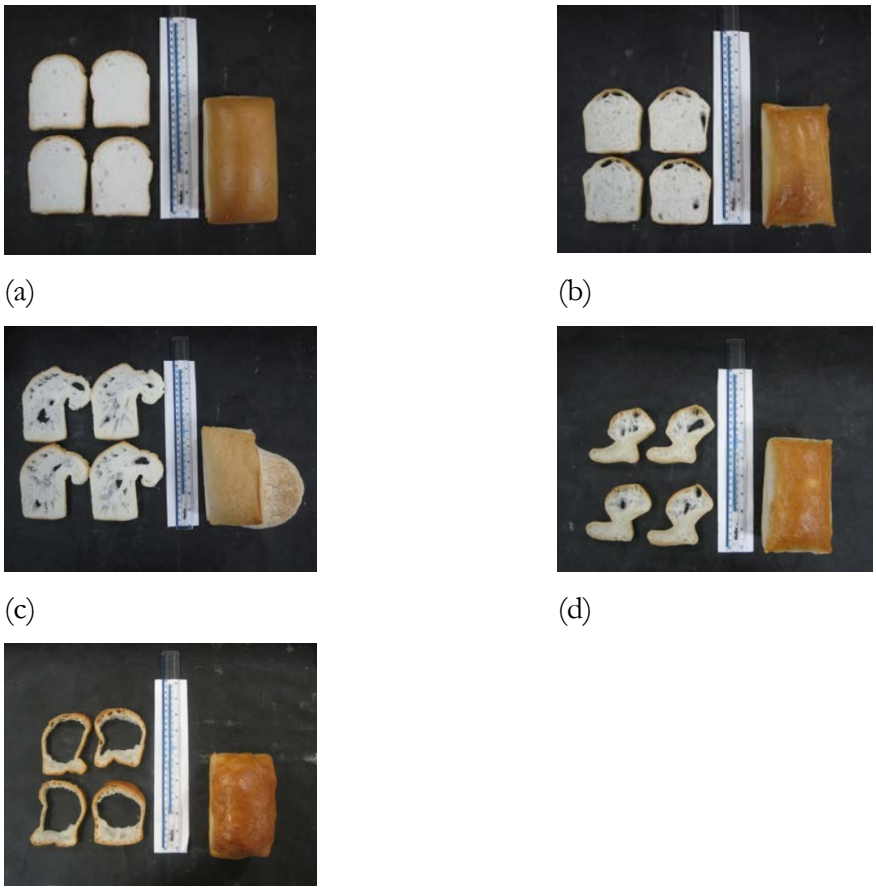
The differences in pasting behaviour between the cereal and root / tuber starches are influenced by the different ratios of amylose and amylopectin in starch, which cause different degrees of gelatinisation. Starch gels are an amylose network, where swollen granules are immersed [16]. Furthermore, the lipid content of starch has an influence on pasting. The results obtained in this work showed that the cereal starches with the higher lipid contents had lower gelation viscosities and started to gelatinise later than the root and tuber starches. Based on these findings the authors hypothesize a correlation between increasing lipid content and increasing gelatinisation temperature. This hypothesis is in agreement with literature, which reported that lipids increased the gelatinisation temperature, thereby retarding granule swelling and prevented leaching of amylose during gelatinization [21,22]. However, the high gelation viscosity of potato starch is believed not only to be related to the absence of lipids. Schirmer et al. [14] demonstrated that potato starches have a high content of phosphate monoesters. The authors further stated that these are covalently bound to the amylose and amylopectin fraction, and that they induce a greater granule swelling causing a higher peak viscosity. Furthermore, the difference in

pasting behaviour could be due to the granule size [14]. Sánchez et al. [23] stated that native starches with large granules, e.g. potato starch, display a unique swelling capability and form highly viscous pastes. However, the peak viscosity (PV) was positively correlated with the amylose content (|r| 0.94). In general, it had been found that large granules (e.g. potato starch) have a greater swelling capacity and therefore form highly viscous pastes [23] and are correlated with a greater breakdown of a viscosity curve. The granule size of the starches had an influence on this breakdown. Only gluten-free wheat starch did not correlate. This is due to the significantly higher damaged starch content, which was shown by Barrera et al. [24], to have an influence on the breakdown and setback. These authors stated that an increase in damaged starch would decrease the breakdown. Hence, for gluten-free wheat starch and rice starch a very small breakdown was found. In this study it was not possible to correlate the pasting behaviour of a starch to a single factor. The authors hypothesize that the pasting properties are dependent on many intrinsic and extrinsic factors instead of one single factor. This is in agreement with Abdel-Aal [2], who reported that, in general, the pasting properties of starch depend on the source and type of the starch, the amylose content, amylose / amylopectin ratio, molecular weight, percentage of starch damage, moisture content, lipid content, shear rate, temperature and period of time during the measurement. The pasting properties could be linked to the firmness of the bread, as the potato starch showed that highest viscosity values compared to corn and wheat, which showed a softer crumb. The lipid content also plays a role in the lowering of starch gelation and softening of bread crumb.

9.4.3. Model bread systems

9.4.4. Bread structure

Pictures of the model breads shown in Figure 9-4 illustrate that gluten-free wheat starch and potato starch gave the best crumb structure.



(a) (b)

(c) (d)

(e)

Figure 9-4 Images of the breads obtained with different starches: (a) wheat starch, (b) potato starch, (c) corn starch, (d) tapioca starch and (e) rice starch

The analysis and correlation of starch characteristics with bread structure revealed that starches containing A and B granules resulted in the best bread structure (|r| 0.92). This is in agreement with Park et al. [25], who analysed the size distribution of gluten-free wheat starch granules in relation to crumb characteristics. These authors found that the breads with better crumb grain contained more A granules with larger sizes. Furthermore, it can be seen that during baking the dough in the corn-starch bread overflowed, which led to

big holes in the crumb. As described earlier higher lipid contents can hinder gelation. Thus, it is hypothesised that a lower lipid content causes a weaker stabilisation of the network interfaces, which leads to partial rupture of the network resulting in big holes. The big holes led to a greater specific volume (|r| 0.83), which in turn led to the high bake loss (|r| 0.89) of corn starch; due to the greater surface of the loaf more water was able to evaporate. Rice starch and tapioca starch produced breads with an irregular structure and large holes. The rice and tapioca starch based breads could not be used for further study due to their poor structure. The SEM pictures, shown in Figure 9-1, revealed that rice starch and tapioca starch had small and agglomerated granules. Due to the fact that granule size correlates with the bread structure, it is hypothesised that the agglomeration of granules could lead to a weak baking performance. Moreover, the inferior performance, particularly in rice starch, is also linked to the high damaged starch content and the high amylase activity. This may have caused a collapse of the interior of the bread, due to liquefying of the starch. The damaged starch can easily be cleaved by the high β-amylase activity [20], which results in a higher amount of maltose, some glucose and loss of water holding capacity [26]. The high amount of maltose is fermented by the yeast during proofing which leads to increased formation of carbon dioxide and alcohol [20]. It is assumed that the carbon dioxide and the alcohol, which evaporates during baking, led to the inflation of the bread. The area of holes and the wall thickness were measured using a c-cell system (Table 9-2). For the area of holes significant differences between the various starch based breads were found. These are most likely linked to the lipid contents in the starches as described earlier. The pictures (Figure 9-4) of the breads confirm the high value of the area of the holes in the corn starch based bread. No significant difference between the starch breads for the wall thickness and moisture content were found.

9.4.5. Bread texture

Crumb hardness is a very important quality characteristic of bakery products. The TPA results are shown in Table 9-2.

Potato starch bread showed the significantly highest hardness values, in comparison to the cereal starches. Correlation analysis showed that these results are linked to its high amylose content (|r| 0.92), which results in higher swelling power and the granule size.

Table 9-2 Overview of the baking results: Results are shown as the mean values ± confidence interval of at least 9 replicates[#]

Starch	Bake loss [%]	Specific volume [ml/g]	Crumb moisture [%]	C-Cell		Texture profile analysis		
				Area of Holes [%]	Wall thickness [mm]	Day of analysis	Hardness [N]	Cohesiveness [-]
Potato	18.3 ± 2.7 a	3.3 ± 0.1 a	49.2 ± 1.8 a	4.1 ± 1.6 a	0.5 ± 0.1 a	Day 0	4.2 ± 0.5 a	0.71 ± 0.02 a
						Day 2	24.2 ± 2.0 a	0.55 ± 0.05 a
						Day 5	28.8 ± 2.0	0.53 ± 0.04 a
Corn	20.9 ± 3.6 b	5.0 ± 0.3 b	48.2 ± 0.3 a	12.0 ± 1.0 b	0.5 ± 0.1 a	Day 0	3.2 ± 0.6 b	0.69 ± 0.02 a
						Day 2	17.7 ± 4.4 b	0.59 ± 0.06 a
						Day 5	20.7 ± 3.4 b	0.54 ± 0.07 a
Wheat	19.1 ± 2.6 ab	4.0 ± 0.1 c	48.3 ± 0.2 a	2.4 ± 1.7 c	0.5 ± 0.1 a	Day 0	3.0 ± 0.4 b	0.75 ± 0.01 a
						Day 2	14.9 ± 0.8 b	0.67 ± 0.05 b
						Day 5	22.5 ± 0.9 b	0.57 ± 0.05 a

[#]Means in the same column (hardness and cohesiveness same row) with different letters are significantly different (≥3 = One-way ANOVA; ≥2 0 =t-Test, $p < 0.05$)

Nevertheless Hug-Iten et al. [6] also mentioned that α-amylase could prevent the amylopectin recrystallization which would hinder bread firming. Moreover, a recent study on gluten-free baking by Mäkinen et al. [31] revealed that α-amylase has indeed a positive influence on the specific volume and on the crumb structure. However, in the current study, as stated above little α-amylase activity was found in the starches (data not shown). It is reasonable to assume that the amylose / amylopectin ratio and the related amylose crystallisation and amylopectin retrogradation of a starch have also have a major influence on the hardness of baked bread, alongside granule size (|r| 1.00) and swelling power (|r| 0.92) and lipid content (|r| -0.71). Potato starch contained the highest amylose content (Table 9-1), which resulted in the highest hardness value. On the other hand, gluten-free wheat starch contained the highest amylopectin content, which resulted in the lowest hardness of the bread crumb (Table 9-2). This result leads to the conclusion that amylose crystallisation, in general, has a higher impact on bread hardening than amylopectin. This effect is probably based on the reason that amylose crystallises over a period of minutes to hours, while amylopectin retrogrades over hours or days [32,33]. The lipid content must also be considered when discussing the hardness / firmness / staling of bread. It is assumed that the lipid content lowers the hardness of the bread crumb by retarding the staling process. This assumption is supported by findings of Copeland et al. [16] and Keetels et al. [34] who mentioned that the developed lipid-amylose complexes retarded the retrogradation of amylose and the recrystallization of amylopectin, respectively. Additional effects on the bread structure and texture could be caused by starch-hydrocolloid interactions. HPMC is reported to delay bread staling and affect the pasting and rheological properties of starch [35]. Such influences are reported to be dependent on the specific starch-hydrocolloid interactions [36]. In this study the water level was kept constant, to focus on the impact of starch. Due to this, the addition of HPMC could have restricted the pasting of starch, by limiting the available water for the pasting.

9.5. Conclusions

This study was conducted to investigate the impact of starches on a simple gluten-free bread system. It was observed that gluten-free wheat starch and potato starch performed better compared to the other starches in this study, in terms of bread loaf volume and crumb structure. It was found that the starches had a significant impact on the gluten-free model breads. Correlation analysis revealed that the granule size of the starches has the highest impact on bread texture and structure. It correlated with the bake loss (|r| -0.88), specific volume (|r|-0.93), crumb moisture (|r| 0.99) and the staling rate (|r| 1.00). For the baked bread analyses the rice starch and tapioca starch were excluded due to their lack of a bread structure. It is suggested that the high β-amylase activity and the high damaged starch content in rice starch lead to this weak performance. Although the results of the characterization of potato and wheat starches showed no similarity (except morphology) between them, the resulting bread structure was very similar. Overall this study showed in a model bread system that gluten-free wheat starch is the best option for the production of gluten-free bread followed by potato starch, in terms of volume and bread structure. This study contributes to the knowledge of gluten-free baking by highlighting the differences of various starches in a simple model bread system. The correlation between granule size and baking characteristics further supports the idea that larger granules are better suited to gluten-free bread production. Although the morphology of the starches has a major impact on the final product, the differences in the composition of the starches should not be neglected. Therefore further research on the effect of starches from the same source, but of different composition could give further insights into the importance of starch source or composition. In addition, further research on interactions between different components and their behaviour in a model bread system could provide a deeper understanding of gluten free systems and help to gain a fundamental understanding of how wheat flour can be replaced by gluten-free ingredients.

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9.7. Author Contributions

Stefan Horstmann carried out the experiments and data processing and wrote the manuscript. Mareile Heitmann contributed her knowledge and expertise in the compositional analysis of the starches. Markus Belz, Emanuele Zannini and Elke Arendt participated in the design of the experimental plan and coordination of its execution.

9.8. Conflicts of Interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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