Technische Universität München

Lehrstuhl für Brau- und Getränketechnologie

Valorizing food byproducts: Distiller's grains and its potential as functional ingredient for cereal based food products

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Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften

genehmigten Dissertation.

Vorsitzende:	Prof. Dr. Mirjana Minceva
Prüfer der Dissertation:	1. Prof. Dr. Thomas Becker
	2. Prof. Dr. Michael Rychlik

Die Dissertation wurde am 15.07.2019 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 27.04.2020 angenommen.

What we know is a drop, what we don't know is an ocean.

Sir Isaac Newton

ACKNOWLEDGEMENT

Writing the Acknowledgement section, one gets able to realize the end of an important stage of life. I am very grateful to many people who supported me during my ambition to attain a doctoral degree and therefore it is about time to show my appreciation.

First and foremost, I would like to express my sincere gratitude to Prof. Dr. Thomas Becker for his generous support and the opportunity to work on this project under his supervision, for our helpful discussions, for the chance to present our work at national and international platforms and most important for his confidence in giving me scientific freedom. Moreover, I want to thank him for giving me the opportunity to work as head of the Chairs chromatography lab. During this time, I have learned to overcome borders and surpass myself.

Further, I would like to thank the examination board, Prof. Dr. Mirjana Minceva in chairing the commission and Prof. Dr. Michael Rychlik for his time and effort in reviewing my thesis as second examiner. Thank you both, for your incredible short-term willingness in being part of my examination board.

I am very grateful to Dr. Mario Jekle, with whom it was a pleasure to work with. His valuable feedback noticeably formed the quality of this dissertation. Mario- thank you for being my mentor and friend, I appreciate your inspiring way of scientific working!

I want to extend my deepest thanks to all the members and doctoral candidates of the Institute of Brewing and Beverage Technology for the great and collaborative atmosphere during work and after hours- it was unique! Moreover, I want to deeply thank Dr. Hubert Kollmannsberger for all of his support in the GC/HPLC lab, for everything I learned from him and for his incredible patience and time in working on my MS data. Thank you to the whole team of the GC/HPLC lab during my time as head- Thanks to Moni, Heike, Ben and all Azubis.

Besides that, I want to thank my students Michael Meiringer, Sabine Haindl, Sebastian Berger, Hanna Schuster, Ann-Marie Winkler und Evgenia Pavlycheva for working with me. Without their contribution many parts of this thesis could not have been accomplished.

A special thank you to Dr. Susanne Procopio, Dr. Clemens Döring and Daniele Stock Leh: Susie- thanks for being my best office mate during this stage of life and for all the dancing and fun in our office. Clemens- thanks for your sympathetic ear and our discussions, for your support during baking and for time in proof reading this thesis. Daniele- thanks for all after hours with sushi at sunset and for proof reading! Thanks to BGT, we can call each other friends.

Ein ganz besonderer Dank gilt meinen Eltern für Ihre fortwährende Unterstützung und Geduld während der Promotion. Mama und Papa- ohne euren Mut wäre mir vielleicht ein anderer Lebensweg beschert gewesen. Danke für alles!

Holger, für immer- Danke.

Meinen Eltern und Holger.

PREFACE

The results and publications of this thesis were developed at Technical University of Munich, Institute of Brewing and Beverage Technology, Research Group Cereal Technology & Process Engineering in the period of August 2011 to January 2016.

PEER REVIEWED PUBLICATIONS

The following peer reviewed publications were generated in the period of this work. Publications with numbers 1-4 are contributing as part of this thesis.

1. **Roth, M.,** Jekle, M., Becker, T.: Opportunities for Upcycling Cereal Byproducts with special focus on Distiller's Grains, Trends in Food Science & Technology, 91 (2019), 282–293, doi: 10.1016/j.tifs.2019.07.041.
2. **Roth, M.,** Schuster, H., Kollmannsberger, H., Jekle, M.; Becker, T.: Changes in aroma composition and sensory properties provided by distiller's grains addition to bakery products. Journal of Cereal Science, 72 (2016), 75–83, doi: 10.1016/j.jcs.2016.10.002
3. **Roth, M.,** Döring C., Jekle M., Becker, T.: Mechanisms behind distiller's grains impact on wheat dough and bread quality, Food and Bioproducts Technology, 9 (2016), 274–284. DOI: 10.1007/s11947-015-1620-y
4. **Roth, M.,** Meiringer, M., Kollmannsberger, H., Zarnkow, M., Jekle, M., Becker, T.: Characterization of key aroma compounds in distiller's grains from wheat as a basis for the utilization in the food industry, Journal of Agricultural and Food Chemistry, 62 (45) (2014), 10873–10880, DOI: 10.1021/jf503281x

5. Jekle, M., Horeld, C., Gratzl, R., **Roth, M.,** Becker, T., Höbel, W.: Aluminium leaching from baking tray materials into surface-alkalized baked products. Cereal Technology 03 (2016), 127-135.
6. Döring, C., Grossmann, I., **Roth, M.,** Jekle, M., Koehler, P., Becker, T.: Effect of rye bran particles on structure formation properties of rye dough and bread. Journal of Food Processing and Preservation (2016). DOI 10.1111/jfpp.12998.

ABBREVIATIONS

2AP	2-Acetyl-1(H)-pyrrolin
AEDA	Aroma extract dilution analysis
BSG	Brewer's spent grain
CBP	Cereal byproducts
CDS	Condensed distiller's solubles
DDG	Dried distiller's grains
DDGS	Dried distiller's grains with solubles
DG	Distiller's grains
DMTS	Dimethyl trisulphide
EFSA	European Food and Safety Authority
EU	European Union
FA	Ferulic Acid
FAO	Food and Agriculture Organization of the United Nations
FD	Flavor dilution
FLW	Food Loss and Food Waste
FSC	Food supply chain
GC	Gas chromatography
GC-O/MS	Gas chromatography olfactometry mass spectrometry
HMF	Hydroxymethylfurfural
HPLC	High performance liquid chromatography
HS-SPME	Headspace solid phase microextraction
OAV	Odor activity value
PC	Principal Component
PCA	Principal Component Analysis
SAFE	Solvent assisted flavor evaporation
SDE	Simultaneous distillation and extraction
SPE	Solid phase extraction
UN	United Nations
US	United States
WDG	Wet distiller's grains
WS	Weizenschlempe

CONTENTS

SUMMARY

A secure food supply chain, diminishing of fossil resources and a growing world population represent divergent issues that globally occupy stakeholders in economy or politics these days. Hence, the improved exploitation of available resources suitable for human nutrition comes to the fore. The restructuration of food manufacturing processes and superficially the management of food processing byproducts are considered as solution approaches, since byproducts entail unexploited potential represented by their nutritionally composition. Cereal byproducts (CBP) such as dried distiller's grains (DDG), the central byproduct of ethanol production, are an untapped source of nutrients, due to high shares of protein and dietary fiber and therefore incorporate huge potential for further utilization in the food industry. In the past, research initiatives recognized this potential. However, it was determined that the application of DDG in food products is connected to some challenges, as the impact on sensory properties, predominantly affecting odor and texture. Causes or impact factors that trigger these negative effects have not been studied by now.

In this context, the aim of this thesis was the in-depth enlightenment of impact factors that accompany the addition of DDG to cereal based food products, using the example of wheat bread. In the first part of the thesis, aroma-related deficiencies, the characteristic odor profile, possible off-flavors, as well as key aroma compounds of DDG were successfully enlightened for the first time. It could be shown, that the aroma composition represents a thermally processed cereal based product. Therefore, the model matrix wheat bread was chosen to investigate and enlighten effects of DDG addition on textural properties as well as on sensory properties and the aroma composition of a food system. Besides common side effects of high fiber additives, the low pH of DDG as well as the impact of reaction products of the drying process could be revealed as important and DDG specific impact factors. Alterations in the aroma composition of wheat bread induced by DDG addition predominantly can be attributed to concentration effects and the absence of 2-acetyl-1-pyrrolin, the key aroma compound of wheat bread crust. In summary, the drying process represents the key step in DDG aroma formation and moreover induces the formation of fermentation inhibitors, which negatively affect the use of DDG in bakery products. With this knowledge, aroma deficiencies can be counteracted due to targeted treatment. Based on present findings, gentle processing after ethanol separation should enable improved utilization of DDG in food products.

ZUSAMMENFASSUNG

Die Versorgung einer stetig wachsenden Weltbevölkerung mit sicheren und gesunden Lebensmitteln ist durch den Rückgang fossiler Ressourcen und landwirtschaftlicher Nutzflächen in der Zukunft nicht mehr vollständig gesichert. Vor diesem Hintergrund rückt die verbesserte Nutzung für die menschliche Ernährung geeigneter Rohstoffe weiter in den Fokus. Lösungsansätze finden sich u.a. in der Restrukturierung der Lebensmittelproduktion und reststofforientiertem Management, da Reststoffe aufgrund ihrer Nährstoffzusammensetzung großes ungenutztes Potential besitzen. Getreidebasierte Reststoffe wie Weizenschlempe stellen eine bisher unentdeckte Nährstoffquelle dar, da diese reich an Proteinen und Ballaststoffen sind und so bedeutendes Potential für eine weitere Nutzung in der Lebensmittelindustrie besitzen. In der Vergangenheit haben zahlreiche Forschungsinitiativen das Potential erkannt und aufgezeigt, dass die Anreicherung von Lebensmitteln mit Getreideschlempen an Herausforderungen gekoppelt ist, etwa dem Einfluss auf sensorische Merkmale wie Geruch und Textur. Die Ursachen und Einflussfaktoren dieser Defizite wurden bisher jedoch nur unzureichend untersucht.

Das Ziel der vorliegenden Studie umfasst die Analyse und Aufklärung der Einflussfaktoren, die eine Verwendung von Weizenschlempe (WS) in getreidebasierten Lebensmitteln wie Weizenbrot begleiten. Im ersten Teil der Arbeit wurde dafür erstmalig ein charakteristisches Aromaprofil von WS erstellt und die Schlüsselaromastoffe ermittelt. Es wurden 8 Schlüsselaromastoffe identifiziert und weiterhin aufgezeigt, dass die Aromastoffzusammensetzung typisch für thermisch behandelte Getreideprodukte ist. Aufgrund der Aromastoffzusammensetzung wurde die Modellmatrix Weizenbrot gewählt. Die Effekte eines Zusatzes von WS auf die Textur, sowie auf sensorische Eigenschaften und die Aromastoffzusammensetzung konnten erfolgreich aufgeklärt werden. Der niedrige pH-Wert von WS, sowie der Einfluss von Reaktionsprodukten aus der Trocknung wurden neben bereits bekannten Effekten, die durch Verwendung ballaststoffreicher Additive ausgelöst werden, als wesentliche und für Weizenschlempe spezifische Einflussfaktoren identifiziert. Veränderungen in der Aromastoffzusammensetzung, die durch den Einsatz von WS im Weizenbrot induziert werden, basieren vordergründig auf Konzentrationsunterschieden, sowie der Abwesenheit von 2-Acetyl-1-pyrrolin, des Schlüsselaromastoffes von Weizenbrot. Der Trocknungsprozess ist ein Schlüsselschritt in der Aromastoffbildung und induziert darüber hinaus die Bildung von

Fermentationsinhibitoren, die eine Verwendung von WS in Produkten wie Weizenbrot limitieren. Mit dem vorliegen Wissen kann Aromadefiziten gezielt entgegengewirkt werden. Basierend auf den Erkenntnissen, ermöglicht die Entwicklung schonenderer Trocknungsverfahren eine verbesserte Nutzung von WS in Lebensmitteln.

1 INTRODUCTION

> *"The extent of FLW" (food loss and waste) "while more than 800 million people still suffer from hunger seems to indicate that something is wrong, that food systems do not function as they should."*
>
> *(HLPE, 2014)*

Food production processes in the 21st century are driven by automation, inter alia targeting on simplifying processes, improving speed and maximizing the output of products. However, global fundamental issues such as population growth by 9 billion by 2050, climate change or decline in agricultural productivity due to barren soil, will limit available resources and dramatically affect food production systems in the long term. These effects will enforce intergovernmental stakeholders and food industry representatives to rethink the way food is produced. The initial stage for a solution might be provided by avoiding byproducts and improved exploitation of existing food resources since these include untapped sources of nutritionally valuable fractions.

In this context, food processing byproducts can play a key role for securing the food supply chain in the near future, since numerous side streams of the food industry can serve as novel raw materials for the development of food products in secondary processes. Especially in developing countries, food processing byproducts can contribute to alleviate hunger and malnutrition, because these byproducts could satisfy the increased demand for proteins, phytochemicals or other important nutrients (Patras, Oomah, & Gallagher, 2011).

The following chapters will introduce to the topic of food loss and waste in food production and will give an overview on food processing byproducts (chapter 1.1) and current and novel approaches for their utilization (chapter 1.2). Subsequently, this thesis will focus on the byproduct distiller's grains and therefore will introduce to the ethanol manufacturing process and its corresponding byproducts (chapter 1.3). Since these byproducts and food products can provide flavor deficits, the physiology of sensory perception and formation of flavor in food products is introduced in chapter 1.4 in the context of distiller's grains (DG). The potential and possibilities to use DG as a novel raw material for food production will be introduced in chapter 1.5 and deepened in a detailed review in chapter 2.2. Studies on the flavor and texture impact of DDG are presented during the chapters 2.3, 2.4 and 2.5, followed by a critical discussion of the presented results in chapter 3.

1.1 FOOD LOSS, FOOD WASTE AND FOOD PROCESSING BYPRODUCTS

In 2011, the food and agriculture organization (FAO) published a study that arrested much attention towards the issue of global food losses and waste (FLW): Almost one third of food that is globally produced for human consumption is lost and wasted along food production (HLPE, 2014). Thus, global enterprises and governmental or non-governmental research initiatives increased efforts for the investigation of efficiencies and the identification of productivity gaps within food production. According to the High Level Panel of Experts on Food Security and Nutrition, a science-policy interface of the UN Committee on World Food Security, FLW definitions must be differentiated by their reflecting perspectives with focus on waste (a) or focus on food (b) (HLPE, 2014): FLW are

(a) part of waste that is food or related to food (including non-edible parts)
(b) edible part of food that is lost or wasted (non-edible parts are not included)

Since (a) is reported as common approach, the food-focused terminology is used in this thesis. FLW happen at all stages of the food supply chain, whereas food loss is defined prior to the consumer (harvest, post-harvest, processing, etc.) and food waste at consumer level (HLPE, 2014). Figure 1 visualizes the complex connection of FLW in the food supply chain and shows the different levels contributing to FLW amounts. The distribution among the different levels strongly depends on the geographical region. In developed countries, more than 80 % of FLW arise at the processing and consumer level, the latter due to behavioral causes as result of choice and overconsumption (Mirabella, Castellani, & Sala, 2014). By contrast, in less developed countries FLW mainly is generated at harvest and post-harvest level (FAO, 2011). Obviously, losses that happen due to insufficient storage or transport conditions (storage loss) must be taken into account as well. Especially in developing countries, inappropriate storage possibilities represent one major cause of post-harvest losses (FAO, 2011). However, the processing level contributes with a huge share of 39 %, which is indicating efficiency deficiencies in food production. According to Pfaltzgraff et al. the food supply chain (FSC) has recently been recognized as being inefficient, also because 38 % of the FLW produced in the European Union is generated by the food processing sector (Pfaltzgraff, De bruyn, Cooper, Budarin, & Clark, 2013). As presented in figure 1, FLW include food processing byproducts as integral part. Food processing byproducts remain as bypass flow during the production of target products among all sectors of food production.

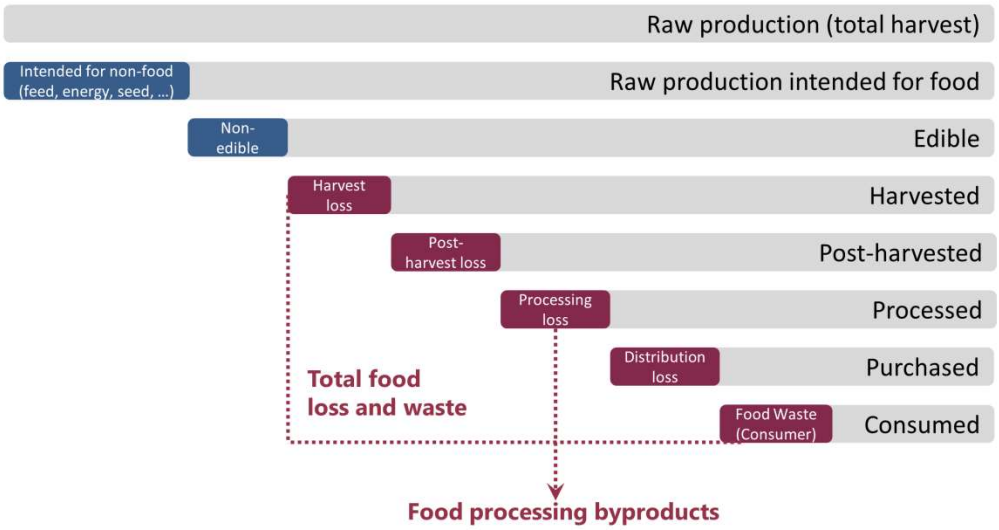

Figure 1: Schematic structure of agricultural resources in the context of total production of food (unscaled). Total food loss occurs at harvest, post-harvest, processing and distribution level. Food waste occurs at consumer level. Storage loss can be part of various levels, e.g. as post-harvest or distribution loss. Food processing by-products are generated in the production level and are part of processing loss. Modified according to (HLPE, 2014)

However, the line between a valuable byproduct with potential for Upcycling and waste with inferior or inappropriate properties is narrow. For instance, peels of citric fruits can serve as byproduct source for D-limonene or pectin production, but can become waste if high amounts of pesticides prevent the utilization. The differentiation between byproduct and waste can be made based on Directive 2008/98/EC, the European Waste Framework Directive, which officially defines the term byproduct: A byproduct is a substance that results from a production process in which the production of this substance is not the primary target. The substance can be seen as byproduct instead of waste, if further use is certain and lawful, doesn't require elaborate processing and is produced as an integral part of the process itself (European Commission, 2008). Cereal bran represents a good example, as it is generated as byproduct during flour production and finds further use in the food and feed sector without complex processing.

Without exception, every sector of the food industry, plant or animal-based, generates food processing byproducts. The dairy industry contributes with liquid wastes such as whey (Kosseva, 2011). The further processing of whey is deeply investigated and consequently whey turned from byproduct to food ingredient and became a common substrate for protein or saccharide recovery (Galanakis, 2012). Besides dairy, the second important industry sector of animal-based byproducts is represented by the meat and fish industries, who produce the major proportion of FLW within the food industry (Kosseva, 2011). As global meat

consumption has been increasing, the demand of edible slaughterhouse residues like entrails, blood or parts of muscles is decreasing, which consequently lead to higher amounts of byproducts (Mirabella et al., 2014). Meat byproducts generally can be utilized as source for proteins (gelatin or collagen), enzymes, trace minerals or nitrogen (Pleissner et al., 2015).

When considering production processes, plant-derived waste represent the main share compared to animal-derived waste (Pfaltzgraff et al., 2013). Plant-based byproducts are composed of both fruit and vegetable residues or are based on side streams out of the cereal sector. Plant-based byproducts are a promising and versatile source for numerous valuable and nutritionally important compounds, such as phenolic compounds, carotenoids or specific carbohydrate polymers (pectin, lignin, hemicelluloses), which make them an ideal raw material for further processing in the scope of Upcycling. Phenols and carotenoids in fruit and vegetable based raw materials can be utilized as natural preservatives in the food sector, due to their ability to extend shelf life and inhibit off-flavor formation (Galanakis, 2012). Berries and other red fruits as well represent important sources for recovery of antioxidants, phenolic acids, flavonoids and polyphenols (Mirabella et al., 2014). According to a detailed review of Galanakis et al., the respectable extractability of relevant compounds was widely investigated and the feasibility of recovery due to soft and weak tissue texture of fruit and vegetables already proven (Galanakis, 2012). Side streams of olive oil or vine production, actually represent up to 60 % of the total starting material and contain high amounts of bioactive phytochemicals that could serve as ingredients for food, cosmetics or the pharmaceutical industry (Pleissner et al., 2015). Even in the production of gluten-free bread, fruit byproducts such as orange pomace can be utilized. The addition can contribute to improved bread properties and fiber content of gluten free bread, due to high water binding and holding capacity, good gelling and thickening properties and a high level of dietary fiber in the feedstock (O'Shea, Roessle, Arendt, & Gallagher, 2014). In general, vegetable byproducts contain significant amounts of dietary fiber, which suggests the utilization as a crude fiber additive for bakery products.

Byproducts utilization in food products like cereal based products could close gaps in the food supply chain, since cereals serve as staple food nearly in every part of the world and remain as most efficient energy source of human nutrition. In poorer regions, where food is scarce and protein consumption is not affordable, consumption of grain based foods represents the core of human diet and lack of grains can be responsible for hunger and

undernourishment (Poutanen, 2012). Due to the high content of starch in common cereals, with more than 2/3 share, the starchy endosperm is the principal part of interest of the cereal kernel. Thus, the cereal processing industry mainly directs its attention to the carbohydrate fraction of starch, which subsequently can be converted to ethanol, energy or be purified as important feedstock for the food manufacturing industry. Commonly, cereal byproducts (CBP) are composed of outer grain layer fractions of the cereal kernel, such as germ, husk and bran, which remain after utilization of the starchy endosperm. In a perfect scenario of Upcycling, CBP could serve as source of nutrients in form of a whole grain additive for the enrichment of nutritionally valuable food products (compare figure 2).

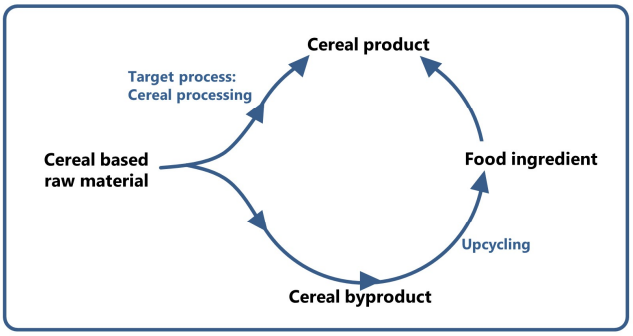

Figure 2: Two alternate ways to produce cereal products: Starch based food is produced within the target process and ultimately leads to the production of a byproduct stream. Utilization of the byproduct stream (usually the outer grain layer fractions) in a secondary process is presented as opportunity to produce novel cereal products as result of byproduct Upcycling

Generally, various industry branches in cereal processing such as the milling industry, with bran or gluten, the brewing industry, with brewer's spent grains (BSG) or the ethanol manufacturing industry, with distiller's grains produce CBP as an integral part of their manufacturing process. These cereal byproducts are characterized by valuable amounts of dietary fiber and hemicelluloses, protein and other bioactive compounds such as phenolic acids. Several attempts for utilizing CBP were made in the past and investigated two different possibilities: Non-targeted approaches, focused on reutilizing the byproduct as a whole; and targeted approaches, focused on the extraction and purification of single valuable compounds in the byproducts. For instance, the latter thereby can be the extraction of proteins, phenolic acids or proanthocyanidins out of BSG (Fărcaş et al., 2014); or the alkaline extraction of proteins out of wheat bran, which represents a common approach for more than 20 years (Roberts, Simmonds, Wootton, & Wrigley, 1985). Direct and non-targeted applications superficially investigated the applicability in cereal-based food systems like extruded snacks or cookies and especially bread for the CBP bran (Curti, Carini, Bonacini,

Tribuzio, & Vittadini, 2013; Lai, Hoseney, & Davis, 1989), BSG (Ktenioudaki, Chaurin, Reis, & Gallagher, 2012; Waters, Jacob, Titze, Arendt, & Zannini, 2012) and dried distiller's grains (Abbott, O'Palka, & McGuire, 1991; Rasco, Hashisaka, Dong, & Einstein, 1989). The feasibility was shown, however, CBP enrichment must be kept within a limit: according to Ktenioudaki et al., the incorporation of BSG in breadsticks was possible up to an amount of 10 %, but food quality suffered with higher amounts (Ktenioudaki, Crofton, et al., 2013). In bran, amounts up to 10 % were reported as suitable (Lai et al., 1989). In studies for cornbread, shares of 20 –25 % dried distiller's grains (DDG) supplementation to corn flour were reported as suitable (Liu et al., 2011). However, the specific causes have not been investigated so far, but the phenomenon was obvious: irrespective of the type of CBP used, high levels of CBP in cereal based products induce deficiencies regarding sensory attributes even up to off-flavors and restrictions in technological performance and textural quality (Ktenioudaki et al., 2013; Ktenioudaki, O'Shea, & Gallagher, 2013; Rosentrater & Krishnan, 2006).

By now, mechanisms and specific causes responsible for these limitations have not been totally enlightened and research gaps especially for distiller's grains, the most relevant byproduct of the ethanol manufacturing process are present. Efforts targeting on the investigation of the application of distiller's grains in the food industry are meanwhile rare. In the past, starting in 1980–1990, research groups began to explore the nutritional potential of DDG as a food supplement. As it was known for other high-fiber additives, the application was possible in only low amounts, since sensory deficiencies, such as bitter character or undefined aftertaste, as well as poor flavor or malty and soapy off-flavor were reported (Bookwalter, Warner, Wall, & Wu, 1984; Rasco et al., 1989). Although the ethanol industry and arising byproducts experience steady growth, interest in food products enriched with DDG or its coproducts decreased considerably due to poor sensory qualities (Rosentrater & Krishnan, 2006). As a consequence, present research studies mainly aim on its utilization as feed or raw material for biorefinery applications and food applications moved to the background (Fonseca, Lupitskyy, Timmons, Gupta, & Satyavolu, 2014; B. Liu, McKinnon, Thacker, & Yu, 2012).

Nonetheless, possibilities to counteract the adverse effects of CBP and especially DDG are needed further on. Therefore, the in-depth investigation of phenomena that limit the range of CBP application to those small amounts became a key topic in CBP research. A structured and elaborate overview on quantitative important cereal byproducts, their special features

and commonalities, and potential ways for their utilization is presented in Chapter 2.2, which implies a detailed Review Paper on cereal byproducts, focused on DG.

1.2 CONVENTIONAL BYPRODUCT PROCESSING MEETS UPCYCLING

In the ethanol manufacturing industry, sales of side streams contribute substantially to the economic viability of ethanol manufacturing plants (Rosentrater & Krishnan, 2006). So, under the economical perspective, the almost complete and efficient processing of byproducts is an important branch for the whole ethanol industry.

Byproducts after ethanol distillation such as thin stillage or wet distiller's grains are characterized by limited shelf life and further processing needs immediate conservation steps. Thus, costs of drying, storage and shipment are not negligible and economically limiting factors (Patras et al., 2011). Inter alia for this reason, agro-industrial byproducts primarily were utilized as feed for local farmers or fertilizer for nearby farms. In the past, food processing byproducts were further processed by conventional methods, such as the generation of energy in form of bioethanol plants, as well as the utilization as fertilizer and landfill. However, until today the most common approach remains the utilization as animal feed, which additionally can be attributed to the high level of nutrients (Rosentrater & Krishnan, 2006). The beneficial nutritive value of DDG regarding energy, protein, neutral detergent fiber and fat, popularized its use in global animal feeding for dairy cows, beef cattle, sheep, goats, horses, pigs and poultry (Westreicher Kristen, 2013). For instance, dried distiller's grains with solubles (DDGS) are an acceptable ingredient up to 15 % in poultry diet (Świątkiewicz & Koreleski, 2008) and can be used as the major source of protein with unaffected and stable milk production in dairy diet (Mutsvangwa, Kiran, & Abeysekara, 2016). Today, the animal feed industry is the only customer for ethanol manufacturing residues like DDGS and DDG. In the long-term, byproduct supply on the market will increase to a greater extent than its corresponding demand (Rosentrater & Krishnan, 2006).

Besides feed, disposing agro-industrial byproducts as landfill was commonly exerted in the last years. Obviously considering the sustainable point of view, removal of byproducts into landfill sites is inappropriate, as the production of food is energy and nutrient demanding (Pleissner & Lin, 2013). Moreover, the environmental footprint of landfills is poor, since emissions of methane as serious greenhouse gas significantly contribute to climate change (Pham, Kaushik, Parshetti, Mahmood, & Balasubramanian, 2015). Byproduct and waste

disposal as landfill as well increased public concerns regarding latent pollution of agricultural soil and possible decrease in environmental quality (Pham et al., 2015). In addition, even authorities in the European Union tightened up legal restrictions, which directed the utilization of byproducts to a more conscious use (Patras et al., 2011).

From this point of view, the production of energy from byproducts is becoming environmentally favorable and diverse research initiatives directed their efforts to conversion technologies of food waste to energy. As a result, pyrolysis or gasification processes were evaluated as useful tools for energy production (Pham et al., 2015). In addition, anaerobic biomethanation was assessed as reasonable treatment method for the valorization of biodegradable solid waste (Elmekawy, Diels, De Wever, & Pant, 2013).

All efforts in byproduct management aim on the improved exploitation of existing resources towards complete utilization. In terms of the almost complete utilization of raw materials, the sustainable design of novel products, including the green production of food products while minimizing its environmental impact, are innovative concepts in food production. Within these concepts, a new consciousness in resource consumption, the cradle to cradle principle, circular economy and the Upcycling of products were recognized as leading principles for successful eco-innovation (Mirabella et al., 2014).

The term Upcycling is a neologism that describes the valorization and the conscious use of global resources within the scope of a circular metabolism of products (McDonough & Braungart, 2013). It was first described by Braungart and McDonough, who developed a model for the design industry to a more efficient and effective use of resources, targeting on cleaner production systems (McDonough & Braungart, 2013). In this model, every biological or technical resource passes stations of production to product, to use, to decomposition and returns to production in a perpetual cycle (Braungart, 2007). This *metabolic cycling* (figure 3 A) differentiates Upcycling to current Recycling practices, wherefore it is also described as upgraded Recycling (Sung, 2015). Current research describes Upcycling as process, in which waste materials or byproducts gain higher value after passing secondary processes (Sung, 2015). However, Upcycling doesn't aim on eliminating waste and emissions. It targets on upgrading resources by increasing their cycles of use and in the long term profits from waste reduction as a side effect (Braungart, 2007). In the context of food production, the Upcycling concept incorporates the reutilization of raw materials, byproducts and side streams out of the food industry, in which high value products can be generated in secondary processes.

This schematic process of integrating Upcycling into existing food production is presented in figure 3B.

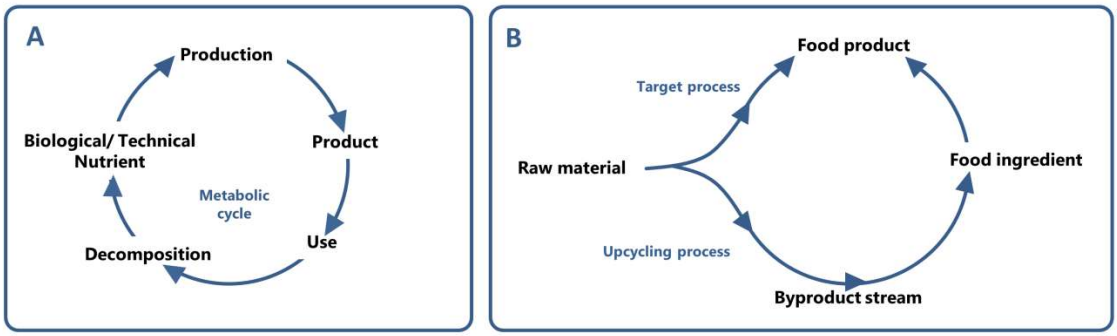

Figure 3: A, Perpetual metabolic cycling of Technical and Biological Nutrients according to the Cradle to Cradle Principle. All resources can serve as nutrients for secondary products. Modified according to (Braungart, 2007). B, Integration of Upcycling approaches into existing food manufacturing processes via utilization of byproduct streams in secondary process streams.

Interestingly, in 2011 the number of commercial products (e.g. art, clothes, furniture, food products not included) generated by product Upcycling increased by more than 400 % on the US market (Sung, 2015), which is already indicating the public and industry attention directed at this topic.

Likewise, the Upcycling concept can be applied for food processing byproducts and especially cereal byproducts. In this context, CBP should not be used in matters such as landfill or energy production, but further processed for their most valuable approach. The development of whole wheat additives, purified plant-based protein created from reconditioned CBP, or merely the direct application of CBP in food products can help parts of food loss to regain the status of being food for human nutrition, which in the long term can lead to manageable supply shortages particularly in developing countries.

1.3 ETHANOL PRODUCTION AND CORRESPONDING BYPRODUCTS

Modern industrial and traditional ethanol production is based on fermentation of monomeric carbohydrates by *Saccharomyces* yeast to the main products ethanol and carbon dioxide. Starting materials predominantly are starchy or sugary feedstocks, with cereal grains or sugarcane as main representatives. The USA produce the biggest share of global ethanol production and process corn as predominant feedstock. Canada processes barley and wheat; in Brazil sugarcane is leading, whereas in Europe, mostly wheat or blended cereals are utilized (Chatzifragkou et al., 2015; Mustafa, McKinnon, & Christensen, 2000). Since the USA is the

main producer of ethanol, distiller's grains (DG) on the market mainly are represented by corn as raw material. During production of ethanol out of corn, two fundamentally different processes have to be distinguished, that vary upon the target compound after processing. 33 % of corn are processed in the wet milling process, targeting besides ethanol also on crude oil, corn gluten meal and corn gluten feed by fractionating the grain prior to processing (Bothast & Schlicher, 2005). The wet milling process does not yield distiller's grains as byproduct. 67 % of corn are processed within the dry grind process, targeting on maximum yields of ethanol and providing distiller's grains as single byproduct (Bothast & Schlicher, 2005). Ethanol production out of wheat and ethanol production for beverage ethanol purposes proceeds according to the dry grind process, thus it can be seen as common method (Liu, 2011). In dry grind processing, the ethanol manufacturing process starts with milling and mixing of grain flour with water in preparation for mashing. During mashing, liquefaction and saccharification takes place, representing degradation of polymeric and oligomeric carbohydrates such as amylose or amylopectin. Degradation of starch occurs by endogenous enzymes, optionally together with addition of exogenous and thermostable amylolytic enzymes like α- and β- amylase as well as glucoamylase. In industrial ethanol production, key performance indicator is maximum ethanol yield, so addition of exogenous amylolytic enzymes is obligated, since increases in the efficiency of carbohydrate polymer digestion can be achieved and higher ethanol yields are supported. Subsequently, alcoholic fermentation of carbohydrates is introduced by addition of *Saccharomyces* yeast, in most cases traditional baker's yeast of species *Saccharomyces cerevisiae*. By dissimilation, glucose is fermented to equimolar amounts of carbon dioxide and ethanol, until concentrations of 9-10 % ethanol content in the slurry are reached. The addition of nitrogen sources such as urea, ammonium sulphate or proteases to enhance the amino acid or peptide content is reported to achieve improved yeast growth (Bothast & Schlicher, 2005). After fermentation, ethanol is distilled using distillation and rectification columns until purities around 95 %. Due to azeotrope formation of ethanol/water-mixtures, higher purities cannot be achieved by using boiling point differences via temperature-based separation processes. Therefore further purification and dehydration of ethanol can be achieved using molecular sieves or nearly critical propane to purities > 99.5 % (Cardona & Sánchez, 2007). Characteristics of a standard process of ethanol manufacturing are presented in table 1. Dried distiller's grains investigated in this thesis were manufactured according to the characteristics presented in table 1.

Table 1: Present temperatures and duration times in ethanol production according to the dry grind process und subsequent byproduct processing from the grain to the byproduct dried distiller's grains.

Process step	Temperature [°C]	Duration
mashing	33–35	2–3 h
liquefaction	75–80	around 135 min
saccharification	50–55	around 10 min
fermentation	33–34	68–72 h
temporary storage	33–34	8–10 h
distillation	78–80	1 h
temporary storage	75–80	2–8 h
concentration of thin stillage to CDS	55–65	2–4 h
drying of WDS to DDG	100–105	1–3 h

Byproduct management and recovery represents the last step in ethanol production. Figure 4 visualizes this complex process of ethanol production beginning with the whole grain and accompanied by its byproduct stream to the end product DDG/DDGS. The remaining residue of distillation contains the non-volatile fraction in form of a whole stillage. The distillation residue as well contains the remaining amounts of yeast cells, which certainly can influence characteristics of the finished product. However, the extent is not investigated by now. Due to its limited shelf life, the whole stillage is divided by centrifuging, decanting or pressing and extruding into a solid rich and solid poor fraction, the thin stillage. Shares of thin stillage around 15 % or more are redirected into the ethanol stream and used as backset to slurry the ground grain as processing water (Liu, 2011). The thin stillage is characterized by 5 to 10 % solids and is concentrated to condensed distiller's solubles (CDS), a syrup-like byproduct with around 30 to 50 % solid content (Westreicher Kristen, 2013). The phase rich in solid material, the wet distiller's grains (WDG), contains around 65 to 70 % of moisture. After drying to a final water amount around 8 to 10 %, the process results in the final side stream dried distiller's grains (DDG). Generally, CDS can be mixed with WDG prior to drying, to enhance the nutritional value by higher shares of solubles. After drying, the process results in the DDGS.

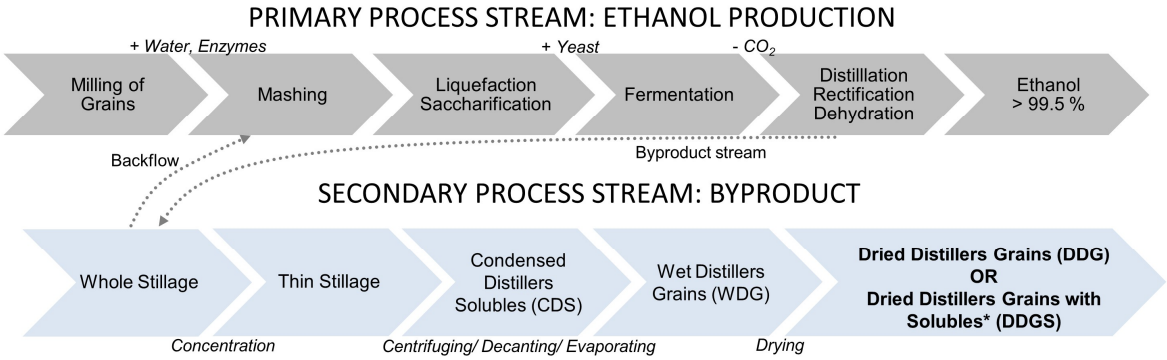

Figure 4: Production process of ethanol in dry grind processing, accompanied by accruing side streams DDG/DDGS and coproducts thin stillage, CDS and WDG. *DDGS results after addition of CDS to WDG prior to drying. DDG results after drying of pure WDG. Adapted from Roth, M., Jekle, M., & Becker, T., Opportunities for Upcycling Cereal Byproducts with Special Focus on Distiller's Grains, (Review Paper, Revision submitted in 04/2019. Trends in Food Science & Technology)

In summary, the major byproducts from dry grind ethanol production are dried distiller's grains (DDG), dried distiller's grains with solubles (DDGS), wet distiller's grains (WDG), condensed distiller's solubles (CDS), thin stillage and whole stillage (Xiang & Runge, 2014).

Ratios of ethanol to DDG are almost stable around 1 to 0.75 and every 100 kg of grain approximately deliver 40 L of ethanol, 32 kg of DDG and 32 kg of CO_2, (Chatzifragkou et al., 2016; Sundquist & Bajwa, 2016). Recently, the global ethanol market experienced remarkable surge in growth, due to increasing demand for fuels (Liu, 2011). Likewise, the utilization of crops for bioethanol production doubled within the last ten years (DBV, 2015; FAO, 2015). As grain-based feedstocks continue to be the main representative in ethanol production, corresponding byproducts experienced growth in the same manner. Estimations predict steady growth for the ethanol sector. The FAO estimates the global ethanol production to reach 134.5 billion liters in 2024 (FAO, 2015).

From the regulatory point of view and important for further processing, DG is a byproduct from the food industry, which is generally used as animal feed. Thus, it is covered by the definition "byproduct" of the European Waste Framework Directive, and therefore is considered to fall outside of the definition of waste (European Commission, 2007).

1.4 BYPRODUCT UTILIZATION IN FOOD AND IMPACT ON FLAVOR

As shortly described in chapter 1.1, the addition of DDG to food products was accompanied by deficiencies in the sensory perception, which made its further utilization difficult. These

deficiencies were not investigated in detail, but related to as bitter character or undefined aftertaste, as well as poor flavor or malty and soapy off-flavor (Bookwalter et al., 1984; Rasco et al., 1989). For a better understanding of the negative impact on flavor, some important characteristics of sensory perception have to be considered.

Interaction of odor, taste and texture generates the sensory impression of a food product, which is defined by the term "flavor" (Belitz, Grosch, & Schieberle, 2009). The complex interplay that causes flavor and the overall perception of a food product is influenced by multiple factors such as odor, taste or chemosensation as visualized in Figure 5. In German language the term flavor often is translated as "Aroma" (Belitz et al., 2009). The term "aroma" in this thesis, is used according to the definition of odor, as described below.

In general, bioactive compounds responsible for the creation of flavor impressions can be divided into two classes that fundamentally differ in their chemical nature and the physiological mechanism of perception:

Non-volatile compounds, predominantly characterized by hydrophilic character, are responsible for taste. The hydrophilic character supports the solution in aqueous food systems and the saliva, which is necessary to reach gustatoric receptor cells in taste buds, located on the tongue.

Volatile compounds, often designated as aroma compounds or aroma volatiles, are responsible for odor. Their hydrophobic character and high vapor pressure is necessary to reach olfactoric receptor cells of the regio olfactoria in the upper throat.

In general, gustatoric impressions are caused by the 5 basic taste qualities sour, salty, bitter, sweet and umami and are perceived by gustatoric receptor cells of the tongue papillae (Chandrashekar, Hoon, Ryba, & Zuker, 2006). Chemosensation, the perception of chemical stimuli by sensory means, complements the impression of the overall flavor and includes the trigeminal somatosensory system (Viana, 2011). These chemesthetic modalities are trigeminally active compounds that can cause impressions such as hot or cool, like capsaicin in chili (*Capsicum sp.*) or menthol in mint (*Mentha sp.*) (Obst, 2014).

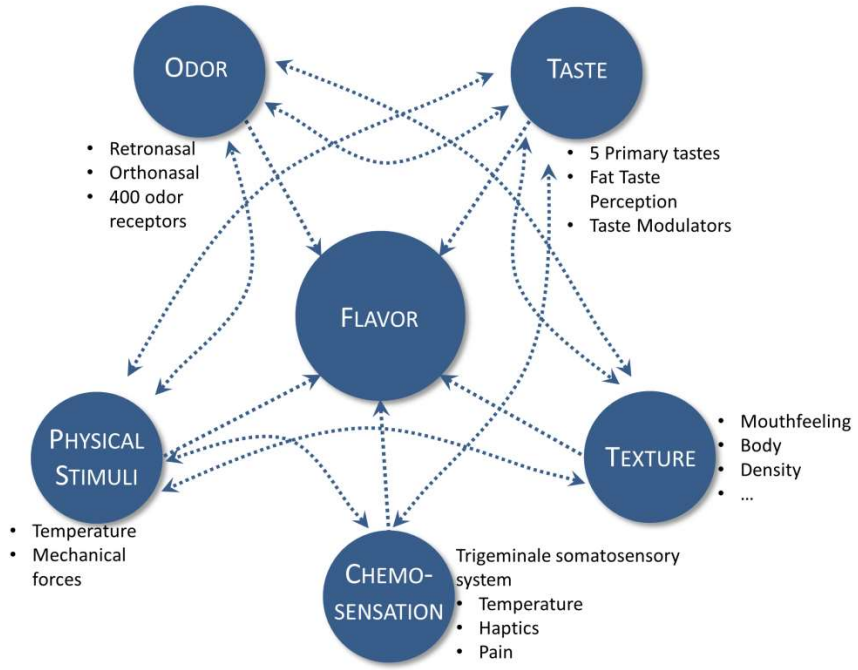

Figure 5: The overall impression of a food product (flavor) is generated by a complex interplay of textural, physical and chemosensational impact factors parameters as well as by odor and taste.

By contrast, odor perception is based on more than hundreds of odor qualities, responsible for the overall aroma impression of food products (Dunkel et al., 2014). Aroma volatiles can be perceived orthonasally through the nose, or retronasally, after release from the food matrix during consumption via the throat. Odor active compounds are perceived by chemoreception via olfactory neurons in the nose, by binding to chemosensory cilia of olfactory cells in the olfactory epithelium, the regio olfactoria. Subsequently, olfactory cells transform chemosensory information into electrical signals and transmit the signal to the olfactory bulb (bulbus olfactorius) in the brain. By now, around 400 odorant receptors are identified, that translate chemical stimuli into neuronal information (Dunkel et al., 2014). Odor active volatiles are characterized by high vapor pressure and volatility, low water solubility and polarity and generally small molecular weight < 300 Da (Kalua et al., 2007). Even though more than hundreds of aroma volatiles are identified, only a few determine the aroma information in form of the aroma profile and fingerprint that is determining every specific food product.

The investigation of aroma profiles of cereal byproducts and especially DDG only played a minor role in aroma and byproduct research. Aroma volatiles in DDG have not been studied by now and so any information on odor active volatiles is still missing. However, a systematic

study on volatile flavor compounds of DG can provide new possibilities for including DG in food products. For the evaluation of the characteristic aroma profile of DDG, which indeed includes potential sensory deficiencies such as off-flavors, the key aroma compounds that significantly influence the overall aroma impression must be investigated. Physiologically speaking, only if a compound's concentration exceeds the odor threshold, a nerve impulse is generated and transmitted from the receptor cells to the brain and consequently an odorant can contribute to the aroma profile of a food product (Tamura, 2012). Thus, odor active compounds present in concentrations above the odor threshold must be distinguished from odor active volatiles that are present in negligible concentrations. For this differentiation, the odor activity value (OAV) is helpful, since it describes the ratio between a compound's specific threshold (a) and the present concentration (c) (Belitz et al., 2009).

$$OAV = \frac{c}{a}$$

In addition, aroma extract dilution analysis (AEDA) performed during gas chromatographic (GC) analysis is a suitable tool for the determination of a compounds sensory impact, i.e. if analytical limitations prevent the determination of the OAV of an odorant, due to coelutions on the analytical column or low concentrations near the limit of detection. Within the dilution analysis, the aroma extract of a food product is diluted stepwise with equal parts of solvent 1:2, 1:4, 1:8, 1:16 etc. as long as activity at the sniffing port as detection unit on the GC is no longer perceivable. The degree of dilution, at which a compound can last be perceived, is defined as flavor dilution (FD) factor. Thus, the longer a component can be perceived at the sniffing port, the greater is its effect in the assessment of the flavor contribution. The elucidation of the negative sensory influence in food products induced by addition of DDG, should focus on determining these aroma active compounds which have the highest impact and are able to significantly affect the aroma of future food products.

1.5 THESIS OUTLINE AND MOTIVATION

In western and developed countries, a public consciousness for valorization of resources became apparent, which in consequence recently increased the demand for green and environmentally friendly produced food. Besides, the food industry recognized the good marketability of sustainable produced food and the emerging of a novel customer group, the lifestyle of health and sustainability (LOHAS) consumers (Kim, Lee, Kim, & Kim, 2013). Remarkably, valorization is linked to the novel healthy life style via the additional benefit of food products providing a health benefit. As an example, the number of food products on the market incorporating bran increased enormously from 52 to 798 between 2001 and 2011 (Prückler et al., 2014). Moreover, the growing demand in healthy products and increased requirements for protein, directed the attention of research to bread protein enrichment out of lupines or legumes (Paraskevopoulou, Chrysanthou, & Koutidou, 2012). This life style change can be an immense chance for the utilization of cereal byproducts such as DDG and suitable solutions for its processing in the food industry are necessary.

Unfortunately, the use of DDG as food ingredient remains challenging, because sensory and technological properties are negatively affected and not totally enlightened up to now. Considering deficiencies on sensory properties, detailed scientific knowledge is missing and no systematic study on odor-active volatiles in DDG has been performed so far, although research efforts identified the negative impact on flavor (Bookwalter et al., 1984; Rasco et al., 1989; Rosentrater & Krishnan, 2006). In 2001, volatile compounds of wet and dried DG from corn were investigated for the first time (Biswas & Staff, 2001). However, the determination of odor activity or odor impact, as well as the contribution to the overall aroma was not part of the study.

Regarding textural quality, negative impact of high fiber additives is known and mainly considers decreased loaf volumes, darker appearance as well as firmer and less elastic crumbs (Ktenioudaki et al., 2013; Rosentrater & Krishnan, 2006). In particular for bran, potential causes were enlightened and the hindered expansion of the gluten network through bran particles was determined as impact factor (Noort, van Haaster, Hemery, Schols, & Hamer, 2010). However, the transferability of known effects from other high fiber and high protein byproducts to DDG, as well as an initial in-depth cause study for DDG and its effects in dough and bread preparation is still missing.

Depending on the presented research knowledge, closing the corresponding research gaps will include studies on aroma composition, sensory properties and off-flavor in DDG, as well as studies on the impact on DDG enriched bakery products. Moreover, the investigation of technological deficits is necessary to assess the suitability for bakery product manufacturing without quality losses. In summary, as presented in figure 6, the following four sections are addressed in this thesis:

(I) Overview on cereal byproducts arising as side streams in the food and bioethanol processing industry: focused on distiller's grains, differences and commonalities, valuable compounds and potential approaches for further utilization of cereal byproducts are presented

(II) Investigation of aroma volatiles of dried distiller's grains, including enlightenment of the characteristic aroma profile and determination of key aroma compounds

(III) Elucidation of technological deficiencies and associated causes that accompany the enrichment of bakery products with dried distiller's grains

(IV) Enlightenment of alterations in aroma composition caused by the enrichment of bakery products with dried distiller's grains that subsequently lead to changes in sensory perception of flavor

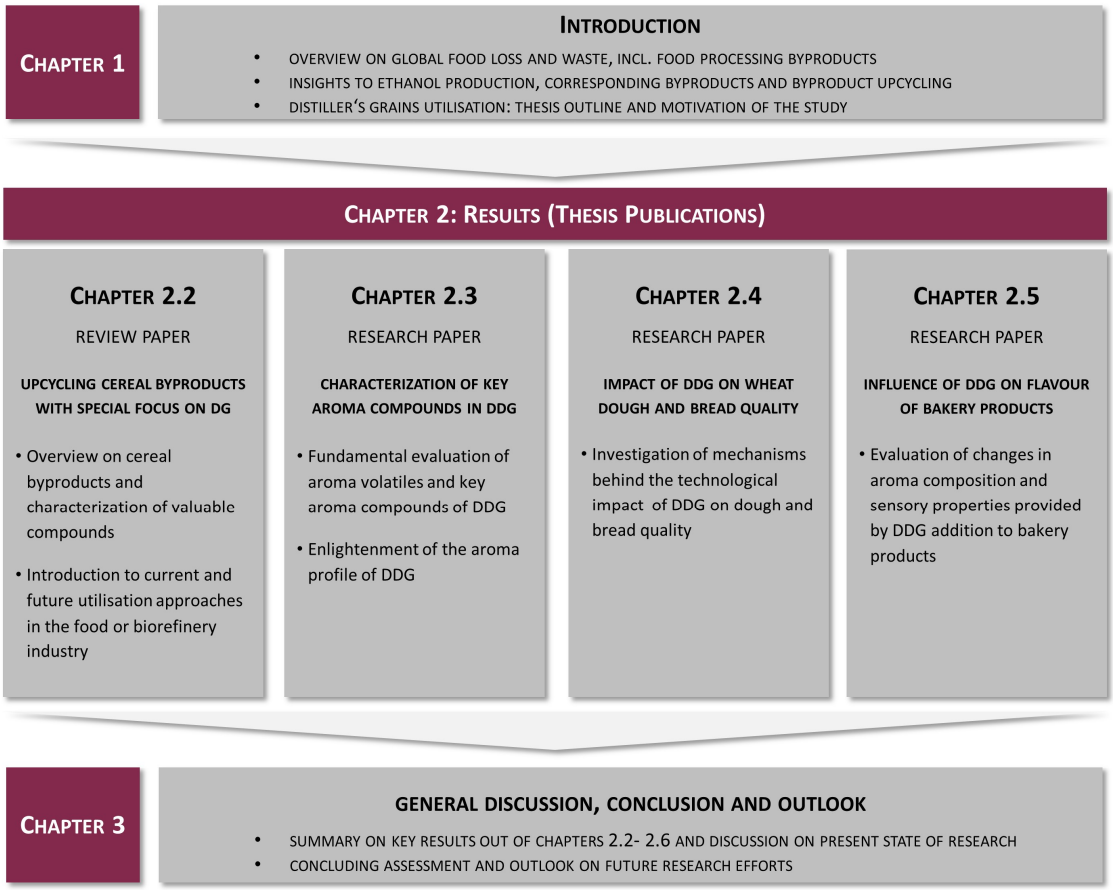

Figure 6: Thesis outline (DG: distiller's grains, DDG: dried distiller's grains)

This knowledge is fundamental to understand the effects of enriching food products with DDG and to enable the development of specific pretreatment methods in future studies, i.e. deodorizing technologies to prevent off-flavor effects in finished goods or recipe management with targeted additives, able to counterbalance technological deficiencies. In a global perspective, the successful utilization of DDG in food products and efficient use of valuable fractions like DDG dietary fiber and protein is an immense chance to provide novel raw materials for future food production. Consequently, raw materials for food production are exploited in a superior way, which is a key factor for the future food supply chain.

2 RESULTS

2.1 SUMMARY OF RESULTS

Within this chapter, a summary of each thesis publication is given and subsequently followed by full copies of the publications in their published version.

Part 1

Chapter 2.2.; page 27-39

Opportunities for Upcycling cereal byproducts with special focus on Distiller's Grains, Review Paper

As introduced in section 1, cereal byproducts (CBP) such as bran, brewer's spent grain or distiller's grain can answer the important question of a secure food and protein supply chain in the near future. The exploitation of CBP for food products however requires in depth knowledge on characteristics and properties, including valuable compounds, accruing and estimated amounts, as well as an overview of potential application fields for their utilization.

The following Review Paper in section 2.2 aims to outline specific characteristics, as well as differences and parallels that occur among the quantitative most important cereal byproducts of the non-glutenfree cereal sector: bran, brewer's spent grain (BSG) and especially distiller's grains (DG). Present-day and future approaches of CBP Upcycling will be presented and food applications are discussed in the context of competing biorefinery approaches. Key fractions in CBP are represented by the carbohydrate fraction that include dietary fiber or nutritionally important hemicelluloses, as well as the non-carbohydrate fraction that includes protein and phenolic compounds such as ferulic acid. All fractions could be utilized as starting materials for further food applications or depending on the Upcycling aim. Component targeted Upcycling that aims on the utilization of particular compounds as well as non-targeted concepts that aim on utilizing CBP as a whole are presented and discussed. Concluding, in a perfect role model of Upcycling, CBP can be completely utilized according to a zero waste approach: depending on the value of the fraction used as food, carbohydrate or energy source, or serve as feedstock for the bioethanol or bulk chemical industry.

Authorship contributions: Roth, M.: Study design, literature search, manuscript conception and writing; Jekle, M.: Critical review of study design and manuscript draft; Becker, T.: Supervision, critical review and approval of manuscript.

Part 2

Chapter 2.3.; page 40-48

Characterization of key aroma compounds in distiller's grains from wheat as a basis for the utilization in the food industry

During the past decades, research initiatives investigated DDG application in food and especially cereal based food systems. The impact of DDG on the overall aroma of finished goods was reported as insufficient (Bookwalter et al., 1984; Rasco et al., 1989; Rosentrater & Krishnan, 2006). However, no investigation to enlighten the aroma composition followed the detection of these sensory deficiencies and consequently food applications were not developed any longer. For this reason, the primary aim of this study was to elucidate the aroma composition in DDG and create detailed knowledge on the key aroma compounds, responsible for the characteristic aroma for the first time. To receive an almost representative odor profile, three different sample preparation techniques Headspace Solid Phase Micro Extraction (HS-SPME), Solvent Assisted Flavor Evaporation (SAFE) and Simultaneous Distillation/Extraction (SDE) were compared. Detection was performed via Gas Chromatography-Olfactometry/Mass Spectrometry (GC-O/MS). In addition, by means of sensory evaluation, the characteristic odor qualities that are perceived must be defined and a sensory profile of DDG aroma was established by a trained and experienced sensory panel. Consequently, the relation of analytical and sensory aroma investigation can be compared.

By sensory evaluation, panellists revealed three impressions seasoning-like, roasty/bread-like and malty/caramel-like as most intensive odors. This initially indicates the domination of thermal process odors in perception. Analytical determination of volatile flavor compounds revealed 42 odor-active compounds in total in dried distiller's grains from wheat. After application of AEDA, eight of these 42 odorants showed the highest FD factors ≥ 32 and consequently could be determined as key aroma compounds in DDG from wheat:

(I) 3-hydroxy-4,5-dimethyl-2(5H)-furanone
(II) 3-hydroxy-4-methyl-5-ethyl-2(5H)-furanone

(III) 4-hydroxy-2,5-dimethyl-3(2H)-furanone
(IV) 2-ethyl-3,5-dimethylpyrazine
(V) 2-phenylethanol
(VI) dimethyl trisulfide
(VII) *(E,Z)*-2,6-nonadienal
(VIII) 3-methylbutanoic acid

In accordance to sensory evaluation, the chemical origin of the eight key odorants DDG from wheat (pyrazine (IV) contributing to roasty character, furaneol (III) contributing to caramel like odor) is represented by typical reactions of thermal food processing, such as Maillard reaction and Strecker degradation. In general, aroma volatiles and key aroma compounds comply with wheat processed products like white wheat bread and no specific off-flavor could be identified. Concluding, DDG represents a thermal processed wheat product, which is supported by its composition of flavor volatiles.

Authorship contributions: Roth, M.: Study design, literature search, analytical method development, data analysis and interpretation, manuscript writing; Meiringer, M.: Data creation and analysis, Kollmannsberger, H.: Support in data analysis (GC-O/MS); Zarnkow, M.: Critical review of manuscript draft; Jekle, M.: Critical review of study design and manuscript draft; Becker, T.: Supervision, critical review and approval of manuscript.

Part 3

Chapter 2.4.; page 49-60

Mechanisms behind distiller's grains impact on wheat dough and bread quality

The negative impact of DDG on the sensory perception is accompanied by textural quality losses. In particular, high fiber additives and DDG provoke challenges to the dough and bread system and decrease loaf volumes, cause darker appearance as well as firmer and less elastic crumbs (Ktenioudaki et al., 2013; Rosentrater & Krishnan, 2006). Already 60 years ago, Jones und Erlander reported interactions of fibre and wheat proteins and some decades later, harder and less elastic dough could be explained by the hindered expansion of the gluten network during fermentation (Jones & Erlander, 1967; Wang, Rosell, & Benedito de Barber, 2002). However, the case study DDG isn't totally enlightened by now and the feasibility to transfer known phenomena from familiar approaches (i.e. bran addition to wheat bread) remains unclear.

The aim of this study was to investigate the mechanism that control the negative impact of DDG on dough and bread characteristics and to work out DDG specific details that affect quality parameters. The study covered the investigation on wheat bread containing 0-20 % DDG and investigated effects of pH, particle size and furfural as important DDG metabolite. As expected, some negative effects could be confirmed: wheat bread incorporating DDG provided decreased volumes from 20 % to 45 %, harder crumb up to a factor of 6 and less elasticity up to 10 %. Conspicuously, DDG enriched dough were characterized by decreasing pH value with increasing shares of DDG. The adjustment of the pH value balanced the negative influences and the low pH of DDG could be revealed as most influential parameter. Significant particle size effects could not be observed. Moreover, furfural as toxic DDG metabolite significantly affects the fermentation performance of *S. cerevisiae* and inhibitory effects were successfully confirmed in a model suspension and dough. Concluding, the incorporation of DDG in bakery products can be an opportunity for the reutilisation of this byproduct, if negative impacts are balanced by suitable treatments (acidity regulators, gentle drying technologies to avoid furfural formation). Shares of 10 % of DDG can be technologically feasible and consequently will provide an additional benefit through valuable amounts of dietary fibre and protein to bakery products.

Authorship contributions: Roth, M.: Study design, literature search, data creation, analysis and interpretation, manuscript writing; Döring, C.: Interpretation of data for dough and bread analysis; Jekle, M.: Critical review of study design and manuscript draft; Becker, T.: Supervision, critical review and approval of manuscript.

Part 4

Chapter 2.5.; page 61-70

Changes in aroma composition and sensory properties provided by distiller's grains addition to bakery products

The elucidation of DDGs characteristic aroma fingerprint, as presented in detail during section 2.3, serves as fundament for further investigations that target specific food products. It could be revealed that DDG as resource didn't cover an off-flavor and the aroma composition complies with wheat processed products like white wheat bread. Consequently, the application of DDG to bakery products can be suitable, as long as no off-flavors develop

as reaction compounds during the dough and bread manufacturing process. Nonetheless, different off-flavors were reported for DDG enriched bakery products and the investigation of alterations in the aroma composition induced by DDG addition is pending.

Therefore, enlightening differences in the aroma composition of a common wheat bread and DDG enriched wheat bread, as well as working out correlations to sensory impressions of DDG enriched and free systems were the objectives of this study. In analogy to DDG characterization in section 2.3, aroma volatiles in wheat bread containing 0 to 20 % DDG were identified with Gas Chromatography-Olfactometry/Mass Spectrometry and sensory properties were evaluated by a trained sensory panel.

In summary, 42 odor active compounds were identified in DDG enriched bread. The DDG enrichment altered the aroma composition of DDG free bread systems predominantly in its quantitative distribution. Two compounds, phenylacetic acid and dimethyltrisulphide, differentiated the aroma composition of the control wheat bread, but their impact is only low, due to low concentrations and therefore negligible aroma contribution. An important difference makes the absence of the key aroma compound 2-acetyl-1-pyrrolin in DDG enriched bread with concentrations > 5 %. Overall aroma perception did not provide relevant off-odors, as long as the supplemented amount did not exceed critical amounts > 20 %. Thus, the absence of the typical bread key aroma compound 2-acetyl-1-pyrrolin significantly contributes to the perception of an off-flavor. Moreover, odor attributes (roasty 2-ethyl-3,5-dimethylpyrazine or seasoning like 3-hydroxy-4,5-dimethyl-2-(5H)-furanone) were transferred from DDG to the bread crumb. However, overall perception was positively affected according to the results of the sensory evaluation. So, DDG addition to wheat bread can positively affect overall aroma and aroma popularity. With PCA analysis it was possible to substantiate the findings for variation of aroma volatiles in DDG enriched bread.

Authorship contributions: Roth, M.: Study design, literature search and evaluation, analytical method development, data analysis and interpretation, manuscript writing; Schuster, H.: Data creation and analysis, Kollmannsberger, H.: Support in data analysis (GC-O/MS); Jekle, M.: Critical review of study design and manuscript draft; Becker, T.: Supervision, critical review and approval of manuscript.

2.2 Opportunities for Upcycling Cereal Byproducts with special focus on Distiller's Grains, Review Paper

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Trends in Food Science & Technology 91 (2019) 282–293

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: www.elsevier.com/locate/tifs

Review

Opportunities for upcycling cereal byproducts with special focus on Distiller's grains

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ARTICLE INFO

Keywords:
Upcycling
Cereal byproducts
Biorefinery
Distiller's grains
Bran
Brewer's spent grain

ABSTRACT

Background: A growing world population and simultaneous diminishing of fossil resources force global stakeholders to provide solution approaches for ensuring the food supply chain in the near future. Within this context, cereal byproducts (CBP) occurring in the food or bioethanol industry are an untapped source of nutritious fractions that could serve as novel feedstock for secondary processes. Besides the utilization as feed, the conscious use of valuable compounds in abundant CBP remains as commitment against the use as landfill or waste.
Scope and approach: This review aims to outline characteristics, differences and parallels that occur among the quantitatively most important cereal byproducts bran, brewer's spent grain (BSG) and especially distiller's grains (DG) and provides an overview of present-day and future approaches of CBP upcycling. CBP include unexplored potential among the fractions of dietary fiber, protein and phenolic compounds that could be utilized as starting materials for food applications. Novel targeted approaches that aim on the valorization of particular compounds in CBP are presented. In addition, the feasibility of non-targeted traditional concepts of utilizing CBP as a whole is presented.
Key findings and conclusions: As a result, by applying upcycling concepts, CBP can be completely utilized based on the upcycling aim: Nutritionally valuable fractions such as hemicelluloses or protein can be fractionated for food applications, whereas insoluble fiber fractions can serve as carbohydrate or energy source for the bioethanol or bulk chemical industry.

1. Introduction

Today, 1.3 billion tons of food loss and waste (FLW) are generated every year among all stages of the food manufacturing process (FAO, 2011; Pham, Kaushik, Parshetti, Mahmood, & Balasubramanian, 2015; Pleissner et al., 2015). FLW primary arise in harvest processing, post-harvest processing, during production and up to the final product at the consumer level. Even though considerable amounts accrue at household level in developed countries due to behavioral causes, a huge share of 39% arises at the production level, indicating that there must be efficiency deficiencies with unexplored potential (Mirabella, Castellani, & Sala, 2014). Per definition, FLW comprise the part of waste that is food or related to food, originally intended for human consumption (HLPE, 2014). Therefore, FLW include food processing byproducts, which remain as sidestreams during the production of target products among all sectors of food production. According to the High Level Panel of Experts on Food Security and Nutrition, a science-policy interface of the UN Committee on World Food Security, food systems do not function as they should, regarding the extent of FLW while more than 800 million people suffer from hunger (HLPE, 2014). Within this discussion, intergovernmental stakeholders and industry representatives of the global food industry have to face and work on topics like zero waste and the "Zero Hunger Challenge". *Zero Hunger* and the *Responsible Consumption and Production* of food belong to the 17 Global Sustainability Goals of the United Nations, which represent an agenda for global action up to the year 2030 and defines sustainable development in its three dimensions — economic, social and environmental (United Nations, 2015). However, besides the strategy of optimizing food manufacturing processes for higher yields of food, the almost complete exploitation of existing food resources has to arouse more attention. The utilization of byproducts from the food manufacturing industry reflects industry branches with underestimated capacity and growing amounts of byproducts are unjustifiable against the background of sustainability. Consequently, leading enterprises in food processing have started to

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https://doi.org/10.1016/j.tifs.2019.07.041
Received 2 April 2017; Received in revised form 18 April 2019; Accepted 18 July 2019
Available online 24 July 2019
0924-2244/

adopt the Life Cycle Assessment (LCA) method for evaluating the environmental impact of their food product as part of their corporate and sustainable image (Chandrasekaran, 2013). With LCA, efficiency in food production can increase by identification and evaluation of high impact factors such as environmental impact compared to product reformulations or alternative raw materials (Miah et al., 2018). In consequence, targeted improvement i.e. higher yields and an improved use of raw materials and reduced output (emissions or waste) can be observed. In the near future, a complete exploitation of raw materials will be inevitable, because a growing world population and decline in agricultural productivity due to fertilizer scarcity and barren soils will limit available resources. Sustainability includes valorization, which represents a novel approach in the sector of byproducts management, in which the idea of a complete utilization of organic material is favored under the angle of sustainable development (Chandrasekaran, 2013). Within valorization, byproducts should not be recycled in any matter, but upcycled for the most valuable approach, e.g. using byproducts and compounds as food instead of feed or energy production. These days, the necessity for valorization of byproducts is driven by fundamental issues such as population growth to more than 9 billion by 2050, climate change or decline in agricultural productivity, due to barren soil (Fig. 1) (Chatzifragkou et al., 2016).

The cereal manufacturing industry covers a lot of nutritional potential among its byproducts. The milling industry provides huge amounts of bran, the brewing industry contributes with brewer's spent grain and the ethanol industry provides distiller's grain. Moreover, the availability of CBP is facing an oversupply on the market and alternative utilization routes, besides the common use as feed, are strongly needed (Liu, 2011; Prückler et al., 2014; Steiner, Procopio, & Becker, 2015). Regarding distiller's grain, the utilization of crops for bioethanol production more than doubled between 2006 and 2016 and continues growth for the ethanol market is estimated (DBV, 2015; FAO, 2015). All byproducts share similarities within their composition: high amounts of dietary fiber and protein remaining as non-utilized raw material. Within this review, a new perspective for the suitability of CBP utilization in the food sector is given. Understanding parallels and special features is needed to develop targeted approaches for CBP utilization. The main aim of this review is to provide a systematic overview on the quantitative most important cereal byproducts (CBP) in the sector of non-gluten-free raw materials. Focused on distiller's grains, the byproduct of the ethanol industry will be discussed within the context of the CBP bran and brewer's spent grain. Valuable compounds, their physiological function and current and potential manners of utilization and valorization within targeted and non-targeted upcycling aims will be pointed out.

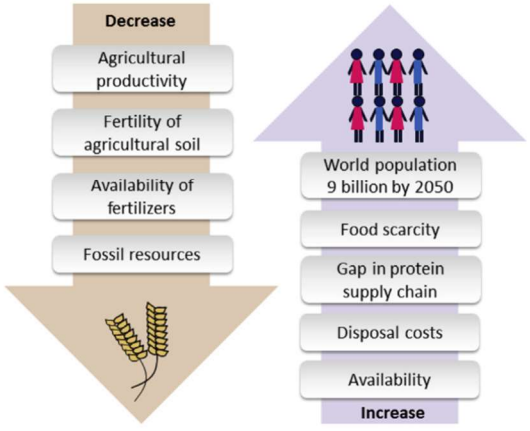

Fig. 1. Reasons for the inevitability of upcycling cereal byproducts (CBP).

2. Characteristics and special features of cereal byproducts

Regarding food products made from common cereals, with more than 2/3 share, the starchy endosperm is the principal part of interest of the cereal kernel. It serves as most efficient energy source for human nutrition. Starch is the main source of carbohydrates for human consumption in its changeability in form of pastry, pasta, biscuits, pizza and most importantly bread. It is a popular ingredient in the paper, textile and whole food processing industry which can be attributed to its unique properties. Due to this exceptional nature, the outer grain layers of the cereal kernel, germ, husk and bran remain as byproducts during cereal processing in the food industry. The quantitatively most important cereal byproducts (CBP) in the sector of non-gluten-free raw materials distiller's grains, brewer's spent grain and bran and will be introduced to point out parallels among CBP and special features.

2.1. Bran

After milling of the grain and sieving of the flour, several valuable fractions such as wheat bran or wheat germ remain as main byproducts of the milling industry. Research focused on bran mainly deals with rice and wheat bran, other cereals like corn or millet seem to be negligible. Bran is a multilayer material and contains the outer and inner pericarp, testa and nucellar layer. The protein-rich aleuron-layer as well belongs to bran. However, this can be depending on the literature source and milling technology (Apprich et al., 2014). In general, wheat bran contains 13–18% protein, around 3–4% lipids, 3–8% ash and more than 57% different carbohydrates (mainly hemicelluloses, cellulose, and around 14–25% starch) (Apprich et al., 2014). Rice bran contains similar amounts of protein and carbohydrates with around 14% and 30–50%, respectively, but up to 19% lipids (Moongngarm, Daomukda, & Khumpika, 2012; Phimolsiripol, Mukprasirt, & Schoenlechner, 2012). According to Tirpanalan et al. around 55% of wheat bran is lignocellulose, however, the remaining fraction which is rich in starch and minerals can be considered as feedstock for food products (Tirpanalan et al., 2015).

For wheat, due to the fact that more than 650 million tons are produced and processed annually, the corresponding amount of wheat bran was estimated to 150 million tons per year (Prückler et al., 2014). In the past, bran was mainly used as feed, however food applications were the foundation for many studies and the bran enrichment of food, especially cereal-based food systems, such as bread have been studied for many years (De Kock, Taylor, & Taylor, 1999; Föste et al., 2014; Heiniö et al., 2016; Salmenkallio-Marttila, Katina, & Autio, 2001). With respect to its composition, bran can serve as protein supplier within the scope of food applications, however, the exploitation of the carbohydrate fraction is the main part of interest for numerous biorefinery approaches (Apprich et al., 2014; Dorado et al., 2009; Reisinger et al., 2013; Tirpanalan et al., 2015). This fact already indicates the gap between applications for food or biorefinery applications: food applications often try to utilize the byproduct as whole, whereas within a biorefinery concept, the strategy targets specific compounds.

Remarkably, consumers' growing demand for healthier food leads to an increase in the consumption of whole grain food products and already indicates the latent potential among fractions that include the outer grain layers, like CBP (Prückler et al., 2014).

2.2. Brewer's spent grain

In 2017, the global brewing industry produced 1.95 billion hL beer (Statista, 2019). Apart from the main product, 20 kg of wet spent grain arise per 1 hl beer (Fărcaş et al., 2014). Assuming this ratio of 1:5, the calculated amount for 1.95 billion hL beer generates 390 million tons of wet BSG annually.

Depending on the type of crops used as raw material, BSG differ in their composition. However, barley is the predominant cereal applied

for beer production. BSG consists of the husk, pericarp and seed coat of the grain, with residual amounts of endosperm and the aleuron layer and therefore is characterized by high amounts of fiber (including cellulose, hemicellulose and lignin) up to 70% and high protein content up to 25–30% (Mussatto, 2014; Mussatto, Dragone, & Roberto, 2006). Important differences to wheat-based byproducts like DG occur due to the presence of lignocellulosic husk, which leads to high amounts of lignin, up to 28%. Since most of the phenolic compounds of barley are contained in the husk, BSG is a potentially valuable source of natural antioxidant compounds (Fărcaş et al., 2014). Moreover, alterations occur due to the content of lipids, which can reach up to 10% (Mussatto et al., 2006). Analogically to bran or DG, the utilization as feed supplement represents the most common application. However, a nutritional benefit in cereals like barley draws attention for applying BSG as a raw material for food products. Hemicelluloses in barley are characterized by health-promoting properties and β-D-glucans and arabinoxylans in BSG became the primary point of interest. Extraction methods for isolation and purification of arabinoxylan (AX) and (1–3,1–4)-β-D-glucan from BSG have widely been studied and will be discussed in chapter 4 (Steiner et al., 2015). Compared to other plant sources, high extraction yields up to 20 mg gallic acid equivalents (GAE) per g of BSG can be achieved (Moreira et al., 2013).

2.3. Distiller's grains

Between 2006 and 2016 the utilization of crops for bioethanol production increased from 60 to 160 million tons (DBV, 2015). According to the literature, every 100 kg of grain approximately delivers ethanol, DDG and CO_2 in equal amounts of around 32 kg (Chatzifragkou et al., 2016). Consequently, around 50 million tons of DDG result out of the bioethanol industry annually.

The chemical composition of DDG is defined by its origin and the main parameters of the ethanol manufacturing and drying process. The biggest impediment of DG available on the market are qualities inconsistent in their composition, especially due to the lack of an exact definition of the designation *distiller's grains* and corresponding coproducts distiller's grains with solubles (DGS, in dried condition DDGS) condensed or wet distillller's grain (CDS or WDG). The fact of adding parts of the soluble fraction which is poor in solid particles to wet distiller's grain or not later decides on the byproduct dried distiller's grains (DDG) or dried distiller's grains with solubles (DDGS).

Another difference occurs due to the grain species of the applied raw material itself. While in the US the main source for ethanol production is corn, Canada processes also barley and wheat; in Brazil sugarcane is predominating, whereas in Europe, mostly wheat or blended cereals are utilized (Chatzifragkou et al., 2015; Mustafa, McKinnon, & Christensen, 2000). Secondly, differences occur due to grain quality of starting materials. DG from bioethanol or beverage ethanol sources differ in the quality of utilized feedstock, since beverage ethanol production is subjected to requirements of food legislation including Good Manufacturing Practice (GMP), hygienic aspects and international or national restrictions concerning toxicological relevant compounds or contaminants (Chatzifragkou et al., 2015). For instance, in Germany the Federal Plant Variety Office defines requirements for wheat intended for food, as a minimum amount of 2% protein or sufficient enzyme activities, as defined by a minimum falling number of 4 (Bundessortenamt, 2015). Thirdly, and what is most important, differences occur due to the ethanol manufacturing process itself. Fig. 2 shows the manufacturing process of ethanol with DDG as side stream, to emphasize this principle. Fourthly, processing properties and yeast contribution are also responsible for variations in DDG qualities (Liu, 2009). In 2011, Liu published a detailed review on the composition of distiller's grains and emphasized the complexity behind the inconsistencies among DDGS qualities (Liu, 2011). For corn, the main difference was found within the milling process. So, a dry milling process can cause higher values of protein, ash and oil against a wet milling process (KeShun Liu, 2011).

Additionally, the amount of Condensed Distiller's Solubles (CDS) added to Wet Distiller's Grains (WDG) (see Fig. 2) during the manufacturing process contributes significantly to the composition of the final product. CDS obviously contain more soluble components (ash, soluble carbohydrates) and emulsified lipids and therefore affects both nutritive value and physical characteristics of the byproduct (Kingsly et al., 2010). Kingsly et al. studied changing ratios of WDG to CDS and reported increasing amounts of insoluble fiber, protein and amino acids with higher WDG amounts, whereas increasing moisture and particle size, amounts of lipids, soluble sugars and glycerol can be found with higher amounts of CDS (Kingsly et al., 2010).

On an average, after almost complete utilization of available fermentable carbohydrates, compounds in DDG are concentrated threefold over the original raw material (Han & Liu, 2010). The conversion from corn to corn DDGS leads to increased concentration of protein by factor 3.6; lipids by factor 3.4; ash by factor 3.3; and total non-starch carbohydrates by factor 2.9 (Liu, 2009). Within the literature there are a numerous studies trying to reveal the wide range of alterations among DDG and DDGS qualities. Cozannet et al. investigated ten DDG qualities and determined ranges for protein (N x 6,25) from 33 to 39%, ash between 4 and 6%, crude fat between 4 and 6%, crude fiber between 6 and 11% and neutral and acidic detergent fiber in a range of 25–34 and 8–18%, respectively (Cozannet et al., 2010).

3. Parallels and differences among cereal byproducts

The composition and properties of remaining CBP are defined by the manufacturing process of the target compound. Primary target compound in cereals used for industrial food or bioethanol and energy applications is starch, serving as energy and carbohydrate source. Consequently, most CBP resemble in their composition, as presented in Table 1. CBP contain outer grain layer components of the grain kernel and therefore are characterized by high amounts of protein and dietary fiber, with limited contents of fermentable carbohydrates. Hence it seems reasonable, that the main parts of interest aim at the reutilization of especially non-starch carbohydrates and protein.

3.1. CBP dietary fiber: basic resource for food and biorefinery

A major component and principal commodity of CBP is represented by the non-starch carbohydrate fraction, a heterogeneous mixture of polysaccharides and lignin, consisting of insoluble fiber, mainly represented by cellulose and lignin, and soluble fiber, mainly represented by hemicellulose. Cellulose is a high molecular linear polymer with β-(1 → 4) linked D-glucose units. Due to its high chain length up to 14000 units, cellulose is insoluble and non-degradable for endogenous enzymes. Already around 1970, a correlation of whole grain products, dietary fiber and health beneficial effects such as a reduced risk of coronary heart disease, diabetes, obesity, and some forms of cancer was reported, which directed the dietary fiber fraction in cereals into the focus of research (Elleuch et al., 2011; Poutanen, 2012). Together with lignin, a phenolic macromolecular biopolymer, the insoluble carbohydrate fraction in CBP holds difficulties for further food applications, since it provides sensory deficits such as a sandy and strawy mouthfeeling to food products, which in consequence reduces the acceptance by the consumer (Prückler et al., 2014; Roth et al., 2014). For this reason, the utilization of the cellulosic fraction for food products is limited and present-day approaches investigate the separation of fiber fractions aiming at separated applications.

Fractions that are sorted out from the food industry can gain new attention within biorefinery concepts. Under the angle of an almost complete utilization of raw materials, the insoluble carbohydrate fraction of CBP can be of value for the bioethanol industry. After a complete degradation to glucose monomers, a conversion to bioethanol and energy can be achieved. This process is known as the production of

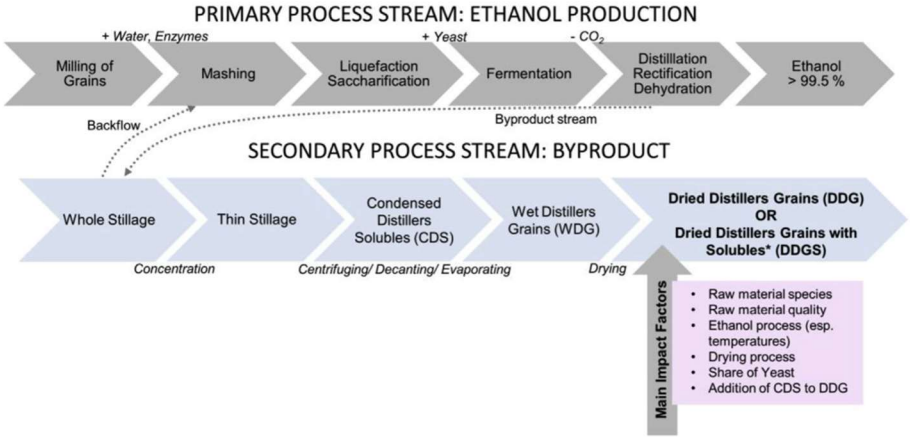

Fig. 2. Impact factors on DDG/DDGS quality and schematic process of ethanol production accompanied by the byproduct stream DDG/DDGS, *Addition of CDS to WDG leads to byproduct DDGS, no addition leads to DDG.

cellulosic ethanol (Koutinas et al., 2014). Indeed, the upcycling of DDG for bioethanol production is described as one of the most studied biotechnological processes for generating additional profit to bioethanol plants via microbial conversion of non-starch carbohydrates (Chatzifragkou et al., 2015). According to the literature, around 93% of cellulose in DDG can be converted to glucose by combining AFEX (ammonia fiber expansion) treatment with enzymatic hydrolysis and increased ethanol yields of 14% can be realized (Chatzifragkou et al., 2015). The cost-intensive production of cellulosic ethanol (0.63 $ per liter) might be counterbalanced by a remaining fraction of protein enriched DDG, which is characterized by higher sales prices due to higher nutritional value (Chatzifragkou et al., 2015; Ladisch et al., 2008).

With respect to their nutritional value, hemicelluloses remain as carbohydrate fraction with higher potential for food applications compared to cellulose. Hemicelluloses are a heterogeneous group of polymers and part of the cell wall, which includes glucans, xylans, arabinoxylans or mannans. One great attribute of several cereal hemicelluloses, like β-glucan or AX, is the recognition of health promoting effects by the European Food Safety Authority (EFSA) and their positive listing on the EU Register on nutrition and health claims of the European Commission.

Particularly in oats, barley and subsequent BSG high amounts of mixed linkages of β-D-glucan are part of the kernel composition. Mixed linkages of β-D-glucan such as (1–3,1–4)-β-D-glucan in barley characterize the properties of the carbohydrate polymer such as its solubility or enhanced ability to bind water and form gels (Steiner et al., 2015). Health promoting effects of β-D-glucan include the ability of lowering postprandial glycemic and insulinemic response, and therefore the reduction of the risk for diabetes. Moreover, β-D-glucans attenuate the level of blood cholesterol, which consequently reduces the risk for cardiovascular diseases such as coronary artery disease (Sullivan, Arendt, & Gallagher, 2013; Topping, 2007). These health promoting effects are correlated to molecular weight and solubility, amount and viscosity of the β-D-glucan polymer (Sullivan et al., 2013; Wood, 2007). In cereal feedstocks, ranges from 1% in wheat, 3–7% in oats and 5–11% in barley were reported (Brennan & Cleary, 2005). With barley as raw material, the suitability of BSG as resource for β-D-glucan extraction becomes reasonable; however, the efficacy of β-D-glucan is strongly determined by the extraction process and molecular and rheological characteristics of the polymer itself, so the development of gentle extraction procedures limits the reutilization success of β-D-glucan out of BSG (Brennan & Cleary, 2005). The food industry is just one representative of interested industry parties for purified cereal β-D-glucan. The cosmetic industry ascribed β-D-glucan to have positive effects on collagen production, cleaner skin, reduction of several skin aging effects and properties as film-forming moisturizer (Zhu, Du, & Xu, 2016). In medicines, β-D-glucan was observed to exert positive effects in wound dressing, wound care, to decrease post injury pain or even to serve as a new vaccine platform (Zhu et al., 2016).

The second important hemicellulose fraction is arabinoxylan (AX), a

Table 1
Composition of wheat bran, BSG from barley and wheat DDG.

Component/Amount	% in BRAN	% in BSG	% in DDG
Water	2.1	n.i.	5.6–10.7
Protein	13.2–25.0	15.4–30.0	32.6–38.6
Lipids	3.5–3.9	10.0	3.6–5.6
Total carbohydrates	56.8	n.i.	n.i.
Total dietary fiber	n.i.	n.i.	46.8
Starch	13.8–24.9	4.0	2.1–18.5
Cellulose	11.0–16.0	16.8–25.4	n.i.
Lignin	6.6	11.9–27.8	1.0–1.2
AX	10.9–26.0	21.8–28.4	n.i.
b-D-Glucan	2.1–2.5	n.i.	n.i.
Ash	3.4–8.1	2.0–5.0	3.6–6.7
Source	(Apprich et al., 2014; Mandalari et al., 2005)	(Mussatto et al., 2006; Steiner et al., 2015)	(Dong & Rasco, 1960; Jarret, Cozannet, Martinez, & Dourmad, 2011; Roth, Döring, Jekle, & Becker, 2016, Roth, Schuster, Kollmannsberger, Jekle, & Becker, 2016; Villegas-Torres, Ward, & Lye, 2015; Westreicher Kristen, 2013)

n.i.: no Information.

pentosan polymer. AX consists of a linear (1–4)-β-D-xylopyranosyl backbone, with α-L-arabinofuranosyl units randomly linked at O2 and/or O3 of xylose units of the backbone. The degree of mono- and disubstitution determines the arabinose to xylose ratio and consequently the solution properties. So, AX can be divided in soluble and water extractable (WEAX), and insoluble and water unextractable carbohydrates (WUAX). Interestingly, the arabinose to xylose ratio is specific for the source of botanical origin. For wheat endosperm, the arabinose/xylose ratio is around 0.50–0.60, whereas higher values around 0.57–1.07 were reported for wheat bran (Döring et al., 2016). Polymer chains can be crosslinked by ferulic acid via ester bonds to xylan/xylan double strands or linked to other cell wall compounds like lignin to xylan/lignin double strands (Döring et al., 2016; Prückler et al., 2014).

Health promoting properties of AX and its hydrolysis products arabinoxylan oligosaccharides (AXOS) include the improvement of the immunostimulatory and antitumor activity (Cao et al., 2011; Mendis & Simsek, 2014). Moreover AX intake leads to delayed gastric emptying, which induces delayed blood glucose and insulin response (Lu et al., 2000). In analogy to β-D-glucan, AX can contribute to lower levels of cholesterol and attenuate the type II diabetes risk, support prebiotic effects by stimulating microbiota growth in the intestine and reduce the risk for intestine cancer and cardiovascular diseases (Döring et al., 2016; Mendis, Leclerc, & Simsek, 2016). Typically, AX in wheat, rye or barley whole meal flour show amounts around 5–7%, whereas in wheat bran AX it can reach amounts up to 20–25%, making it an excellent source for extraction and purification of AX (Döring et al., 2016).

3.2. CBP protein: closing the gap in the protein supply chain

A corporate benefit among CBP is high amounts of protein, mainly originating from outer grain layer components like the aleuron layer. Protein residues out of the endosperm can be found in CBP, as well. Endosperm and outer grain layer protein differ in their composition: Whereas endosperm protein mainly contains glutenins and gliadins, albumins and globulins dominate in bran (Apprich et al., 2014). In general, bran protein has higher nutritional value, making bran protein more valuable compared to BSG or DDG protein. The higher biological value can be ascribed to higher amounts of essential amino acids in albumins and especially globulins, which serve as amino acid resource during germination. Due to its nature, bran protein is mainly consistent in its composition and consists of high-value protein of the grain covering layers. Since the milling process does not provoke high temperatures or other adverse conditions to the protein itself, bran protein can be seen as native high-quality protein. By contrast, DDG protein is composed rather intricately. DDG protein contains outer grain layer protein, endosperm protein and residual amounts of yeast protein, since after distillation of ethanol no fractionation or purification steps take place. Moreover, the processing of mashing, fermentation and distillation leads to partial aggregation and possible denaturation of DDG proteins. For BSG, the mashing process can lead to similar consequences: proteins are partially degraded to polypeptides and amino acids (Mussatto et al., 2006). Therefore the same restriction in protein quality can be found for BSG and DDG protein: whereas bran protein is available in its native form, BSG and DDG protein are degraded, aggregated, or even denatured and in some cases (as after drying) can be useful as amino acid or nitrogen source after hydrolysis.

Hitherto CBP were mainly applied as feed due to the nutritional value of CBP protein. However, supply and demand of protein for human consumption will suffer from a strong imbalance in the near future. In order to meet the increasing demand in future protein sources for human nutrition, existing resources have to be exploited in the best possible way. The utilization of protein from CBP appears as a viable option to support the ensuring of a global protein supply chain (see also 4.2.1).

3.3. Phenolic compounds

An additional feature, typical for outer grain layers of cereals and consequently part of CBP are high amounts of phenolic compounds, which can be found due to their location in the aleuron layer, testa, pericarp or husk. Phenolic compounds comprise alkylresorcinols, phenolic acids or lignans serving as the grain's protector against oxidation as natural antioxidant and action as free radical scavenger. Alkylresorcinols are mainly located in the testa, whereas phenolic acids primarily can be found in the pericarp and the aleuron layer (Prückler et al., 2014). Hydroxycinnamic acids are the most abundant phenolic compounds in cereals, with ferulic acid as main representative, followed by diferulic acids, p-coumaric acid, sinapic acid and others (Vitaglione, Napolitano, & Fogliano, 2008). In grains, phenolic acids are mainly connected to lignin in the cell wall matrix or linked to AX via ester bonds.

CBP have already been recognized as source for phenolic acids: Inglett et al. reported around 6 mg/g ferulic and coumaric acid in DDGS (Liu, Singh, & Inglett, 2011). For wheat and rye bran, amounts of 3–6 mg/g (measured as gallic acid equivalents, GAE) were reported (Vitaglione et al., 2008). McCarthy et al. reviewed existing literature and summarized ferulic acid as the most abundant hydroxycinnamic acid ranging around 2 mg/g, while the p-coumaric levels ranged around 0.7 μg/g in BSG (McCarthy, O'Callaghan, Piggott, FitzGerald, & O'Brien, 2013). So, the enrichment of food products with phenolic acids out of CBP can be a possible method of CBP upcycling. Moreover industry branches such as food, pharmaceutical or cosmetics have already recognized their potential benefit from sources of phenolic acids out of cereal byproducts, which represent inexpensive feedstocks due to their excessive supply on the market.

3.4. Minerals

Recovered nutrients from CBP can be of interest for various fields, such as feed for microbes in biotechnological processes, simple animal feed or the fertilizer industry. The amount of minerals is almost comparable in bran and DDG around 3–8% and slightly lower for BSG with 2–5%. However, an interesting benefit in DDG is the composition of minerals. Depending on the literature source, nearly 0.5–1% of phosphor can be found, which is considerable higher than in other grains and the third most expensive nutrient in animal diet (Liu, 2011). Moreover, the fertilizing character of distillery stillages is well known and so almost the entire volume of stillage generated in Brazil is used for the fertigation of sugarcane fields (Fuess & Garcia, 2014). According to Fuess, the high nutrient and especially phosphor content in distillery stillage such as DG can reduce mineral fertilization in the fields by 50–80% (Fuess & Garcia, 2014). This utilization route remains interesting for serving the potential phosphor shortage that challenges sustainable farming and protecting crop yields in the future (Herrera-Estrella & López-Arredondo, 2016). Therefore the utilisation of phosphor in byproduct streams and the improved management of existing resources can be useful for struggling the phosphor crisis in the long term.

4. Component-targeted upcycling

4.1. Carbohydrate fraction

Unfortunately, without intermediate steps of extraction, purification and hydrolysis, CBP upcycling remains difficult. With regard to this complexity for DDG, the fermentation and distillation residue remain as complex mixture of celluloses, hemicelluloses, proteins and some minor components such as fermentation byproducts (acetic acid, glycerol) and lipids, completely independent from the DDG manufacturing process itself. The future application of carbohydrate material out of DDG requires extraction procedures, able to deliver one-component polymer

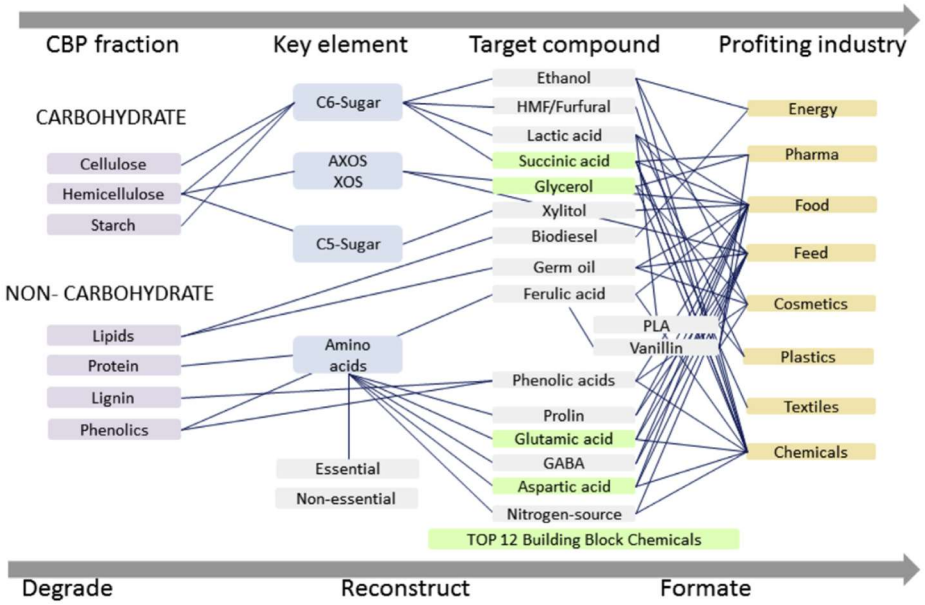

Fig. 3. Possibilities for upcycling of CBP fractions and potential profiting industry branches (based on the model of a biobased product flowchart for biomass feedstocks (Werpy & Petersen, 2004) and data from (Apprich et al., 2014; Chandrasekaran, 2013; Mussatto et al., 2006; Villegas-Torres et al., 2015), abbreviations: AXOS: arabinoxylan oligosaccharides, XOS: xylooligosaccharides, HMF: Hydroxymethylfurfural, PLA: Polylactic acid, GABA: γ-aminobutyric acid).

fractions. Fortunately, and in contrast to bran or BSG, DDG is amenable to relative mild hydrolysis conditions, due to the absence of a rigid lignocellulosic structure (Chatzifragkou et al., 2015). Once hydrolyzed, carbohydrate monomers are changeable multifunctional molecules, which can be transformed to a huge number of further medium- and high-value chemicals (Fig. 3). In 2004, the US Department of Energy identified twelve types of chemicals, the so-called top twelve building block chemicals that can be produced from sugars via biological or chemical conversions. These building blocks can be subsequently converted to numerous secondary high-value chemicals and polymer intermediates, which will be applicable in different industry branches (Werpy & Petersen, 2004). Carbohydrate monomers, pentoses or hexoses, can be a source for C3-compounds like lactic acid or glycerol, C4 compounds like succinic or fumaric acid, or C5-compounds like furfural or glutamic acid (Fig. 3). Prior to polymer degradation, carbohydrates have to be separated from the remaining material. Srinivasan et al. investigated this separation task for DDG and DDGS (Srinivasan et al., 2005, 2008). After milling for reduction of particle sizes and polymer chain lengths, fiber can be separated by combining sieving and elutriation. Within this so called Elusieve process, fractions of DDG/DDGS with reduced fiber, increased lipid and protein content and an enriched fiber fraction with 11.9% yield can be realized (Srinivasan et al., 2008). Considering the only low extraction yields, further research efforts for improving separation approaches are necessary.

After simplifying the complex polymer mixture by targeted extractions, the degradation of carbohydrate polymers out of CBP can be reached with different approaches. In general, chemical (acidic or alkaline), biological (enzymatic) or physical (hydrothermal) treatments are known for degradation of every specific carbohydrate polymer and shall not be discussed in detail within this review (Elleuch et al., 2011; Kosseva, 2011; Prückler et al., 2014). However, studies targeted at CBP will be presented to show the feasibility of CBP upcycling potential. As already mentioned, the hydrothermal treatment of CBP suspensions can be a promising tool for carbohydrate fractionation with the choice of focus on oligomers or monomers. Yields of XOS or monomers can be controlled by alterations in temperature and duration of hydrothermal treatment and therefore be directed to the upcycling aim (Carvalheiro, Esteves, Parajó, Pereira, & Gírio, 2004; Steiner et al., 2015). Higher temperatures and longer periods of hydrothermal treatment lead to higher yields of carbohydrate monomers. However, temperatures around 180 °C can decrease carbohydrate monomer yields and favor the formation of undesired sugar degradation products, such as furfural out of pentoses, or hydroxymethylfurfural out of hexoses (Reisinger et al., 2013). For wheat bran it was shown that a combination of hydrothermal treatment and subsequent enzymatic hydrolysis can deliver glucose yields of 65% and 90%, respectively, and hemicelluloses could be degraded to monomers only to approximately 50% (Reisinger et al., 2013). For BSG similar approaches are known. Carvalheiro et al. showed the viability of XOS production out of BSG with yields up to 50–60% of the initial xylan content and yield shifts with increasing temperatures and reaction times in favor of monomers (Carvalheiro et al., 2004). For DDG, no studies concerning hydrothermal treatments and utilization in the food industry have been published so far. However, according to present knowledge, the transferability to DDG or other byproducts seems feasible and could be topic of future research studies.

4.1.1. The xylan fraction and pentose production

For the extraction of AX out of bran or BSG media, processes for extraction and purification have been investigated and there are numerous studies and literature reviews available, that deal with definite feedstocks and targeted applications for specific carbohydrate polymers. Apprich et al. summarized this topic for wheat bran (Apprich et al., 2014). Due to the weak binding of AX to the cell wall, WEAX can be extracted under gentle conditions with water. WUAX extraction requires more drastic efforts: after removal of starch and protein (i.e. enzymatic via amylases and proteases), barium hydroxide is the method of choice for alkaline extraction of AX, since a arabinoxylan-specific complexing behavior was observed (Apprich et al., 2014). Steiner et al. reviewed extraction methods of AX out of BSG and presented the hydrothermal treatment as suitable tool for the release and partial

degradation of xylans to monomers or xylooligosaccharides within a hydrothermal fractionation (Steiner et al., 2015). In addition, alkaline or microwave-assisted procedures were reported. Viera et al. introduced a process, in which around 70% of AX could be successfully extracted by applying alkaline extraction with increasing concentrations (0.1 M–4.0 M) for 24 h and recovery of AX by precipitation with ethanol (Vieira et al., 2014). Besides, a cellulose-rich residue aroused, which could serve as feedstock for further biorefinery steps, as the production of cellulosic ethanol. Research on extraction of AX from DDG sources is rare, although amounts of 35–40% have been reported for bioethanol DDGS (Chatzifragkou et al., 2015). The conversion of xylan to AXOS/XOS out of DDG is a possible manner of utilization, since the low amounts of lignin (and what is a marked benefit against BSG or bran) prevent a delignification step. Yields up to 25% have already been reported after alkaline treatment and subsequent ethanol precipitation (Chatzifragkou et al., 2015; Xiang, Watson, Tobimatsu, & Runge, 2014). Concerning enzymatic methods, it was reported that xylanase and protease increase the solubilization of non-starch polysaccharides of DDGS, whereas wheat DDGS degradation yields are higher for wheat than corn DDGS (Pedersen et al., 2015). Moreover, Fonseca et al. recognized the potential of DDG due to its abundant availability and high level of hemicellulose and investigated pentose production by dilute acid hydrolysis in a perculation reactor (Fonseca, Lupitskyy, Timmons, Gupta, & Satyavolu, 2014). After a pretreatment step of screening for fines and surface roughening by ultrasonication, arabinose and xylose yields > 80% could be achieved. In summary, alkaline extraction and recovery via ethanolic treatment can be seen as method of choice for AX recuperation out of CBP.

4.1.2. Glucan fraction and hexose production

In analogy to AX extraction, literature data indicates that extraction of β-D-glucan is feasible based on their solubility in water and alkaline solutions and subsequent alcoholic precipitation (Zhu et al., 2016). According to Zhu, it has to be considered that the extraction of β-D-glucan out of cereals is more difficult and expensive than the extraction out of other sources (Zhu et al., 2016). In addition, no specific extraction process has been reported targeted on β-D-glucan out of BSG or DDG by now and the transferability of present methodologies has yet to be substantiated. The extraction out of bran, and due to comparable high amounts of β-D-glucan especially, extractions out of oat or barley were reported (Du, Zhu, & Xu, 2014; Limberger-Bayer et al., 2014). Du et al. investigated and compared extractions tools such as accelerated solvent extraction, ultrasound-assisted, microwave-assisted, and reflux extraction for their performance (Du et al., 2014). Highest yields could be obtained at 9 min, 70 °C, 10 MPa and four extraction cycles. However, with only 16.4%, extraction yields were comparably low. Limberger-Bayer et al. reported an extraction with aqueous solution of calcium carbonate and ethanolic precipitation of β-glucans and optimization of yields via response surface methodology (RSM) (Limberger-Bayer et al., 2014). In this study, a maximum concentration of 53.4% at pH 7.6 and temperature of 45.5 °C could be achieved. However, residual amounts of starch and protein could not be avoided.

4.1.3. Organic acids: production of succinic and lactic acid

The bio-based economy and bio-based production of chemicals is a key topic of recent research, due to the unstoppable diminishing of fossil resources. It has been shown, that there are platform chemicals, which could be produced via alternative pathways to preserve existing resources. As important platform chemical and one of the top twelve building block chemicals, succinic acid (SA) finds utilization as additive in the pharma and food industry, but more importantly within the plastics industry, since it can be a precursor for numerous polymers, e.g. polybutylene terephthalate after conversion of SA to 1,4 –butandiol. By now, market volume for SA is relatively small, however market potential is estimated to a multiple billion market considering bulk chemicals that could be produced from SA as intermediate (McKinlay, Vieille, & Zeikus, 2007). Traditionally, SA is synthesized out of the C4 fraction of naphtha by catalytic hydrogenation of maleic anhydride (Bechthold, Bretz, Kabasci, Kopitzky, & Springer, 2008). Crude oil as fossil resource is finite and therefore the biotechnological pathway could be an alternative for SA production. After suitable pretreatment of biomass (e.g. of byproducts), different microorganisms as fungi, yeast or bacteria are able to produce SA via fermentation of C5/C6 sugars (Bechthold et al., 2008).

CBP include huge potential for producing SA within the carbohydrate fraction after hydrolysis of polymers to fermentable monosaccharides. There are numerous organisms able to produce SA, however, highest yields can be obtained with *Anaerobiospirillum succiniciproducens* and *Actinobacillus succinogenes* (Bechthold et al., 2008). In 2009, Dorado et al. introduced a process, where wheat bran was first subjected to hydrolysis by solid state fermentation and secondly exposed to *A. succinogenes* fermentation for SA production (Dorado et al., 2009). Within this process, 50 g/L SA could be produced by fermentation of glucose and maltose with an overall production yield up to 30% referring to 1 kg wheat flour milling by-products. According to the authors, the developed process could prove as an opportunity for upcycling wheat milling by-products such as bran, due to the suitable and simple ability to be integrated into the wheat-milling processes. In 2013, Zhang et al. presented a procedure to produce SA out of bakery waste (Zhang et al., 2013). Similar to the process of Dorado et al., cakes and pastries were subjected to fungal autolysis and hydrolysis with subsequent fermentation of *A. succinogenes*. SA yields reached around 30 g/L. SA was recovered after cation-exchange resin-based separation, vacuum distillation, crystallization from the fermentation broth and purified, delivering SA crystal purity of 96–98% (Zhang et al., 2013). A perfect showcase of a successful commercial and sustainable production of chemicals via alternative and sustainable pathways is Biosuccinium. Biosuccinium represents a bio-based succinic acid, produced by yeast-based fermentation of plant-based resources as corn or sugarcane (Cok, Tsiropoulos, Roes, & Patel, 2014; Theunissen, Leemans, & Janssen, 2014).

The fermentation of sugars with lactic acid bacteria (LAB) represents a simple approach for the generation of lactic acid, which was also classified as an important top 30 building block chemical for the future (Werpy & Petersen, 2004). In recent years, the development of biodegradable plastics made from renewable resources and available biomass have gained much attention. Within a biorefinery approach, the production of polylactic acid (PLA) by polycondensation of lactic acid (LA) represents a showcase for the total exploitation of raw materials. PLA is a thermoplastic polyester and represents a promising green biopolymer for the packaging, textile and due to non-toxic monomer and its characteristics even medical industry (Lasprillaa, Martineza, Lunellia, Jardinia, & Filhoa, 2012). Currently, the production of LA for PLA production is based on the fermentation of food carbohydrate substrates, however, alternative resources such as agro-food wastes should also be considered (A. Djukic-Vukovic et al., 2016). The suitability of generating LA out of CBP has already been shown. Djukic-Vukovic et al. successfully examined distillery stillage (2014) and wastes from bioethanol and beer production (bread, potato stillage and brewer's' spent grain hydrolysate, 2016) for their suitability as substrate for LAB and subsequent LA production (A. Djukic-Vukovic et al., 2016; A. P. Djukic-Vukovic et al., 2014). LA concentrations around 50 g/l could be achieved and all waste materials were classified as appropriate for LA and biomass production (A. Djukic-Vukovic et al., 2016; A. P. Djukic-Vukovic et al., 2014). In 2015, Tirpanalan et al. effectively investigated the production and extraction of LA with wheat bran as feedstock (Tirpanalan et al., 2015). In this study, a LA yield of 15% of initial wheat bran dry mass could be obtained. In consequence, utilizing non-fermentable carbohydrates for production of organic acids can be a suitable way for utilization, insofar as industrial processes are improved for higher yields and purities.

4.2. Non-carbohydrate fraction

4.2.1. Protein fraction

One of the biggest bottlenecks for the generation of successful upcycling is the reliable prediction of feedstock quality. Numerous intrinsic and extrinsic variables can influence protein quality: soil, weather, breeding, location of growth and ultimately varieties can be mixed, e.g. for bioethanol production. Furthermore, the processing of CBP strongly influences protein properties. If the protein is exposed to high temperatures, alterations in protein degradability can be observed (Villegas-Torres et al., 2015). Ultimately, a prediction of protein quality or composition is hardly possible and so, potential processes need a high grade of robustness to buffer protein variabilities.

For DDG, most of the recent literature has focused on the valorization of carbohydrate fractions, since these represent the main share. Besides the consideration of DDG protein as animal feed, only minor attention has been paid to the protein fraction (Villegas-Torres et al., 2015). However, with amounts of up to 40% protein, DDG has a huge potential as feedstock for protein extraction (Rosentrater & Krishnan, 2006; Roth et al., 2014). The composition of protein fractions and amino acid distribution in DG is strongly connected to that of the native grain. Moreover, and what is unique for DDG in contrast to other by-products, there are significant amounts of yeast in the final material since there is no separation of yeast after the fermentation process. In regard to the starting material protein and amino acids are concentrated 3.0-fold and 2.0–3.5 fold, respectively (Han & Liu, 2010). By now, there is limited data available, revealing the exact share of yeast protein in wheat DDG. However in the case of corn, around 20% of yeast protein was estimated as share in total protein of corn DDGS by regression analysis (Han & Liu, 2010). Exact amounts are not well documented (Villegas-Torres et al., 2015). Though, the impact of yeast protein on the amino acid composition seems to be negligible for both corn and wheat DDGS, since the distribution of amino acids is not affected significantly (Villegas-Torres et al., 2015).

Above all, the utilization of DDG protein depends on its extractability and ability of purification. According to Chatzifragkou et al., the extraction of proteins from DDG remains a considerable difficulty, due to the decreased solubility of protein aggregates formed during DDG processing (Chatzifragkou et al., 2016). The intensive thermal treatment during drying favors aggregation and denaturation of proteins. However, the authors were able to recover 55% of proteins, with purities of 58% (w/w) (Chatzifragkou et al., 2016). Even though development of methods able to deliver higher extraction yields and purities might fail, research can focus on alternative ways for DDG protein upcycling. According to Villegas-Torres et al., an alternative strategy to valorize wheat DDGS protein without prior hydrolysis and purification steps is represented by the application within the biomaterial sector and its utilization for biodegradable polymers, edible films or coatings (Villegas-Torres et al., 2015).

Bran protein possesses a noticeable advantage in the protein composition compared to DDG or BSG:

In general bran proteins stand out for higher nutritional value than endosperm proteins, which can be attributed to higher amounts of essential amino acids and a more equilibrated amino acid composition (Apprich et al., 2014). Already in 1985, extraction methods for wheat bran proteins were presented, revealing the alkaline extraction as method of choice with protein yields up to 83% (Roberts, Simmonds, Wootton, & Wrigley, 1985). Also tap water as neutral medium was able to deliver extraction yields up to 72% (Roberts et al., 1985). Up to now, alkaline extraction remains a suitable process, however, protein modifications, such as molecular cross-links or rearrangements resulting in the formation of toxic compounds such as lysinoalanine were reported as well (Prückler et al., 2014). Moreover, it is possible to produce free amino acids such as lysine or glutamine and γ-aminobutyric acid (GABA) by simple water-soaking treatment (Nogata & Nagamine, 2009).

Considering the amount of 20% protein, BSG is a valuable feedstock for protein extraction, as well. BSG protein contains a high level of essential amino acids, which accounts for one third of the total protein content, and a valuable proportion of around 14% of lysine among the amino acid distribution (Waters, Jacob, Titze, Arendt, & Zannini, 2012). Since lysine is the limiting factor for the biological value of protein in cereal foods for human nutrition, BSG protein has high potential to close this gap by enriching food products with high value protein. The feasibility of protein extraction was already shown by Viera et al., who developed an integrated process for protein and AX extraction from BSG via alkaline extraction (Vieira et al., 2014). According to the authors, around 80% of BSG protein can be extracted.

Even though CBP might not suffice regulatory or nutrionally needs, the chemical industry could benefit as well. Valorization of CBP protein could serve as an important source of amino acids. According to Sheldon, in a perfect scenario of an integrated biorefinery approach, after isolating and hydrolyzing the protein, essential amino acids are utilized as feed, whereas non-essential amino acids serve as feedstocks for platform chemicals (Sheldon, 2014).

4.2.2. Phenolic acids: approaches for ferulic acid and vanillin production

Due to its highest amounts in CBP and its various fields of applications, FA is the primary target compound among phenolic acids. Mussato et al. investigated the extractability of ferulic and coumaric acid (CA) after alkaline treatment of BSG (Mussatto, Dragone, & Roberto, 2007). It was reported that a hydrolysis with 2% NaOH and 90 min at 120 °C was able to deliver around 140 mg/L each ferulic acid and p-coumaric acid, which equals to around 10 mg FA or CA per gram of solubilized lignin. For BSG, even extraction yields up to 20 mg gallic acid equivalents per g of BSG can be achieved (Moreira et al., 2013).

Commonly, phenolic acids can be released by alkaline or acidic conditions, because of their native embedding into the cell wall structure via ester bonds. However, alkaline hydrolysis was reported to be more efficient (Apprich et al., 2014). Modern approaches for phenolic acid extraction investigated alternative approaches such as microwave or ultrasonic treatments (Inglett, Rose, Stevenson, Chen, & Biswas, 2009; Moreira, Morais, Barros, Delerue-Matos, & Guido, 2012; Wang, Sun, Cao, Tian, & Li, 2008). Ultrasonic pretreatment appears as promising tool, as increased yields of up to +14% more phenolic compounds could be extracted from DDG (Inglett et al., 2009). According to the authors, ultrasonic treatment exerts beneficial changes on the surface structure of DDG particles, so that damaged cell walls and larger pores favor the release of phenolic compounds. For wheat bran, ethanolic extraction for 25 min at 60 °C and ultrasound-assisted extraction at 40 kHz delivered around 3 mg gallic acid equivalents/g of wheat bran (Wang et al., 2008). In 2012, Moreira et al. introduced a method, in which polyphenols out of BSG can be extracted by microwave-assisted treatment (Moreira et al., 2012). It was shown, that 15 min at 100 °C can increase yields of FA fivefold compared to conventional methods.

Developing processes that are able to deliver fractions rich in phenolic acids would raise considerable interest for the industry, since FA is the main precursor of vanillin, one of the most crucial flavoring agents used in food, cosmetics and pharmaceuticals. For instance, the aroma industry has a marked interest in alternative resources for ferulic acid, since it exists as most important precursor for the commercial production of vanillin (Chatzifragkou et al., 2015).

The feasibility of vanillin production out of CBP was investigated by Di Gioia et al. for BSG (Di Gioia et al., 2007). By microbial conversion of FA almost 50% of FA could be converted to vanillin by a genetically modified *E. coli* strain. Yields of vanillin can be increased if isolation of free ferulic acid from carbohydrates hydrolysates can be achieved (Apprich et al., 2014). With solid phase extraction (SPE) remarkable yields of 95% FA are reported (Di Gioia, Sciubba, Ruzzi, & Fava, 2008).

5. Non-targeted approaches

Investigating byproducts and their components for finding maximum value applications is an important concept for fulfilling the needs of sustainable use of raw materials. However, the development of elaborate extraction and purification steps can ultimately complicate the feasibility and applicability of evolved processes for enterprises or will produce costly high purity raw materials that will unbalance the life-cycle assessment of the finished product. Therefore, the possibility of non-targeted approaches aiming at the byproduct as a whole, instead of utilizing particular byproduct components should also be considered.

The feasibility of non-targeted approaches of CBP for the use in food products was a topic of several studies and chances and limitations for their application were worked out. A strong connection of the addition of high fiber fractions to bakery products with parameters of product quality such as appearance, texture and taste represents the hardest challenge for CBP utilization in the cereal manufacturing industry (Ktenioudaki, Chaurin, Reis, & Gallagher, 2012). According to Ktenioudaki et al., the incorporation of BSG in breadsticks was possible up to an amount of 10%, but food quality suffered with higher amounts (Ktenioudaki, Crofton, et al., 2013). Moreover, Waters et al. reported for a level of 10% BSG in bread increased nutritional properties of BSG enriched products regarding protein, fiber, minerals and noticed the overall acceptability suitable as food ingredient (Waters et al., 2012). Results are similar in bran, as amounts up to 10% were reported as suitable (Lai, Hoseney, & Davis, 1989). Starting in 1980–1990, research groups began to explore the nutritional potential of DDG as a food supplement. Especially cereal food products such as bread, cookies or other bakery products served as media for DDG utilization (Abbott, O'Palka, & McGuire, 1991; Liu et al., 2011; Rasco, Hashisaka, Dong, & Einstein, 1989; Roth, Schuster et al., 2016). DDG also caused the darkening of products, though this phenomenon is beneficial for the acceptability of the consumer, since the appearance of a whole meal product is supported (Roth, Döring, et al., 2016, Roth, Schuster et al., 2016). For cornbread, shares of 20–25% DDGS supplementation to corn flour were reported as suitable (Liu et al., 2011). In wheat bread, the enrichment of 10% DDG can provide food products with higher nutritional value with respect to protein and dietary fiber, high acceptance and respectable food quality attributes (Roth, Döring, et al., 2016, Roth, Schuster et al., 2016).

The application of high level of CBP in food products behaves quite similar irrespective of the type of CBP used. Higher amounts cause deficiencies regarding sensory attributes and restrictions in technological performance and textural quality (Ktenioudaki, Crofton, et al., 2013; Ktenioudaki, O'Shea, & Gallagher, 2013; Rosentrater & Krishnan, 2006). Even off-flavors were reported (Rosentrater & Krishnan, 2006). Textural quality is deteriorated due to firmer and less elastic dough, decreased loaf volumes and sticky products (Roth, Döring, et al., 2016). Gluten dilution effects, hindered expansion of the gluten network due to fibrous macromolecules of increased shares of dietary fiber were identified as impact factors. For wheat bran, fiber-gluten interactions are considered to be the main cause (Noort, van Haaster, Hemery, Schols, & Hamer, 2010). Possibilities to counteract these effects are still needed. In consequence, more research has to be targeted on enlightening the phenomena and mechanisms in detail that limit the range of CBP application to those small amounts. These phenomena include studies on sensory properties and off-flavor coming along with the application of CBP in food products, as well as the investigation of technological deficits in food rheology and food structures. The in-depth enlightenment of accompanying effects of CBP application can provide solutions for end-users to tackle these challenges and higher amounts of CBP might be applicable in food systems without elaborate pretreatment steps in the future.

Considering non-targeted approaches of byproduct streams, the energetic exploitation is certainly worth to mention and can be seen as upcycling and last chance route if materials might be prevented from rotting. Energetic recovery of byproducts is represented by further treatments such as pyrolysis, biogas, combustion or gasification. The methods differ based on the out coming products (e.g. syngas, bio-oil or ash) but show a common bottleneck in the needed energetic input and economic attractiveness (Arvanitoyannis, Tserkezou, Varzakas, & Ladas, 2006).

6. Conclusion

The upcycling of byproducts remains an important task for securing the food supply chain of a growing world population due to inevitable diminishing of fossil resources. This circumstance includes the necessity for developing ways for more efficient exploitation of raw materials.

In this review, it was shown that cereal byproducts (CBP) include numerous opportunities for reasonable reutilizations. Quality characteristics among ingredients, in addition to low cost and high level of availability qualify CBP appropriate as functional food ingredient. CBP hold enormous potential as feedstock for novel approaches. The versatility of valuable compounds can fit specific industry branches and diverse application fields. It is a duty for research to reveal this hidden potential among CBP for food manufacturing industries and a challenge to provide robust extraction methods that are able to deliver consistent raw materials (Brennan & Cleary, 2005). In future, successful processes should not be dependent on feedstock variability. In any case, the economic view on the processability of byproduct management should arrest more attention, because only profitable processes will prevail on the market. Additionally, and what is decisive in the long term is that the developed processes must be evaluated for their environmental impact, sustainable character and influence on the balance of a products life cycle assessment (Chandrasekaran, 2013). Independent of the availability of low cost byproducts, developing high cost and elaborate extraction und purification steps might be difficult to implement in current processes. In addition, costs of CBP transport in wet form and drying are big impediments in a sustainable upcycling approach (Mussatto et al., 2006). Therefore, more efforts must be directed to finding alternative economically sustainable and gentle drying methods, which in addition will not change protein quality due to denaturation or aggregation.

Ideally, the development of simple one-step procedures that can be included in existing processes is necessary. Whereas between 1980 and 2000, direct non-targeted applications were a topic of numerous research studies, present-day studies focus on targeted approaches of one-component systems. Besides optimizing compound-targeted concepts, basic and non-targeted approaches, where CBP can be utilized without elaborate procedures, should not be underestimated. By now, research on the use of CBP and especially the use of DDG in food products is limited. Potentially, the food sector can be a high-volume upcycling option that must return in the focus of future studies to fulfill the obligation of a secure food supply chain in the future.

Abbreviations

AFEX	Ammonia fiber expansion
AX	Arabinoxylans
AXOS	Arabinoxylan oligosaccharides
BSG	Brewer's spent grains
CA	Coumaric acid
CBP	Cereal byproducts
CDS	Condensed distiller's solubles
DNA	Deoxyribonucleic acid
DDG	Dried distiller's grains
DDGS	Dried distiller's grains with solubles
DG	Distiller's grains
EFSA	European Food and Safety Authority
FAO	Food and Agriculture Organisation
FLW	Food Loss and Food Waste

GABA	γ-aminobutyric acid
GAE	Gallic acid equivalents
GMP	Good Manufacturing Practice
HMF	Hydroxymethylfurfural
LA	Lactic acid
LAB	Lactic acid bacteria
LCA	Life cycle assessment
PLA	Polylactic acid
RSM	Response surface methodology
SA	Succinic acid
SME	Small and medium-sized enterprises
SPE	Solid phase extraction
UDP	Undegraded dietary protein
UN	United Nations
US	United States
WDG	Wet distiller's grains
WEAX	Waterextractable arabinoxylans
WUAX	Waterunextractable arabinoxylans
XOS	Xylooligosaccharides

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2.3 CHARACTERIZATION OF KEY AROMA COMPOUNDS IN DISTILLER'S GRAINS FROM WHEAT AS A BASIS FOR UTILIZATION IN THE FOOD INDUSTRY

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Characterization of Key Aroma Compounds in Distiller's Grains from Wheat as a Basis for Utilization in the Food Industry

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ABSTRACT: The limited use of distiller's grains (DG) in the food industry depends occasionally on the characteristic odor of DG. For a better understanding of this typical odor, a sensory evaluation was performed first. The impressions seasoninglike, roasty/breadlike, and malty/caramellike were revealed as the most intensive odors. Furthermore, analysis of volatile flavor compounds was applied on dried DG from wheat. Isolation was performed by means of headspace solid-phase microextraction, solvent-assisted flavor evaporation (SAFE), and simultaneous distillation/extraction and identification with gas chromatography-olfactometry/mass spectrometry. As a result, 42 odor-active compounds could be identified in total. Among 24 of the 42 odor-active compounds obtained by SAFE, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (seasoninglike) showed the highest flavor dilution (FD) factor, and 7 compounds (3-methylbutanioc acid, dimethyl trisulfide, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 2-ethyl-3,5-dimethylpyrazine, 2-phenylethanol, 2,6-nonadienal, and 5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone) with a FD factor ≥ 32 were identified as key aroma compounds in DG from wheat.

KEYWORDS: *distiller's grains, wheat, flavor, HS-SPME, SAFE, SDE, GC-O/MS, AEDA*

INTRODUCTION

Distiller's grains (DG), the cereal byproduct coming up during the production process of fuel or beverage ethanol, arise commonly from cereal grains such as wheat or corn. While in the United States corn is the predominant cereal for DG, in Europe, wheat and rye represent typical DG cereals. It is prevalently used as feed additive or material for biogas plants, but the main part remains as waste material, where disposal problems become more relevant. During the fermentation process of milled and mashed cereal kernels, starch is converted to glucose, fermented to ethanol, and distilled. Subsequently, the remaining product is dried (dried distiller's grains, DDG). Except starch, DDG contain all ingredients of the whole grain in a concentrated form, especially high amounts of protein and dietary fiber.[1,2] The nutritional properties of DDG and the oversupply on the market provide a new interest in marketing DDG as a food ingredient for commercial food uses. Up to now, the application of DDG as a food ingredient still is not usual, although the nutritional profile provides a valuable amount of protein and dietary fiber to food products. During the last 30 years, there have been several trials for using DDG as a food ingredient, especially in cereal food products such as bread and noodles, but the results were not sufficient, because there was a lack of acceptance regarding texture and flavor.[3−6] As an example, baguettes containing DDG were described with an aftertaste and more sour, salty, and bitter than the control.[6] Additionally, blended foods containing DDG were unsuitable, because of the poor flavor.[3] One reason for the sensory deficiencies is the characteristic and special aroma of DG, which is described as an off-flavor and therefore lead to products marginally acceptable to not acceptable.[1] However, aroma volatiles of DDG have not been studied by now, and so, any information on odor active volatiles is still missing. Biswas and Staff were the first to investigate volatile compounds of wet and dried DG in corn, but it was neither distinguished between odor-active and inactive compounds nor reported about the relevance of selected components.[7] A systematic study of volatile flavor compounds of DG can simplify the selection of food products, where odor compounds of DG emphasize and match the flavor profile of a food product. This knowledge can provide new possibilities for including DG in food products. Structured knowledge on flavor volatiles in DDG can offer opportunities to work on neutralization, modification, and masking steps and therefore create new ways for the utilization of this byproduct. The aim of this study was to establish an aroma profile of DDG from wheat for the first time. Therefore, volatile flavor compounds were isolated by means of headspace solid-phase microextraction (HS-SPME), solvent-assisted flavor evaporation (SAFE), and simultaneous distillation/extraction (SDE) and identified with gas chromatography-olfactometry/mass spectrometry (GC-O/MS). Finally, key aroma compounds were investigated using aroma extract dilution analysis (AEDA). The simultaneous application of three different methods can ensure the formation of a representative odor profile of DDG from wheat.

MATERIALS AND METHODS

Distiller's Grains. DDG from wheat were kindly provided by Euro-Alkohol GmbH (Lüdinghausen, Germany). DDG were composed of 38.2% protein (AACC 46-10, N × 6.25), 3.6% fat (AACC 30-25), 3.5% ash (AACC 08-01), 46.8% total dietary fiber (AACC 32-05) on a dry basis and 7.8% water (AACC 44-01). Flavor analysis was carried out using DDG of the same batch, to exclude influences through different composition or dryness. Before extraction

Received: July 9, 2014
Revised: September 17, 2014
Accepted: September 22, 2014

dx.doi.org/10.1021/jf503281x | J. Agric. Food Chem. XXXX, XXX, XXX−XXX

of the volatile fraction, DDG was milled to particles less than 500 μm using an Ultra Centrifugal Mill ZM200 from Retsch (Haan, Germany) by 5000−6000 rpm.

Chemicals. Chemicals were obtained from the following sources: diethyl ether (≥99.5%) and anhydrous sodium sulfate (≥99.0%) from Sigma−Aldrich (Taufkirchen, Germany), sodium carbonate (≥99.5%) from Merck (Darmstadt, Germany), sodium chloride from Avantor Performance Materials (Deventer, Netherlands), and hydrochloric acid (37%) from Roth (Karlsruhe, Germany). Reference standards of aroma compounds were purchased from commercial sources: Alfa Aesar, Karlsruhe, Germany; Merck, Darmstadt, Germany; Sigma−Aldrich, Taufkirchen, Germany; others were kindly provided from flavor companies (Firmenich, Switzerland; Symrise, Holzminden, Germany).

Sample Preparation for HS-SPME. A 3.0 g amount of DDG was weighed in a 20 mL headspace vial and sealed with flanged caps. The septum was pierced with the SPME needle, and subsequently, the fiber was manually exposed to the sample for a normal adsorption time of 1 h and a prolonged adsorption time of 6 h for a better enrichment of medium and low volatile compounds. The adsorption process was performed in a tempered water bath at 30 and 70 °C. SPME fiber material was 50/30 μm divinylbenzol/carboxen/PDMS (Supelco Inc., Bellefonte, PA, USA). Fibers were conditioned in a GC injector at 270 °C for 1 h. After adsorption, the fiber was directly transferred to the injection port of the gas chromatograph. Volatiles then were desorbed at 250 °C for 30 s. Before the next analysis, the fiber was reconditioned at 250 °C for 15 min in the GC injector to avoid carry-over of compounds from previous samples.

Sample Preparation for SDE. SDE was performed in a Likens−Nickerson apparatus, as modified by Schultz et al.[8] using 100 g of DDG suspended in 500 mL of water. The sample−water mixture and 100 mL of diethyl ether were boiled for 2 h. After cooling down to room temperature, diethyl ether was dried over sodium sulfate and concentrated to 1 mL using a Vigreux column (30 cm × 1 cm i.d.).

Sample Preparation for SAFE. A 150 g amount of DDG was mixed with diethyl ether (2 × 100 mL) and extracted for 2 h. For quantitation, 500 μL of methyl decanoate (c = 0.81 mg/L) as the internal standard was added to the DDG−diethyl ether mixture. After filtration, the volatile fraction of the diethyl ether extract was isolated by means of the SAFE technique,[9] dried over sodium sulfate, and subsequently, concentrated to 1 mL using a Vigreux column.

Separation into Neutral-Basic and Acidic Fraction. The distillate obtained by SAFE was fractionated into a neutral-basic fraction (NBF) and an acidic fraction (AF) by treatment with 0.5 M sodium carbonate solution (2 × 50 mL, pH 10.0). After drying over anhydrous sodium sulfate, the NBF was concentrated to 1 mL using a Vigreux column. The AF was treated with a solution of 100 mL of saturated sodium chloride, and the pH was adjusted to pH 1.0 with hydrochloric acid. After treatment with diethyl ether, the organic phase was dried over anhydrous sodium sulfate and concentrated to 1 mL as described above.

GC-Olfactometry and GC/MS. Identification of aroma compounds was carried out on a SiChromat II gas chromatograph (Siemens, München, Germany) directly coupled to a Finnigan MAT 8222 magnetic sector mass spectrometer. At the end of the capillary column, the effluent was split into two equal parts using a Life-T effluent splitter to the mass spectrometer and a sniffing port.[10] The sniffing port was heated to 250 °C and rinsed with humidified air, to avoid dehydration of nasal membranes of assessors. Samples were separated using a silica capillary column SPB5 (Supelco) (30 m × 0.53 mm i.d., 1.5 μm film thickness). The column carrier gas was helium at a constant flow of 3 mL/min. A 2 μL amount of samples was applied with a split ratio of 1:10. The injector temperature was 250 °C, and the transfer line temperature was 200 °C. The oven program started with an initial temperature of 100 °C and subsequently was raised to finally 250 °C at a rate of 5 °C/min. MS detection was performed with an electron impact (EI) energy of 70 eV. The analyzed mass range was 35−350 amu in EI mode. For quantitation, an internal standard was added (500 μL of methyl decanoate with c = 0.81 mg/L) at the beginning of the sample preparation step. Calculated concentrations are semiquantitative, since no response factors were determined.

Aroma Compound Identification. Identification of aroma compounds was based on the following criteria: odor description, linear retention indices (RIs), comparison with reference substances, and mass spectrometric data from the literature and the NIST library. In the case that MS data were too weak for unequivocal identification, aroma compounds were tentatively identified based on the remaining criteria. Linear RIs were determined after van Den Dool et al.[11] using a mixture of linear alkanes C_6−C_{20} under the same chromatographic conditions described above.

Sensory Analysis. The sensory analysis of DDG was performed twice by a group of at least 10 panelists from the Institute of Brewing and Beverage Technology on different days. The analysis took place in a room for sensory analysis at room temperature. Panelists were trained weekly after DIN 10961 in general odor and taste perception and especially on cereal-based food products. In a preliminary session, panelists were asked to define attributes, which describe the odor of DDG. Subsequently, in the following sessions, panelists evaluated the intensity of the prior defined attributes on a scale of 0 (not present) to 5 (very intensive).

RESULTS AND DISCUSSION

Sensory Analysis of DDG from Wheat. The incorporation of DDG in food products was a topic for many studies during the late 1980s, but unfortunately, the investigated products did not have a sufficient odor for being a popular food product.[1] Since no research has been done on the systematic study of flavor volatiles of DDG, a sensory analysis of the aroma profile was performed first.

For the development of an aroma profile, panelists were asked to list and evaluate perceptible odor impressions and the overall odor impression. In the following sessions, panelists rated these impressions in their intensity on a scale from 0 (not present) to 5 (very intensive). The three impressions caramellike, roasty, and brothlike were rated as most frequently mentioned impressions. The evaluation of the intensity during the following sessions emphasizes these results as it is shown in Figure 1 with seasoninglike and roasty, breadlike as the most

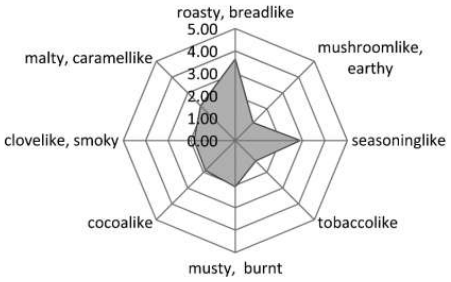

Figure 1. Flavor profile of DDG from wheat after sensory analysis; intensities of descriptors on a scale from 0 (not present) to 5 (very intensive), $n \geq 20$, results where averaged.

intensive odor impressions in DDG from wheat. High intensities could be determined for malty, caramellike and musty, burnt as well. During the production of DDG, fermentation, distillation and drying steps play an important role. In the distillation process, highly volatile compounds can be distilled together with ethanol, and new components can be formed during the drying process. Thus, odor-active compounds from Maillard reaction, byproducts of yeast fermentation, or thermal reaction compounds of the drying process are expected to contribute to the odor of DDG. The results of

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Table 1. Odor-Active Compounds in DDG from Wheat

odor-active volatiles identified with SPME, SDE, and SAFE					
odor-active compound	odor quality[a]	RI (DB-5)[b]	SPME	SDE	SAFE
butanoic acid[d]	cheesy	743	x	x	x
3-methylbutanoic acid[d]	cheesy	810	x	x	x
2-methylbutanoic acid[d]	cheesy, fruity	830	x	x	x
1-octen-3-one[c]	mushroomlike	978	x	x	x
1,5-octadien-3-one[c]	metallic	986	x	x	x
dimethyl trisulfide[d]	cooked, cabbagelike	995	x	x	x
2-phenylacetaldehyde[d]	flowery	1055	x	x	x
2-ethyl-3,6-dimethylpyrazine[d]	breadlike	1089	x	x	x
2-ethyl-3,5-dimethylpyrazine[d]	roasty	1095	x	x	x
2-methoxyphenol[d]	smoky	1102	x	x	x
3-hydroxy-4,5-dimethyl-2(5H)-furanone[c]	seasoninglike	1106	x	x	x
2-phenylethanol[d]	flowery	1124	x	x	x
(*Z*)-2-nonenal[c]	cucumberlike, fatty	1149	x	x	x
(*E*,*Z*)-2,6-nonadienal[c]	cucumberlike	1159	x	x	x
3-hydroxy-4-methyl-5-ethyl-2(5*H*)-furanone[c]	fatty, brothlike	1191	x	x	x
4-vinylphenol[c]	clovelike, phenolic	1222	x	x	x
2-methoxy-4-vinylphenol[d]	clovelike	1329	x	x	x
β-damascenone[d]	fruity, cooked applelike	1402	x	x	x
4-hydroxy-3-methoxybenzaldehyd[d]	vanillalike	1419	x	x	x
odor-active volatiles identified with specific technique (marked with an "x")					
odor-active compound	odor quality[a]	RI (DB-5)[b]	SPME	SDE	SAFE
acetic acid[d]	acetic	589	x		
2,3-butanedione[d]	buttery	600	x		
3-methylbutanal[d]	breadlike	675	x		
2,3-pentanedione[d]	mushroomlike, cheesy	707	x		
2-methylpropionic acid[d]	sweet	716		x	
hexanal[d]	green	798		x	
n.i.[e]	caramellike	883	x	x	
n.i.[e]	mushroomlike, seasoninglike	898		x	x
3-methylthiopropanal[d]	cooked potato	913	x	x	
2,5-dimethylpyrazine[d]	breadlike, roasty	915		x	
n.i.[e]	musty, burnt	920		x	
n.i.[e]	musty, earthy	926	x		
2-ethyl-5-methylpyrazine[d]	roasty	1011		x	
4-hydroxy-5-methyl-3(2*H*)-furanone[c]	caramellike, sweet	1039	x		x
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone[c]	caramellike	1054	x		x
n.i.[e]	caramellike, sweet	1083	x		
(*E*)-2-nonenal[d]	dusty, rancid	1164	x		
2-phenylacetic acid[d]	honeylike	1248	x		x
2-phenylethyl acetate[d]	flowery	1264		x	x
n.i.[e]	roasty, nutty	1283	x	x	
(*E*,*Z*)-2,4-decadienal[d]	fatty	1298		x	
(*E*,*E*)-2,4-decadienal[d]	fatty, rancid	1324		x	
3-methylindole[c]	fecal	1409	x	x	

[a]Odor quality perceived at the sniffing port. [b]RIs on a DB-5 column. [c]Tentatively identified by RIs, odor quality, and comparison with reference substances. [d]Identified by RIs, odor quality, and mass spectra obtained in EI. [e]n.i. = not identified.

sensory analysis indicate the presence of volatiles from thermal processing such as pyrazines (roasty and breadlike, burnt) or sugar degradation products (caramellike, seasoninglike). The analysis of odor-active volatiles was carried out in the next step to substantiate the results of the sensory evaluation.

Analysis of Odor-Active Volatiles in DDG. Sample preparation and isolation procedures applied before the analysis can determine the composition of aroma extracts. Since there is no knowledge concerning odor-active compounds in DDG, the sample preparation step is performed with multiple techniques to avoid misinterpretations and to work out a representative classification of odor-active volatiles. For this purpose, one headspace technique (HS-SPME) and two solvent-extraction techniques (SDE, SAFE) were carried out.

With the three applied methods, 42 odor-active compounds could be found in total in DDG from wheat. Nineteen of the 42 compounds were identified in accordance with the application of every specific method (upper part, Table 1). Twenty-three compounds (lower part, Table 1) could only be identified with one respectively two specific techniques and confirms the dependence of traceability and the sample preparation step. Classification of identified aroma compounds reveals four

Table 2. Peak Areas of Characteristic Mass Fragments of Selected Compounds after HS-SPME at Different Enrichment Conditions (1 and 6 h; 30 and 70 °C)

odor-active compound	RI (DB-5)[a]	*m/z*	30 °C, 1 h[b]	30 °C, 6 h[b]	70 °C, 1 h[b]	70 °C, 6 h[b]
acetic acid	589	60	1540.0	1890.0	1800.0	1470.0
2,3-butanedione	600	86	9.5	6.6	21.5	13.7
3-methylbutanal	675	58	47.8	28.0	19.0	30.0
2,3-pentanedione	707	100	5.7	2.7	10.0	3.9
butanoic acid	746	60	15.2	13.9	16.9	5.2
hexanal	798	82	7.1	2.6	5.0	3.5
3-methylbutanoic acid	810	60	53.2	35.7	19.0	10.8
2-methylbutanoic acid	830	74	31.3	30.4	14.7	7.9
dimethyl trisulfide	995	126	21.0	10.7	14.8	10.0
2-phenylacetaldehyde	1055	120	3.3	9.1	25.9	19.9
2-ethyl-3,5-dimethylpyrazine	1095	135	12.0	30.7	20.5	6.5
2-phenylethanol	1124	120	1050.0	1720.0	1760.0	1390.0
2-phenylacetic acid	1248	136	0.0	0.0	4.0	5.6
2-phenylethyl acetate	1287	104	18.2	96.9	113.0	55.4
2-methoxy-4-vinylphenol	1329	150	0.9	7.0	72.5	204.0
4-hydroxy-3-methoxybenzaldehyde	1419	151	0.0	24.9	90.2	307.0

[a]RIs on a DB-5 column. [b]Values show peak areas × 10^8.

furanones (3(2*H*)- and 2(5*H*)-furanones), four pyrazines (2,5-dimethylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-3,6-dimethyl-, and 2-ethyl-3,5-dimethylpyrazine), and six acids (acetic and butanoic acids, 2-methylpropanoic acid, 3- and 2-methylbutanoic acids, and 2-phenylacetic acid). Moreover, four phenolic compounds (2-methoxyphenol, 2-methoxy-4-vinylphenol, 4-vinylphenol, and 4-hydroxy-3-methoxybenzaldehyd), two sulfur-containing compounds (dimethyl trisulfide and 3-methylthiopropanal), and a number of aldehydes and ketones (2,3-butanedione, 3-methylbutanal, 2,3-pentanedione, hexanal, 1-octen-3-one, 1,5-octadien-3-one, 2-phenylacetaldehyde, (*E*)- and (*Z*)-2-nonenal, (*E*,*Z*)-(2,6)-nonadienal, (*E*,*E*)- and (*E*,*Z*)-2,4-decadienal, and *β*-damascenone) were determined. Besides 2-phenylethanol and 2-phenylethyl acetate, no alcohols and esters were determined. 3-Methylindole was determined as single N-heterocyclic compound.

Unequivocal MS data could not be obtained for 10 compounds ((*Z*)-2-nonenal, (*E*,*Z*)-2,6-nonadienal, 5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone, 4-vinylphenol, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, 1-octen-3-one, 1,5-octadien-3-one, 3-methylindole, 4-hydroxy-5-methyl-3(2*H*)-furanone, and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone), so the identification was based on the remaining criteria. Further, six odor-active regions in the GC chromatogram could not be identified by any of the applied methods, because of low concentrations and insufficient MS data.

Identification of Odor-Active Compounds in DDG from Wheat via SPME. To get a first impression of volatile compounds in a matrix, HS-SPME is a reasonable technique for a qualitative overview. Moreover, HS-SPME is a proven method for the analysis of volatiles in food and wheat products such as partially baked bread.[12,13] The application of SPME on DDG from wheat revealed 33 odor active regions in the gas chromatogram, of which 29 could be identified. The four compounds acetic acid, 2,3-butanedione, 3-methylbutanal, and 2,3-pentanedione could only be determined by HS-SPME, due to the coelution and overlay with diethyl ether used as the solvent in the distillation methods. Odor-active compounds eluting before or simultaneously with the solvent cannot be determined. Therefore, a headspace method complements information caused by solvent delay.

Furthermore, the isolation of flavor compounds by SPME reveals strong dependence of adsorption behavior on the SPME fiber from adsorption time and temperature as it is shown in Table 2. Low-temperature adsorption (30 °C) and prolonging of adsorption time from 1 to 6 h results in lower peak areas for highly volatile compounds (e.g., dimethyl trisulfide) and increased peak areas for medium and low volatile compounds (e.g., 4-hydroxy-3-methoxybenzaldehyde). The prolonging of adsorption time shifts the spectrum of volatiles to medium and low volatile compounds. High-temperature adsorption (70 °C) and prolonging of adsorption time leads to a reduction of peak areas of high volatile compounds (e.g., 2-phenylethyl acetate, 2-phenylethanol, and acetic acid). The odor perception of 4-hydroxy-5-methyl-3-(2*H*)-furanone, 3-methylindole, and an unknown compound with green, metallic odor at the sniffing port (RI_{DB5} = 1197) is enabled only at a longer adsorption time and a higher temperature (6 h and 70 °C). Moreover, prolonging of adsorption time to 6 h made the odor perception of 1,5-octadien-3-one, 2-phenylacetaldehyde, (*Z*)-2-nonenal, 2-phenylacetic acid, and two unknown compounds (RI_{DB5} = 1083, sweet, caramellike; RI_{DB5} = 1283, roasty, nutty) possible. Basically, an enhanced intensity of odor perception could be obtained by higher temperature and prolonging of adsorption time (70 °C and 6 h); nevertheless, artifact formation at these conditions cannot be excluded.

Identification of Odor-Active Compounds in DDG from Wheat via SDE. SDE, as developed by Likens and Nickersson[14] and modified by Schultz et al.,[8] is a method for continuous and concurrent vapor distillation and solvent extraction. For the isolation of volatiles from a food matrix, SDE still represents a standard procedure.[15−17] In the case of DDG from wheat, 32 odor-active areas could be located in total in the GC chromatogram obtained by an aroma extract derived by means of SDE, of which 29 could be identified. The isolation of volatile compounds with SDE revealed an extract with intensive roasty and breadlike aroma. Besides 2-ethyl-3,6-dimethyl- and 2-ethyl-3,5-dimethylpyrazine, two additional pyrazines (2-ethyl-5-methylpyrazine and 2,5-dimethylpyrazine) were determined. Since it is well known that pyrazines contribute to the roasty odor of processed food, the increased

number of pyrazines in the SDE extract might contribute to the intensified roasty odor.

Further, the aldehydes hexanal (green), (*E*,*Z*)- and (*E*,*E*)-2,4-decadienal (fatty, rancid) were detected in the SDE extract. Since these aldehydes were not detectable or perceptible with SPME or SAFE, the artifact formation during the distillation process cannot be excluded. Moreover, lipid oxidation products are enriched due to the temperature treatment during extraction. After isolation with SDE and in accordance to SPME, 3-(methylthio)-propanal, 3-methylindole, and two additional unidentified compounds (RI_{DB5} = 883, caramellike; RI_{DB5} = 1283, roasty) could be determined. In accordance to SAFE, SDE was able to determine (*Z*)-4-heptenal and 2-phenylethylacetat. In addition, an unidentified compound (RI_{DB5} = 920) was determined. Because of the musty and burnt odor quality, it might be an artifact. SDE was not able to isolate 2-phenylacetic acid, as well as the two furanones 4-hydroxy-5-methyl-3(2*H*)-furanone and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone. The good solubility in water leads to poor extractability and retention in the water matrix, which was already reported by Siegmund et al.[18] Since highly hydrophilic compounds are prevented from extraction out of the water suspension and thus remain in the extraction residue during SDE, an important part for a responsible aroma profile is missing. For this reason, SAFE was applied as an additional extraction procedure on DDG from wheat.

Identification of Odor-Active Compounds in DDG from Wheat via SAFE. SAFE as developed in 1999 by Engel et al.[9] was chosen as the second solvent-extraction method, since SAFE is known as a gentle isolation process preserving the native profile of odor-active compounds in a food product. An extract derived with SAFE generally neither contains or enriches oxidation products and thermal reaction compounds nor supports the formation of artifacts. The isolation of volatile compounds of DDG from wheat with SAFE revealed an extract with strong seasoninglike, breadlike, and spicy odor impression. By contrast to SDE, the aroma of the extract was less roasty and breadlike but showed a strong seasoninglike odor impression. Twenty-four odor-active areas were located in the gas chromatogram after injection of an extract derived via SAFE. Present results confirm that SAFE represents a gentle isolation process of volatile compounds, because Strecker aldehydes (e.g., 3-methylthiopropanal) and possible artifacts isolated via SPME and SDE could not be confirmed with SAFE under moderate conditions. Because of the native odor profile, the SAFE extract was chosen for further analysis.

In the last step of aroma analysis, the extract derived via SAFE was fractionated into an AF and NBF, to determine coelutions and improve the separation behavior on the capillary column. The fractionation revealed five additional odor-active areas in the GC chromatogram. One could be identified as 3-hydroxy-2-methylpyran-4-one (RI_{DB5} = 1123, caramellike), already identified as an odor-active volatile in the crust of wheat and rye bread.[19] Because of the coelution with 2-phenylethanol (RI_{DB5} = 1124) and the similar odor impression, 3-hydroxy-2-methylpyran-4-one could not be determined without fractionation. Four of the five additional compounds could not be identified due to unequivocal MS data.

Key Aroma Compounds in DDG from Wheat. To investigate key odorants in DDG from wheat, the distillate obtained by SAFE was analyzed by means of AEDA. Therefore, the gas chromatogram was compared to the flavor dilution (FD) chromatogram (Figure 2), and eight compounds with a

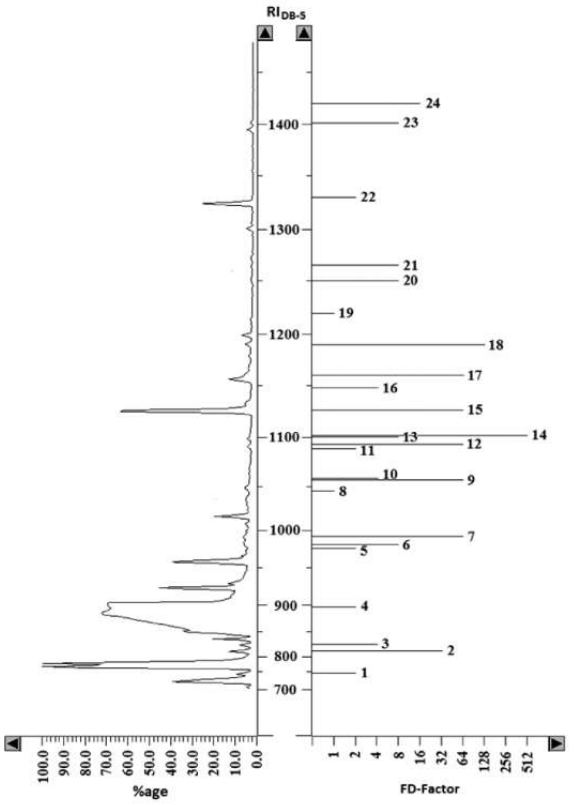

Figure 2. FD chromatogram compared to the gas chromatogram of a SAFE distillate from DDG from wheat; numbers indicate flavor-active compounds as identified in Table 3.

FD factor ≥ 32 could be determined. The highest FD factor of 512 was determined for 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon) with seasoninglike odor and a slightly lower FD factor of 128 for the ethyl-homologue 5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone (brothlike, spicy). These furanones were revealed as the most influential odor-active volatiles in DDG from wheat, which is in accordance with the results of the sensory evaluation shown in Figure 1. 3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone is the character impact compound of fenugreek and has also been identified in white and whole wheat flour and Bavarian wheat beer as an important odor-active volatile with high FD factors of 256 for the formers and 512 for the latter.[20−22] Blank et al.[20] proposed that, in the case of fenugreek, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone originates from thermally induced oxidative deamination of 4-hydroxyisoleucine and its lactone 3-amino-4,5-dimethyl-3,4-dihydro-2(5*H*)-furanone as shown in Figure 4. Moreover, it was revealed that the yield is improved by boiling several hours at acidic conditions. Regarding the thermal conditions of the drying process and the acidic pH of 3.8 of DDG, it can be concluded that the formation of 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone can be enhanced.

Further, a high FD factor of 64 could be determined for 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol) with caramellike odor. 3(2*H*)-Furanones are an important group of hydroxyl furanones, usually responsible for the sweet and caramellike odor impression of processed food. 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone could already be identified in wheat

dx.doi.org/10.1021/jf503281x | J. Agric. Food Chem. XXXX, XXX, XXX−XXX

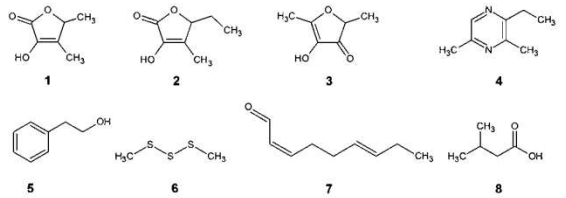

Figure 3. Chemical structures of key aroma compounds in DDG obtained by AEDA with FD factor ≥ 32: 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**1**), 3-hydroxy-4-methyl-5-ethyl-2(5*H*)-furanone (**2**), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**3**), 2-ethyl-3,5-dimethylpyrazine (**4**), 2-phenylethanol (**5**), dimethyl trisulfide (**6**), (*E*,*Z*)-2,6-nonadienal (**7**), and 3-methylbutanoic acid (**8**).

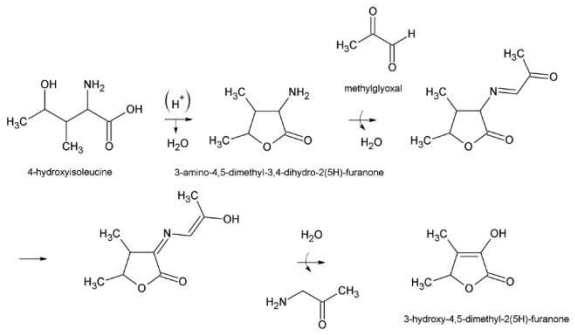

Figure 4. Formation pathway leading to 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone from 4-hydroxyisoleucine, modified according to Blank et al.[20]

beer and wheat bread crumb.[19,22] The formation pathway of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone in wheat bread crust is known to descend from yeast as the most important source, with fructose-1,6-diphosphate as the predominant precursor.[23] The roasty and breadlike odor impression revealed during sensory analysis can be attributed to 2-ethyl-3,5-dimethylpyrazine with a FD factor of 64. Besides the seasoning and caramellike odor impression, the roasty character represents a central odor impression of DDG. It is well known that a number of volatile pyrazines contribute to the flavor of thermal processed foods originating from nonenzymatic browning of food products. Schieberle and Grosch identified 2-ethyl-3,5-dimethylpyrazine as an odor-active compound in wheat bread crust[19] and rye bread crust,[24] with FD factors of 16 and 64, respectively. Moreover, 2-ethyl-3,6-dimethylpyrazine with a FD factor of 4 and the identification of 2,5-dimethylpyrazine and 2-ethyl-5-methylpyrazine substantiate the contribution of pyrazines to the roasty odor impression of DDG from wheat. With a FD factor of 32, dimethyl trisulfide, the cabbagelike odor typically arising in plants of the genera *Brassica* and *Allium*, plays an important role in DDG odor as well. In processed wheat products, dimethyl trisulfide has not been found as a relevant key odorant by now. Nevertheless, Lee at al. showed that the analysis of volatiles from a wheat gluten hydrolysate thermally processed with glucose or fructose revealed dimethyl trisulfide as a product from Maillard reaction.[25] Because of the high FD factor and very low odor threshold of 0.01 μg/L in H_2O, dimethyl trisulfide could be a possible source for the off flavor in DG from wheat described previously.[26] The differences in DDG flavor to the flavor of wheat products might occur especially due to the presence of dimethyl trisulfide, which is not known as a key odorant of processed

Table 3. Odor-Active Volatiles and Corresponding FD Factors Obtained by AEDA in DDG from Wheat

no.[a]	odor-active compound	odor quality[b]	RI (DB-5)[c]	FD factor[d]
1	butanoic acid[f]	rancid	743	2
2	3-methylbutanoic acid[f]	cheesy	810	32
3	2-methylbutanoic acid	cheesy	830	4
4	n.i.	spicy, brothlike	900	2
5	1-octen-3-one[e]	mushroomlike	978	2
6	1,5-octadien-3-one[e]	metallic	986	8
7	dimethyl trisulfide[f]	sulphurous	995	32
8	4-hydroxy-5-methyl-3(2*H*)-furanone	caramellike	1045	1
9	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone[e]	caramellike	1054	64
10	phenylacetaldehyde[f]	flowery	1055	4
11	2-ethyl-3,6-dimethylpyrazine[f]	breadlike	1089	4
12	2-ethyl-3,5-dimethylpyrazine[ff]	roasty	1095	64
13	2-methoxyphenol[f]	smoky	1102	8
14	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone[e]	seasoninglike	1100	512
15	2-phenylethanol[f]	flowery	1126	64
16	(*Z*)-2-nonenal[e]	cucumberlike	1160	2
17	(*E*,*Z*)-2,6-nonadienal[f]	fatty	1189	64
18	5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone[e]	brothlike	1196	128
19	4-vinylphenol[f]	phenolic, sweet	1222	1
20	2-phenylacetic acid[f]	honeylike	1250	8
21	2-phenylethyl acetat[f]	flowery	1263	8
22	2-methoxy-4-vinylphenol[f]	clovelike	1329	2
23	β-damascenone[f]	applelike	1404	8
24	4-hydroxy-3-methoxybenzaldehyd[f]	vanillalike	1422	4

[a]Numbering refers to Figure 2. [b]Odor quality perceived at the sniffing port. [c]RIs on a DB-5 column. [d]Flavor dilution factor. [e]Tentatively identified by RIs, odor quality, and comparison with reference substances. [f]Identified by RIs, odor quality, and mass spectra obtained in EI; n.i. = not identified.

wheat products by now. 2-Phenylethanol with a FD factor of 64 is also one of the eight most important odor-active volatiles in DDG and consequently contributing to its sweet and floral odor. It is formed as byproduct in the fermentation of yeasts by enzymatic reactions via Ehrlich pathway.[27,28] The presence of 2-phenylethanol in processed wheat products is already known, since it was revealed as an important odor-active volatile in wheat bread crumb.[29] Likewise, 3-methylbutanoic acid with a FD factor of 32 was enlightened as an important odor-active volatile in DDG from wheat. Together with 2-methylbutanoic acid, the two methyl-substituted butanoic acids are responsible for the rancid off flavor in wheat bread.[30] (*E*,*Z*)-2,6-Nonadienal, a degradation product of linolenic acid, is another of the most important odorants of DDG from wheat with a FD factor of 64. It is already identified in wheat bread crust and white wheat flour.[19,21] A number of other unsaturated aldehydes such as (*E*)-/(*Z*)-2-nonenal and (*E*,*Z*)-/(*E*,*E*)-2,4-decadienal were also identified in DDG from wheat. These unsaturated aldehydes are well known to contribute to the fatty and rancid odor impression.

In summary, 42 odor-active compounds could be identified in total in DDG from wheat. Ranges of concentrations and odor activity values of selected important odor-active volatiles can be seen in Tables 4 and 5. Eight compounds were determined as key aroma compounds with a FD factor ≥ 32 as shown in Figure 3. These eight key odorants of DDG from wheat point out that the overall aroma of DDG is based on reaction compounds and precursors from thermal food processing, in particular Maillard reaction and Strecker degradation. This is also supported by a view on the production process of DDG. Regarding the further processing of the yeast fermented grain matrix, the mash is distilled for separation of ethanol at 78 °C. After a temporary storage (75−80 °C, 2−8 h), the thickening process of the thin stillage to syrup (55−65 °C, 2−4 h) follows. Subsequently, the main drying process takes place, where the stillage is dried at 100−105 °C for 1−3 h. These conditions markedly favor the formation of oxidation products and the continuation of Maillard and Strecker reactions. Aroma volatiles and key aroma compounds in DDG from wheat show high compliance to wheat processed products such as white wheat bread. DDG represents a thermal processed wheat product, which is supported by its composition of flavor volatiles.

Table 4. Odor-Activity Values Greater Than 1 of Selected Compounds in DG from Wheat

odor-active compound	odor quality[a]	RI (DB-5)[b]	OAV[c]
butanoic acid	cheesy	743	3.0
3-methylbutanoic acid	cheesy,	810	15.5
2-methylbutanoic acid	cheesy, fruity	830	4.55
dimethyl trisulfide	cooked, cabbagelike	995	965.0
2-phenylacetaldehyde	flowery	1055	22.0
2-ethyl-3,5-dimethylpyrazine	roasty	1095	90.5
2-methoxyphenol	smoky	1102	1.8
2-phenylethanol	flowery	1124	8.7
2-phenylethyl acetate	flowery	1264	2.7
2-methoxy-4-vinylphenol	clovelike	1329	2.3
β-damascenone	fruity, cooked applelike	1402	50.0

[a]Odor quality perceived at sniffing port. [b]RIs on a DB-5 column. [c]Odor-activity value based on odor thresholds known from literature and concentrations calculated with methyl decanoate as the internal standard.

Table 5. Concentrations of Selected Compounds in DDG from Wheat

odor-active compound	odor quality[a]	RI (DB-5)[b]	conc[c] (μg/kg)
2-methylpropionic acid	sweet	716	>100
butanoic acid	rancid	746	>100
hexanal	green	798	>100
3-methylbutanoic acid	cheesy	810	>100
2-methylbutanoic acid	cheesy, fruity	819	>100
2,5-dimethylpyrazine	breadlike, roasty	915	>100
dimethyl trisulfide	cooked, cabbagelike	995	10−100
2-phenylacetaldehyde	flowery	1055	10−100
2-ethyl-3,6-dimethylpyrazine	breadlike	1089	1−10
2-ethyl-3,5-dimethylpyrazine	roasty	1095	10−100
2-methoxyphenol	smoky	1102	1−10
2-phenylethanol	flowery	1124	>10 000
2-phenylacetic acid	honeylike	1248	10−100
2-phenylethyl acetate	flowery	1264	10−100
2-methoxy-4-vinylphenol	clovelike	1329	10−100
β-damascenone	fruity, cooked applelike	1402	1−10
4-hydroxy-3-methoxybenzaldehyde	vanillalike	1419	10−100

[a]Odor quality perceived at sniffing port. [b]RIs on a DB-5 column. [c]Concentration in μg/kg.

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Notes
The authors declare no competing financial interest.

ABBREVIATIONS USED

DG, distiller's grains; DDG, dried distiller's grains; HS-SPME, headspace solid-phase microextraction; SAFE, solvent-assisted flavor evaporation; SDE, simultaneous distillation extraction; AEDA, aroma extract dilution analysis; GC-O/MS, gas chromatography-olfactometry/mass spectrometry; AACC, American Association of Cereal Chemists; PDMS, polydimethylsiloxane; NBF, neutral-basic fraction; AF, acidic fraction; RI, retention index; DIN, Deutsches Institut für Normung; FD, flavor dilution

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2.4 MECHANISMS BEHIND DISTILLER'S GRAINS IMPACT ON WHEAT DOUGH AND BREAD QUALITY

Reproduced with permission from Roth, M., Döring C., Jekle, M., Becker, T.: Mechanisms behind distiller's grains impact on wheat dough and bread quality, Food and Bioproducts Technology, 9 (2016), 274–284. DOI: 10.1007/s11947-015-1620-y

Food Bioprocess Technol
DOI 10.1007/s11947-015-1620-y

ORIGINAL PAPER

Mechanisms Behind Distiller's Grains Impact on Wheat Dough and Bread Quality

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Received: 10 April 2015 / Accepted: 5 October 2015

Abstract Distiller's grains, by-product from ethanol production, can be a new source for nutritionally enriched bakery products, particularly because of its high amount of dietary fibre and protein. Ingredients rich in fibre provoke challenges to the dough and bread system; therefore, mechanisms behind dried distiller's grains (DDGs) impact on wheat bread must be evaluated. So, dough and bread characteristics were analysed in bread containing 0–20 % DDG, and effects of pH, particle size and furfural as DDG metabolite were studied. As a result, wheat bread incorporating DDG provides smaller volume from 20 to 45 %, firmer crumb up to a factor of 6 and reduced springiness up to 10 %. However, pH adjustment balanced the negative influence, and the low pH of DDG was revealed as the most influential parameter. The variation of particle sizes could not influence dough or bread characteristics significantly. While the low pH of DDG and the high amount of dietary fibre do not completely explain the negative impacts, inhibiting effects on the activity of *Saccharomyces cerevisiae* were evaluated. DDGs contain 2.7 ppm furfural as a consequence of the drying process, so its role as inhibiting compound was investigated in a model suspension and dough. It was confirmed that furfural is contributing to structure weakening effects in dough.

Keywords Fibre enrichment · Dough development · Rheofermentometer · CO_2 · Furfural · Dough weakening

Highlights • DDG is a sustainable opportunity for dietary fibre and protein enrichment of bread.
• Low pH of DDG hinders fermentation, what can be balanced by pH adjustment.
• Thermal treatment during drying of DG induces formation of furfural.
• Furfural inhibits fermentation and leads to deficits in texture quality parameters.

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Abbreviations

AACC	American Association of Cereal Chemists
$Ba(OH)_2$	Barium hydroxide
$BaCO_3$	Barium carbonate
BSG	Brewers spent grain
CO_2	Carbon dioxide
DG	Distiller's grains
DDG	Dried distiller's grains
DDT	Dough development time
DS	Dough softening
DST	Dough stability
FU	Farinograph units
HCl	Hydrochloric acid
Hm	Maximum dough height
H'm	Maximum height of gaseous release
HPLC	High pressure liquid chromatography
ICC	International Association for Cereal Science and Technology
$NaCO_3$	Sodium carbonate
$NaHCO_3$	Sodium hydrogen carbonate
NaOH	Sodium hydroxide
TPA	Texture profile analysis
UV	Ultraviolet
WA	Water absorption

Published online: 14 October 2015

Introduction

Due to increasing amounts of by-products and waste materials of the food industry, suitable solutions and conscious effort to the use of raw materials are needed. From the nutritional and sustainable point of view, some of these by-products are not exhausted and consequently useful for further food applications. Therefore, distiller's grains (DG) can be a source for enriching food products with dietary fibre and protein, since the amounts in dry matter are described up to 45 % for neutral detergent fibre and 35 % for protein (Rasco and Rubenthaler 1990).

DG belong to agricultural waste and commonly arise during the production process of fuel or beverage ethanol of cereal grains like wheat or corn. The cereal by-product is prevalently used as feed additive or material for biogas plants, but the main part remains as waste material, where waste management and disposal problems become more relevant. In the production process of ethanol from raw materials rich in carbohydrates, starch is converted to glucose during mashing, fermented to ethanol and distilled; subsequently, the remaining product is dried to prolong its shelf life (dried distiller's grains, DDG). Except starch and fermentable carbohydrates, DDG contain all ingredients of the whole grain in a concentrated form, especially high amounts of protein and dietary fibre (Rosentrater and Krishnan 2006; Liu 2011). Besides, DDG contain a significant amount of dead yeast cells and intracellular compounds released after autolysis of the yeast. The nutritional properties of DDG and the oversupply on the market provide a new potential for commercial food uses, particularly in baked goods. DDG is the remnant of the wheat kernel after utilisation of the starchy endosperm and mainly consist of outer grain layer components. Its composition is comparable to bran or other cereal by-products like brewers spent grain (BSG) and thus might provide similar effects to the dough and bread system. The addition of high fibre fractions to bakery products is connected to some challenges, mostly associated to end product quality and appearance, texture and taste (Ktenioudaki et al. 2012). During the last 20 years, especially during 1980 and 1990, there have been several trials for using DDG as food ingredient and as ingredient in bakery products, but the results were not sufficient, because there was lack of acceptance regarding texture and flavour (Rasco and Rubenthaler 1990; Rasco et al. 1989; Reddy et al. 1986; Tsen et al. 1983). As an example, baguettes containing DDG provide an aftertaste and are more sour, salty and bitter than the control (Rasco et al. 1989), and DDGs in blended foods are unsuitable because of poor flavour (Bookwalter et al. 1984). Nevertheless, typical off-flavours in DDG could not be identified so far (Roth et al. 2014).

Up to now, the application of DDG as a food ingredient still is not usual, although a steadily growing interest in food products providing a health benefit through functional ingredients can be observed (Arvanitoyannis and Van Houwelingen-Koukaliaroglou 2005). By now, mechanisms behind the negative impacts of DDG on food and especially bakery products are not fully explained yet. Targeted knowledge can help to understand quality loses caused by the addition of high-fibre and high-protein by-products like DDG, bran or BSG. It can provide new possibilities for including such by-products in food and improving the final product quality. This paper enlightens the background of influences of wheat-based DDG on a wheat dough and bread matrix. The evaluation of its potential as functional ingredient in bakery products by producing a fibre and protein-enriched product with comparable textural and sensory properties to those of traditional bakery products can provide sustainable solutions for the utilisation of this by-product. For this reason, amounts of 5, 10, 15 and 20 % wheat flour were replaced by DDG from wheat, and effects of DDG on dough and bread texture, volume, and sensory qualities were assessed. The effect of particle size and low pH of DDG were studied for a better understanding of the quality loses.

Material and Methods

Ingredients for Dough and Bread Preparation

Distiller's Grains Dried distiller's grains from wheat were kindly provided by Euro-Alkohol GmbH (Lüdinghausen, Germany). DDG was composed of 38.2 % protein (AACC 46–16, N×6.25), 3.6 % fat (AACC 30–25), 3.5 % ash (AACC 08–01), 46.8 % total dietary fibre (AACC 32–05) on dry matter and 7.8 % water (AACC 44–01) and is characterised by a water retention capacity of 54.1 % (AACC 56–11). DDG was milled to particles of different sizes (<250, <500, <750 and unground <1250 μm) using a Ultra Centrifugal Mill of type ZM200 from Retsch (Haan, Germany) at 5000–6000 rpm.

Wheat Flour Wheat flour type 550 was kindly provided by Rosenmühle (Ergolding, Germany) and was composed of 1.2 % fat (AACC 30–25), 0.6 % ash (AACC 08–01) on dry matter and 14.1 % water (AACC 44–01). Further ingredients were sodium chloride (NaCl, Südsalz GmbH, Germany) and dry yeast of species *Saccharomyces cerevisiae* (fermipan red, Casteggio Lievitii srl, Casteggio, Italy). Analysis of flour and DDG was conducted in duplicate and presented as mean.

Chemicals

Sodium hydrogen carbonate ($NaHCO_3$, p.a.), sodium carbonate ($NaCO_3$, 99.9 %), sodium hydroxide (NaOH, 99.9998 %) and phenolphthalein were obtained from Merck (Darmstadt, Germany). Furfural (>98 %) was obtained from Sigma-

Aldrich (Taufkirchen, Germany). Methanol (100 %), formic acid (>99.5 %), hydrochloric acid (HCl, 37.0 %) and acetonitrile (>99.9 %) were from VWR International (Darmstadt, Germany). Barium hydroxide was purchased from AppliChem ($Ba(OH)_2$, Darmstadt, Germany) in p.a. quality.

Preparation of Dough and Bread Samples

Preparation of dough and bread samples was performed according to the procedure of Schirmer et al. with slight modifications (Schirmer et al. 2011). The recipe for control wheat bread preparation was 60.0 % water, 2.0 % sodium chloride and 1.6 % dry yeast based on 100-g wheat flour (corrected to 14 % moisture). To evaluate the influence of DDG on a control wheat bread, different amounts of wheat flour (0, 5, 10, 15 and 20 %) were replaced by DDG of four different particle sizes (<250, <500 and <750 μm and unground <1250 μm). Water temperature was adjusted for dough end temperature of 28 °C, and water amount was corrected for each DDG content and particle size for a maximum dough consistency of 500 Farinograph Units (FU). All ingredients were blended for 120 s at 100 rpm and mixed for 360 s at 200 rpm in a spiral kneader type 12 A-3 (Diosna Dierks & Söhne GmbH, Osnabrück, Germany). After resting for 15 min at room temperature, 250-g pieces of dough were hand-molded, weighed in baking tins, kept in a proofing chamber for 30 min (30 °C, 80 % relative humidity) and subsequently baked for 30 min at 230 °C in a multiple hearth-oven (Matador Store 12.8, Werner & Pfleiderer Lebensmitteltechnik Sachsen GmbH, Sohland, Germany). Each recipe was performed twice on two different days, providing eight independent bread loaves for analysis (n=8).

Evaluation of Dough Water Absorption and Structural Characteristics

Optimum water absorption and structural characteristics of different dough were evaluated according to AACC method 54–70.01 using a doughLAB with torque measuring Z-kneader (doughLAB; Perten Instruments, Germany). Dough development time (DDT) characterises the time interval needed to reach the maximum (torque) of the kneading curve. Time between exceeding the 500 FU-line and the first fall below 500 FU is described by dough stability (DST). Dough softening (DS) represents the decrease of consistency 12 min after reaching the maximum of the curve. All dough were analysed in duplicate.

Evaluation of Dough Development

Dough development and gaseous release characteristics were studied using a Rheofermentometer F3 (Chopin, Tripette & Renaud, Villeneuve-la-Garenne, France). All ingredients were mixed in the same manner as for the baking trials using a doughLAB z-kneader at mixing speed of 63 rpm. Subsequently after kneading, dough samples of 315.0 g were placed into the fermentation basket and the proofing chamber was sealed hermetically. Dough development and gaseous release of two samples were analysed at 30 °C for 180 min (n=2). Characteristics of interest were maximum dough height (Hm), maximum height of gaseous release (H'm) and total CO_2 volume ($V(CO_2)$).

Evaluation of Bread Characteristics

Volume of bread samples was analysed after the baking process and a cooling time of 120 min on a wooden rack using a laser-based volumeter (BVM-L370, TexVol Instruments, Vieken, Sweden) according to AACC method 10–14.01. The specific bread volume then was calculated as quotient of volume and weight of each bread sample. Crumb characteristics firmness and springiness were evaluated using a Texture Profile Analyzer (TPA) type TVT-300 XP (TexVol Instruments, Vieken, Sweden) according to AACC method 74-09-01 with slight modifications. Two bread slices of 12.5 mm each were compressed by 40 % with a plunger of 25-mm diameter in two subsequent cycles with 15-s intermediate rest. Firmness was defined as peak force of the first compression cycle. Springiness was defined as ratio of distance recorded during second compression to that of first compression and represents how a product springs back after being deformed. Four replicates of two different baking sets were analysed and averaged (n=8).

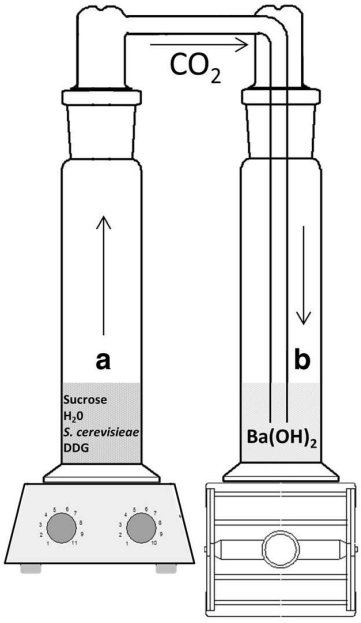
Quantification of Furfural by HPLC-UV

Furfural in DDG was extracted with slight modifications according to the extraction procedure of Durmaz and Gökmen (2010). Therefore, a 0.3-g sample of DDG was transferred into a 1.5-ml Eppendorf test tube and mixed with 1 ml 70 % methanol in water (n=3). Furfural was extracted in a vortex mixer for 1 min, and the mixture was centrifuged at 9500×g for 5 min in an Eppendorf mini spin centrifuge. After centrifugation, the liquid phase was separated, and the extraction was repeated two times with 0.6 ml of 70 % methanol in water under the same conditions. The combined upper phases were filtered through a 0.45-μm Chromafil PET 45/25 membrane filter (Macherey Nagel, Düren, Germany). Ten microliter of the final extract were injected to a Dionex U3000 HPLC system equipped with a quaternary pump, an auto sampler, a temperature-controlled column oven and a diode array detector. A Phenomenex Luna C18 column (Luna 5 μm C18(2) 100 Å, 250×4.6 mm) was used as stationary phase with an isocratic mixture of 1.0 % aqueous formic acid and acetonitrile (90/10, v/v) used as mobile phase for final UV detection of furfural at 270 nm.

Quantification of CO_2 in a Model System

To exclude influences caused by other unknown compounds in dough, the amount of total CO_2 produced by *S. cerevisiae* during fermentation was determined in a model suspension in the absence and presence of DDG. Arising CO_2 during fermentation was trapped into a solution of barium hydroxide where CO_2 precipitates as barium carbonate. After this simulated fermentation in a modified gas-washing bottle system (Fig. 1), CO_2 was determined by back titration of remaining barium hydroxide with 0.1 M HCl and phenolphthalein as indicator. For determination of CO_2 in samples, bottle A was filled with a suspension of 1.0 g *S. cerevisiae*, 100 ml of 8.0 % aqueous solution of sucrose and 0.0–5.0 % of DDG. Bottle B was filled with 40 ml of 1 % aqueous $Ba(OH)_2$. The simulated fermentation was carried out 90 min at 30 °C. All fermentations were carried out in duplicate. After fermentation, 10 ml of the $Ba(OH)_2$/$BaCO_3$ suspension was used for titration with 0.1 M HCl and phenolphthalein as indicator (n=2). For preparation of a blanc sample, 10 ml of 1.0 % aqueous $Ba(OH)_2$ was spiked with phenolphthalein and titrated under the same conditions. The fermentation of mixtures was carried out in duplicate. The calculated CO_2 amounts are considered as semi-quantitative, since a CO_2 free atmosphere and contaminations cannot be excluded. The amount of CO_2 then can be calculated according to the following formula.

$$m(CO_2) = [c(HCl) \times V(HCl)\,blank) - (c\,(HCl) \times V(HCl)\,sample)] \times M(CO_2)$$



a $C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2\uparrow + 6\,H_2O$

b $Ba(OH)_2 + CO_2 \rightarrow BaCO_3\downarrow + H_2O$

Fig. 1 Schematic diagram of CO_2 determination in a model suspension after fermentation of 90 min at 30 °C. Arising CO_2 (bottle A) was trapped in $Ba(OH)_2$ (bottle B) and precipitated as $BaCO_3$

Statistical Analysis

Statistical analysis was performed with Prism 5 (Version 5.03, GraphPad Software, Inc.). Significant differences were detected with one-way analysis of variance (ANOVA, $p<0.05$). The Tukey's test was used to detect statistical differences between means ($p<0.05$).

Results and Discussion

Water Absorption and Structural Characteristics of Dough

Dough development is one of the key stages within the breadmaking process and understanding its characteristics in that early stage of manufacturing can help to comprehend subsequent deficits of the final product. For this reason, the characteristics of dough development of DDG-enriched dough were analysed by doughLAB, and results are shown in Table 1.

As anticipated, water absorption (WA) of DDG-enriched dough increased linear with growing amount of wheat flour replaced by DDG from lowest WA of 59.4±0.3 % to highest WA of 63.8±0.8 % for 20 % DDG. Since DDGs contain about 46.8 % total dietary fibre, the addition of 10 % DDG leads to an increased content of 4.04 % more fibre in the dough matrix based on dry matter of 100 % flour/DDG mixture. The increased WA is typically caused by the high amount of fibre in DDG competing with starch and protein for free water in the matrix. Already in the late 1980s, Lineback and Rasper determined fibre as predominant source for increased water absorption (Lineback and Rasper 1988). Due to an increased number of hydroxyl groups, fibre absorbs more water than starch and protein, which allows more interaction with water via hydrogen bonds (Rosell et al. 2001).

The competition of fibre for free water can lead to incomplete hydration of starch and gluten and thus favour deficits during processed dough development, which is also supported by differences in dough development time (DDT). While DDT for standard wheat bread is 2.5±0.3 min, prolonged DDT for DDG-enriched dough between 3.3 and 5.9 min can be observed. The effects on DDT are independent from particle sizes or amount of replaced DDG, supporting the

Table 1 Dough characteristics (water absorption, dough development time, dough softening and dough stability) of 0, 10, 15 and 20 % DDG-enriched matrices with four different particle sizes (unground, <750, <500, <250 μm) as analysed by dough lab

	Water absorption (WA) (%)	Dough development time (DDT) (min)	Dough softening (DS) (FE)	Dough stability (DST) (min)
0 % DDG	59.4±0.3a	2.5±0.3a	30.1±2.6a	15.7±0.3a
10 % DDG				
Unground	61.1±0.1b	5.9±0.1b	116.6±1.8b	6.9±0.2b
<750 μm	61.1±0.7ab	5.0±0.1a	157.9±3b	6.2±0.2bcd
<500 μm	61.2±0.1b	5.0±0.2a	150.4±1.7b	6.5±0.2bcd
<250 μm	60.6±0.0b	3.3±2.1a	135.4±13b	6.5±0.1bcd
15 % DDG				
Unground	61.5±0.2bc	5.5±0.5b	143.2±38b	6.5±0.5bcd
<750 μm	62.5±0.2c	5.3±0.4b	167.3±7.2b	5.5±0.1cd
<500 μm	62.6±0.1c	5.0±0.2a	153.3±2.5b	5.9±0.1cd
<250 μm	61.7±0.2bc	4.9±0.2a	145.9±12b	6.6±0.2bc
20 % DDG				
Unground	62.7±0.8cd	5.1±1.4b	129.6±31b	5.8±0.3cd
<750 μm	63.4±0.4de	4.8±0.3a	142.1±5.4b	5.0±0cd
<500 μm	64.0±0.0e	4.7±0.1a	144.9±4.3b	6.0±0.2cd
<250 μm	63.8±0.8d	4.4±0.4a	156.1±6.4b	5.5±0.1cd

Replicates are means with standard deviation (n=2); different letters indicate significant differences between means in the same column (p<0.05)

theory that there is no significant relationship between particle size and dough characteristics. According to Auffret et al., there must be an influence of different particle sizes, since lower values for water absorption can be observed with increasing particle sizes (Auffret et al. 1994). Nevertheless, this phenomenon could not be confirmed in the present study, since no significant differences could be detected among different particle sizes. As also observed by Zhang and Moore, a connection between particle size and water absorption could not be found (Zhang and Moore 1997). However, particles in general can interfere the gluten network reaggregation and destabilise the dough system during prolonged kneeding (Noort et al. 2010). Additionally, dough softening increases rapidly to 150 FE for DDG-enriched bread, whereas 0 % DDG dough represents a system more stable with dough softening values of only 30 FE.

Moreover, the replacement of flour by DDG leads to reduced dough stability times. While the 0 % DDG dough reaches stability times up to 15 min, DDG-enriched bread suffers from poor dough stabilities less than 6.9 min. This can be attributed to a less stable and diluted gluten network, so DDG particle addition accelerates gluten break down. Tsen et al. observed lower dough stabilities for 10 and 20 % DDG-enriched bread than breads made from white flour and concluded that DDG provide weakening effects to the dough system and behaves more like whole wheat flour than white flour (Tsen et al. 1983). However, possible reasons were not revealed, and a systematic study has not been done so far. For the cause study of DDG's destabilising effects to the dough system, a closer look to the gaseous development and gas holding properties is needed.

Dough Development of DDG-Enriched Bread

Total CO_2 Volume

The level of CO_2 is the foundation of a respectable loaf volume and spongy crumb structure and contributing to this important quality feature of bread. Too large amounts of CO_2 can cause a structure collapse during baking, too small amounts lead to poor volumes and a hard and dense crumb. Fractions rich in fibre are well known to reduce specific loaf volumes as a consequence of insufficient amounts of arising CO_2 during fermentation. So, the influence of DDG on fermentation parameters such as CO_2 formation, total CO_2 volume and gas holding parameters was investigated by the Rheofermentometer.

While the total CO_2 volume of a 0 % DDG control bread is 1398 mL±6 mL, the amount of total CO_2 formed during fermentation decreases linear with increasing amount of DDG (Table 2). Lowest amounts of 1089±3 mL CO_2 could be found for 20 % DDG replacement, not dependent on the particle size used. Already, the replacement of 10 % DDG shows a significant decrease of CO_2 volume (p<0.05), which can be attributed to a number of reasons caused by the addition of outer grain layer components in combination with simple

Table 2 Dough characteristics (Hm, H'm and total CO_2 volume) of 0, 10, 15 and 20 % enriched DDG dough, corresponding dough with adjusted pH to 0 % DDG dough and dough spiked with furfural as analysed during 180 min of fermentation by Rheofermentometer

	Hm (mm)	H'm (mm)	Total CO_2 volume (ml)
0	59.8±0.3a	72.7±1.4a	1398±6ab
10	43.4±0.8b	59.1±1.6a	1232±10b
10pH+	51.1±2.8ab	72.3±1.2a	1473±17a
15	36±2.5b	52.9±4b	1180±59b
15pH+	40.4±2.6b	66.1±5.4ab	1339±61b
20	27.3±0.9c	55.6±0.6bc	1089±3b
20pH+	33.2±0.1c	53.3±1.6bc	1096±18b
fur10+	59.15±0.8a	66.1±3.5a	1366±72a

Replicates are means with standard deviation (n=2); different letters indicate significant differences between means in the same column (p<0.05)

pH+ pH of dough standardised to 0 % DDG dough, *fur10+* control dough spiked with furfural in equivalent amount to 10 % DDG dough

gluten dilution effects. According to Wang et al., the total amount of gluten is reduced due to the partial exchange of flour on the one hand; additionally, properties of the gluten network are influenced on the other hand (Wang et al. 2002). Already in the late 1960s, Jones und Erlander noticed an interaction between fibre structure and wheat proteins (Jones and Erlander 1967). Due to these interactions, free expansion of the gluten network during fermentation can be hindered, and it becomes harder and less elastic (Wang et al. 2002). Such interactions also might take place in DDG-enriched dough, since DDG used in the present study contains about 46 % fibre.

Another influential factor of DDG and other outer grain layer supplements can be aleurone intracellular compounds like alkylresorcinols, phytate or glutathione, which are released during the grinding process caused by the breakage of aleurone cells (Noort et al. 2010; Rosa-Sibakov et al. 2015). Aleurone cells are not the only source of glutathione, since autolysed yeast cells can also release glutathione into their environment. In 2015, Verheyen et al. confirmed a relationship between glutathione and structure weakening effects in wheat dough (Verheyen et al. 2015). In this study, standard dough of wheat flour was used, and dead yeast cells of dry yeast used as leavening agent were determined as responsible source. The content of dead yeast cells so can determine the amount of glutathione being released to the fermentation medium, consequently contributing to the degree of polymerisation of the gluten network. DDG is the remnant of ethanol production after fermentation of glucose by yeast, and consequently, DDG might contain a significant amount of dead yeast cells. So, glutathione in DDG, originating from aleurone cells or as intracellular compound released after autolysis of yeast might also contribute to the destabilising effects of DDG to wheat dough.

Gaseous Development During Fermentation

For an acceptable end product quality, not only total CO_2 but also even gaseous development is an important criterion. It is known from literature that gluten reveals its functionality mainly during the dough phase, so dough phase and especially gas holding properties must be in the focus of this study due to their strong influence on bread volume and final bread quality (Gujral and Singh 2000; Jekle and Becker 2012).

Figure 2a shows the development of CO_2 during 180 min of fermentation of 0 % DDG control dough related to 10, 15 and 20 % DDG dough of particle sizes <500 μm. The gaseous development curves of DDG-enriched dough process markedly less steep than the one of 0 % DDG dough, which might be attributed to a decelerated fermentation, diluted gluten and especially fibre/gluten interactions weakening the gluten network. Decreasing maximum dough height and maximum gaseous release with increasing amounts of DDG can also be observed (Table 2). The addition of 15 % DDG reduces maximum dough height by almost 40 %. Similar results were also reported for BSG or other additives rich in fibre (Wang et al. 2002; Ktenioudaki et al. 2013). The incorporation of 15 % BSG reduced maximum dough height by almost 50 %. Wang et al. found a reduction of 10 % for the addition of 3 % fibre (carob, inulin, pea) and attributed these findings to the interactions between gluten and fibre, preventing the free expansion of the network during the proofing stage.

In DDG-enriched dough, significantly less CO_2 is produced at the beginning of the fermentation already by replacing 10 % flour, probably due to less available yeast substrate and reduced enzymatic activities. However, dough development of DDG-enriched dough shows higher amounts of retained CO_2 at the end of fermentation time, which can be ascribed to decelerated fermentation and the requirement of prolonged fermentation times. Wang et al. reported similar findings, since the addition of special fibres decreases values of volume loss and so can improve dough characteristics, allowing longer proofing times (Wang et al. 2002). Also, internal structures of dough can be responsible for different retention capacities of dough. After Martínez et al. (2014), this phenomenon might be attributed to soluble fibre. Soluble fibre can help to create a mesh-like structure that encloses flour particles and starch, making the dough more cohesive and so favour gas retention (Martínez et al. 2014).

Influence of pH and Furfural

The influence of particles and the high amount of dietary fibre do not completely explain the negative impacts, indicating that other DDG components or properties must contribute to

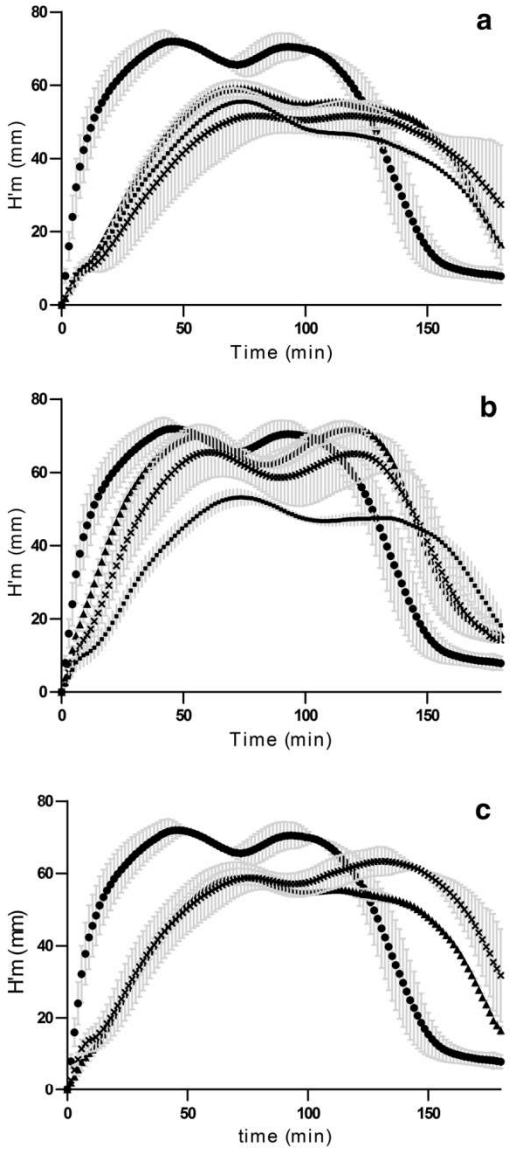


Fig. 2 Gaseous development of DDG-enriched and furfural enriched dough. **a** Native pH and **b** pH standardised to 0 % DDG. 0 % DDG (*black circle*), 10 % DDG (*black triangle*), ×15 % DDG, 20 % DDG (*black square*), $n=2$, and **c** furfural enriched dough, 0 % DDG control (*black circle*), 10 % DDG (*black triangle*), ×0 % DDG control spiked with furfural in equivalent amount to 10 % DDG, $n=2$

the destabilising effects in the dough. A possible reason for a decelerated fermentation may originate in the lower pH of DDG dough, as pH 5.5 for 0 % and 4.3 for 20 % DDG dough was determined at the beginning of fermentation. It is well known that wheat dough can be sensitive to pH changes, subsequently causing effects up to the final product (Jekle and Becker 2012). Lower pH values do not influence yeast activity directly, since the pH optimum of *S. cerevisiae* is about pH 4.0–4.5 (Buzás et al. 1989). Nevertheless, lower pH values can influence enzyme activities and be the origin for limited baking properties of wheat flours. For α-amylases and β-amylases, the pH optima are pH 5.5–5.7 and pH 5.4–6.2 (Belitz et al. 2009), respectively, which can be a reason for reduced substrate availabilities and subsequently less CO_2 formed during fermentation. Besides amylases, pentosanases can be inhibited under acidic conditions, as present in DDG dough medium. Pentosanases degrade pentosan molecules to lower polymerisation degrees, which then become more soluble. Soluble pentosans can contribute to improved dough relaxation and increased specific volume. While DDG low pH could inhibit pentosanases and amylases, the enzyme activities of some proteases could be favoured. Proteases degrade peptide branches and so can promote the breakdown of gluten.

Hindered fermentation through acidic conditions and the effect of lactic acid on CO_2 production was already reported by Gujral and Singh (2000). Acidic conditions in the dough system significantly influence the denaturation of protein and gluten strands, lead to the inhibition of metabolic enzymes and influence the uptake of amino acids into the yeast cell. Moreover, decreased pH values result in a change of protein conformation leading to more water-binding capacity and less water available for the matrix (Jekle and Becker 2012). Additionally, changes in hydrophobicity due to charged side strains of amino acids and also disruption of the gluten network caused by weakened hydrogen bonds between gliadin and glutenin were reported (McCann et al. 2009).

To verify this hypothesis, the pH of DDG-enriched dough was raised to the pH of 0 % DDG dough and analysed under the same conditions by Rheofermentometer. By raising the pH of DDG-enriched dough with sodium hydroxide, these negative effects can be balanced. As Fig. 2b shows impressively, raising the pH of DDG-enriched dough to the 0 % DDG control of pH 5.5 positively affects CO_2 formation, gaseous release and dough height, with the strongest effect for 10 % DDG addition (Table 2). In the case of 10 % DDG, the pH adaption increased total CO_2 volume by 19.6 %, gaseous release by 22.3 % and max. dough height by 17.7 %. Since characteristics of the 0 % DDG control wheat bread could not be achieved, other factors than the low pH of DDG, e.g. particle size effects or yeast inhibiting metabolites in DDG must have an influence on fermentation and dough development. However, the occurrence of particle size effects could not be confirmed in the present study.

From bioethanol production and cultivation processes of *S. cerevisiae*, toxic effects of furfural on fermentation and growth are a known phenomenon. In the presence of furfural, glucose uptake and fermentation rate can be inhibited (Palmqvist et al. 1999; Horváth et al. 2001). However, the mechanism of inhibition is not fully explained yet. Furfural originates from five carbon sugars when heated under acidic

conditions, as present during the drying step of DG to DDG. As a consequence of the conditions during the drying process, 2.7-ppm furfural was found in DDG. Since the European Food Safety Authority established an Acceptable daily intake (ADI) of 0.5-mg furfural per kg body weight based on a no-observed-adverse-effect level (NOAEL), this amount is not relevant for human consumption (EFSA 2004). Nevertheless, effects on S. cerevisiae cannot be excluded. Therefore, the role of furfural as inhibiting compound was investigated in a model suspension and dough. Figure 2c visualises the dough development curves of 0 % DDG control in direct evaluation to 10 % DDG dough and dough spiked with furfural in equivalent amount to the 10 % DDG dough (fur10+). Even though the absolute values of gaseous development of fur10+ dough and the differences in total CO_2 or maximum dough height are not significantly different to the 0 % DDG control (Table 2), a negative impact cannot be excluded when comparing course of dough development. The development of gaseous release in fur10+ dough containing no DDG and 10 % DDG dough reveals a progress, not significantly different ($p<0.05$) to each other but both significantly different to 0 % DDG dough. So, fur10+ and 10 % DDG dough are developing mutually comparable supporting the theory that there is an influence on dough development caused by furfural (Fig. 2c). This emphasises the interaction of multiple effects like pH, furfural and potentially even other components in DDG dough which are not fully investigated by now. Even synergistic effects among the disruptive factors cannot be excluded.

Thus, inhibiting effects of DDG on the metabolism of *S. cerevisiae* were verified in a model suspension to exclude effects by other unknown dough components. The model set-up is presented in Fig. 1. By comparing of the model suspensions after 90 min of simulated fermentation, a strong precipitation of $BaCO_3$ in DDG free suspension and less precipitation of $BaCO_3$ in DDG containing suspension was detected visually. After titration, in the DDG free suspension, 127.9±4.1 mg CO_2 could be calculated, whereas in the presence of 2.0 or 4.0 % DDG, only 89.2 ±13.3 or 36.38±10.75 mg CO_2 could be calculated, respectively. Trials with 0 to 5 % DDG in suspension pointed out that there is a linear correlation between the concentration of present DDG in the fermentation media and the amount of produced CO_2 after fermentation (R^2= 0.9520, $p<0.05$), revealing inhibiting effects of DDG on the metabolism of *S. cerevisiae*.

Effects on Specific Loaf Volume and Texture Profile Characteristics

Loaf volume and texture profile characteristics form the key quality features for characterisation of the end product quality. For this case, specific bread volume of bread loaves, in which 5 to 20 % of flour was replaced by DDG from wheat, was investigated. In addition, the influences of four different DDG particle sizes (<250, <500, <750 μm and unground) were evaluated. In the case of wheat bran, the addition significantly reduces the specific bread volume (Pomeranz et al. 1977), and smaller bran particles lead to increased effects on reduced volume (Noort et al. 2010). Nevertheless, for quinoa, amounts of quinoa bran up to 20 % do not necessarily support negative impacts on gas holding properties in gluten-free dough but provide volume increase when higher amounts of substrate are available (Föste et al. 2014).

Regarding DDG, the analysis of specific bread volume of DDG-enriched bread showed comparable results to those of wheat bran, as the higher amount of flour replaced, the lower values for specific bread volume were achieved (Table 3, Fig. 3). Compared to the 0 % DDG control, the replacement of 10 and 20 % DDG resulted in 21.1 and 37.3 % reduced volume, respectively. This phenomenon was also observed by Schmiele et al. (2012). It was reported that significant decreases were observed when 20 % of flour was replaced by wheat bran. Already in 1991, Abbott et al. assumed that

Table 3 Impact of DDG on specific volume and texture profile parameters (hardness and elasticity) in DDG-enriched bread matrices as analysed by volumeter and texture profile analyser

% DG	Specific volume (mL/g)	Firmness (N)	Springiness (−)
0	3.43±0.06a	3.6±0.43a	0.92±0.02a
Unground			
5	2.70±0.04bc	5.86±0.68b	0.86±0.01bc
10	2.69±0.12c	6.22±0.19b	0.88±0.01ab
15	2.48±0.08d	8.52±0.90bc	0.87±0.02bc
20	2.15±0.04f	12.7±2.18d	0.83±0.02cde
<750 μm			
5	2.82±0.03b	5.73±0.92b	0.90±0.01ab
10	2.61±0.08c	8.94±1.14c	0.88±0.01bc
15	2.29±0.04e	13.47±1.86d	0.84±0.02cd
20	2.01±0.04fg	18.56±2.40e	0.82±0.01e
<500 μm			
5	2.81±0.03bc	5.56±0.48b	0.89±0.01a
10	2.66±0.05c	8.84±0.56c	0.88±0.01bc
15	2.37±0.05e	12.47±1.86d	0.84±0.02bc
20	2.10±0.04fg	20.65±1.98f	0.79±0.03de
<250 μm			
5	2.64±0.07c	6.87±0.63bc	0.85±0.02bc
10	2.38±0.05ed	9.34±1.56c	0.87±0.02bc
15	2.18±0.03f	14.02±1.36d	0.85±0.01bc
20	1.88±0.03h	20.3±1.50f	0.82±0.02de

Replicates are means with standard deviation ($n=8$); different letters indicate significant differences between means in the same column ($p<0.05$). Presented are four different particle sizes (unground, <750, <500, <250 μm) and 0, 10, 15 and 20 % enriched DDG matrices

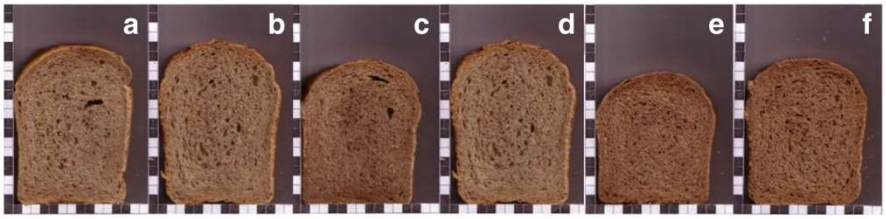


Fig. 3 Images of DDG bread slices, **a** 10 % DDG<500 μm, **b** 10 % DDG<500 μm with raised pH to 0 % DDG pH, **c** 15 % DDG<500 μm, **d** 15 % DDG<500 μm with raised pH to 0 % DDG, **e** 20 % DDG<500 μm, and **f** 20 % DDG<500 μm with raised pH to 0 % DDG. Scale: one square is 5 mm

acidity of DDG may contribute to volume problems, also because the addition of sodium bicarbonate significantly increased the volume (Abbott et al. 1991). However, these increases in volume could also be attributed to CO_2 being released out of sodium bicarbonate under acidic conditions. For this reason, sodium hydroxide was chosen as pH raising agent in this study. The raise of the low pH of DDG contributes to better product volumes as a consequence of improved CO_2 formation and dough height as discussed before. Regarding different particle sizes, Noort et al. reported that reducing the particle size increases the negative effects caused by an increased surface area and physical or chemical interactions (Noort et al. 2010). In addition, a dilution of gluten by adding bran should also be considered.

For DDG, the reduction of particle size surface area cannot provide a significant negative correlation but supports the hypothesis from Noort et al. since there are significant differences in specific volume between the smallest and largest particles of bread containing equal amounts of DDG. Moreover, it was mentioned that high fibre content of DDG contributes to reduced product volumes by fibre cutting gluten strands, interfering gluten network formation and providing gluten dilution (Abbott et al. 1991). As de Kock investigated in 1999, bran reduces loaf volume of 0 % bran control wheat bread. Nevertheless, this could be reduced by the application of heat treatment to bran (De Kock et al. 1999). In this case, bran was dry-treated 90 min at 121 °C, and loaf volumes were significantly increased due to inactivation of lipases and decline in reducing substances, especially glutathione. Regarding the production process of DDG, the stillage is dried during the main drying process at 100–105 °C for 1–3 h, which can probably be compared to the heat treatment of bran described before. The decrease in specific volume demonstrates the synergetic relationship between volume and texture profile parameters. Further consequences are a denser bread crumb and smaller pores, leading to higher crumb firmness. A negative linear correlation of firmness and specific volume with R^2=0.868 (p<0.05) and positive correlation of springiness to specific volume with R^2=0.757 (p<0.05) was found for these synergetic relationships. As it was reported by Schmiele et al. (2012), the addition of wheat bran did not affect the firmness of pan bread when adding 10 % but significantly increases with higher amounts of wheat bran, while the effects were stronger for wheat bran than for whole grain wheat flour (Schmiele et al. 2012). In the case of DDG, the addition of already 5 % significantly increases the firmness. As Table 3 shows, the addition of 5 % unground DDG particles leads to an increase in firmness of more than 60 % and for smallest particles <250 mm of more than 90 %. The highest increase up to 450 % can be observed for the addition of 20 % DDG with particles <500 and 750 μm. These enhanced effects of DDG in comparison to bran might be due to the additional structure weakening effects caused by the low DDG pH and influences through furfural as described before.

Conclusion

Upcycling of by-products, especially novel sources for dietary fibre or protein, has received much attention in the literature recently. This study has shown that the incorporation of DDG in bakery products like wheat bread can be an opportunity for the reutilisation of this by-product. The enrichment of food products with DDG as alternative plant-based protein and fibre source is possible. In conclusion, DDGs provide similar effects to a standardised wheat bread than the addition of bran, based on its nutritional composition. The addition of DDG induces the reduction of maximum dough height and gaseous release, leading to deficits in bread quality. The low pH in DDG affects dough development and bread characteristics negatively but could be balanced by pH adaption. Additionally, the content of furfural negatively affects dough development and bread characteristics. So, furfural is contributing to weakening effects in dough. However, already 10 % of DDG can provide a valuable amount of dietary fibre to bakery products, so the additional benefit should not be overlooked. There is no standardisation among DDG by-products on the market, and the available products are not consistent in their composition. The drying process is a decisive step for DDG characteristics. Choosing a procedure more gently can help to preserve nutritionally valuable ingredients (protein, vitamins) and avoid the formation of toxic

components (furfural, acrylamide). To find solutions for improving the quality features of high fibre-baked products, further research on the impact of DDG, its sensory characteristics and its techno functional properties is necessary.

Acknowledgments This work was based on research project AiF Projekt GmbH (Project KF2271406SK1) supported by the German Ministry of Economics and Technology.

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2.5 CHANGES IN AROMA COMPOSITION AND SENSORY PROPERTIES PROVIDED BY DISTILLER'S GRAINS ADDITION TO BAKERY PRODUCTS

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Journal of Cereal Science 72 (2016) 75–83

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Changes in aroma composition and sensory properties provided by distiller's grains addition to bakery products

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ARTICLE INFO

Article history:
Received 27 April 2016
Received in revised form
9 August 2016
Accepted 1 October 2016
Available online 5 October 2016

Keywords:
Distiller's grains
Bread
Flavour
AEDA

ABSTRACT

The limited use of distiller's grains (DG) in the food industry result out of negative effects on texture and flavour. To investigate the odour, aroma volatiles in the common cereal based food system bread were analyzed. Therefore aroma volatiles in bread containing 0–20% DG were identified with Gas Chromatography-Olfactometry/Mass Spectrometry. Likewise, sensory properties were evaluated. As a result, 42 odour active compounds were identified in DDG bread. Phenylacetic acid and dimethyl-trisulphide are transferred from DG to bread crust and crumb, but not interfering bread aroma. After comparison of highest flavour dilution (FD) factors, 4-hydroxy-2,5-dimethyl-3(2H)-furanone (FD = 128) and 2-ethyl-3,5-dimethylpyrazine (FD = 128) were revealed in control bread crust, whereas 3-hydroxy-4,5-dimethyl-2-(5H)-furanone (FD = 512) and 4-hydroxy-23,5-dimethyl-3-(2H)-furanone (FD = 512) were revealed in 20% DG bread crust. Regarding bread crumb, 3-methylthiopropanal and 2-phenylethanol provided highest FD factors (FD = 32) in the control, whereas in 20% DG bread crumb 3-methylbutanoic acid, 2-ethyl-3,5-dimethylpyrazine and 3-hydroxy-4,5-dimethyl-2-(5H)-furanone provided FD factors ≥ 32 as well. Principal component analysis (PCA) of bread samples correlated to sensory attributes and important aroma volatiles revealed differences in odorant perception to the presence of 3-hydroxy-4,5-dimethyl-2(5H)-furanone and phenylacetic acid, with simultaneous absence of 2AP.

1. Introduction

In the 21st century sustainable engineering and a zero waste society become central topics of the global food industry. The growing world population will lead to a growing demand in food products and will cause deficits in the food supply chain. Within this discussion, the upcycling of byproducts gained much attention, due to the fact that 39% of food losses occur in the food manufacturing industry (Mirabella et al., 2014; van der Goot et al., 2016). Among the cereal manufacturing industry byproducts like bran from the milling industry, brewers spent grain out of the brewery or distiller's grains from the ethanol industry arise next to the main product.

With respect to its composition, distiller's grains (DG) represent a byproduct, not fully exhausted by now, since the amounts in dry matter are described up to 45% for neutral detergent fibre and 35% for protein (Rasco and Rubenthaler, 1990; Roth et al., 2014). To add value to this byproduct, DG can be a source for enriching food products with dietary fibre and protein. DG arise as main byproduct in the manufacturing process of fuel or beverage ethanol out of cereals like wheat or corn. Milled cereals are mashed and enzymes convert starch to yeast digestible carbohydrates. Subsequently, yeasts metabolise digestible carbohydrates to ethanol during fermentation. After distillation of ethanol, distiller's grains remain as a stillage, which is concentrated and subsequently dried to prolong its shelf life (dried distiller's grains, DDG). With regard to the production process, DDG mainly consist of outer grain layer components including the embryo and amounts of yeast cells remaining after the fermentation and distillation process. DDG contains especially high amounts of protein and dietary fiber, but only residual amounts of digestible carbohydrates (Rosentrater and Krishnan, 2006; Liu, 2011).

From a nutritional and sustainable point of view the utilization of DDG as food ingredient seems obvious. However, its application

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http://dx.doi.org/10.1016/j.jcs.2016.10.002
0733-5210/

still isn't usual. The incorporation of DDG in food products was the topic of numerous studies during the 1980s and 1990s, especially regarding integration in cereal food products like bread and noodles. The results were not sufficient, because the addition of high fiber fractions is connected to some challenges and often linked to deficits in consumer acceptance of texture and flavour (Abbott et al., 1991; Bookwalter et al., 1984, 1988; Rasco et al., 1989). For instance, DDG addition in baguettes provides an aftertaste and leads to increased perception of sour, salty and bitter taste in comparison to the control (Rasco et al., 1989). Additionally, poor flavour was the reason for deficits of DDG in blended foods (Bookwalter et al., 1984). These sensory deficiencies were attributed to the characteristic and special aroma of DG (Rosentrater and Krishnan, 2006). However, a study providing information concerning sensory and chemical analysis on aroma composition in DDG enriched food products is still missing. In previous studies it was shown that the typical DDG odour shows high compliance to wheat processed products such as white wheat bread (Roth et al., 2014). The composition of flavour volatiles depends on the fact that DDG represents a thermal processed wheat product. So, the composition of flavour volatiles can match the bakery product and can provide new possibilities for including DG in food products.

The aim of this work was to provide insights into the sensory deficits occurring along with the application of DDG in bakery products and to identify components responsible for the typical flavour. For this reason amounts of 5, 10, 15 and 20% wheat flour were replaced by DDG from wheat and effects of DDG on sensory properties and the composition of flavour volatiles were investigated. After evaluation of sensory characteristics, volatile flavour compounds were isolated by means of Solvent Assisted Flavour Evaporation (SAFE) and identified with Gas Chromatography-Olfactometry/Mass Spectrometry (GC-O/MS). Key aroma compounds were classified by aroma extract dilution analysis (AEDA).

2. Materials and methods

2.1. Dried distiller's grains and raw materials for bread preparation

Dried distiller's grains (DDG) from wheat were purchased from Euro-Alkohol GmbH (Lüdinghausen, Germany). DDG were composed of 38.2% protein (AACC 46-16, N × 6.25), 3.6% fat (AACC 30-25), 3.5% ash (AACC 08-01), 46.8% total dietary fiber (AACC 32-05) on dry basis and 7.8% water (AACC 44-01) and is characterized by a water retention capacity of 54.1% (AACC 56-11). Flavour analysis was carried out using DDG of the same batch, to exclude influences through different composition or dryness. Before extraction of the volatile fraction, DDG was milled to particles <500 μm using an Ultra Centrifugal Mill of type ZM200 from Retsch (Haan, Germany).

Wheat flour type 550 was purchased from Rosenmühle (Ergolding, Germany) and was characterized by 10.6% protein (AACC 46-16, N × 6.25), 1.1% fat (AACC 30-25), 0.6% ash (AACC 08-01) on dry matter and 14.2% water (AACC 44-01). Further ingredients were sodium chloride (NaCl, Südsalz GmbH, Germany) and dry yeast of species *Saccharomyces cerevisiae* (fermipan red, Casteggio Lievitii srl, Casteggio, Italy). Analysis of flour and DDG were conducted in duplicate and presented as mean.

2.2. Chemicals

Chemicals were obtained from the following sources: Diethyl ether (≥99.5%) and anhydrous sodium sulfate (≥99.0) from Sigma-Aldrich (Taufkirchen, Germany), sodium carbonate (≥99.5%) from Merck (Darmstadt, Germany), sodium chloride from Avantor Performance Materials (Deventer, Netherlands) and hydrochloric acid (37%) from Roth (Karlsruhe, Germany). Reference standards of aroma compounds were purchased from commercial sources: Alfa Aesar, Karlsruhe, Germany; Merck, Darmstadt, Germany; Sigma-Aldrich, Taufkirchen, Germany; others were kindly provided from flavour companies (Firmenich, Switzerland; Symrise, Holzminden, Germany).

2.3. Preparation of dough and bread samples

Preparation of dough and bread samples was performed according to the procedure of Schirmer et al. with slight modifications (Schirmer et al., 2011). The recipe for control wheat bread preparation was 60.0 parts water, 2.0 parts sodium chloride and 1.6 parts dry yeast based on 100 g wheat flour (corrected to 14% moisture). To evaluate the influence of DDG on a control wheat bread, different amounts of wheat flour (0, 5, 10, 15, and 20%) were replaced by DDG. Water temperature was adjusted for dough end temperature of 28 °C and water amount was corrected for each DDG content for a maximum dough consistency of 500 Farinograph Units (FU). All ingredients were blended for 120 s at 100 rpm and mixed for 360 s at 200 rpm in a spiral kneader type 12 A-3 (Diosna Dierks & Söhne GmbH, Osnabrück, Germany). After resting for 15 min at room temperature, 250 g pieces of dough were hand-moulded, weighed in baking tins, kept in a proofing chamber for 30 min (30 °C, 80% relative humidity) and subsequently baked for 30 min at 230 °C in a multiple hearth-oven (Matador Store 12.8, Werner&Pfleiderer Lebensmitteltechnik Sachsen GmbH, Sohland, Germany). Each recipe was performed twice on different days, providing 4 independent bread loaves for analysis (n = 4).

2.4. pH measurement of bread samples

10.0 g of bread crumb were milled in a rotor mixer type GK 900 (Rotor Lips AG, Uetendorf, Switzerland) and mixed with 90 ml water. After homogenization the pH-value of the suspension was measured using a pH meter.

2.5. Isolation of flavour volatiles

30 min after baking, bread samples were separated in crumb and crust, cut into small pieces, frozen with liquid nitrogen and milled in a rotor mixer type GK 900 (Rotor Lips AG, Uetendorf, Switzerland). DDG enriched bread was exposed to an extraction with diethyl ether (DEE), in prior to distillation by SAFE. Therefore, 210 g of crumb or crust were mixed with 300 ml DEE, 0.5 ml of an internal standard (methyl decanoate in DEE with concentration of 0.83 g/L) were added for quantification. After 60 min the solvent was changed and the extraction continued for another 60 min. After filtration and concentrated to 50 ml. Subsequently the volatile fraction of the diethyl ether extract was isolated by means of Solvent Assisted Flavour Evaporation technique (Engel et al., 1999), dried over sodium sulfate and concentrated to 1 ml using a Vigreux column.

2.6. GC-Olfactometry-MS

Analysis of aroma extracts produced under 2.5 was carried out on a Trace 1300 GC directly coupled to an ISQ QD single quadrupole MS (Thermo Fisher Scientific, Dreieich, Germany). At the end of the capillary column, the effluent was split into a proportion of 2:1 using a 2-way-μ-split device (Gerstel, Munich, Germany) to the MS and the sniffing port (ODP 3, Gerstel, Munich, Germany). The sniffing port was heated to 250 °C and rinsed with humidified air, to avoid dehydration of nasal membranes of assessors. Samples were separated using a silica capillary column TG-5-MS (Thermo

Scientific, 60 m × 0.25 mm i. d., 0.25 μm film thickness). Column carrier gas was helium at a constant flow of 1.85 mL/min 1 μL of samples were injected in split mode with a split ratio of 1:10. Injector temperature was 250 °C; transfer line temperature was 250 °C. The oven program started with an initial temperature of 60 °C, held for 4 min and subsequently was raised to 220 °C at a rate of 5 °C/min. After holding for 5 min at 220 °C the oven was raised with a rate of 10 °C/min to a final temperature of 250 °C and held for 2 min. MS detection was performed with electron impact energy of 70 eV. The analyzed mass range was 35–350 amu in EI-mode. For quantification an internal standard was added at the beginning of the sample preparation step. Calculated concentrations are semi-quantitative, since no response factors were determined. Identification of aroma compounds was based on the following criteria: odour description, linear retention indices (RI), comparison with reference substances and mass spectrometric data from the literature and the NIST library. In the case MS data were too weak for unequivocal identification, aroma compounds are tentatively identified based on the remaining criteria. Linear retention indices (RI) were determined after Van Den Dool and Kratz (1963). using a mixture of linear alkanes C_6-C_{20} under the same chromatographic conditions described above.

2.7. Sensory analysis

The sensory analysis of DDG bread samples was performed twice by a group of at least 10 panelists from the Institute of Brewing and Beverage Technology on two different days (n = 20). The analysis took place in a room for sensory analysis at room temperature. Panelists were trained weekly after DIN 10961 in general odour and taste perception and especially on cereal based food products. Bread samples were provided with a numerical code. In part 1 of the sensory analysis, panelists evaluated bread characteristics with respect to the attributes pore size, crumb hardness, acceptance of look and texture, acceptance of mouth feeling, sour odour, off-odour, off-taste, acceptance of odour and taste and overall acceptance on a scale from 0 (not present) to 10 (very intensive).

To get more insights into the composition of present odour active volatiles, odour qualities were defined and assessed on a scale from 0 (not present) to 5 (very intensive) within part 2 of the sensory evaluation.

2.8. Statistical analysis

Statistical analysis was performed with Prism 5 (Version 5.03, GraphPad Software, Inc.). Significant differences were detected with ANOVA (one way ANOVA, $p < 0.05$). The Tukey's test was conducted to detect statistical differences between means ($p < 0.05$). Principal Component Analysis was carried out using XLStat (Addinsoft XLSTAT, Version 2016.04.32525).

3. Results and discussion

3.1. Sensory characteristics of DDG enriched bread

Sensory analysis of general odour, taste and texture perception was performed in prior to analytical investigation (part 1). Panelists evaluated texture, odour and taste perception as well as overall impression and acceptance (Table 1).

A marked difference was found for the rating of sour impression, with intensity ratings of 1.2 for 0% DDG bread, and 4.4 for 20% DGG bread. DDG addition undoubtedly affected sour smell and taste already when 10% of flour was replaced. The perception of sour impression can be attributed to higher amounts of organic acids in the DDG samples. pH measurement in bread samples revealed pH values of 5.7 for 0% and 4.2 for 20% DDG. Besides the sour character, off-odour and taste was enhanced by DDG addition. However, panelists were not able to define this off-odour or off-taste.

For this reason the aroma profiling of DDG enriched bread crumb and crust in direct relation to a control wheat bread (0% DDG bread) was performed, to get insights into intensities of present odor qualities and the overall popularity of crumb and crust aroma. Results of part 2 are shown in Fig. 1. The addition of DDG increased the impression of single aroma impressions and also the overall aroma. In bread crust typical bread flavour attributes like malty/caramel-like and roasty/bread-like were enhanced with addition of 20% DDG by ratings from 1.6 to 2.1 and 2.0 to 3.3, respectively. Moreover, the attributes seasoning-like and musty/burnt were markedly intensified in bread crust, which must be interpreted as transfer effects accompanying DDG addition. In bread crumb, cocoa-like (0.5–1.8), malty/caramel-like (1.4–2.5) and roasty/bread-like (1.9–3.4) impressions were enhanced most. Remarkably, this aroma intensification positively affected the overall perception, since panelists rated the popularity of bread crust aroma in the second session with 5.7 for 0% DDG and 6.0 for 10% DDG. For bread crumb, the popularity of aroma was rated 5.5 for 0% DDG and 5.8 for 10% DDG. Higher amounts decreased popularity ratings. So, the aroma enhancement seems to contribute to the overall aroma in a positive way, if amounts of DDG do not exceed critical amounts of 15–20%. Lower ratings for higher DDG amounts might be attributed to the intensified sour character of the loaves, which is not common in traditional wheat bread made from refined flour. Nevertheless this can easily be overcome by additives like acidity regulators or other taste enhancing ingredients like malt flour. Likewise, the application in sourdough media or rye dough should be considered. For these potential applications, the acidity of DDG can fit the product. Another circumstance, which must be taken into account are nonvolatile ingredients e.g. lactic acid, which can negatively affect flavour release and influence flavour perception (Pico et al., 2015). DDG changes the matrix composition by its high content of dietary fiber and protein, and moreover contains high amounts of lactic acid and glycerin and so might influence flavour perception besides its effects on volatiles.

Regarding effects on texture and taste, assessors classified slightly finer distribution of pores and denser crumb structure for higher DDG amounts. These crumb characteristics can be attributed to the weakened and underdeveloped network and lacking gas holding properties (Roth et al., 2016). As a consequence of the denser crumb, panelists evaluated hardness of DDG enriched bread significantly harder already with addition of 10% DDG. These findings are in accordance to instrumental crumb texture analysis by TPA, which was part of a previous study (Roth et al., 2016). Moreover, these effects are comparable to the effects of bran addition (Noort et al., 2010; Zhang and Moore, 1999).

The addition of 10% DDG to standard wheat bread also affected the appearance of look and texture of the bread loaf positively, with a slightly higher rating. It can be assumed that with crumb and crust darkening, DDG addition supports the look of whole meal bread. DDG bread suggests being a healthier product and so can lead to a higher rating in popularity which is also supported by the higher rating of the intensified aroma. Similar results were found for brewers spent grain addition to baked snacks. A 10% BSG addition resulted in highly acceptable products and provided similar texture as the control. Nevertheless all BSG formulations altered the odour profile of the snacks (Ktenioudaki et al., 2013). In conclusion, the addition of DDG seems to enhance typical bread flavour attributes and does not enhance singular off-odour impressions, in particular. The application of DDG and its special aroma character can fit the flavour profile of a bakery product.

Table 1
Sensory characteristics of DDG enriched breads.

	0% DDG	10% DDG	15% DDG	20% DDG
Pore size	5.1 ± 1.6^{bcdef}	5.2 ± 1.9^{bcde}	5.2 ± 1.8^{bcd}	5.7 ± 1.8^{bc}
Crumb firmness	2.5 ± 1.5^{e}	3.8 ± 1.3^{bcd}	4.7 ± 1.6^{b}	6.4 ± 1.6^{a}
Acceptance of look and texture	5.9 ± 2.3^{a}	6.1 ± 1.9^{a}	5.5 ± 1.6^{a}	4.6 ± 1.6^{bc}
Acceptance of mouth feeling	6.2 ± 2.2^{a}	5.9 ± 1.8^{ab}	5.5 ± 2.0^{ab}	4.7 ± 2.1^{bc}
Sour odour	1.2 ± 1.2^{bcdef}	3.2 ± 2.0^{a}	3.8 ± 2.3^{a}	4.4 ± 2.7^{a}
Off-flavour	0.4 ± 0.7^{d}	3.4 ± 1.5^{cb}	4.2 ± 2.0^{a}	5.1 ± 2.4^{a}
Off-taste	0.5 ± 0.8^{f}	2.9 ± 1.6^{d}	4.3 ± 1.4^{cb}	6.0 ± 2.0^{a}
Acceptance of odour and taste	6.3 ± 2.5^{a}	4.9 ± 2.1^{bc}	3.5 ± 1.5^{dc}	2.8 ± 2.3^{cdef}
Overall Acceptance	6.4 ± 2.4^{a}	5.1 ± 1.9^{abc}	3.4 ± 1.6^{de}	2.9 ± 2.3^{ef}

Replicates are means with standard deviation ($n \geq 30$), different letters indicate significant differences between means in the same line (ANOVA, followed by Tukey test, $p < 0.05$). Sensory attributes were evaluated on a scale from 0 to 10 as follows: pore size fine (10), crumb hardness high (10), Acceptance high (10), sour odour high (10), Off-flavour or taste high (10).

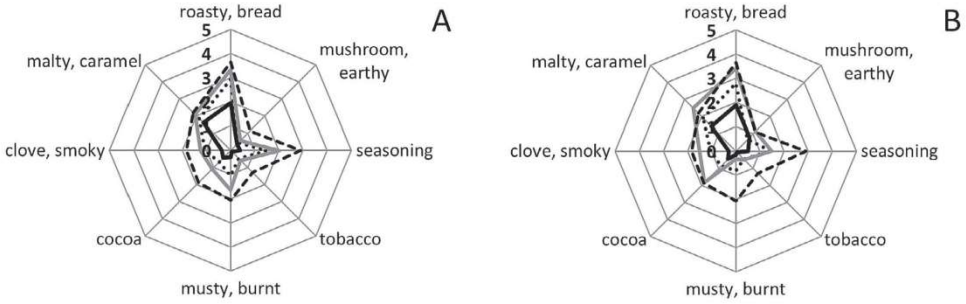

Fig. 1. Flavour profile of DDG enriched bread crust (A) and crumb (B) in comparison to the flavour profile of pure DDG; intensities of descriptors on a scale from 0 (not present) to 5 (very intensive), $n \geq 20$, results were averaged, black line: 0% DDG control; dotted black line: 10% DDG; grey line: 20% DDG; dashed black line: 100% DDG.

Systematic insights into flavour composition of DDG enriched bakery products will be topic of the next chapter.

3.2. Identification of odour active volatiles in DDG enriched bread crumb and crust

Subsequently to sensory analysis bread crust and crumb samples were exposed to chemical analysis of aroma volatiles. During this analysis 42 odour active regions were detected in total in the GC-chromatogram of the control and DDG enriched wheat bread. Among all odour active regions, 17 heterocyclic compounds (11 N-heterocyclic, 7 O-heterocyclic), 10 carbonyl compounds, 4 alcohols, 6 acids and 3 phenols, one sulfur compound and one unknown compound were found.

For crumb and crust, in each case 41 odour active regions could be detected with only slight differences in the qualitative composition (Table 2). Due to odorant transfers from the crust to the crumb, differences within the qualitative composition are only subordinate (Onishi et al., 2011). Moreover, insufficient division of crumb and crust within sample preparation could be a reason for limited differences within the qualitative composition. Aroma composition in DDG enriched bread crumb and crust is dominated by heterocyclic pyrazines and furanones providing roasted and caramel like odour. The composition of flavour volatiles shows high concordance with the evaluated sensory attributes, were malty (3- and 2-methylbutanal, phenylacetaldehyd), caramel-like (4-hydroxy-5-methyl-3(2H)-furanone, 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 3-hydroxy-2-methyl-4(4H)-pyranone) and roasty (2-acetyl-1-pyrroline and pyrazines, mainly 2-ethyl-3,5-dimethylpyrazine) impressions dominated.

Marked differences of DDG enriched bread to the control wheat bread were found within the qualitative composition. With higher amounts of DDG 2AP and 5H-5-methyl-6,7-dihydrocyclo-penta-pyrazine could no longer be identified. Among all odour active compounds only phenylacetic acid and dimethyltrisulphide (DMTS) were found additionally in DDG enriched bread and differentiate the aroma composition of the control wheat bread. In general, aroma compounds in bread origin in fermentation, lipid oxidation, out of enzymatic reactions or autolysis of yeast, as well as Maillard reaction for bread crust, additionally (Schieberle and Grosch, 1985, 1991, 1994). However, phenylacetic acid (honey like) does not interfere with the overall aroma perception. The corresponding alcohol and aldehyde of phenylacetic acid, the aroma molecules 2-phenylethanol and 2-phenylacetaldehyd with similar floral and honey like odour are positively correlated with bread aroma (Hansen and Hansen, 1996; Paraskevopoulou et al., 2012). Moreover, the additional compound DMTS should not interfere with the overall aroma, because it could not be identified in bread containing amounts less than 20% DDG. In one experiment it could be enlightened that activity at Sniffing Port could initially be perceived with amounts of 50% DDG in bread. Concluding, the additional compounds phenylacetic acid and dimethyltrisulphide in DDG should not contribute to the overall aroma of bakery products, as long as DDG amounts up to 20% are applied. Consequently, no particular off-odour in DDG is interfering with the typical bread crust or crumb flavour. During sensory evaluation, panelists were not able to define the present off-odour. Changes in overall aroma must be attributed to concentration effects and influences provided by absence of important key aroma compounds (2-acetyl-1-pyrrolin, 5H-5-methyl-6,7-dihydrocyclo-pentapyrazine). These compounds could not be detected in breads with higher amounts of DDG. Similar findings are known for whole wheat products. According to Jensen et al. alterations in flavour composition of white and whole wheat bread can be explained by

Table 2
Odour-active compounds in crumb and crust of DDG enriched bread.

No	RI (DB-5)[e]	Odour active compound	Odor quality[f]	Crumb	Crust
1	565	acetic acid[a]	acetic	x	x
2	575	2,3-butandione[a]	buttery	x	x
3	647	3-methylbutanal[a]	malty	x	x
4	658	2-methylbutanal[a]	malty	x	x
5	729	3-methylbutanol[a]	malty	x	x
6	733	2-methylbutanol[a]	malty	x	x
7	753	isobutanoic acid[a]	cheesy	x	x
8	779	butanoic acid[a]	rancid	x	x
9	801	hexanal[a]	green	x	x
10	833	3-methylbutanoic acid[a]	cheesy	x	x
11	844	2-methylbutanoic acid[a]	cheesy, fruity	x	x
12	871	methylpyrazine[a]	nutty	x	x
13	872	unknown	seasoninglike	x	x
14	908	3-methylthiopropanal[a]	cooked potato	x	x
15	924	2,6-dimethylpyrazine[a]	nutty	x	x
16	924	2,5-dimethylpyrazine[a]	nutty	x	x
17	925	ethylpyrazine[a]	roasty	x	x
18	925	2,3-dimethylpyrazine[a]	nutty	x	x
19	924	2-acetyl-1-pyrroline	roasty, popcorn		x[c]
20	980	dimethyltrisulphide[d]	sulfury	x	x
21	982	1-octen-3-ol[a]	mushroomlike	x	x
22	984	1-octen-3-on[b]	mushroomlike	x	x
23	1026	3-methyl-1.2-cyclopentanedione[a]	roasty	x	x
24	1046	4-hydroxy-5-methyl-3(2H)-furanone[a]	caramellike	x	x
25	1050	2-phenylacetaldehyd[a]	floral	x	x
26	1063	4-hydroxy-2,5-dimethyl-3(2H)-furanone[a]	caramellike	x	x
27	1082	2,3-diethylpyrazine[a]	musty	x	x
28	1087	2-ethyl-3,6-dimethylpyrazine[a]	roasty	x	x
29	1094	2-ethyl-3,5-dimethylpyrazine[a]	roasty	x	x
30	1095	2,5-diethylpyrazine[a]	musty	x	x
31	1096	3-methoxyphenol[a]	smokey	x	x
32	1121	3-hydroxy-4,5-dimethyl-2(5H)-furanone[c]	seasoninglike	x	x
33	1122	3-hydroxy-2-methyl-4(4H)-pyranone[a]	caramellike	x	x
34	1123	2-phenylethanol[a]	floral	x	x
35	1151	5H-5-methyl-6,7-dihydrocyclopentapyrazine[a]	roasty	x[g]	x[g]
36	1167	2-*E*-nonenal[a]	fatty	x	x
37	1248	phenylacetic acid[d]	floral	x	x
38	1298	*E,Z*-2,4-decadienal[a]	rancid	x	x
39	1323	*E,E*-2,4-decadienal[a]	rancid	x	x
40	1325	3-methoxy-4-vinylphenol[a]	clovelike	x	x
41	1372	y-nonalacton[a]	cocoslike	x	x
42	1412	4-hydroxy-3-methoxybenzaldehyd[a]	vanillalike	x	x

[a] Identified by retention indices, odor quality and mass spectra obtained in EI.
[b] Tentatively identified by retention indices, odor quality and comparison with reference substances.
[c] Mass spectra and odour activity could only be obtained in 0, 5 and 10% DDG bread.
[d] Mass spectra/odour activity could only be obtained with DDG amounts ≥ 10%.
[e] Retention indices on a DB 5 column.
[f] Odor quality perceived at sniffing port, n.i. not identified.
[g] Not detectable with amounts ≥15% DDG.

concentration effects (Jensen et al., 2011). In conclusion, DDG addition does not transfer specific off-odours to wheat bread, which can be one reason for inability of assessors to define the off-odour. DDG addition must support differences in concentration ranges of common bread flavour. For this reason, deeper insights into key aroma compounds and odour activity values are needed.

3.3. Evaluation of key aroma compounds in DDG enriched bread by AEDA

Characterizing the aroma contribution of odour active compounds by AEDA helps to enlighten compounds, were quantification is not possible because of insufficient chromatographic conditions or MS data, as it was present for 2AP and 3-hydroxy-4,5-dimethyl-2(5H)-furanone. For further studies on aroma changes accompanying DDG addition, 18 compounds with odour activity at sniffing port were chosen. Therefore all bread samples were separated into crust and crumb and aroma extract dilution analysis was performed separately.

3.3.1. Bread crust

18 odour active compounds with FD-factors between 1 and 512 were found in DDG free and enriched bread crust (Fig. 2A and B). Among these 18 odour-active compounds in bread crust, 6 compounds with roasty, bread-like and caramel-like odour dominated and provided high FD factors ≥16 (3-methylthiopropanal, 2,5-/2,6-dimethylpyrazine, 2-acetyl-1-pyrroline, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 2-ethyl-3,5-dimethylpyrazine and 2-phenylethanol). With addition of DDG, important alterations in the composition of volatiles were observed.

Regarding the development of odorants from 0% DDG free up to highest DDG amounts of 20%, some sensory findings were confirmed. Enhanced caramel-like odour was supported by increasing FD factors for 4-hydroxy-5-dimethyl- and 4-hydroxy-2,5-dimethyl-3(2H)-furanone, from FD = 8 to 32 and FD = 128 to 512, respectively. Moreover, 3-hydroxy-4,5-dimethyl-2(5H)-furanone, the seasoning-like key aroma compound of DDG increased markedly from FD = 8 to the highest FD = 512 with 20% DDG addition. Increases could also be found for (*E,E*)-2,4-decadienal and

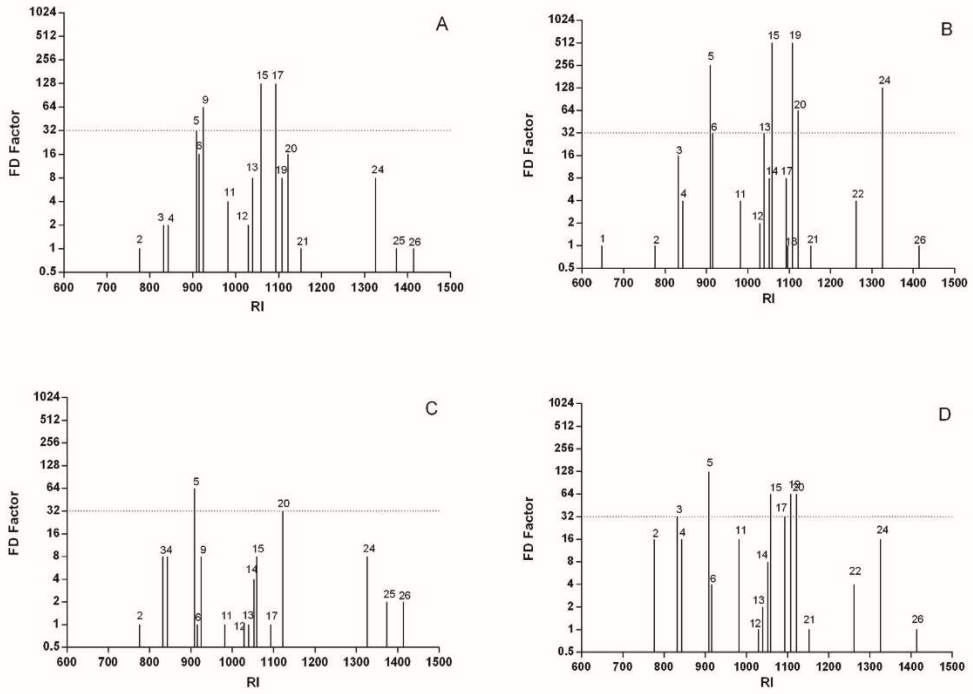

Fig. 2. Aroma extract dilution analysis of DDG enriched bread crust and crumb with different amounts of DDG (A: 0% DDG crust, B: 20 DDG % crust %, C: 0% DDG crumb %, D: 20% DDG crumb). Odour active compounds numbers as followed: 1: 3-methyl butanal, 2: butanoic acid, 3: 3-methyl butanoic acid, 4: 2-methyl butanoic acid, 5: 3-methylthiopropanal, 6: 2,5-, 2,6-dimethyl pyrazine, 9: 2-acetyl-1-pyrroline, 11: 1-octen-3-ol, 12: acetyl pyrazine, 13: 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 14: 2-phenyl acetaldehyde, 15: 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 16: 2-ethyl-3,6-dimethylpyrazine, 17: 2-ethyl-3,5-dimethylpyrazine, 18: 2-methoxyphenol, 19: 3-hydroxy-4,5-dimethyl-2-(5H)-furanone, 20: 2-phenylethanol, 21: 5H-5-methyl-6,7-dihydrocyclopentapyrazine, 22: phenylacetic acid, 24: EE-2,4-decadienal, 25: γ-nonalacton, 26: 4-hydroxy-3-methoxybenzaldehyd.

3-methylthiopropanal from FD = 8 to 128 and FD = 32 to 128, respectively. These increases might be a reason for the musty and bread-like character of DDG enriched bread. Likewise, 2-phenylethanol showed higher FD-factors from 16 to 128 and therefore contributed to the enhanced malty and bread-like character of DDG enriched bread.

2-Acetyl-1-pyrrolin (2AP), the key aroma compound of wheat bread crust could only be identified in control wheat bread crust (FD = 64) and 5% (FD = 16) DDG bread crust with decreasing FD factors. Surprisingly, in 10–20% DDG 2AP could not be detected. This leads to significant effects on flavour perception of DDG enriched bread, because absence of 2-acetyl-1-pyrrolin is connected to freshness of bread aroma. Absence of 2AP favours stale off-odour, which arises due to rapid evaporation of 2AP as a phenomena of staling (Schieberle and Grosch, 1992). This phenomenon of lower amounts or absence of 2AP is already known for whole wheat bread. Ferulic acid (FA), free or released out of outer grain layers, can influence mechanisms during Maillard reaction and inhibit the formation of 2-acetyl-1-pyrrolin by competitive reaction with its precursor methylglyoxal (Moskowitz et al., 2012). Then methylglyoxal is shorter or even no longer available for formation of 2AP. Due to the fact that DDG contains residual amounts of bran, this phenomenon might be transferable. Moreover high temperature and fermentation can increase the bioaccessibility of bonded FA, supporting the fact that the production process of DDG and also manufacturing process of bread delivers suitable conditions for the release of bonded FA into the dough media (Moskowitz et al., 2012). The absence of 2AP might be a possible source, that panelists attributed an off-odour to DDG enriched bread, but were not able to define the nature or characteristics of this off-odour. In this case, the characteristic odour is not defined by the presence of an additional key odorant, but defined by absence of the most important key aroma compound of bread, 2-acetylpyrroline.

3.3.2. Bread crumb

16 odour active compounds with FD factors between 1 and 128 were found in DDG free and 17 in DDG enriched bread crumb (Fig. 2C and D). In general, FD factors in crumb appear with lower intensities in relation to the crust. Similarly in this study, FD factors decreased in relation to the corresponding crust samples. Aroma composition of DDG free and enriched bread crumb showed slight differences. In control wheat bread crumb, 3-methylthiopropanal (FD = 64) and 2-phenylethanol (FD = 32) provided the highest FD factors. With addition of DDG, increases in the intensity of numerous compounds were observed. 3-methylthiopropanal reached values of FD = 128 for 20% DDG and represents the compound with the highest FD factor and most relevant aroma contribution in DDG enriched bread crumb.

Another important difference was found for 3-hydroxy-4,5-dimethyl-2(5H)-furanone. Conspicuously, the seasoning-like 3-hydroxy-4,5-dimethyl-2(5H)-furanone could not be perceived at Sniffing Port in 0% DDG crumb, but arose with FD = 32 in 20% DDG bread crumb. So, 3-hydroxy-4,5-dimethyl-2(5H)-furanone constitutes the seasoning-like odour in DDG bread crumb, which was described during sensory evaluation. 3-methylbutanoic acid, 2-ethyl-3,5-dimethylpyrazine and 3-hydroxy-4,5-dimethyl-2-(5H)-furanone provided FD factors of 32 for the priors and 64 for the latter. Moreover, butanoic acid, 2-methylbutanoic acid, 1-octen-3-

ol and *(EE)*-2,4-decadienal were determined with FD factors of 16. The higher impact of *(EE)*-2,4-decadienal can enhance a fatty or musty off-odour and must be attributed to higher amounts of lipids in the DDG raw material, since DDG contains 3.6% lipids in contrast to 1.1% for wheat flour. As already found in bread crust, 2AP could only be detected in samples with low DDG amounts up to 10%. Likewise, 2-phenylacetic acid was only found in DDG enriched bread crumb. 2-ethyl-3,5-dimethylpyrazine shifted from FD = 1 to FD = 32 from 0 to 20% DDG. This fact might have important influences on overall aroma perception, due to the fact that bread crumb generally not contains Maillard products such as 2-ethyl-3,5-dimethylpyrazine in aroma relevant amounts. 2-ethyl-3,5-dimethylpyrazine and 3-hydroxy-4,5-dimethyl-2(5H)-furanone were revealed as key aroma compounds in DDG so the presence can be attributed to odorant transfers as carry over effects out of the bread crust and DDG (Roth et al., 2014). These findings provide an explanation for enhanced malty, bread like and roasty character of DDG bread and are in compliance with the sensory evaluation.

3.4. Quantification and evaluation of odour activity values (OAV's)

Quantitative data and odour activity values of chosen compounds are shown in Table 3. Alterations between DDG free and enriched bread were found, which can help to understand the differences in flavour perception.

The total concentration of odorants with OAV ≥1 was calculated to 581.6 μg/kg for control bread crust in contrast to 4185.8 μg/kg for 20% DDG bread crust. Unfortunately, only insufficient spectroscopic data could be obtained for 3-hydroxy-4,5-dimethyl-2(5H)-furanone. Consequently, the calculation of 3-hydroxy-4,5-dimethyl-2(5H)-furanones contribution was not possible. Tenfold increase for malty 3-methylbutanal was determined and explained the enhanced malty character of DDG enriched bread together with five- and tenfold increases for the malty and floral notes of 2-phenylacetaldehyd and 2-phenylethanol in direct relation to the control wheat bread crust. Amounts of 2- and 3-methylbutanoic acid increased tenfold, and corresponding OAVs exceeded the aroma relevant value of OAV>1 in DDG enriched bread. So, 2- and 3-methyl butanoic acid are contributing to the rancid and musty off-odour in DDG enriched bread, as they are known to contribute in wheat bread (Grosch and Schieberle, 1997). Likewise, twofold increase could be observed for fatty *(EE)*-2,4-Decadienal. These increases can be explained as transfer effects out of DDG. Higher amounts of lipid degradation products resulted due to higher amounts of lipids material in the DDG feedstock. For lupin protein enriched wheat bread it was also reported that higher amounts of aldehydes and ketones in general were found, which were ascribed to the higher amount of lipids in the raw material (Paraskevopoulou et al., 2012). There are similar effects known for addition of brewers spent grain (BSG) (Ktenioudaki et al., 2013). According to Ktenioudaki et al., the addition of BSG provided a characteristic odour markedly influencing the aroma of baked snacks and lead to slight increases for 3-methylbutanal and 2,3-butandione. For pyrazines 6, 8, 16 and 17 quantification delivered no significant differences between amounts for 0 and 20% DDG bread crust. Nevertheless sensory analysis and AEDA revealed increased perceptions and higher FD factors of the roasty character. This could be attributed to altered or hindered flavour release and different flavour perception. Induced by microstructural changes flavour release could be affected negatively. The high amount of fibre in DDG typically causes increased water absorption and consequently reduces water availability. During bread preparation water amount was corrected for each recipe with increasing DDG amounts for a maximum dough consistency of 500 FU, nevertheless changes in water distributions during baking might influence flavour release negatively and therefore cannot be excluded. Moreover, enzyme activities can be affected. Due to the high water absorption of fiber, water is mostly present in bound form and so only limited water amounts can be available for enzymes, e.g. amylase. As a consequence, this effect will markedly influence the formation of aroma compounds originating from monosaccharides and their degradation products, like 4-hydroxy-2,5-dimethyl-3(2H)-furanone. The activity of amylase is decisive for the release of maltose and glucose, necessary as initial products for flavour compounds out of Maillard reaction or fermentation (Ktenioudaki et al., 2013). According to Jensen et al. flavour of whole wheat bread is perceived more intense; although the total concentration of volatiles in wheat bread is higher (Jensen et al., 2011). Whole wheat matrix provides stronger retention and delayed release due to a firmer crumb. There's only limited data on key odorants and changes in the aroma composition with application of whole wheat flour, so a comparison with usual changes coming along with application of additives containing outer grain layer components (bran, BSG, DDG) must be topic of future studies.

3.5. Relationship between sensory and analytical data

To emphasize significant differences between analytical data

Table 3
Concentrations of selected compounds in DDG bread crust with 0% DDG compared to bread crust with 20% DDG.

Nr	RI (DB 5)[a]	Odour active compound	m/z[d]	Concentration [μg/kg][b]		OAV[c]	
				0%[e]	20%[e]	0%[e]	20%[e]
1	647	3-methylbutanal	58	33.3 ± 3.0	374.9 ± 36.7	83	937
3	833	3-methylbutanoic acid	60	11.5 ± 0.7	101.7 ± 30.3	0.3	3.1
4	844	2-methylbutanoic acid	74	6.6 ± 0.3	68.1 ± 27.5	0.1	1.4
5	908	3-methylthiopropanal	104	7.3 ± 0.6	49.4 ± 5.7	36.5	247
11	982	1-octen-3-ol	72	nd	8.5 ± 1.0	nc	8.5
14	1050	2-phenylacetaldehyd	120	27.0 ± 2.4	139.8 ± 17.0	27.0	140
15	1063	4-hydroxy-2,5-dimethyl-3(2H)-furanone	128	338.5 ± 25.3	276.0 ± 17.0	5.6	4.6
17	1094	2-ethyl-3,5-dimethylpyrazin	136	2.2 ± 0.1	2.4 ± 0.2	55.4	60
18	1096	3-methoxyphenol	124	nd	2.9 ± 0.4	nc	1.2
20	1123	2-phenylethanol	122	133.7 ± 4.9	2867.6 ± 464.9	0.1	2.6
23	1323	*(E,E)*-2,4-decadienal	81	4.7 ± 0.7	13.1 ± 1.1	23.4	65
24	1325	3-methoxy-4-vinylphenol	135	16.8 ± 2.5	281.4 ± 68.1	3.4	56

[a] Retention indices on a DB 5 column.
[b] Concentration in μg/kg (calculated with methyl decanoate as internal standard).
[c] Odour activity value based on odor thresholds in air known from literature, nd = not detected, nc = not calculated.
[d] Mass to charge ratio of fragment used for quantification.
[e] Amount of DDG contained in bread crust sample.

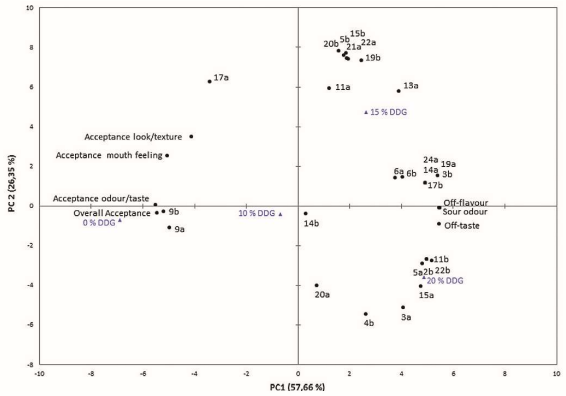

Fig. 3. PCA biplot of PC1 and PC2. DDG bread samples are correlated to sensory attributes and FD factors of aroma volatiles. 2: butanoic acid, 3: 3-methyl butanoic acid, 5: 3-methylthiopropanal, 6: 2,5-, 2,6-dimethyl pyrazine, 9: 2-acetyl-1-pyrroline, 11: 1-octen-3-ol, 13: 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 14: 2-phenyl acetaldehyde, 15: 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 17: 2-ethyl-3,5-dimethylpyrazine, 19: 3-hydroxy-4,5-dimethyl-2-(5H)-furanone, 20: 2-phenylethanol, 21: 5H-5-methyl-6,7-dihydrocyclopentapyrazine, 22: phenylacetic acid, 24: EE-2,4-decadienal); FD factors in crust and crumb are labeled with "a" and "b", respectively.

and sensory findings and to substantiate present investigations, PCA was carried out between sensory attributes and FD-factors of important aroma volatiles in crumb and crust. Fig. 3 shows the biplot, which overlays scores and loadings of the first two principal components of the PCA model. PCA clearly discriminated between samples with different DDG amounts and PC 1 and PC 2 explained 86% of data variability. Acceptance (overall, odour and taste, mouth feeling) is negatively correlated to PC 1 with $r < -0.91$ and mainly associated to 0% DDG. For acceptance of look and texture, correlation to PC 1 is slightly lower, underlining the preference of assessors to the look of a whole meal product. In accordance to the findings within the analytical investigations, the key aroma compound 2AP (9a and b) is negatively correlated to PC 1 with a low value for PC 1. With increasing amounts of DDG the trend becomes positive, so the absence of 2AP correlates inversely with % DDG.

By contrast, off-flavour, off-taste and sour odour are positively correlated to PC 1 ($r > 0.99$) and predominantly related to higher amounts of DDG. This confirms the hypothesis that the characteristic aroma of DDG is related to the absence of 2AP. Evidently, the attribute off-flavour is strongly correlated to sour odour ($r > 0.99$, $p < 0.001$). This detail indicates an explanation for flavour deviations within DDG enriched food samples found in common literature. In bread crust, similar to the sensory attributes off-taste and off-flavour, aroma compounds 3a, 5a, 14a, 15a, 19a and 24a are positively correlated to PC 1. In bread crumb, aroma compounds 2b, 3b, 11b, 17b and 22b are positively correlated to PC 1. These compounds represent volatiles, which were mainly transferred to the bread matrix as a consequence of carry over effects out of DDG. In bread crumb, the highest correlation was found for phenylacetic acid, whereas in bread crust, the effect was strongest for 3-hydroxy-4,5-dimethyl-2(5H)-furanone, key aroma compound of DDG ($r < -0{,}98$). Therefore, alterations within odorant perception of DDG free end enriched bread samples can principally be attributed to the presence of 3-hydroxy-4,5-dimethyl-2(5H)-furanone and phenylacetic acid, with simultaneous absence of 2AP.

4. Conclusion

The enrichment of wheat bread with DDG alters the aroma composition. However, overall aroma perception did not provide relevant off-odours, as long as the supplemented amount did not exceed critical amounts >20%, then the aroma composition can fit the bakery product. The sour character of DDG influences aroma and taste perception, but can be counteracted with acidity regulators. Alterations in aroma composition predominantly originated in absence of the key aroma compound 2-acetyl-1-pyrrolin and concentration effects of compounds usual present in bread crumb and crust. Only 2 compounds phenylacetic acid and dimethyltrisulphide differentiated the aroma composition of the control wheat bread, but are not contributing as key aroma compounds. These additional compounds were not influencing the overall aroma substantially, but were part of the background aroma. Besides, transfer effects could be observed. Key aroma compounds of DDG, especially Maillard products, which are usually not part of the crumb, like 2-ethyl-3,5-dimethylpyrazine or 3-hydroxy-4,5-dimethyl-2-(5H)-furanone were transferred from DDG to the bread crumb. They contribute to the roasty and seasoning like character in a positive way according to the results of the sensory evaluation. So, DDG addition to wheat bread can positively affect overall aroma and aroma popularity. With PCA analysis it was possible to substantiate the findings for variation of aroma volatiles in DDG enriched bread. The transferability to other outer grain layer additives, such as BSG or bran, should be the topic of future studies. This could enable the tackling of flavor deficiencies coming along with the addition of cereal high-fiber byproducts.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgement

The authors thank Iain Whitehead for his advice in statistical issues.

Abbreviations

2AP	2-Acetyl-1-pyrrolin
DG	Distiller's grains
DDG	Dried distiller's grains
0% DDG	control crumb or crust, with no additional amount of DDG
5-20% DDG	crumb or crust, with selected amounts of DDG
HS-SPME	Headspace solid phase micro extraction
SAFE	Solvent Assisted Flavour Evaporation
SDE	Simultaneous distillation extraction
AEDA	Aroma extract dilution analysis
GC-O/MS	Gas chromatography-olfactometry/mass spectrometry
AACC	American Association of Cereal Chemists
PDMS	Polydimethylsiloxan
NBF	Neutral-basic fraction
AF	Acidic fraction
RI	Retention index
DIN	Deutsches Institut für Normung (German Institute for Standardisation)
FD	Flavour dilution
PCA	Principal Component Analysis
PC	Principal Component

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3 DISCUSSION

> *"Considering how distillers grains ultimately will be utilized is essential to the future of the industry, and as a result, we face a pressing need for research into and development of value-added uses for these coproduct streams. Food products currently are an untapped but potentially high-volume utilization avenue for these materials."*
>
> (Rosentrater & Krishnan, 2006)

By designing novel food products, both textural as well as sensory properties have to be considered, since they fundamentally influence the purchase decision of the consumer. Moreover, mouthfeeling and taste are crucial criteria because they immediately decide on the willingness on repeated purchasing and consumption. Thus, new ingredients for the food industry either must improve textural or sensory properties or at least provide no significant negative impact but support beneficial health effects via its composition of ingredients. In this context, the aim of this thesis was the in depth investigation on the byproduct distiller's grains combined with the evaluation of its potential as functional ingredient for cereal based food products. Achieving these objectives included the assessment of aroma and sensory characteristics of DDG and the detailed enlightenment of the aroma composition. Moreover, to proof the feasibility of the application in food systems such as bakery products, a common wheat bread enriched with different shares of DDG was investigated regarding the technological performance, followed by the elucidation of mechanisms that trigger the adverse effects on the dough and bread system. Research objectives also involved the investigation of alterations in the aroma composition that accompany DDG addition and induce aroma deficiencies as observed in former studies (Rosentrater, 2006).

Sensory deficiencies and enlightenment of DDG aroma

Sensory deficiencies of odor arising with DDG enrichment of food products were firstly described during the 1980s. Bookwalter et al. described the flavor of pure DG from corn as sour and fermented, further processed in blended foods as rancid and fermented (Bookwalter et al., 1984). Some years later, the research group around Bookwalter proposed solvent-extraction to remove pungent fermentation flavors and lipid oxidation products (Bookwalter, Warner, & Wu, 1988), although no knowledge on responsible compounds was available. For wheat DDG, Rasco et al. detected similar odors and described baguettes enriched with DDG

as sour, malty and yeasty, bitter and soapy (Rasco et al., 1989). In the present study, sensory properties of DDG (section 2.3) were investigated, as well as alterations that appear after utilization and further processing in wheat bread (section 2.5). By sensory assessment of DDG, panellists evaluated three impressions seasoning-like, roasty/bread-like and malty/caramel-like as most intensive odors. Comparing odor impressions with literature data for odor qualities in reference to corresponding odor active volatiles (i.e. Czerny & Schieberle, 2002), indications to thermal process flavourings had to be considered. This seems not remarkable, considering the production process of DDG after distillation. Distiller's grains represent a yeast fermented grain matrix which experiences alterations in the volatile composition during processing: highly volatile fermentation odorants are separated along with ethanol during distillation and new odor active volatiles can be built during drying. The central drying process provides temperatures > 100 °C for several hours (DDG used in this study: 100-105°C for 1-3 h) and consequently favors the formation of oxidation products and the continuation of Maillard and Strecker reaction markedly. For instance, carbohydrate containing heat processed food frequently promotes caramel-like odor, provoked by 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) (Schieberle, 1992). Due to its low odor threshold of 0,01 mg/l water, furaneol contributes as key aroma compound to the aroma of numerous food products, such as strawberry, popcorn and most important wheat bread crust (Schieberle, 1992). In wheat bread crust furaneol is known to descend from yeast as central source with fructose-1,6-diphosphate as predominant precursor, therefore its formation in DDG from wheat seems likely. As caramel like odor was predominant, furaneol could be identified during analytical investigation and determined as important key odorant in DDG. An important point as well is represented by the impact of residual amounts of dead yeast cells on the overall aroma of DDG, which was not taken into consideration of this study and therefore should be reflected in future studies.

Key aroma compounds of DDG

According to section 2.3, the analytical determination of aroma volatiles revealed 42 odor-active compounds in total in dried distiller's grains from wheat. After application of aroma extract dilution analysis (AEDA), eight of these 42 odorants showed highest FD factors ≥ 32 and therefore could be determined as key aroma compounds that substantially built up the characteristic aroma of DG (figure 7). These eight key odorants of DDG from wheat are compliant to the findings during sensory evaluation, since their chemical origin is related to

lipid oxidation or typical reactions in thermal food processing, such as Maillard reaction and Strecker degradation. In accordance to the results from sensory analysis, key aroma compounds correspond to perceived odor impressions: 3-hydroxy-4,5-dimethyl-2(5H)-furanone induces seasoning like odor (supported by the ethyl homologue 3-hydroxy-4-methyl-5-ethyl-2(5H)-furanone), 2,5-dimethyl-4-hydroxy-3(2H)-furanone induces caramel like odor and 2-ethyl-3,5-dimethylpyrazine is responsible for roasty and bread like odor. A detailed discussion on the chemical classes of odorants and key aroma compounds in DDG, their origin and reaction mechanisms can be found in section 2.3.

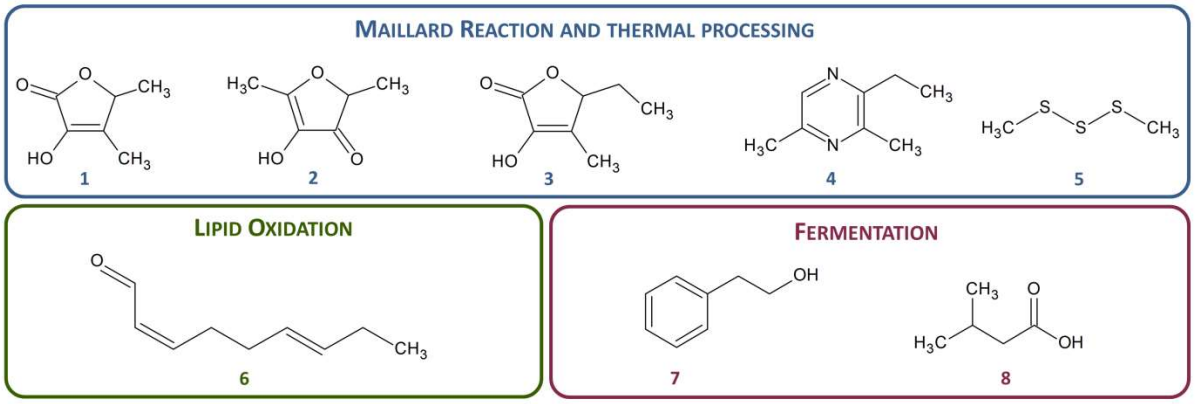

Figure 7: Key aroma compounds in DDG obtained by AEDA with FD factor ≥ 32; Reaction compounds from thermal processing: 1 3-hydroxy-4,5-dimethyl-2(5H)-furanone, 2 3-hydroxy-4-methyl-5-ethyl-2(5H)-furanone, 3 2,5-dimethyl-4-hydroxy-3(2H)-furanone, 4 2-ethyl-3,5-dimethylpyrazine, 5 dimethyl trisulfide; reaction products from lipid oxidation: 6 *(E,Z)*-2,6-nonadienal; odorants from fermentation: 7 2-phenylethanol, 8 3-methylbutanoic acid

As generally known, fatty and soapy off-flavor in DDG and DDG enriched products can be ascribed to unsaturated aldehydes resulting out of lipid oxidation. In DDG *(E,Z)*-2,6-nonadienal significantly contributes as key aroma compound and furthermore additional unsaturated aldehydes such as *(E)*- and *(Z)*-2-nonenal or *(E,Z)*- and *(E,E)*-2,4-decadienal were identified. These unsaturated aldehydes are well known to contribute to the fatty and rancid odor impression of food products. Even though these aldehydes do not contribute as key aroma compounds or exceed their specific OAV, synergistic effects of unsaturated aldehydes are likely and so, the contribution of key aroma compound *(E,Z)*-2,6-nonadienal and the impression of the fatty odor can be enhanced. In analogy, whole wheat bread likewise exhibits higher amounts of 2-*(E)*-nonenal and 2,4-*(E,E)*-decadienal than wheat bread made from refined flour. Moreover, 2- and 3-methylbutanoic acid were determined in DDG, which as well contribute to rancid odor impressions, as their aroma contribution is already known for common wheat bread (Grosch & Schieberle, 1997). Key aroma compounds in DDG from

wheat clearly determine a thermal processed wheat product. Interestingly, the distribution of aroma volatiles is related to other wheat processed products like white wheat bread and therefore the examination of alterations that accompany DDG enrichment of wheat bread was part of this thesis in section 2.5.

Aroma of DDG enriched wheat bread

During sensory evaluation of DDG enriched bread products, crucial differences in comparison to DDG free products were found for sour impression and intensified off-odor and taste. Interestingly, panelists were not able to describe this off-odor or off-taste or define its odor quality. The enhanced sour character can be ascribed to around 4 % of lactic acid, which was determined in untreated DDG used in the present study (unpublished data) and other organic acids in DDG, which arise along with ethanol as fermentation byproduct. During drying in DG processing, non-volatile compounds and fermentation byproducts that are present in the fermented mash, such as organic acids or glycerol are concentrated. On an average, concentration increases around factor 3 are reported (Han & Liu, 2010). As strong sour character is not common in traditional wheat bread, the viability to overcome sour perception was proven by simple treatment of dough with additives like acidity regulators or by masking effects that simulate decreased sour character via taste enhancing ingredients like malt flour (unpublished data). Thus, sour character in DDG processing and application in bakery products should not represent a burden for the utilization in the food industry. As side effects, pH treatment positively affected specific volume and firmness, as well as appearance and popularity of bread loaves (see figure 7).

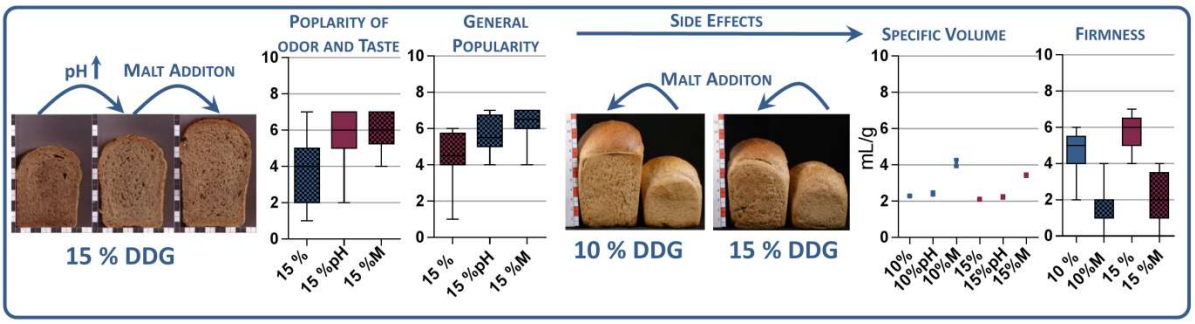

Figure 8: Exemplary presentation of DDG enriched bread in amounts of 10 and 15 % (right) and after treatment with alkali and baking malt as additive (left). By simple recipe management, deficiencies of sour character and specific volume can be handled in industrial applications (unpublished data, 10 and 15 %pH= dough pH value raised to pH of control dough, 10 and 15 %M= malt addition to DDG enriched bread samples, popularity and firmness rated by panelists on a scale from 0-10, with 10= very popular/very firm).

Aroma extracts, prepared out of freshly baked DDG enriched bread crumb and crust samples predominantly supported roasty and caramel-like odor, which could be reflected in the composition of aroma volatiles as reported in section 2.5: 3- and 2-methylbutanal and phenylacetaldehyd induced malty odor, 4-hydroxy-5-methyl-3(2H)-furanone, 2,5-dimethyl-4-hydroxy-3(2H)-furanone and 3-hydroxy-2-methyl-4(4H)-pyranone induced caramel like odor and roasty 2-acetyl-1-pyrroline and several pyrazines, mainly 2-ethyl-3,5-dimethylpyrazine induced roasty odor impression. The surely most crucial difference of DDG to the control wheat bread is represented by the absence of 5H-5-methyl-6,7-dihydrocyclopentapyrazine and more important 2-acetyl-1-pyrrolin (2AP) in the crust of DDG enriched bread > 5 % DDG share. 2AP represents the key aroma compound of wheat bread crust and absence of 2AP contributes to stale off-odor and is known as a phenomena of staling due to rapid evaporation of 2AP (Schieberle & Grosch, 1992). By means of AEDA, 2AP was determined with FD 64 in control wheat bread crust. The FD factor rapidly fell to FD 16 with 5 % DDG bread crust, until FD 0 and no perceivable odor activity in 10 to 20 % DDG bread crust. Since 2AP is significantly correlated to the aroma and freshness of wheat bread crust, its absence markedly affects aroma perception of DDG enriched bread and consequently induces uncharacteristic impression of odor. In this context, it seems transparent that during sensory evaluation panelists assigned an off-odor to DDG enriched bread with increased shares of DDG, but were incapable to define the corresponding odor quality.

In 2012, Moskowitz et al. observed similar effects for whole wheat bread and ascribed the cause to hydroxycinnamic acids such as ferulic acid (FA), influencing Maillard-type flavor generation in bread (Moskowitz, Bin, Elias, & Peterson, 2012). In crust made from whole wheat flour, lower amounts of Maillard compounds, i.e. 2AP, 4-hydroxy-2,5-dimethyl-3(2H)-furanone and higher amounts of lipid oxidation products 2-*(E)*-nonenal and 2,4-*(E,E)*-decadienal were determined. The effects on 2AP formation were attributed to competitive reaction of FA with 2APs precursor methylglyoxal. Free or released out of outer grain layers during fermentation and baking, FA inhibits the formation of 2-acetyl-1-pyrrolin (Moskowitz et al. 2012). The transferability to DDG seem feasible, inter alia since DDG contain all non-fermentable parts of the wheat kernel including outer grain layer parts in concentrated form. In a preliminary trial, tenfold higher amounts of free FA could be determined in DDG

compared to wheat flour (unpublished data) [1]. Moreover, amounts of FA in DDG enriched bread can further increase, since bioaccessibility of bonded FA is favored during fermentation and baking and both DDG processing and bread manufacturing deliver suitable conditions for the release of bonded FA into the dough media (Moskowitz et al. 2012). The quantification of FA in DDG enriched dough as well as studies on the release of FA during fermentation and baking were not included in this study. Since determining higher amounts of free FA could contribute to proof the suppression of 2AP formation in DDG enriched dough, future studies should take this topic into consideration.

Further differences of DDG enriched systems to control samples were the additional presence of two odor active compounds, phenylacetic acid and dimethyltrisulfide (DMTS). The impact of DMTS remains negligible, since DMTS initially was perceived with amounts of 50 % DDG in bread. Nevertheless, DMTS is a serious off-flavor and enlightening its origin could help to prevent aroma deficiencies if amounts increase due to diverse factors in DG processing. Presence of DMTS in lysed yeast cells or yeast extracts is already reported in the literature (Nishibori et al., 2014; Zhang, Song, Li, Yao, & Xiong, 2017). To clarify this, further research should take the impact of dead yeast cells on DDG aroma with focus on enlightening the DMTS source into account.

The impact of phenylacetic acid is correlated to the corresponding alcohol and aldehyde (2-phenylethanol and 2-phenylacetaldehyd), which are positively correlated with bread aroma (Hansen & Hansen, 1996; Paraskevopoulou et al., 2012). By means of principal component analysis (PCA), relations between different levels of DDG enriched and free bread samples, seven sensory attributes considering acceptability and off-flavor/-taste and 27 FD factors of important odor active volatiles in crumb and crust and results could be statistically confirmed (figure 9). Detailed discussion of the PCA model can be found in section 2.5; however, key findings shall be pointed out shortly. As it could be shown during analytical investigation, the divergence between 2AP and DDG enriched systems was emphasized by PCA: 2AP (figure 9, numbers 9a and b) is negatively correlated to PC1, whereas with increasing amounts of DDG the trend becomes positive, so the absence of 2AP correlates inversely with % DDG.

[1]Measured after solid phase extraction (SPE) using a SPE column Chromabond EASY by Macherey-Nagel, polar modified polystyrene-divinylbenzene copolymer with a weak anion exchanger), elution with methanol and subsequent high performance liquid chromatography (HPLC) measurement on a common C18-column and UV detection at 310 nm

Simultaneously, off-flavor, off-taste and sour odor are positively correlated to PC 1 (r > 0.99) and predominantly related to higher amounts of DDG. Concluding, the characteristic aroma of DDG in bakery products, often related to as off-flavor, is evidently linked to the absence of 2AP. Remarkably, off-flavor is significantly correlated to sour odor, supporting the hypothesis that besides concentration effects and partial absence of important aroma compounds, no off-odor is present in DDG besides the one triggered by sour impression, which can be counteracted in a simple way.

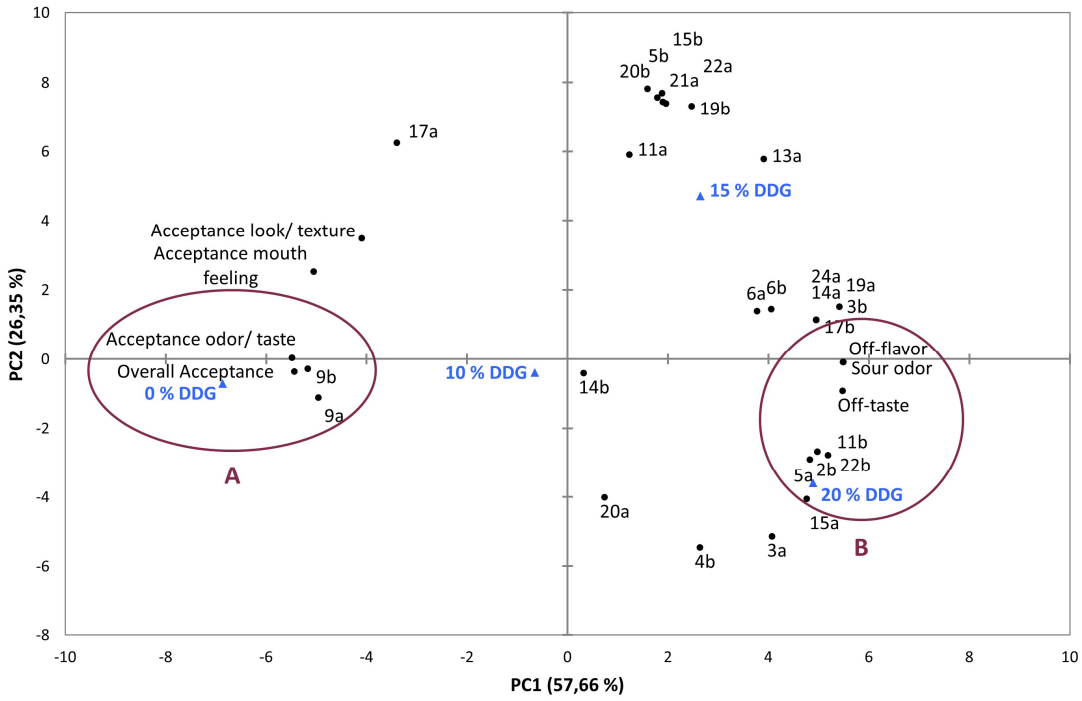

Figure 9: PCA biplot of PC1 and PC2. DDG bread samples are correlated to sensory attributes and FD factors of aroma volatiles. A: Correlation of 2AP, 0 % DDG sample and overall or odor and taste acceptance To PC 1, B: Correlation of off-flavor, sour odor, off-taste and 20 % DDG sample to PC2, Odorants: 2 butanoic acid, 3 3-methyl butanoic acid, 5 3-methylthiopropanal, 6 2,5-, 2,6-dimethyl pyrazine, 9 2-acetyl-1-pyrroline, 11 1-octen-3-ol, 13 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 14 2-phenyl acetaldehyde, 15 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 17 2-ethyl-3,5-dimethylpyrazine, 19 3-hydroxy-4,5-dimethyl-2-(5H)-furanone, 20 2-phenylethanol, 21 5H-5-methyl-6,7-dihydrocyclopentapyrazine, 22 phenylacetic acid, 24 EE-2,4-decadienal); FD factors in crust and crumb are labeled with "a" and "b", respectively. Adapted from Roth et al. (Roth et al. 2016).

Textural deficiencies of DDG enriched dough and bread systems

The aroma side is just one fragment of sensory deficiencies that come along with the addition of DDG to food products. The application of high protein and high fiber additives as bran or BSG and DDG is accompanied by textural deficiencies as well. The enrichment of bakery products with fractions rich in fiber is known to provoke challenges to the system, especially linked to end product quality and appearance, texture and taste (Ktenioudaki et al.,

2012). For DDG no scientific knowledge was available so far that investigated causes and mechanisms connected to negative impacts of DDG to food and especially bakery products and therefore were central topic of section 2.4. The utilization of DDG in food products is possible, until critical amounts around 10-15 % are not exceeded (figure 8 and 10). In summary, increased water absorption, prolonged dough development time, reduced dough stability, as well as reduced volume of total carbon dioxide (CO_2) produced during fermentation could be determined in DDG enriched systems.

Figure 10: Feasibility of enriching bakery products such as common wheat bread with DDG. Left and center: 20 and 10 % DDG shares, right: common wheat bread. The addition of DDG supports the look of a whole meal product and therefore increased sensory ratings were observed considering the appearance of bread loaves during sensory evaluation.

A detailed discussion and cause study can be found in section 2.4. Lastly, simple gluten dilution effects and a significant effect of dietary fiber, inducing competitive reactions for free water, hindering the free expansion of the gluten network and consequently provoking a less stabilized gluten network are ascribed responsible for textural deficiencies in DDG enriched dough. Since fiber and particle influences could not completely explain the negative impact, the study in section 2.4 as well concentrated on DDG specific impact factors on the dough system (Roth et al., 2016). Thus, the low pH value of DDG remains exceptional and its impact causes a decrease to the dough pH from pH 5.5 for 0 % DDG via steady decline to pH 4.3 for 20 % DDG dough. Restrained fermentation induced by acidic conditions of lactic acid leads to decreased production of CO_2 and can be ascribed to denaturation of protein and gluten strands and inhibition of metabolic enzymes (Gujral & Singh, 2000). Thus, it could be successfully shown that the neutralization of organic acids in DDG enriched dough to the pH level of standard wheat dough reduces the negative impact of DDG on CO_2 formation, gaseous release and dough height and consequently allows the production of improved end products.

The impact of thermal reaction compounds

Another important finding of DDG specific impact factors is connected to the drying process of DDG and the presence of typical thermal reaction compounds, such as furfural. The

presence of furfural was firstly detected during gas chromatographic (GC) analysis of aroma extracts of section 2.3 and consequently quantified by targeted HPLC measurement to an amount of 2.7 mg/kg. Furfural originates from five carbon sugars, after heating under acidic conditions. The present conditions of the DG to DDG drying process (pH value, amounts of lactic acid, 1 to 3 h, 100 to 105 °C) consequently favor the formation of furfural. Since toxic effects of furfural on fermentation and growth of *S. cerevisiae* by inhibition of glucose uptake and fermentation rate were reported for bioethanol production (Horváth, Taherzadeh, Niklasson, & Lidén, 2001), its relevance for the production of bakery products under fermentation conditions of aerobic respiration had also to be considered. Examinations in model suspensions, furfural enriched wheat dough and DDG enriched dough merged indications for furfural out of DDG directly affecting dough development by evoking hindered fermentation. Additionally, to furfural, 11.7 mg/kg hydroxymethylfurfural (HMF), could be determined in DDG (unpublished data). HMF represents the corresponding metabolite to furfural, which arises out of six carbon carbohydrates like glucose after heating under acidic conditions. The reaction pathway processes via enolisation and multiple dehydration to the heteroaromatic HMF, as visualized in figure 11.

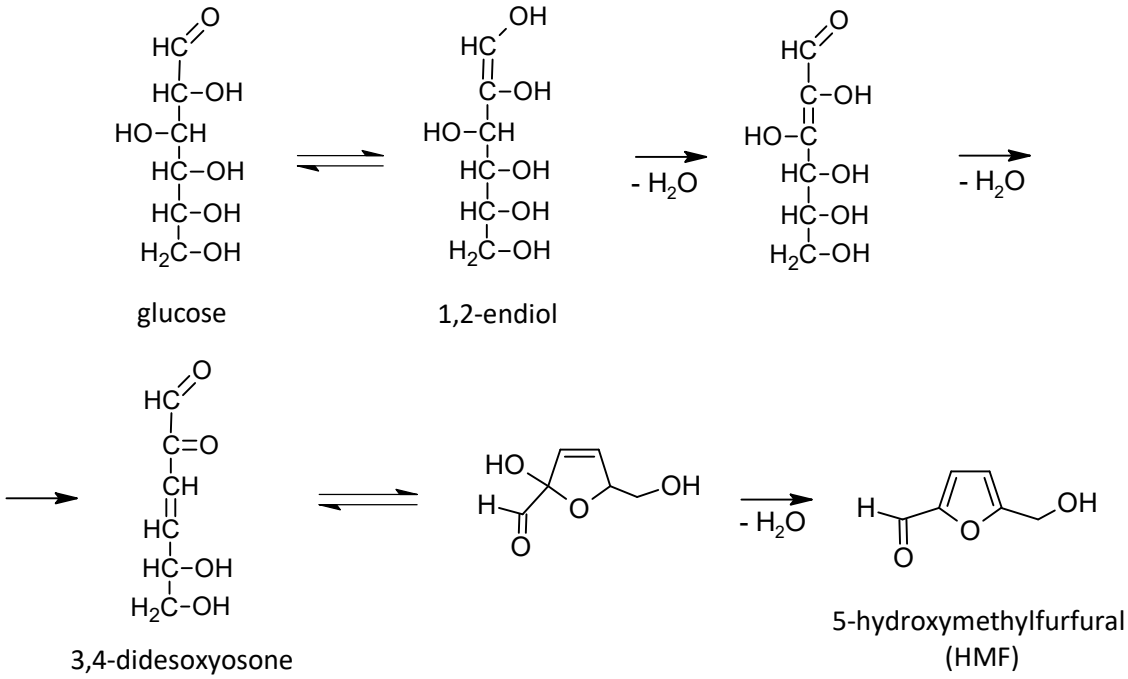

Figure 11: Furaldehyd formation under acidic heating conditions. HMF is generated after enolisation from glucose to 1,2-endiol, twofold dehydration to 3,4-didesoxyoson and subsequent cyclisation and dehydration. Furfural is generated via the same pathway, with pentoses as starting material

HMF as well is proposed as fermentation inhibitor, moreover synergistic effects and the influence of furaldehydes in general have to be considered (Iwaki, Kawai, Yamamoto, & Izawa, 2013; Sanchez, 1988). Figure 12 shows the effect of DDG on the fermentation performance in a model suspension characterized by the amount of total CO_2. With increasing amounts of DDG, the amount of CO_2 steadily declines[2]. Worth to mention, furfural might not be the only reason for decreased CO_2 formation and hindered fermentation. Future studies should as well take the impact of other furaldehydes such as HMF into account.

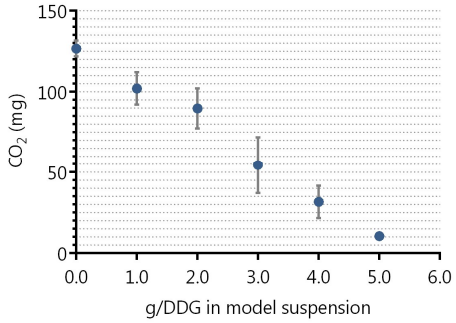

Figure 12: Production of CO_2 during fermentation in a model suspension of saccharose in water, *S. cerevisiae* and increasing amounts of DDG (0.0-5.0 g), n=2 for 0.0-4.0 and n=1 for 5.0 g DDG (unpublished data)

In summary, the cause study of DDG in bakery products revealed a complex interplay of multiple effects such as lower pH, lower enzyme activities and limited substrate availabilities as well as impact through thermal reaction compounds, such as furfural and HMF. Furthermore, common side effects accompanying the utilization of high fiber additives provoked by the dietary fiber fraction are not negligible.

The interrelationship of texture and flavor

Besides the isolated consideration of both texture and flavor, the interrelationship of these two important impact factors should also be taken into account. Undoubtedly, the flavor of a food product is affected by its texture and consequently changes in important textural characteristics can induce alterations in aroma release before or during consumption as a consequence. It is known for decades, that there are interactions between volatile compounds and non-volatile macromolecules and the existence of hydrogen bonds between carbohydrates, water and odor active volatiles, as well as hydrophobic interactions between proteins and volatiles must be considered (Le Thanh, Thibeaudeau, Thibaut, & Voilley, 1992).

[2] Quantification methodology according to section 2.4

To generate a nerve impulse necessary for the transformation of chemosensory information into electrical signals that subsequently are transmitted to the bulbus olfactorius, an odor active compound must be present in adequate concentration and additionally show sufficient volatility to reach the regio olfactoria. However, compared to the volatility in pure water, the presence of proteins, polysaccharides or lipids can reduce the volatility of an aroma compound (Druaux & Voilley, 1997). Consequently, the alteration in the composition of ingredients, that is provoked by the addition of DDG to food products obviously induces changes in the microstructure of food matrices and ultimately in the release of aroma volatiles. Besides, non-volatile compounds, i.e. sodium chloride, lactic acid, ethanol and organic acids are reported to affect the perception of other compounds, which ultimately will lead to modified bread aroma (Pico, Bernal, & Gómez, 2015). According to Thanh et al., the volatility and sorption of volatile compounds by substrates such as proteins or carbohydrates is likewise influenced by the water content (Le Thanh et al., 1992). In the context of DDG enriched systems, the reduced availability of free water in the system, as well as the higher amount of fat, protein and dietary fiber changes the microstructure and therefore might be crucial for the release of volatiles. Moreover, the rate and amount of odorants released into the oral cavity depends both on the retention of flavor compounds in the food matrix, as well as on the interplay of volatiles and main ingredients of the system (Harrison & Hills, 1997). Consequently, the degree of aroma release from a food product can change and even determine the sensory perception of a food product (Clawson, Linforth, Ingham, & Taylor, 1996). In this context, future investigations should take the relationship of aroma release and texture of DDG enriched products, including aroma recombination trials in the food matrix into account.

The drying process of DDG

After illuminating the multiple aspects that accompany DDG addition to food products and gathering novel knowledge on the aroma composition of DDG, as well as on the alterations appearing during its utilization, there is growing evidence that the drying process plays a key role for future properties and the utilization potential of DDG. During section 2.3 und 2.5 aroma volatiles in DDG and transfer effects of odorants from DDG to the food product were investigated and it was proven that the composition of odor active volatiles was directly reflected by the impact of the high temperatures during the drying process (Roth et al., 2016; Roth et al., 2014). To confirm this hypothesis, the aroma composition of freeze-dried samples

of DDG was investigated (unpublished data). As expected, freeze-dried DDG predominantly supported sour and rancid aroma, but did not support roasty odor impressions. During analysis of freeze-dried DDG enriched bread crumb and crust samples, the absence of several pyrazines, as determined in heat-dried DDG, could be confirmed. However, additional degradation products of the lipid metabolism (hexanal, *(E)*-2-nonenal) could be determined, as well as higher concentrations of lipid oxidation products in general. One reason for these alterations is reflected in the higher activity of lipoxygenases, due to a missing heat treatment step. Though, it could be successfully shown that the drying step is a decisive step for the formation and composition of aroma volatiles in DDG enriched food products.

During section 2.4, it could be revealed that heat induced formation of HMF & furfural is triggered by present temperatures and acidic conditions during the drying process. The content of furaldehydes subsequently affects the processing in fermented foods such as bakery products, by inhibitory effects on the fermentation which consequently leads to inferior end product quality. With the choice of a gentle and low-temperature drying procedure, such as lyophilization, the formation of toxic reaction components (HMF, furfural, acrylamide) can be avoided in a simple way. Besides, nutritionally valuable ingredients (protein, vitamins) are preserved and beneficial for the further utilization of DDG as a positive side effect. According to Liu et al. the poor quality of protein, which appears especially in dark heat treated samples of DDG, likewise depends on the low content of lysin (Liu, 2011). By reaction at the ε-amino group of lysin with reducing carbohydrates such as glucose during Maillard reaction, a significant decline in the availability of lysin is induced during excessive heating (Liu, 2011). By gentle drying, the protein would not aggregate or denature to that extent, would remain better digestible and gain a higher nutritional value due to higher amounts of lysin, which in consequence would enhance its marketability (see section 2.2). Therefore further research is needed on the development of gentle and eco-efficient drying technologies for improved end product quality of DDG.

Functional fractions of DDG

For the successful exploitation of DDG in the near future, non-targeted and whole byproduct utilization approaches as examined in this study most not necessarily remain the way of Upcycling DDG in food products (see also section 2.2, targeted and non-targeted approaches). In 2006, Rosentrater et al. suggested the production of DDG with high-protein, low-fiber fractions and high-fiber, low-protein fractions (Rosentrater & Krishnan, 2006).

Tailored fractions can be of special interest for food applications, i.e. to supply the demand in low calorie, low carb and high protein foods (Rosentrater & Krishnan, 2006). The theory of enriched functional fractions instead of pure ingredients in food production and byproduct valorization was also reported by van der Goot et al. (van der Goot et al., 2016). According to the authors, shifting the focus from purity on functionality is necessary for a more efficient and sustainable production of food. Moreover, enriched fractions are beneficial, since native structures of the raw material might be preserved and bioavailability of micronutrients (vitamins, trace elements) will increase. Generally, food production will become more sustainable, avoiding high energy and high cost-purification steps (van der Goot et al., 2016). Functional fractions can balance the bottleneck of inconsistent composition and qualities of different DDG sources available on the global market, that happen due to grain species, grain quality, growth conditions such as weather and soil and lately conditions of the ethanol manufacturing process and byproducts processing, i.e. drying.

To conclude, this study provides detailed knowledge on the aroma composition of DDG, as well as on the effects that accompany DDG addition in the cereal based food product using the example of wheat bread. The enrichment of DDG in wheat bread is possible in limited amounts, as it is known for other high fiber additives such as bran. Higher amounts strongly affect the dough and bread system negatively and support alterations in the aroma composition that induce uncharacteristic aroma impressions. Due to the high amount of dietary fiber, already 10 % of DDG provide a nutritional benefit to food products. This knowledge should encourage the development of gentle drying technologies of DG that avoid the formation of undesirable reaction compounds, preserve important nutrients and enable the targeted treatment of DDG for an improved utilization. Characteristics, strengths and weaknesses of DDG were determined and commonalities and differences to other cereal byproducts were presented in detail. These insights can support the transfer of this knowledge to other food systems and can help to enable the development of food products enriched with DDG. In future, the value of this byproduct can increase in metabolic cycling of food materials by being a resource for novel products in secondary process streams.

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5 APPENDIX

5.1 NON-PEER REVIEWED PUBLICATIONS

Roth, M., Jekle, M., Becker, T.: Undervalued byproduct streams- Opportunities for the sustainable use of distillers grains residue in the baked products industry, Part 2, baking+biscuit international 3 (2016), 55-57

Roth, M., Jekle, M., Becker, T.: Undervalued byproduct streams- Opportunities for the sustainable use of distiller's grains residue in the baked products industry, baking+biscuit international 2 (2016), 56-57.

Roth, M., Jekle, M., Becker, T.: Unterschätzte Nebenströme - Möglichkeiten zum nachhaltigen Einsatz des Reststoffs Weizenschlempe in der Backwarenindustrie. Bäckereitechnologie: Forschung und Innovationen, 2015, f2m food multimedia GmbH, 72-79, ISBN 978-3-9817514-0-6

Rückert, M., Zarnkow, M., Jekle M., Becker, T.: Young Cereal Scientists Explore Nottingham and the Robin Hood Legend at 12th EYCSTW, Technische Universität München, Freising, Germany. Cereal Foods World 58(4):214-215. dx.doi.org/10.1094/CFW-58-4-0214.

5.2 CONFERENCE CONTRIBUTIONS

Oral

Roth, M., Jekle, M.; Becker, T.: Food ingredient or by-product? Distiller's grains potential as functional ingredient in bakery products, 12th International Congress on Engineering and Food, Québec City, Canada, 2015-06-14.-18.

Roth, M., Jekle, M.; Becker, T.: Underestimated byproducts: Upcycling distiller's grains as functional ingredient in bakery product, 5th C&E Spring Meeting, 27.-29.04.2015, Budapest, Hungary

Roth, M., Zarnkow, M., Becker, T.: Weizenschlempe - ein anspruchsvoller Grundstoff für alkoholfreie Getränke, 47. Technologisches Seminar, Freising, Germany, 2014-02-18.

Roth, M., Zarnkow, M., Jekle, M., Becker, T.: Potential und Hintergründe zum Einsatz von Weizenschlempe in Backwaren, 64. Tagung für Bäckereitechnologie, Detmold, Germany, 2013-11-14.

Rückert, M., Zarnkow, M., Jekle M., Becker, T.: Potential of distiller's grains from wheat for the application in functional bakery products, 12th European Young Cereal Scientists And Technologists Workshop (EYCSTW), Nottingham, United Kingdom, 2013-04-11.

Rückert, M., Zarnkow, M., Jekle M., Becker, T.: Weizenschlempe – Praxisnahe Einsatzmöglichkeiten eines protein- und ballaststoffreichen Restestoffes in Backwaren, 2.Frühjahrstagung des Weihenstephaner Instituts für Getreideforschung, Freising, Germany, 2013-03-21.

Rückert, M., Zarnkow, M., Becker, T.: Charakterisierung des Potenzials von Weizenschlempe als Rohstoff für funktionelle Lebensmittel, 46. Technologisches Seminar Weihenstephan 2013, Freising, Germany, 2013-02-19.

Poster

Rückert, M., Kollmansberger, H., Zarnkow, M., Becker, T.: Potential of aroma-modified distiller's grains for the application in cereal based functional beverages, European Brewery Convention, Luxembourg, 2013-05-26.

It always seems impossible until it's done.

Nelson Mandela