# INVESTIGATION OF THE USE OF SPROUTED GRAINS AS FEEDSTOCK FOR DIRECTLY EXPANDED CEREALS


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# ABSTRACT

The use of sprouted grains in the production of directly expanded breakfast cereals is a promising opportunity to produce health-promoting, newly flavored, and colored products with less additive sugar. Hardly any knowledge about the effect of the variation of the sprouting conditions on properties of extrudate products produced on the basis of these differently sprouted samples and methods to evaluate the progress of the sprouting process exists. Therefore, this work aimed to get a deeper insight into these issues.

For this purpose, a simple and standardized system, the *degree of sprouting* method, was developed to characterize the sprouting progress by a visual inspection of the lengths of the coleoptile and radicles. Eight degrees of sprouting were defined. To test this method, wheat and oat grains were sprouted for different periods and at different temperatures. The grains were then processed by drying, milling, and extruding them using a twin-screw extruder. The sprouted grains were assigned a *degree of sprouting* to determine the average *degree of sprouting* of all grains sprouted under the given conditions. The average *degree of sprouting* was subsequently correlated with the flour and extrudate properties.

The presented study indicated that the *degree of sprouting* concept is a reliable method to predict product properties and is, therefore, of use for product development and specification. Furthermore, the results of the investigation of different sprouting times and temperatures on the properties of the resulting flours and extrudates provided new valuable insights.

By sprouting the grains at 20 °C and/or for long sprouting periods of up to 9 days the most significant changes in the flour and extrudate properties were determined. Extrudates based on sprouted wheat were found to expand less, have bigger pores, a darker color, a softer structure, and were more water-soluble. These findings were attributed to the reduction in the starch content as the result of the enhanced enzyme activity during sprouting, the accompanying increase in the reducing sugar content, and reduction in the peak viscosity while heating the sprouted grain flour suspension. Furthermore, the increase in reducing sugars and amino acids enhanced the formation of colorants and flavors as part of the Maillard reaction and provided sweeter products without the need for additional sugar. A sensory panel confirmed these findings and also preferred the extrudates based on sprouted grain flour.

Furthermore, the newly produced extrudates have a higher nutritional value as they are based on whole grain flour and show a higher vitamin content. The nutritional improvement of the sprouted grains compared to native grains was indicated by the increase in vitamin C content. Even after the high-temperature-short-time extrusion process most of the vitamin C content was preserved.

By use of another new method developed in this research work, the average molecular weight changes of the starch molecules isolated from sprouted grain flour were shown. Due to the sprouting process, the average molecular weight of the starch decreases. The results indicated a degradation of the amylopectin into smaller molecules. The reduction was accompanied by significant changes in thermal and rheological characteristics of the sprouted grain flours, which is important for future product applications.

Furthermore, this study demonstrated significant differences in the sprouting behavior of oat and wheat. Wheat was found to have a higher enzyme activity compared to oat and hence, a higher degradation rate of polysaccharides.

In summary, the present thesis shows a promising new method to evaluate the progress of the sprouting process and discusses the application of sprouted grains in directly expanded breakfast cereals.

# ZUSAMMENFASSUNG

Die Verwendung von gekeimten Getreide bei der Herstellung von direkt expandierten Frühstückszerealien stellt eine vielversprechende Möglichkeit dar, gesundheitsfördernde Produkte mit neuen Aromen und Farbe und mit weniger Zuckerzusatz herzustellen. Es gibt jedoch keine Forschungserkenntnisse über den Einfluss der unterschiedlichen Keimungsbedingungen auf die Eigenschaften von Extrudatprodukten, die auf der Basis dieser unterschiedlich gekeimten Proben hergestellt werden. Des Weiteren existiert keine standardisierte Methode zur schnellen Beurteilung des Keimfortschritts. Das Ziel dieser Arbeit war es ein tieferes Verständnis für diese Thematiken zu erhalten.

Zu diesem Zweck wurde ein einfaches und standardisiertes System, der sogenannte Keimungsgrad, entwickelt, um den Keimungsfortschritt durch eine visuelle Beurteilung der Länge des Wurzel- und Blattkeims zu beschreiben. Dazu wurden acht Keimungsgrade definiert. Um diese Methode zu testen wurden Weizen- und Haferkörner für unterschiedliche Zeiten und bei unterschiedlichen Temperaturen gekeimt. Daraufhin wurden die Körner getrocknet, gemahlen und mit Hilfe eines Doppelschneckenextruders extrudiert. Den gekeimten Körnern wurde ein Keimungsgrad zugeordnet, um anschließend den durchschnittlichen Keimungsgrad aller unter den bestimmten Bedingungen gekeimten Körner zu bestimmen. Der durchschnittliche Keimungsgrad wurde anschließend mit den Mehl- und Extrudateigenschaften korreliert.

Die hier präsentierten Ergebnisse zeigten, dass das Konzept des Keimungsgrades eine belastbare Methode zur Vorhersage von Produkteigenschaften ist und daher für die Produktentwicklung und -spezifikation von großem Nutzen sein kann. Des Weiteren, lieferten die Ergebnisse der Untersuchung der unterschiedlichen Keimungszeiten und -temperaturen auf die Eigenschaften der resultierenden Mehle und Extrudate neue, interessante Erkenntnisse.

Durch das Keimen der Körner bei 20 °C und/oder Keimen bis zu 9 Tagen konnten in dieser Arbeit die signifikantesten Veränderungen der Mehl- und Extrudateigenschaften gefunden werden. Es wurde festgestellt, dass Extrudate auf der Basis von gekeimtem Weizen weniger expandieren, sowie größere Poren, eine dunklere Farbe, eine weniger feste Struktur und eine bessere Wasserlöslichkeit aufweisen. Diese Ergebnisse werden auf die Verringerung des Stärkegehalts durch die starke Zunahme der Enzymaktivitäten während der Keimung, die damit einhergehende Erhöhung des reduzierenden

Zuckergehalts und die daraus resultierende Verringerung der Heißviskosität der Suspensionen von gekeimten Getreide zurückgeführt. Darüber hinaus verstärkte die erhöhte Konzentration der reduzierenden Zucker und der Aminosäuren die Bildung von Farb- und Aromastoffen als Teil der Maillard-Reaktion und lieferte süßere Produkte ohne die Notwendigkeit des Zusatzes von weiterem Zucker. Ein Sensorik-Panel bestätigte diese Ergebnisse und bevorzugte ebenfalls die Extrudate auf der Basis von gekeimten Getreide.

Des Weiteren weisen die neu hergestellten Extrudate einen höheren Nährwert auf, da sie auf der Basis von Vollkornmehl hergestellt sind und einen höheren Vitamingehalt besitzen. Die ernährungsphysiologische Verbesserung des gekeimten Getreides im Vergleich zu nativem Getreide wurde durch die Erhöhung des Vitamin-C-Gehalts bewiesen. Auch nach dem Hochtemperatur-Kurzzeit-Extrusionsprozess blieb der Vitamin-C-Gehalt weitgehend erhalten.

Mit Hilfe einer neuen Methode, die ebenfalls in dieser Forschungsarbeit entwickelt wurde, konnte die Veränderung im mittleren gewichteten Molekulargewicht der aus gekeimten Getreide isolierten Stärkemoleküle gezeigt werden. Durch den Keimungsprozess nimmt das mittlere gewichtete Molekulargewicht der Stärke ab. Die Ergebnisse zeigten einen Abbau des Amylopektins in kleinere Moleküle. Dieser Abbau ging mit signifikanten Veränderungen der thermischen und rheologischen Eigenschaften des gekeimten Getreidemehls einher, was von großer Bedeutung für zukünftige Produktanwendungen ist.

Darüber hinaus zeigte diese Arbeit signifikante Unterschiede im Keimungsverhalten von Hafer und Weizen auf. Es wurde festgestellt, dass Weizen im Vergleich zu Hafer eine höhere Enzymaktivität und damit eine höhere Abbaurate der Polysaccharide aufweist.

Zusammenfassend stellt die vorliegende Arbeit eine vielversprechende neue Methode zur Bewertung des Keimungsfortschritts vor und diskutiert die Anwendung von gekeimten Getreide in direkt expandierten Frühstückszerealien.

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# LIST OF PUBLICATIONS

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2. Krapf, J., Arysanto, A., Walther, G., & Flöter, E. (2019). Effect of sprouting conditions on the properties of direct expanded extruded wheat. *Journal of Food Process Engineering*, 42.

3. Krapf, J., Ding, L., Brühan, J., Lorimer, L., Walther, G., & Flöter, E. (2020). Effect of sprouting temperature on selected properties of wheat flour and direct expanded extrudates. *Journal of Food Process Engineering,* 43.

4. Krapf, J., Majocco, V., Walther, G., & Flöter, E. (2019). Effect of sprouting conditions on molecular changes of wheat and oat starch. *Cereal Technology,* 73.

**Oral presentation**

1. Krapf, J. (2018). Einführung einer neuen quantifizierenden Methode zur Beurteilung der Haferkeimung (A new approach to quantify the sprouting process of oat). *D-A-CH-Tagung für angewandte Getreidewissenschaften*, Vienna, Austria.

# CO-AUTHORSHIP

The scientific work of this thesis was partially carried and discussed with the collaboration of other researchers. All experiments were conducted at the Department of Food Process Engineering, TU Berlin, Germany.

Prof. Dr.-Ing. Eckhard Flöter supervised this thesis and all chapters were scientifically discussed with him. Dr. Susanne Rudolph-Flöter helped to outline the results in form of publications and the results were discussed together. Prof. em. Dr.Dr.e.h. Friedrich Meuser provided scientific input. Dr. Goeran Walther gave scientific input for all publications and provided the opportunity to analyze some samples at Medallion Lab, Minneapolis, US.

**Chapter 4: Sprouting of oats: a new approach to quantify compositional changes**

Julia Krapf designed, planned, conducted, supervised, and analyzed the experimental work. The experimental work was partially carried out by Franziska Kandzia and Juliane Brühan. The results were discussed with Susanne Rudolph and Friedrich Meuser.

**Chapter 5: Effect of sprouting conditions on the properties of direct expanded extruded wheat**

Julia Krapf designed, planned, conducted, supervised, and analyzed the experimental work. The experimental work was partially carried out by Arysthina Arysanto.

**Chapter 6: Effect of sprouting temperature on selected properties of wheat flour and direct expanded extrudates**

Julia Krapf designed, planned, conducted, supervised, and analyzed the experimental work. The experimental work was partially carried out by Linda Ding, Juliane Brühan, and Luisa Lorimer. The results were discussed with Susanne Rudolph.

**Chapter 7: Effect of sprouting conditions on molecular changes of wheat and oat starch**

Julia Krapf designed, planned, conducted, supervised, and analyzed the experimental work. The experimental work was partially carried out by Verena Majocco and Juliane Brühan. The results were discussed with Susanne Rudolph.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| CWS | Cold-water Solubility Index |
| DM | Dry Matter |
| DoS | Degree of Sprouting |
| DSC | Differential Scanning Calorimetry |
| $F_{max}$ | Maximum Force to break Extrudate |
| FV | Final Viscosity |
| GT | Gelatinization Temperature |
| ΔH | Gelatinization Enthalpy |
| HPLC | High Performance Liquid Chromatography |
| HTST | High-Temperature-Short-Time |
| LEI | Longitudinal Expansion Index |
| LOQ | Limit of Quantitation |
| $\dot{m}$ | Total Feed per Hour |
| $M_w$ | Average Molecular Weight |
| $M_{act}$ | Calculated Torque |
| $M_{id\%}$ | Calculated idling Torque |
| $N_{act}$ | Screw Speed |
| $N_{max}$ | Maximum Screw Speed |
| $P_{max}$ | Maximum Motor Power |
| PV | Peak Viscosity |

| | |
|---|---|
| Rel. SD | Relative Standard Deviation |
| REPEAT | Repeatability |
| REPROD | Reproducibility |
| SD | Standard Deviation |
| SEC-MALS | Size Exclusion Chromatography – Multi Angle Laser Light Scattering |
| SEI | Sectional Expansion Index |
| SEM | Scanning Electron Microscope |
| SME | Specific Mechanical Energy |
| $T_{off}$ | Offset Temperature |
| $T_{on}$ | Onset Temperature |
| $T_{peak}$ | Peak Temperature |

# 1 INTRODUCTION

Nutrition has always been one of the most important concerns of mankind. The discussion about food and food production focuses more and more on the improvement of food concerning their overall healthiness and the creation of taste experiences. This is confirmed by a recent survey. According to the German food report (2020), 90% of Germans attach importance to healthy and 98% to tasty food. Furthermore, 86% of respondents endorse that less sugar is added to finished products.

Sprouted grains can satisfy those desires of consumers, because sprouts were found to be more nutritious in terms of vitamins, antioxidants, bioavailability of minerals compared to the native grains (Harmuth-Hoene, Bognar, Kornemann, & Diehl, 1987).

It is the aim of the sprouting process to transform steeped grains into sprouts by a controlled germination process. These are often kiln-dried later in order to avoid food spoilage. The sprouting process generates and activates enzymes in the grains, especially amylolytic, cytolytic and proteolytic enzymes, and leads to the molecular degradation of grain substances (Narziß & Back, 2012). However, the health promoting cell wall material, β-glucan, is also degraded as part of the sprouting process (Anttila, Sontag-Strohm, & Salovaara, 2004). Furthermore, the increased microbiological activity during sprouting is another problem arising (Wilhelmson et al., 2001).

Sprouts can be consumed in different forms. Predominantly in Asia sprouts are consumed unprocessed and raw, for example, sprouted soybeans (Schillinger & Becker, 1997).

As a special form of processed sprouts, malt is used in beer brewing and as enzymatic baking agent in bread baking (Backmittelinstitut e.V., 1999).

Over the last years, a big trend has been towards the consumption of sprouted grains and their addition to several food products such as bread, pizza and bars, especially in the US. In 2016, 683 new food products containing sprouted grains were launched (Pagand, Heirbaut, Pierre, & Pareyt, 2017).

Another interesting application of sprouted grains can be found in puffed breakfast cereals.

Puffed breakfast cereals are a popular and quick to prepare breakfast meal. They have a wide market share and are especially popular among kids. In 2009 the yearly consumption of breakfast cereals was about 3 kg per capita of the EU (Moscicki, 2011). These cereals are often produced by use of the direct extrusion technique and the application of a twin-screw extruder.

Over the last years, there was a public debate on nutrition policy at the European level on the improvement of formulas for the production of breakfast cereals. The *Grain, Mill and Starch Industry Society* made a press notice expressing the goal to reduce the sugar content in breakfast cereals for kids by at least 20% until the end of 2025 compared to 2012. They aim for an increase in the whole grain amount in their products (Verband der Getreide-, Mühlen- und Stärkewirtschaft VGMS e.V., 2018).

The application of sprouted grains in the production of directly expanded breakfast cereals could serve as a promising idea to reduce the added sugar content, because sprouts provide a natural sweetening power due to the degradation of starch in the sprouting process. Also, it could help to increase the whole grain amount, since sprouted grains are added without removal of the embryo and bran. The sprouted grain flour exhibits special physical and chemical, as well as nutritional properties, when used as a recipe component in food products. Especially by choosing proper extrusion process conditions, the newly generated sugars and amino acids can contribute to the formation of new flavors and colors as part of the Maillard reaction. Moreover, the high-temperature-short-time extrusion process could prevent the substantial thermal degradation of vitamins and offer a long shelf-life to sprouted products which are usually susceptible to spoilage.

Due to the development of many new products based on sprouted grains, this topic is increasingly becoming the focus of scientific attention as more and more scientists are conducting or already have conducted research about this topic, e.g. Mäkinen and Arendt (2012), Richter, Christiansen, and Guo (2014), Singkhornart, Edou-ondo, and Ryu (2014) and Zhu, Adedeji, and Alavi (2017). They mainly focused on testing the effect of the use of sprouted grains in the production of several food products. Their studies vary in the added levels of sprouted grains and the applied process conditions. Thereby they used sprouted grains that were produced based on a specific single set of sprouting conditions. However, the property changes of sprouted grains, which are caused by the synthesis and degradation of grain substances significantly depend on the steeping and sprouting conditions, e.g. steeping and sprouting time, sprouting temperature. The chosen grain variety and analytical methods also have a large effect on the results (Merx, Seibel, Rabe, & Menden, 1994).

Therefore, it is difficult to compare results from different authors. Until now no methods are known which evaluate the sprouting process quickly and standardized.

Unfortunately, the extended research of a controlled sprouting or seedling growth process in malting for brewing purposes cannot be directly used as the requirements for the new application differ.

This thesis presents and discusses the new approach on the production of directly expanded breakfast cereals by the use of sprouted grains. Thus, this thesis focuses on the following issues:

(1) Finding a characterization method for the sprouting progress
(2) Studying the effect of the addition of sprouted grain flour to directly expanded cereals at constant extrusion conditions and levels of sprouted grain flour by the variation of the sprouted material:
    a. Variation of the sprouting conditions (sprouting temperature and time)
    b. Variation of cereal grain (wheat and oat)

This thesis proposes to characterize the progress of the sprouting process systematically by a defined *degree of sprouting* through a visual assessment of the length of the growing coleoptile and radicles. The new method would have the considerable advantage that the sprouting conditions can be defined in order to produce a specific product without the need for complex chemical and technical analysis of the sprouted grains. Building on this, this study hence aims to find a correlation between the average *degree of sprouting* and the flour and extrudate properties in order to use the *degree of sprouting* concept for product development.

First, as a basis for this work, a standard sprouting and extrusion procedure was developed to ensure comparable results in terms of good repeatability, reproducibility, and process stability.

In order to answer the research question of this thesis, the sprouting material was altered by the use of grains which were sprouted at different sprouting temperatures (10, 14, 20, 25, 30 °C) and for different sprouting times (1 to 9 days). After completion of the different sprouting processes, the *degree of sprouting* of the grains was determined. The sprouted grains were dried and milled. First, the resulting flours were analyzed in order to understand how the raw material changes during the sprouting process. This is necessary to understand the possible consequences of the incorporation of sprouted grains in extruded breakfast cereals.

Later, the extrudates were produced on the basis of the sprouted grain flour at constant extrusion conditions. In contrast to the malting process for brewery purposes, the whole sprouted grain including the roots was used. Hence losses can be significantly reduced. The sprouted grain flours and

extrudates produced from these flours were analyzed with regard to characteristic properties of the starch, the nutritional improvement, enzyme activity, extrudate properties including textural, sensory and color changes, and key system parameters of the extrusion process.

At the same time, it was of special interest whether the use of long-time sprouted grains in extrusion leads to insufficient low expandability and product quality due to the advanced starch degradation. Longer sprouting periods have the chance to maximize the concentration of vitamins and new flavors etc. in final products (Yang, Basu, & Ooraikul, 2001). However, long sprouting processes are usually associated with high process costs, lower production capacities, increased dry matter losses, and progressing β-glucan degradation.

In this thesis oat and wheat were used in an exemplary manner because they were often used in breakfast cereal production in Europe and the United States of America (Moscicki, 2011). Furthermore, these cereal grains significantly differ in their fat, carbohydrates, and mineral content, which makes it especially interesting to compare these. In this thesis, the differences of these cereals regarding their sprouting behavior and product application characteristics were investigated and can later be transferred to other cereals.

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# 2 FUNDAMENTALS

This chapter deals with important aspects related to cereal grains, starch, sprouting, and extrusion. First, the general structure of cereal grains and starch are presented. Moreover, the sprouting process is defined and characteristic changes in the grain were presented. Afterward, the application of sprouted grain flour is discussed and some important aspects of the extrusion process are presented. Hereby, emphasis is given to the role of the extrusion parameters, especially the effect of sugar on the extrusion process is discussed.

## 2.1 Cereal grains

Cereal grains are the most important plants for human consumption. In Europe and America especially wheat, corn, rice, rye, barley, and oat are consumed. Cereal grains convince due to their high nutrient content, long shelf life, and widespread use, e.g. in breakfast cereals, bread, and cakes (Tscheuschner, 1996).

The structure of a cereal grain, in this case wheat, is shown in Figure 1. Cereal grains are basically composed of three parts: the bran, the endosperm, and the germ. The bran is the protective outer layer of the grain and makes an absolute amount of 6-8% DM of the wheat grain. It is particularly rich in dietary fiber and minerals and hence nutritionally beneficial. The endosperm is the biggest part of a cereal grain (wheat: 83-92% DM, oat: 62-65% DM). It provides nutrition and energy especially in the form of starch. The starch granules are usually embedded in a protein matrix. The outer layer of the endosperm, which is called aleurone, is mainly composed of proteins. The germ or embryo is with 2-4% DM the smallest part of a cereal grain and is rich in protein, fat, and vitamins (Tscheuschner, 1996).

Even though the basic structure in cereal grains resembles, there are still some differences. Especially oat differs in the structure and nutrient content most significantly from other cereal grains. The oat grain has a husk, which protects the grain from the environment. The husk is not present in other grains such as wheat. It has to be removed before oat grains can be consumed. Furthermore, oat has the highest fat content of all common cereals (oat: 7%, wheat: 2%), a lower carbohydrate content (oat: 62.5%, wheat: 69.0%) and a higher mineral content (oat: 2.9%, wheat: 1.8%) (Klingler, 2010).

As described, cereal grains have a complex composition of substances. Therefore, analytical methods are often complicated and the substances have to be separated using elaborate methods.

The typical moisture content in cereal grain is 10-14% which contributes to the long shelf life of grains.

In most milling operations the bran and germ are removed in order to achieve a better color and prevent spoilage, because the shelf life of flour is reduced due to the formation of oxidations products with atmospheric oxygen (Tscheuschner, 1996). Moreover, the flour properties are altered when using whole grain flour instead of white flour.

Whole grain flour contains all the three parts and nutrients of the entire cereal grain in their original amounts (AACC International, 2008). Since the bran and embryo are rich in vitamins and minerals, whole grain flour is nutritionally beneficial compared to white flour.

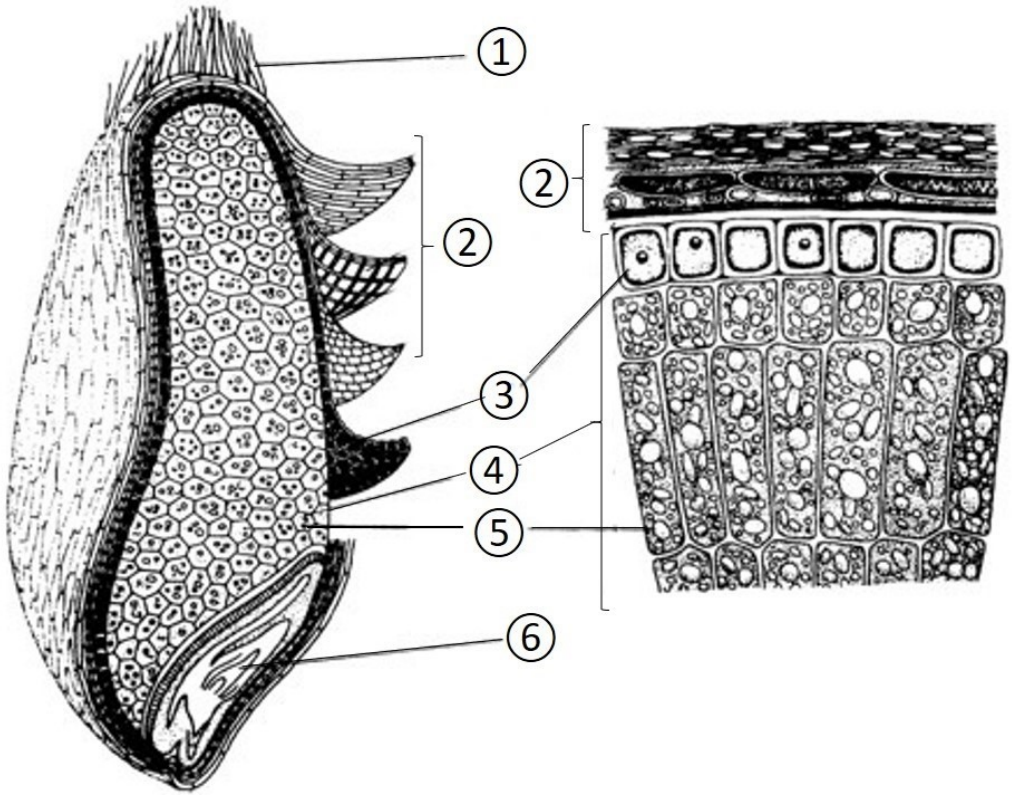


*Figure 1 Structure of a cereal grain: (1) hairs of brush, (2) bran, (3) aleurone layer, (4) endosperm (5) endosperm cells filled with starch, (6) germ (modified from Tscheuschner (1996)).*

## 2.2 Starch

The principal component of cereal grains is the biopolymer starch which decisively affects chemical and physical properties of grain material (Harper, 1981).

Starch is the dominant food reserve substance in plants and naturally occurs in particles, called granules (Whistler & BeMiller, 1999). Their size and shape vary among the starches of the different plants, e.g. wheat starch has two granular fractions, big wheat starch granules (30-40 µm) are

lenticular and oval, and small granules (2-15 µm) are spherical (Tegge, 2007), whereas oat starch granules are small (< 20 µm) and roundish (Lineback & Ponpipom, 1977).

Starch is basically composed of amylose and amylopectin with a proportion of around 28% amylose and 72% amylopectin in wheat starch. Similar proportions of amylose and amylopectin were also found in oat. However, the exact proportions differ according to the variety (Arendt & Zannini, 2013). Amylose molecules have linear chains of α (1→4) glycosidic links, allowing to form strong films and to retrograde. Whereas amylopectin is composed of α (1→4) glycosidic links, but also have 4-5% α (1→6) glycosidic links. Thus, the amylopectin has a branched structure having amorph and crystalline parts. The chemical structure of the polymers amylopectin and amylose is depicted in Figure 2.

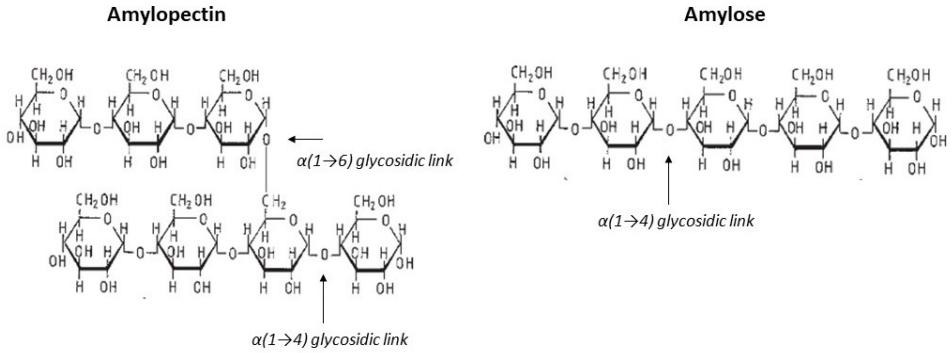


*Figure 2 Chemical structure of the amylopectin and amylose molecules (modified from Schuchmann and Schuchmann (2012)).*

In Figure 3 the structure of a starch granule is given, showing the semi-crystalline character. This fact substantially affects starch properties. In addition, amylose and amylopectin differ in their average molecular weight which is much higher in the case of amylopectin ($10^7$-$5 \cdot 10^8$) compared to amylose ($10^6$) (Whistler & BeMiller, 1999).

Starch molecules can be hydrolyzed by the action of heat, enzymes or acids. Thereby dextrins, hydrolyzed starch strands with less than 100 glucose units, are formed (Harper, 1981).

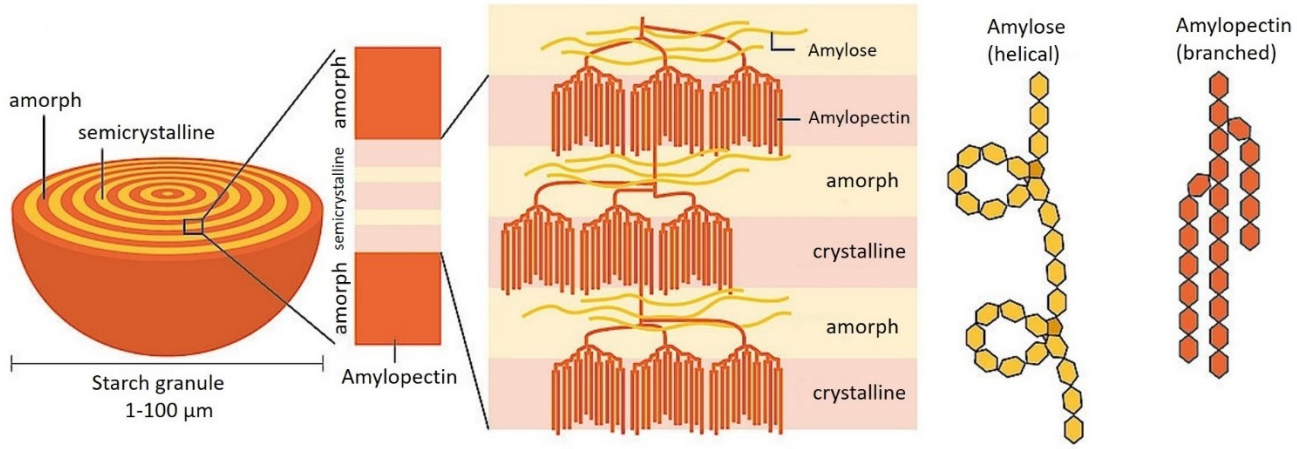


*Figure 3 Structure of a starch granule, amylopectin, and amylose (modified from SimplyScience Stiftung (2019)).*

A typical property of starch is its gelatinization and pasting behavior. In cold water, starch is not soluble. On heating of starch in the presence of excess water, the molecular arrangement of the starch is disrupted. It comes to granule swelling, the loss of the birefringence and crystallinity. This results in a significant increase in viscosity above the gelatinization temperature *GT*. As can be seen in Figure 4, if the starch solution is further heated, soluble material, especially amylose, diffuses out of granules into the paste. The gelatinization behavior is, inter alia, affected by the starch-water ratio, granule type, heterogeneity within the starch granule population, added amounts of sugar. When cooling the paste, it comes to the formation of a viscoelastic gel (Whistler & BeMiller, 1999).

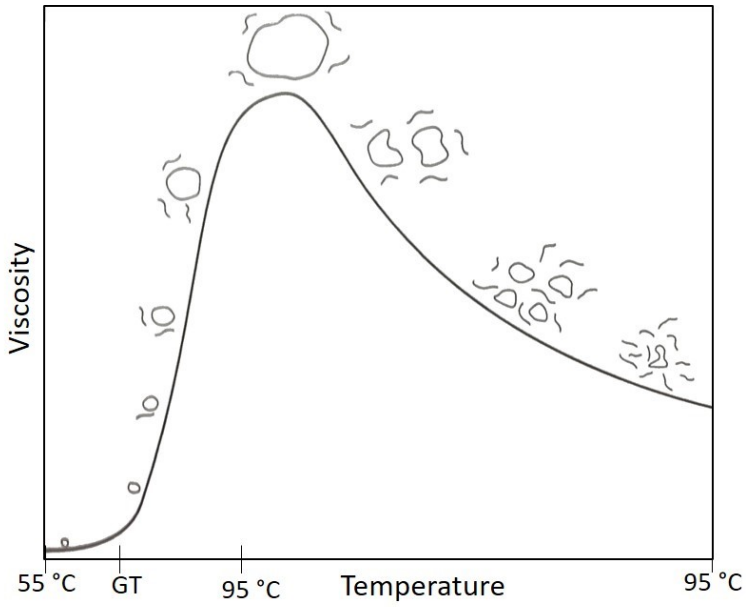


*Figure 4 Viscogram shows the cooking process of a starch granule (Whistler & BeMiller, 1999).*

In contrast to the gelatinization of starch in excess water, starch can also be gelatinized using low moisture contents in the extruder (see chapter *2.4 Extrusion cooking*). Based on stoichiometry, a minimum of 33% water is needed to achieve complete starch gelatinization.

Upon cooling and storage of starch gels the starch polymer chains start to reassociate into a more ordered structure (Atwell, Hood, Lineback, Varriano-Marston, & Zobel, 1988). This is indicated by a loss of consistency and secretion of water from the gels. Thereby, especially the crystallinity of the amylose molecules rises because of their linear structure. Between two starch chains, hydrogen bonds are formed and water molecules are expelled (Harper, 1981).

In addition to the changes in the starch structure due to thermal energy, the structure of starch can also be modified mechanically. It is observed that due to the mechanical energy input, the molecular structure of the starch breaks down and also the semi-crystallinity. The energy input results in a break of the hydrogen bonds. Thus, the reactivity of the starch in water is altered, starch becomes soluble in cold water, and the swelling and water binding capacity increases. When it comes to higher mechanical energy inputs, also starch polymer chains can be affected and degraded (Meuser, Klingler, & Niediek, 1978).

## 2.3 The sprouting process

### 2.3.1 Definition

The definition and distinction of the terms germination, sprouting and malting vary in literature. Bewley (2001) defined germination as a process, which starts with the water uptake and ends with the radicle emergence. At this point, the embryo starts to grow into a seedling and the so-called visible germination (Bewley, 1997) or sprouting process begins. Other authors (e.g. Hübner, O'Neil, Cashman, & Arendt, 2010; Singkhornart, Edou-ondo, & Ryu, 2014; Tian et al., 2010; Singkhornart et al., 2014) use the term '*germination*' for the process where the radicula is already visible and growing.

Malt is produced for brewery purposes and thereby the grains are steeped, sprouted, and kiln-dried. It is the aim of the malting process to synthesize and activate enzymes that help to convert the endosperm structures. The coleoptile of green malt (undried malt) is only allowed to grow to a maximum of two-thirds of the grain length in order to reduce the loss of dry matter. Kilning-drying comprises a two-stage thermal process. At the first stage, the green malt is dried at a temperature of 30-55 °C to a moisture content of 10% (w/w). At the second stage depending on the kind of malt to

be produced, the dried green malt is heated to 70-120 °C and dried to a maximum water content of 4% (w/w). The malted grains are then exempted from their sprouts (Kunze, 2011). Despite these relatively high temperatures, the generated and activated enzymes are largely preserved at the low water content in the dried green malt. This latter performance is of paramount importance for the brewing of beer and is also significant for the use of malt as a baking ingredient.

In contrast to the traditionally used malt, the new products based on sprouted grains, which are discussed in this work, contain grains, which are sometimes sprouted for longer times and hence differ in their properties. During sprouting of grains further growth of the seedling is tolerated up to the onset of the photosynthetic metabolic activity. Sprouting, hence a further progressed germination, gives rise to significant metabolic changes.

The AACC International (2008) established the following definition of the term *malted or sprouted whole grain*: "*Malted or sprouted grains containing all of the original bran, germ, and endosperm shall be considered whole grains as long as sprout growth does not exceed kernel length and nutrient values have not diminished. These grains should be labeled as malted or sprouted whole grain*".

In this work, the term '*sprouting*' is used notwithstanding the advances of the sprouting process in the grains and the chosen drying process.

### 2.3.2 Characteristics of the sprouting process

The production of sprouted grains can be divided into three main steps: steeping, sprouting, and drying.

Basically, due to germination and sprouting the vitals in the embryo start to generate a new plant by developing a leaf (coleoptile) and roots (radicles) (Kunze, 2011). The steeping and sprouting processes are overlapping and can be divided into three phases. The quiescent seed is wet with water resulting in a resumption of the metabolic activities. All the structures and enzymes needed for this reactivation are already present in the seed. In this phase, the solutes, e.g. ions and low-molecular weight metabolites are leaked. However, the membrane is more and more converted to a more stable configuration resulting in a stop of the leakage of the solutes in phase II. Phase II is also marked by a hydrated matrix and a plateau value of the water content in the grain. A further increase of the water content introduces phase III which also marks the completion of the germination and initiates the post-germination and embryo growing phase (Bewley, 2001).

If the grain's growth is continued, the grain will grow from the seedling into a new plant near the onset of the photosynthetic metabolic activity. This begins when the coleoptile changes its color from neutral to green.

Sprouting can only take place under a sufficient moisture content exceeding 30% (w/w) water in the grain. Due to the setting of the conditions, the biological processes in the grain can be directly regulated (Narziß & Back, 2012). The effects of the sprouting conditions on grain properties are further discussed in *2.3.8 Effect of sprouting conditions*.

In Figure 5 a sprouted wheat grain is shown. As can be seen, the embryo organs are the coleoptile, the radicula (embryonic root), the two side roots, the plumule, the mesocotyl, and the scutellum, which absorbs the nutrients for the growing seedling (Leubner, 2000).

The sprouting process is based on metabolizing carbohydrates, allowing the growth of the radicles and coleoptile (Kunze, 2011). The mobilization process of the degradation products is shown in Figure 6. The hormone gibberellic acid activates the hydrolases e.g. amylases and proteinases in the aleurone layer. The enzymes are released to the endosperm of the grain and the starch and proteins of the grains are degraded into transportable sugars, e.g. peptides, amino acids, and glucose. These low molecular weight substances are absorbed by the scutellum of the embryo where the glucose is converted to sucrose. Sucrose, glutamine, and asparagine are transported into the growing regions of the grain were they are needed for the synthesis of a first leaf and roots (Bewley, 2001).

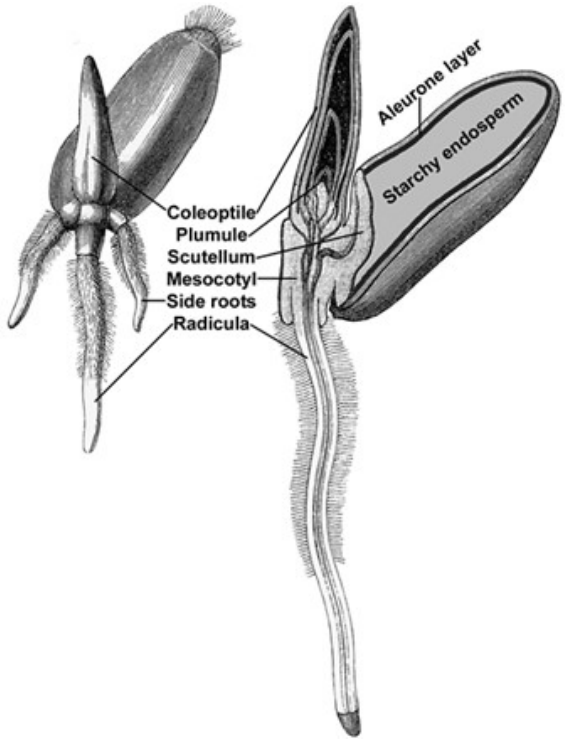


*Figure 5 Structure of a sprouted wheat grain (Leubner, 2000; Sachs & Julius, 1887).*

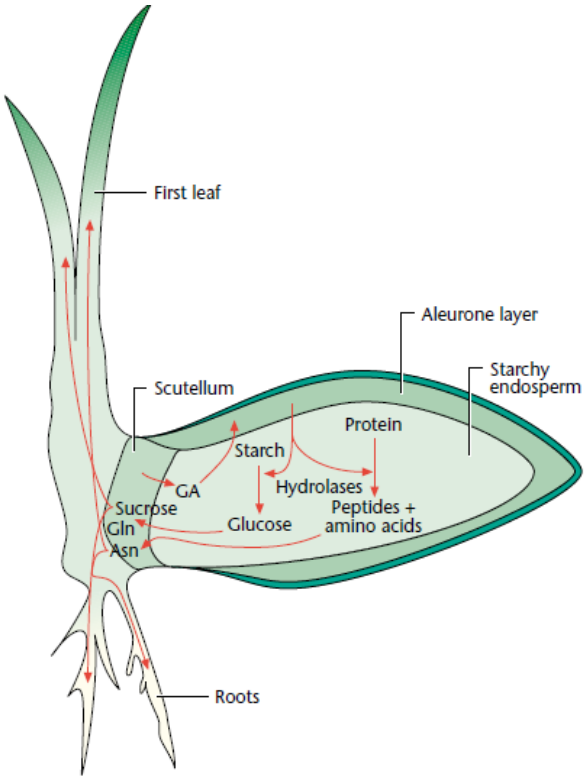


*Figure 6 Mobilization processes during sprouting (GA-gibberellic acid, Gln-glutamine, Asn-asparagine) (Bewley, 2001).*

### 2.3.3 Starch degradation

This work mainly focuses on the changes of the carbohydrates, namely starch, sugar, and β-glucan, due to sprouting, because they are the main components of the grains beside proteins, and hence most significantly affect grain properties. Therefore, starch degradation during sprouting is further discussed in this chapter.

The starch in the grain's endosperm is mainly degraded by α- and β-amylases during sprouting. α-amylase can break α(1→4) glycosidic links between glucose molecules of the starch also inside the starch molecule (Figure 7) and is called an endoenzyme (Harper, 1981). Due to this hydrolysis limit dextrins, glucose and maltose are produced. Limit dextrinase can further cleave the α(1→6) glycosidic links of the amylopectin molecule. In contrast, β-amylase degrades only from the non-reducing end of the starch molecule and releases maltose, which is subsequently degraded by maltase to glucose (Bewley, 2001).

The limit dextrinase, α-amylase, and maltase are newly synthesized during sprouting, whereas β-amylase is already present in the grain before sprouting (Bewley, 2001). Due to the increase of sweetness coming along with the formation of maltose, β-amylase is called a saccharifying enzyme. Because maltose and glucose have one reducing group per molecule, the reducing sugar content increases drastically during sprouting (Harper, 1981).

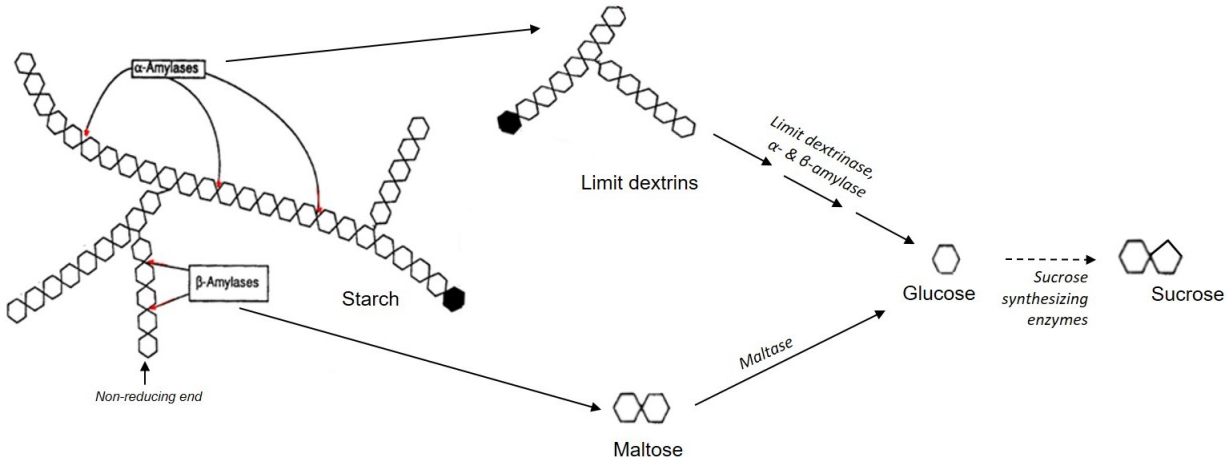


*Figure 7 Hydrolysis of starch by different enzymes.*

Sutcliffe and Baset (1973) showed a close correlation between the starch disappearance and the amylase activity changes during sprouting.

As part of the sprouting process mostly large starch granules are affected. The attack of the wheat starch granules in the endosperm is visible after 2 days of sprouting. With regard to longer sprouting durations, starch granules isolated from the sprouted wheat flour showed successive enzymatic erosion on the surface and hole formations. In contrast, no channeling or pitting on the surface of the oat starch granules was visible in the first days of sprouting. Oat starch granules from long-time sprouted flour disintegrated into small granules and after 14 days of sprouting oat starch granules showed a slightly corroded surface. It seems that oat starch is less susceptible to enzyme attacks and in addition to this, the enzyme concentration in oat is lower compared to wheat (Lineback & Ponpipom, 1977).

Starch and proteins give the grain the main functionality. With regard to the usage of sprouted grains for baking or extrusion, the changes in the starch molecule are crucial. The degradation of starch

during sprouting affects viscosity and gelatinization properties (Malleshi & Desikachar, 1986). Changes in the amylose molecule result in an altered gel formation and retrogradation (Imeson, 2009).

#### 2.3.3.1 Nonenzymatic browning reactions

As described during the starch breakdown reducing sugars are formed, e.g. maltose and glucose. They are characterized by their ability to form aldehydes or ketones in basic solutions. The reactive carbonyl group of these sugars can react with free amino groups of proteins in the Maillard reaction and produce roasting products in the temperature range of 150-200 °C (Hodge, 1953; Schuchmann, 2012). As described in chapter *2.3.5 Protein degradation during sprouting* the amount of free amino acids and peptides is also enhanced due to sprouting. The Maillard reaction and the formation of new colors and flavors are affected by the water activity, heating temperature, and time (Whistler & BeMiller, 1999).

In contrast, the sucrose molecule is a non-reducing sugar, because the glycosidic bond between glucose and fructose is formed between the reducing ends of the monomers. Hence, sucrose could not react in the Maillard reaction, though in caramelization processes. Caramelization is the removal of water from sugars, after which isomerization and polymerization are followed. This reaction can be facilitated by the presence of small amounts of salts and acids (Whistler & BeMiller, 1999).

The reaction takes place in a temperature range of 140-150 °C (Schuchmann, 2012). Besides the above described Maillard reaction of reducing sugars, the caramelization contributes to color and flavor formation in heated or roasted cereal products (Rausch, 2009).

However, these reactions bring along some disadvantages. The Maillard reaction can have toxicological implications, for example, the formation of acrylamide (Mottram, Wedzicha, & Dodson, 2002), and has a negative effect on the nutritional value of proteins due to the decreased availability of amino acids (Singh, Gamlath, & Wakeling, 2007). Moreover, the overproduction of Maillard reaction and caramelization products results in a bitter and burnt product taste (Rausch, 2009).

The formed sugars also sweeten cereal products. Hence, less additional sugar is needed. However, the different sugars also exhibit different sweetening power and relative sweetness compared to sucrose. This means that if a certain amount of sucrose equals 100% of sweetness, the same amount of another sugar can reach a different sweetness. For example, glucose reaches a sweetness of 69% and maltose 46% (Belitz & Grosch, 2013).

On the basis of the described reactions new products containing sprouted grains can be developed. The effect of the addition of sprouted grains to food products is shown in chapter *2.3.9 The sprouted grain trend and product applications.*

#### 2.3.3.2 Effect of sprouting on flavor formation

Generally, due to sprouting the flavor is intensified. The flavor is strongly changed by the drying of the sprouted grain. The flavor and odor of undried, fresh sprouts are described as '*vegetable like*' (Kunze, 2011) and was found to have a more intense odor (earthy, musty, and moist) than unsprouted grains or dried sprouts. Due to drying the flavor of the sprouted grain, e.g. oat gets more roasted, intense, sweet, nutty, and bitter, and the texture is crispier, and more brittle. Moreover, the temperature profile during the drying of the sprouted grains affects the sensory profile. Grains dried at high temperatures (65-85 °C) have a strong roasted odor, a more intense flavor and a crispier texture. In contrast, drying sprouted grains at low temperatures (30-50 °C) results in a mustier and more rancid flavor and a harder texture (Heiniö, Oksman-Caldentey, Latva-Kala, Lehtinen, & Poutanen, 2001).

As already described, the degree of starch degradation and the amount of degradation products clearly affects the flavor evaluation.

During the sprouting process, the triglycerides are hydrolyzed by lipases and hence the concentration of free fatty increases (Bewley, 2001) resulting in a more rancid flavor especially in oat.

#### 2.3.3.3 Losses during sprouting

Another issue coming along with sprouting and vegetative growth are losses. Even though the radicles and coleoptile are used in new product approaches and are not removed as it is the case in malting for brewery purposes, there are still losses due to respiration, the partial degradation of carbohydrate material and the leaching during steeping (Faltermaier, Waters, Becker, Arendt, & Gastl, 2014). These material losses come along with financial losses.

The malting losses are associated with the enzyme activity and the degradation in the grain. Those are affected by the sprouting temperature and time. Suhasini and Malleshi (1995) found malting losses of around 18% (w/w) for wheat which was sprouted for 5 days at 15 °C, and the malting losses almost doubled while sprouting wheat at 35 °C for the same time. Tian et al. (2010) found slightly lower dry matter losses of 14% (w/w) for oat sprouting at 16 °C for 5 days.

### 2.3.4 β-glucan degradation during sprouting

In contrast to wheat, oat contains huge amounts of β-glucan (Delcour & Hoseney, 2010). β-glucan is the main cell wall component of the oat endosperm and is composed of D-glucopyranose units, which are connected by β (1→4) glycosidic links (70%) and β (1→3) glycosidic links (30%). The β (1→3) glycosidic links are separated by two or three β (1→4) glycosidic links (Whistler & BeMiller, 1999). Because of the combination of the different links, the β-glucan molecule is flexible and can bind huge amounts of water and provide a high viscosity.

Oat has excellent health-related properties due to the high content of β-glucan (El Khoury, Cuda, Luhovyy, & Anderson, 2012). The health benefits of β-glucan are mainly based on two effects. The first is the increase of the viscosity in the intestine by β-glucan, which retards the absorption of glucose and lowers blood sugar levels. The second is the positive effect on the cholesterol level (Anttila, Sontag-Strohm, & Salovaara, 2004).

During sprouting, β-glucan is degraded by endo-β-1,4-glucanase, endo-β-1,3-glucanase, exo-β-glucanase, and β-glucan-solubilase. As a result of the breakdown of the cell wall, which is composed of β-glucan, the other enzymes are able to penetrate into the endosperm and start the enzymatic degradation of the macromolecules. This process is called cytolysis and results in the friability of the sprouted grain (Kunze, 2011).

Due to this reduction of the β-glucan content, the health-promoting effect of oat β-glucan is obviously reduced.

Wood et al. (1994) studied the acid hydrolysis of oat gum drinks for 15 and 60 min. The acid hydrolyzed drinks and the reference had the same β-glucan concentration, however, differences in the viscosity and glucose responses were found. Due to the acid hydrolysis, the viscosity was reduced and, consequently, the glucose response increased. Hence, not only the total β-glucan concentration is of interest also molecular degradation.

### 2.3.5 Protein degradation during sprouting

Asparagine and glutamine are two of the most important amino acids needed for the seedling's growth. Therefore, the proteins of the grains have to be degraded by proteinases into amino acids during sprouting. Thereby, endopeptidases cleaving the proteins internally into oligopeptides which

were further degraded into amino acids or aminopeptidases and carboxypeptidases cleaving the amino acids from the terminal of the protein (Bewley, 2001).

In addition to the significant changes of the starch molecules in the cereal grain due to sprouting, the protein matrix is also affected. In matters of baking, the dough forming properties of gluten is crucial. Based on the degradation of proteins during the sprouting process, the dough quality is affected (Agrahar-Murugkar, Gulati, Kotwaliwale, & Gupta, 2015), see chapter *2.3.9 The sprouted grain trend and product applications*. Furthermore, protein concentration and composition also affect extrudate properties (Allen, Carpenter, & Walsh, 2007). However, this is out of the scope of this thesis.

### 2.3.6 Nutritional changes during sprouting

One of the main advantages of the use of sprouted grains is their enhanced nutritional value.

Due to physiological changes during sprouting, stress is induced in the grain, and the redox system is imbalanced. Thereby, secondary metabolites are formed, including the synthesis of antioxidants like phenolics and vitamins (Swieca & Dziki, 2015). Osawa, Narasimhan, Kawakishi, Namdci, and Tashiro (1985) found that rice, which has greater sprouting ability, also had a higher antioxidative activity. These antioxidants are essential to protect the new seedling.

Oat grains contain high amounts of phenols in the aleurone layer, which are part of the defense system against microorganisms (Skoglund, 2008). In contrast, the vitamins are mainly found in the bran and germ of the grain (Kunze, 2011).

Many research efforts focused on the effects of sprouting on the vitamin content in grains. However, the results differ, e.g. Žilić et al. (2014) found a 300% increase and Plaza, Ancos, and Cano (2003) a 50% decrease in the vitamin E content during the sprouting process. The reason for these differences may be the different sprouting conditions, e.g. the variation of the steeping time and temperature, sprouting durations and temperatures, light and air conditions, and the use of different grain varieties. Moreover, there are many different analytical procedures to determine the vitamin contents. Therefore, literature results are hard to compare.

In Table 1 the results of the vitamin content analysis in sprouted grains of different researchers are presented. One can see that for the vitamins B1, B3, B6 and E in sprouted grains different results were found. The sprouting activity was found to be enhanced in the presence of vitamin B1 (Merx, Seibel, Rabe, & Menden, 1994). In contrast, the researchers presented affirmative findings for vitamin B2 and

C. These vitamins significantly rise due to the sprouting process. Since no vitamin C is present in native wheat, its formation through biosynthesis seems to contribute highly to the enhanced nutritional value of sprouted grains. Furthermore, the vitamin C content seems to be a good marker for the increase of the vitamin content during sprouting.

*Table 1 Comparison of results of different studies concerning the vitamin changes during sprouting of wheat*

| Reference | Harmuth-Hoene, Bognar, Kornemann, & Diehl, 1987 | Suhasini & Malleshi, 1995 | Yang, Basu, & Ooraikul, 2001 | Plaza et al., 2003 | Žilić et al., 2014 |
|---|---|---|---|---|---|
| Vitamin: | | | | | |
| B1 | ↗ | → | | ↑ | ↘ |
| B2 | ↑ | ↑ | | ↑ | ↑ |
| B3 | | → | | | ↑ |
| B6 | ↑ | | | ↑ | ↓ |
| C | ↑ | ↑ | ↑ | ↑ | |
| E | ↑ | | ↑ | ↓ | ↑ |

In the first hours of sprouting the embryo of the grains is protected by ascorbic acid which has been formed by the reduction of already present low amounts of dehydroascorbic acid. With progressing sprouting, reducing sugars were formed (see chapter *2.3.3 Starch degradation*) and converted into ascorbic acid (Gara, Pinto, & Arrigoni, 1997).

However, the synthesis of the vitamins is complex biochemical reactions that can only be assessed taking the specific sprouting conditions into account (Merx et al., 1994).

Besides, ascorbic acid is of technological importance. It is used in the bread and flour industry as a redox agent. The addition of only 10 ppm ascorbic acid improves the rheological properties of doughs.

Further, it was found that ascorbic acid increases the gas retention capacity, the dough strength, and the volume of biscuits (Grosch, 1986). The amounts of ascorbic acid formed during sprouting seem to be sufficient to achieve similar positive effects which would make further addition of ascorbic acid redundant.

Furthermore, a significant increase in total phenolics in sprouted oat was pointed out. The increased amount of antioxidants is protecting the product, which contains sprouted grains, from rancidity and changes in color and taste (Xu et al., 2009; Tian et al., 2010; Khang, Vasiljevic, & Xuan, 2016).

In addition to the increase in vitamins and antioxidants, using sprouted grains is also favorably for diabetes patients. It was shown that the consumption of sprouted grain bread improves glycemia by lowering the glucose response and increasing glucagon-like-peptide-1 response, which is a hormone regulating blood sugar (Mofidi et al., 2012).

Another advantage of the usage of sprouted grains is the reduction of anti-nutrients like phytic acid and trypsin inhibitors. Phytic acid binds minerals and proteins and thus affects their solubility and bioavailability. In plants, phytic acid helps to store phosphorous and cations such as calcium and magnesium, which the seedling needs during sprouting (Dendougui & Schwedt, 2004). The sprouting process increases the phytase activity in the grains which results in a reduction of the phytic acid content of around 62% for wheat and 69% for oat after 5 days of sprouting (Centeno, Viveros, Brenes, Lozano, & La Cuadra, 2003; Tian et al., 2010). It has been found that some minerals such as iron, zinc, calcium, and manganese have, therefore, increased bioaccessibility in sprouted grains (Platel, Eipeson, & Srinivasan, 2010).

Moreover, due to the degradation of the macromolecules, sprouted grains are easier to digest (Pagand, Heirbaut, Pierre, & Pareyt, 2017).

### 2.3.7 Microbiological growth during sprouting

Common sprouting conditions like long steeping stages, high moisture contents during sprouting, and sometimes also high temperature (20-25 °C) are favorable conditions for microorganism growth. Thereby, the growth of bacteria, mold, and yeast can be observed and dormant spores can be activated (Wilhelmson et al., 2001; Helland, Wicklund, & Narvhus, 2002).

In order to minimize the risk of microorganism growth, the following options can be taken into consideration: a disinfection step before steeping and sprouting preventing microorganism

contamination on the grain surface (Yang et al., 2001), lower steeping and sprouting temperatures, and the use of protective cultures like lactic acid bacteria (Back, 2008). However, the use of disinfection is not allowed in Germany (Schillinger & Becker, 1997).

Moreover, multiple washing of the sprouts helps to remove microorganisms from the surface and the use of slightly acidified water inhibits the growth of molds and yeasts. Due to intensive heating of the sprouts after completing sprouting, the microorganism content can be reduced significantly and health damages can be prevented (Schillinger & Becker, 1997).

### 2.3.8 Effect of sprouting conditions

As described before, the production process of sprouted grains consists of three main parts, which all affect the final product properties. Sprouting starts with the rise of the water content in the grain during steeping. Thereby, the final steeping degree is mainly affected by the steeping time, steeping temperature, and ventilation or air rests in which the grains are able to respire.

The main step is the sprouting process, which is significantly affected by the air conditions, the sprouting time and temperature. During this step, the water content in the grains should not be reduced. That is why high humidity and frequent spraying of water is desirable.

During the last step, the grains are dried and become storable. Thereby, the grains can be kiln-dried or freeze-dried. The freeze-drying process is more gentle and preserves vitamins (Ali, Yusof, Chin, & Ibrahim, 2016). Malt is usually kiln-dried. The kiln-drying process of the grains is affected by kiln-drying temperatures and duration. In malting for brewery purposes, the grains are dried at 80-105 °C to a final moisture content of a maximum of 4% (w/w) (Narziß & Back, 2012) which is accompanied by long kiln-drying times and high stress on the grain. Higher moisture content and lower temperatures are also conceivable for other product applications, also in order to preserve vitamins and to obtain new flavors.

This work mainly focuses on the sprouting temperature and time effect. That is why the effect of these two parameters is further discussed in this chapter.

Multiple studies suggest that the formation of synthesis and degradation products can be maximized by prolonging the sprouting process (Yang et al., 2001). Thus, the starch in the grain is progressively degraded by allowing increased sprouting times and more and more short-chain sugars are formed (Nirmala, Subba Rao, & Muralikrishna, 2000). This also has a significant effect on the viscous

properties of the sprouted flour (Malleshi & Desikachar, 1986). Though, increasing sprouting times also result in increasing dry matter losses (Tian et al., 2010), progressing degradation of β-glucan (Wilhelmson et al., 2001), and higher process costs coming along with rising energy costs and lower production capacities.

The variation of the sprouting temperature shows different effects on the grain properties. It is hard to find a temperature in the literature that maximizes the progress of the sprouting process. The data are not consistent. Furthermore, there are only a few data available that show the effect of the sprouting temperature on the vitamin content in grains (Koehler, Hartmann, Wieser, & Rychlik, 2007).

However, a significant effect of the sprouting temperature on grain properties was found. It seems that the sprouting temperature variation mainly affects enzyme activity and synthesis. Thus, starch and protein degradation and dry matter losses are also affected by the sprouting temperature (Suhasini & Malleshi, 1995; Swieca & Dziki, 2015). Wilhelmson et al. (2001) showed the changes in enzyme activity due to altered sprouting temperatures. They found an increase in α-amylase activity at 15 °C and 25 °C compared to 5 °C in oat, whereas Suhasini and Malleshi (1995) found the highest α-amylase activity in sprouted wheat at 25 °C, followed by 30 °C, 20 °C, 15 °C, and 35 °C.

Moreover, the sprouting temperature was found to have an effect on the nutritional value of the grains as it affects the β-glucan, dietary fiber, and phenol content (Wilhelmson et al., 2001; Koehler et al., 2007).

The standard sprouting process of malt production for brewery purposes is performed at a sprouting temperature of 14 °C (Jacob, 2016). The application of higher sprouting temperatures also results in a reduction of the energy consumption because of a lower cooling demand (C. Müller, 2015).

As described in chapter *2.3.7 Microbiological growth during sprouting* there is also an adverse effect of long sprouting times at enhanced temperatures on the microbiological growth (Dziki, Gawlik-Dziki, Kordowska-Wiater, & Doman-Pytka, 2015). Temperature and time conditions are two of the main growth factors of microorganisms.

### 2.3.9 The sprouted grain trend and product applications

Sprouts are already consumed for thousands of years. Since 2800 B.C. the Chinese know about the positive health effects of sprouts. In western countries, the consumption of sprouts started in the 18$^{th}$ century (Schillinger & Becker, 1997).

The sprouts of many seeds such as wheat, oat, amaranth, rice, sunflower, pumpkin seed, etc. are edible.

In recent years, the trend towards the addition of sprouted grains to many food products such as pizza, flakes, crackers was observed. In the year 2018 the US retail sales of sprouted grain products reached $267.2 million (Watson, 2018). In the years between 2012 and 2016, the number of products containing sprouted grains increased every year by about 26%. In 2016, already 683 new products containing sprouted grains were launched. 22% of these new products were snacks, followed by 19% meals and 15% bakery products (Pagand et al., 2017).

In the production process, sprouted grains can be used in two ways. After sprouting, the grains can be mashed with water and then used in doughs (e.g. bread, tortillas, muffins). Alternatively, the grains can be dried. Before adding them to different products, the whole grains can be cooked or milled into flour.

Many researchers studied the application of sprouted grains in food products such as bread, tortillas, pasta, breakfast cereals, baby food, fermented beverages, biscuits, bars, sweets, and porridge (Mosha & Svanberg, 1983; DE3741991C1, 1987; Muñoz-Insa, Gastl, Zarnkow, & Becker, 2011; Richter, Christiansen, & Guo, 2014; Singkhornart et al., 2014; Agrahar-Murugkar et al., 2015; Liu, Hou, Cardin, Marquart, & Dubat, 2017). However, in these studies, only a specific single set of sprouting conditions was used and the important effect of the sprouting time and temperature, see chapter *2.3.8 Effect of sprouting conditions*, was not considered. In some studies, flour from grains, which were only sprouted for a short period or commercial flour without any information about sprouting conditions was used (Liu et al., 2017). The researchers mainly focused on the replacement of different levels of flour by sprouted flour. Thereby, different cereals were tested such as wheat, oat, and millet.

The application of sprouted grains in food products such as bread, biscuits, and tortillas was found to affect product properties significantly.

Bread with added sprouted grain flour has a higher specific volume, a longer shelf life, a softer crust, and an improved sensory quality. The results depend on the used cereal grain and the added level of sprouted flour. However, the addition of sprouted flour to bread is limited by high levels of sprouted flour because it may result in the formation of a sticky and coarse crumb. The improvements in the bread quality can be explained by the increased amounts of sugars due to the sprouting process. The

added yeasts are fermenting these sugars and the produced gas increases the volume of the loaf. Moreover, the kilning process after sprouting matures the flour and results in an increased dough gas retention and stability (Mäkinen & Arendt, 2012; Bellaio, Kappeler, Rosenfeld, & Jacobs, 2013; Richter et al., 2014). Furthermore, it was shown that the nutritional value of bread is enhanced due to the addition of sprouted flour (Alvarez-Jubete, Wijngaard, Arendt, & Gallagher, 2010).

Also, in the production of tortillas, the addition of sprouted flour increases the specific volume and diameter of the tortillas. This is probably due to the weaker protein network and the easier spreadability, resulting from the rise in the enzyme activity. Furthermore, tortillas with sprouted flour were found to be brighter in color, have a less firm texture and achieve a higher sensory score with regard to the color, flavor, and overall acceptance (Liu et al., 2017; Zhu, Adedeji, & Alavi, 2017).

Another interesting idea was presented by Mosha and Svanberg (1983). The aim of their work was to produce gruel with a high energy and nutrient density and a reduction in the viscosity because a high viscosity is not appropriate for child feeding. They mixed flour with water and boiled this gruel. The researchers found that an application of sprouted sorghum is convenient due to the amylolytic activity in sprouted grains. In order to achieve a desired viscosity of the gruel, only a certain amount of flour can be added to the gruel. By using sprouted flour, much larger quantities of cereals can be added to the gruel to maintain the same viscosity. Thus, this gruel has a higher energy and nutrient density and the child has to consume smaller amounts.

It is particularly interesting to study the use of sprouted grains in extruded breakfast cereals, also see chapter *2.4 Extrusion cooking*. By the use of sprouted wheat flour as a feed for the production of extruded breakfast cereals the specific mechanical energy, expansion index, bulk density, breaking strength, and water absorption index were found to decrease and the specific length and water solubility index of the extrudates increase. The results are associated with the starch degradation during the sprouting process and accompanying lower dough viscosities and an increase in reducing sugar levels. The extrusion process results in a further increase in the reducing sugar content due to starch hydrolysis and increases starch digestibility. These studies are based on a specific single set of sprouting conditions and focused primarily on the variation of the parameters of the extrusion process (die temperature, screw speed, and $CO_2$ injection) (Singkhornart et al., 2014; Zhu et al., 2017).

A more established application of sprouted grains is the usage in beverages. For centuries the application of malted grains for beer production is common practice. Muñoz-Insa et al. (2011) showed

the nowadays unusual application of oat for malting. Goebel & Hitze patented a ''*Keimgetreidetrunk*'' (malted grain drink) (DE 3741991). They claimed a drink with an increased vitamin level as a result of a sprouting process at 25 °C. Moreover, the amount of soluble dietary fiber and protein is quite high.

## 2.4 Extrusion cooking

HTST (high-temperature-short-time) extrusion is a process, which is often used in food processing. It combines several process technologies such as kneading, mixing, gelatinization, shaping in one device. This device is called an extruder and is depicted in Figure 8. Here a twin-screw extruder is shown. Thereby the two screws (3) are driven by an engine (1) and material is given into the extruder by use of the feeder (2). The screws are embedded in temperature-controlled barrels (5). At the end of the extrusion process, material is forced through a die (6) (van Lengerich, Meuser, & Ng, 2007).

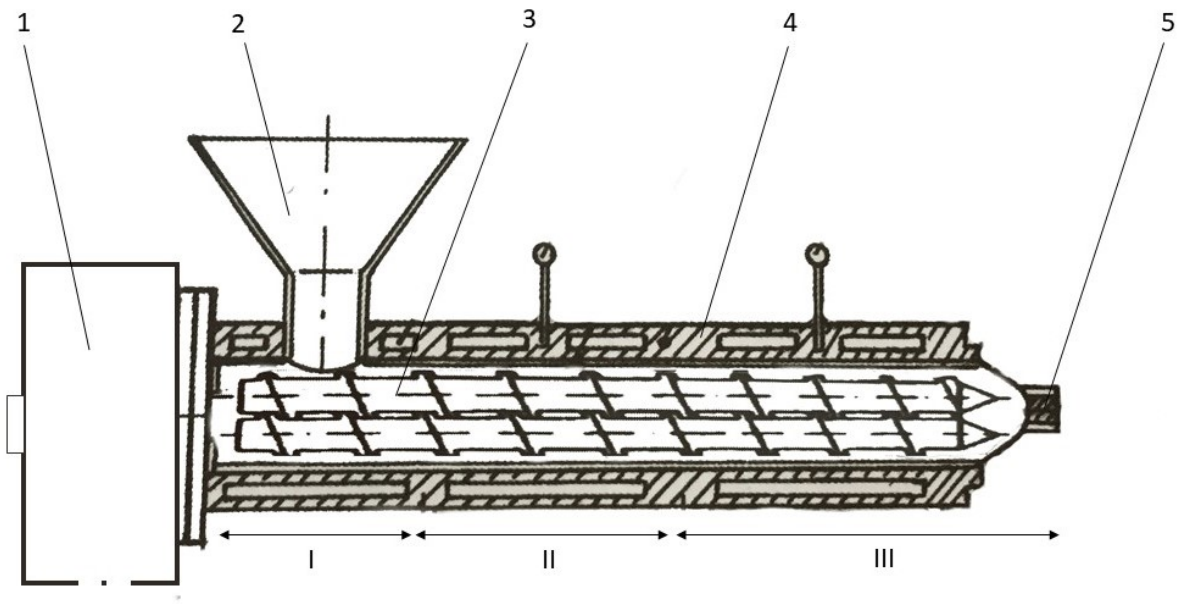


*Figure 8 A cross-section of a twin-screw extruder: 1 – engine, 2 – feeder, 3 – screws, 4 – heating/cooling barrel, 5 – die, I – transport zone, II – compression zone, III – melting and plasticizing zone (modified from Moscicki (2011)).*

There are great numbers of applications for the extrusion process. Typically produced food products are directly expanded breakfast cereals and snacks. Thereby, components rich in starch were mixed with low amounts of water in the extruder (zone I). It is also possible to add other ingredients to improve the flavor, taste, color, or nutritional value. Thermal energy can be introduced into the mixture by conductive heating of the extruder barrels, by the injection of steam, or due to viscous

dissipation from the rotating screws (zone II) (van Lengerich et al., 2007). Due to the temperature increase, the mass first exceeds the glass transition temperature which makes the mass plastic elastic. A further increase in the temperature results in melting and cooking of the mass (zone III), also see chapter *2.2 Starch*. After emerging through the die the viscous dough-like mass suddenly relaxes, the moisture flashes and the mass expands. Due to this temperature and moisture decrease, the product temperature falls below the glass transition temperature and the final product assumes a porous structure (Schuchmann, 2012).

The starch-based extrudates are often described as crunchy and brittle due to their porous structure and complex failure mechanism (Barrett, Cardello, Lesher, & Taub, 1994).

Extrudate product properties can be affected by the variation of extrusion parameters during the extrusion process. The most important parameters that affect the extrudate properties are the choice of the engine and screw length, design and speed, the temperature profile of the barrels, the mass throughput, and the moisture content of the feed. These aspects are further discussed in chapter *2.4.1 System analytical approach*.

In general, the density and shape of the extrudates are affected by a combination of the growth and subsequent shrinkage of water vapor bubbles in the extrudate and the die swell. Even in the absence of water vapor, the die swell can cause the expansion of the extruded mass. The driving force for the bubble growth is the pressure difference between the pressure inside the bubble and the environment (Fan, Mitchell, & Blanshard, 1996).

### 2.4.1 System analytical approach

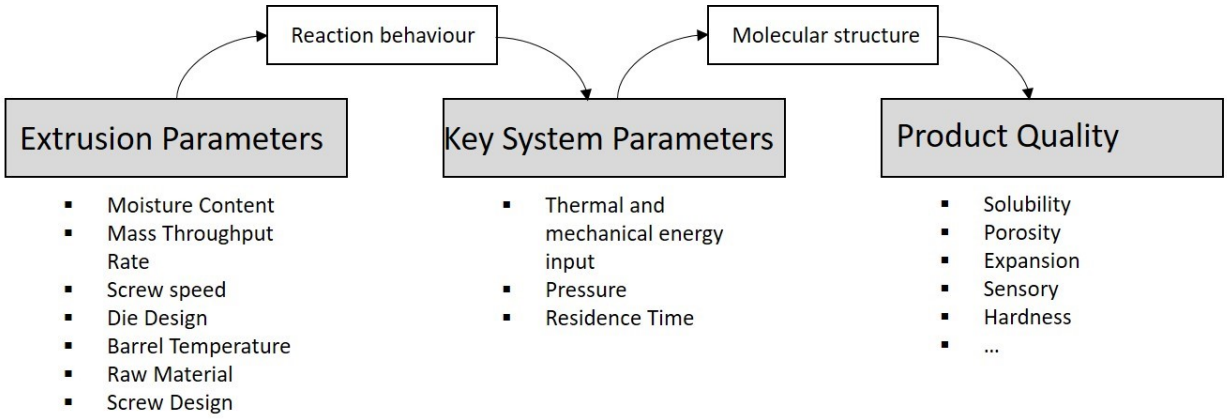


*Figure 9 Systems analytical model (modified from Meuser, van Lengerich, and Reimers (1984)).*

The system analytical approach for extrusion was presented by Meuser et al. (1984) and is shown schematically in Figure 9. Thereby, independent extrusion process parameters such as screw speed and barrel temperature alter the reaction behavior of the material and affect the thermal and mechanical energy input as well as the residence time. The total amount of introduced mechanical energy during the extrusion process into the mass is expressed as SME (specific mechanical energy).

The SME is calculated based on the equation (1) (van Lengerich et al., 2007), whereas $N_{act}$ is used screw speed (rpm), $N_{max}$ is the maximum screw speed (rpm), $M_{act}$ is the calculated torque (Nm), $M_{id\%}$ is calculated idling torque (Nm) depending on the used screw speed, $\dot{m}$ is the total feed per hour (kg/h) and $P_{max}$ is maximum motor power (kW).

$$SME = \frac{\left(\frac{N_{act}}{N_{max}} \cdot \frac{M_{act}}{100}\right) - \left(\frac{N_{act}}{N_{max}} \cdot \frac{M_{id\%}}{100}\right)}{\dot{m}} \cdot P_{max} \quad \left[\frac{Wh}{kg}\right] \qquad \text{(Equation 1)}$$

Depending on the SME input during the extrusion process, the granular structure of the starch is completely or partially dissolved and the starch loses the semi-crystalline character. This is accompanied by the disruption of hydrogen bonds between the starch molecules, which can be determined thermodynamically by determining the remaining gelatinization enthalpy using DSC measurements. Furthermore, high energy input also breaks down starch polymer chains. This effect can be verified by viscosity measurements. After the treatment at high SME levels, only a small torque

increase, this means a low cold paste viscosity, and a low gel formation capacity can be found (van Lengerich, 1984).

The pore wall thickness of the extrudates also depends on the energy input. A high SME results in extrudates with a high expansion index and a low pore wall thickness (van Lengerich, 1984).

The key system parameters must be kept constant when scaling-up the extrusion process. Depending on the energy history to which the material was exposed during the extrusion process, the properties of the extrudates, such as expansion rate and extrudate hardness, are affected (van Lengerich et al., 2007).

The effect of the extrusion process parameters is further discussed in the following part as these parameters are the key levers to obtain the required product properties. Here the parameters are discussed separately. However, there is an interaction of many parameters (van Lengerich, 1984).

When it comes to the impact on the key system parameters the water content of the extruded mass is of particular importance. Due to lower moisture contents, the viscosity of the mass is enhanced which affects the energy absorption of the mass. At low moisture contents, it is hence possible to introduce more mechanical energy into the mass and it comes to the formation of frictional heat (van Lengerich, 1984).

Another important parameter is the barrel temperature. The increase in the barrel temperature decreases the specific mechanical energy input due to the reduction of the viscosity of the mass. Hence, the variation of the barrel temperature is of special importance when developing products that require a high temperature and low energy input (van Lengerich, 1984).

Furthermore, the energy input in the extruded mass is affected by the screw speed. The screw speed affects the residence time of the mass in the extruder. An increased screw speed reduces the residence time and degree of filling of the extruder. At lower residence times the mass comes faster into the front zone of the extruder where the mechanical energy input into the mass is hence increased. Thus, the formation of frictional heat is changed and the temperature of the mass is enhanced (van Lengerich, 1984).

The increase of the mass throughput rate lowers the SME and also affects the residence time of the mass in the extruder. Up to a certain point, the increase of the mass throughput rate also results in a

further temperature increase of the mass in the extruder due to more friction. However, when this point is exceeded the heat transmission is too low (van Lengerich, 1984).

The variation of screw element configurations and die designs also affects the energy input in the extruder.

### 2.4.2 Effect of sugars on the extrusion process

Several factors are affecting the extrudate properties, as discussed in chapter *2.4.1 System analytical approach*. However, this chapter focuses on the effect of sugar in the extruded mass on extrudate properties, because it is the main issue when it comes to the extrusion of sprouted grains.

Breakfast cereals have a sugar content of up to 45% (Kühne, 2004). The most pronounced effects of the sugar addition to the extruded mass are the changes in the taste and texture of the extrudates (Harper, 1981).

In the presence of greater amounts of sugars, the SME and die pressure in the extruder are reduced due to suppressed starch-starch interactions and the reduction of the starch fraction. These facts result in a lower melt viscosity and elasticity of the mass in the extruder, which hence decreases the SME and die pressure. Furthermore, the decrease in the melt viscosity lowers the conversion of mechanical energy into heat energy, which hence reduces the temperature inside the extruder. Both results in a reduced driving force for expansion which is reflected in lower sectional expansion indices for extrudates containing higher amounts of sugars. Whereas the longitudinal expansion index was found to be increased for these extrudates due to the decreased elasticity (Barrett, Kaletunç, Rosenburg, & Breslauer, 1995; Fan et al., 1996).

The changes in the expansion mechanism due to the presence of sugar are also reflected in structural changes. For example, the mean area size of the pores is reduced and the bulk density increased due to the sugar content increase. Furthermore, the extrudates with a high sugar content cracked abruptly during compression tests (Barrett et al., 1995).

The addition of reducing sugars to the extruded mass results in a browning of the extrudates, if free amino acids are present. This reaction is part of the Maillard reaction, as described in chapter *2.3.3.1 Nonenzymatic browning reactions*.

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# 3 MATERIAL, METHODS AND ERROR ANALYSIS

In this chapter, the main experimental methods and materials used in this thesis are described and discussed. Additionally, an error analysis is presented.

## 3.1 Materials

This study focuses on the use of wheat and oat for sprouting experiments. In this chapter, the materials employed are presented and the devices used are listed.

Wheat and oat were supplied by *General Mills* (USA) and *Nestlé* (Switzerland). For the experiments, the wheat variety *Runal* and the huskless oat variety *Gehl* were used. Both were cultivated in 2016. Grains were stored at 10 °C and aerated regularly. In Table 2 the specifications of the respective flours are given. The data were generated based on analyses performed by *Medallion Lab*, Minneapolis, USA, in July 2018.

*Table 2 Specification of the wheat and oat flours used for the experiments, data based on w/w*

| | **Wheat (*Runal*)** | **Oat (*Gehl*)** |
|---|---|---|
| **Ash** | 1.71% | 2.13% |
| **Fat** | | |
| Total Fat | 2.23% | 7.41% |
| Saturated Fat | 0.42% | 1.51% |
| Monounsaturated Fat | 0.31% | 2.81% |
| cis-cis Polyunsaturated Fat | 1.40% | 2.77% |
| trans Fat | <LOQ% | <LOQ% |
| **Dietary fiber** | | |
| Dietary Fiber Gravimetric | 11.40% | 13.10% |
| Dietary Fiber HPLC | 2.00% | 0.90% |
| Total Dietary Fiber | 13.40% | 14.00% |
| **Moisture** | 8.66% | 9.93% |
| **Protein** | 14.10% | 16.20% |
| **Starch** | | |
| Total Starch | 59.30% | 56.30% |
| Resistant Starch | <2.00% | <2.00% |
| **Sugars** | | |
| Galactose | <0.10% | <0.10% |
| Fructose | 0.12% | 0.11% |
| Glucose | 0.15% | 0.11% |
| Sucrose | 0.57% | 0.78% |
| Maltose | 1.84% | 0.13% |

| | | |
|---|---|---|
| Lactose | <0.10% | <0.10% |
| Total Sugar | 2.68% | 1.14% |
| **Tocopherols and Tocotrienols** | | |
| α-Tocopherol (ppm) | 7.07 | 4.40 |
| α-Tocotrienol (ppm) | 2.50 | 15.10 |
| β-Tocopherol (ppm) | 7.73 | 4.17 |
| γ-Tocopherol (ppm) | <0.10 | <0.10 |
| γ-Tocotrienol (ppm) | <0.10 | <0.10 |
| δ-Tocopherol (ppm) | <0.10 | <0.10 |
| δ-Tocotrienol (ppm) | <0.10 | <0.10 |
| Total Tocopherols (ppm) | 17.30 | 23.70 |
| **Vitamins** | | |
| Vitamin B1 (Thiamine) mg/100g | 0.48 | 0.95 |
| Vitamin B2 (Riboflavin) mg/100g | 0.20 | 0.22 |
| Vitamin B3 (Niacin) mg/100g | 5.30 | 0.93 |
| Vitamin B6 mg/100g | 0.36 | 0.15 |

Moreover, the devices used for the manufacturing process, and the analyses are given in Table 3 and Table 4.

*Table 3 Devices used for the manufacturing process*

| **Device** | **Labeling** | **Manufacturer** |
|---|---|---|
| **Malting plant (sprouting and drying)** | A1-2008 | *Seeger* |
| **Climate/drying cabinets:** | | |
| • Climate cabinet for sprouting experiments | 220 P-02 | *Lovibond* |
| • Drying cabinet for sprouting experiments | UT 6120 | *Heraeus Instruments* |
| • Drying cabinet for extrudate drying | T 6420 | *Thermo Scientific* |
| **Mills:** | | |
| • Hammer mill | - | *Siemens Schuckertwerke AG* |
| • Rotor speed mill | Pulverisette 14 | *Fritsch* |
| • Coffee mill | MZ12.35 | *Petra* |

| | | |
|---|---|---|
| **Extrusion:** | | |
| • Dosage | DDW-MS5-FW40/5$^{PLUS}$-50 | *Brabender* |
| • Extruder | ZE 25 | *Berstorff* |
| • Multifunction I/O Device | USB-6002 (782606-01) | *National Instruments* |

*Table 4 Devices used for the analyses*

| **Device** | **Labeling** | **Manufacturer** |
|---|---|---|
| **Balance** | Entris | *Satorius* |
| **pH meter** | 765 calimatic | *Knick* |
| **Spectrophotometer** | Spekol 1300 | *Analytik jena* |
| **Centrifuge** | HERAEUS MEGAFUGE 40R | *Thermo Scientific* |
| **Bench centrifuge** | Minifuge RF | *Heraeus* |
| **Water bath** | 1022 | *GFL* |
| **DSC** | DSC204 F1 | *Netzsch* |
| **Rheometer** | MCR 302 | *Anton Paar* |
| **SEC-MALLS** | SEC-310 | *WGE Dr. Bures* |
| **HPLC** | Chromaster<br>Bi-MwA | *VWR Hitachi*<br>*Brookhaven Instruments Corporation* |
| **Freeze drying** | Beta 1-16 | *Christ* |
| **Chroma meter** | CR-400 | *Konica Minolta* |
| **Moisture analyzer** | MA 30 + 35 | *Satorius* |
| **SEM** | | |
| • **Extrudate** | Gemini SEM500 | *Zeiss* |
| • **Flour** | DSM 982 | *Zeiss* |

## 3.2 Experimental Procedure

In order to obtain comparable results, a standard sprouting procedure was developed. To this end, different steeping times and air rest cycles were tested. It was found that a moisture content of 40-42% (w/w) of the grains after steeping has to be achieved. Otherwise, an even distribution of the moisture in the grain could not be ensured.

For all experiments of this thesis, the same steeping regime was used. Grains were washed under running tap water for 30 mins and were then steeped in water so that all grains were covered with water: 4.5 h wet steeping, 19 h air rest, and again 4 h wet steeping, all at 20 °C (Jacob, 2016). Further details on the steeping, sprouting and drying process conditions are given in Table 5.

*Table 5 Settings of devices used for sprouting, drying, and milling*

| **Unit** | **Settings** | **Time** |
|---|---|---|
| Small-scale method | -grains were washed once a day using tap water<br>-temperature 10-30 °C<br>-grains were covered with cling film | 1-3 days |
| Sprouting in malting unit of *Seeger* | -grains were turned four times daily and sprayed with water twice a day<br>-30% (v/v) of the air was recirculated<br>-air was conditioned to be saturated at used temperature<br>-temperature 14-26 °C | 1-9 days |
| Self-constructed malting unit | -sprouting in a box which was aerated permanently with humidified air<br>-temperature 10-30 °C | 1-9 days |
| Freeze-drying | -0.1 mbar | 60 h |

| | | |
|---|---|---|
| Drying oven | -drying temperature: 65 °C | Until a final moisture content of 12% was reached |
| Drying in malting unit of *Seeger* | -drying temperature: 65 °C | Until a final moisture content of 12% was reached |
| Hammer mill | -mesh size 0.5 mm | |
| Speed rotor mill | -mesh size 0.5 mm<br>-rotor speed 8000 rpm | |

As part of this study, three different sprouting methods and units were used. These relate to different experimental campaigns and hence the different chapters given in Table 6. The small-scale experiments (chapters 4 & *6*) were conducted by sprouting the grains on metal sheets which were placed in a temperature-controlled cabinet.

For the extrusion tests (chapters *5, 6* & 7) and the study of the starch degradation (chapter 7) bigger quantities of sprouted grains were needed and, therefore, the small-scale experiments were scaled up. The following two sprouting plants were used:

1. The automatic malting plant of Seeger: consisting of an integrated steeping and sprouting unit and a separate drying unit.
2. The self-constructed steeping and sprouting unit: hereby the grains were steeped and sprouted in a plastic container which was placed in a climate-controlled cabinet and the grains were aerated with conditioned, moistened air permanently.

*Table 6 Steeping, sprouting, drying and milling methods used in different chapters of this study*

| | **Steeping and Sprouting** | **Drying** | **Milling** |
|---|---|---|---|
| **Chapter 4**: Sprouting of oats: a new approach | Small-scale method | Freeze-drying | Speed rotor mill |

| | | | |
|---|---|---|---|
| to quantify compositional changes | | | |
| **Chapter 5**: Effect of sprouting conditions on the properties of direct expanded extruded wheat | Malting unit of Seeger | Malting unit of Seeger | *Wheat flour:* hammer mill<br>*Extrudates*: coffee mill and rotor speed mill |
| **Chapter 6**: Effect of wheat sprouting temperature on selected properties of wheat flour and direct expanded extrudates | *Small-scale:* small-scale method<br>*Up-scale:* self-constructed malting unit | *Small-scale:* freeze-drying<br>*Up-scale:* malting unit of Seeger | *Small-scale:* speed rotor mill<br>*Up-scale:* hammer mill |
| **Chapter 7**: Effect of sprouting conditions on molecular changes of wheat and oat starch | Self-constructed malting unit | Drying oven | *Flour:* hammer mill<br>*Extrudates:* pre-grinding in a coffee mill and rotor speed mill |

As part of this study, a concept to evaluate the sprouting process, the *degree of sprouting*, was developed. In order to evaluate the concept, oat grains were sprouted up to 3 days at five different temperatures. Grains were analyzed with regard to their *degree of sprouting* and resulting flours were studied in order to correlate the results with the *degree of sprouting* of the grains. Further details on the analytical methods can be found in chapter *3.2.2 Analytical methods*. The results are presented in chapter *4*.

In a second step, directly expanded extrudates were produced on the basis of sprouted wheat flour. Therefore, the wheat was sprouted for different periods and temperatures.

The resulting flours were extruded at standard extrusion conditions, which were established in preliminary tests, see Table 7. The extruder was thereby used as a reactor. The sprouted grains, the

sprouted flour, and the extrudates were studied and characteristic properties were correlated with the *degree of sprouting* of the sprouted grains. Results are presented in chapters *5* and *6*.

In the last step, the changes in the starch molecules as affected by different sprouting times were studied. On that account, oat and wheat grains were sprouted up to 9 days. New methods were developed to get a deeper understanding of the molecular starch changes. Furthermore, these sprouted wheat grains were extruded and the molecular changes of the starch of the extrudates were analyzed. The results are presented in chapter *7*.

In Table 7 the technical data of the extruder are presented. For all experiments, standardized settings were chosen. These settings resulted in a maximum sectional expansion index in preliminary tests. Flour and water were used exclusively as feedstock in order to emphasize the differences between the sprouted wheat flours.

*Table 7 Technical data of extruder ZE 25 and chosen conditions*

| **Feature** | **Specification** |
|---|---|
| Screw length | 870 mm |
| Screw Speed | 200 rpm |
| Throughput | 6 kg/h |
| Motor power | 6000 kW |
| Max Torque | 120 Nm |
| Moisture Content | 27% |
| Die hole diameter | 1 x 3 mm |

The SME of the different extrusion tests was calculated by using Equation 1, see chapter *2.4 Extrusion cooking,* and by use of the data given in Table 7.

In Figure 10 the temperature profile in the extruder is shown. The final temperature of 140 °C was chosen based on preliminary tests, on the one hand, to protect the nutrients, and on the other, because it enables to observe Maillard reactions in this specific temperature range (Rausch, 2009).

The screw was assembled of forward elements with two different pitches (13 standard and 15 short forward screw elements). During the extrusion process, the pressure in the die was measured 10 times per second after the process was stabilized.

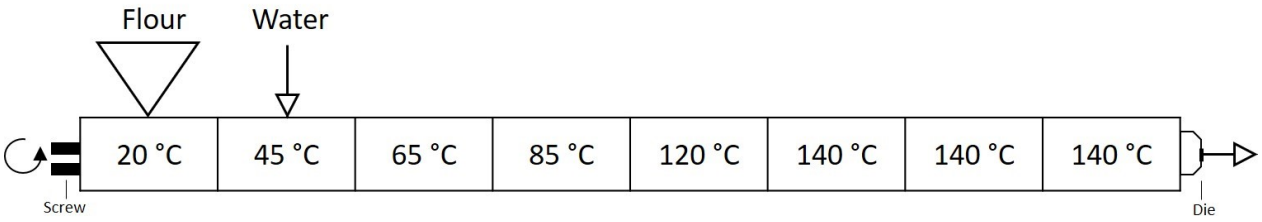



*Figure 10 Schematic diagram of the twin-screw extruder including the chosen temperature profile.*

The extruded strands were cut into pieces of about 50 cm in length after leaving the die. They were dried at 65 °C for 1.5 h in an oven (*Thermo Scientific T 6420*) to a water content of less than 10% (w/w) to receive a crunchy texture. Analyses were performed either directly on the extrudates or by using the milled extrudates. Extrudates were milled by exposing them for 30 s to a kitchen blender (*Petra MZ12.35*). Subsequently, the coarse flour was milled in a speed rotor mill (*Pulverisette 14, Fritsch*) with a sieve ring of 0.25 mm at 8000 rpm.

***Residence time in the extruder***

The residence time was investigated at standard extrusion conditions by the addition of a red dye (*Birkmann 50134, concentrated powder*) to the solid feed in the extruder. Every 5 s a black point was added to the extrudate strand coming out of the extruder die. Afterward the strand was broken at every black point and the different strands were milled into flour. The Lab-values of the flours were determined using a chromameter. The a-value was plotted against the residence time. The determination was done in triplicate.

### 3.2.1 Preparation of samples for analysis

While characterizing the changes in the grain during sprouting, special attention was paid to the separation of the substances, such as starch and sugars. These substances were separated, from the complex matrix of the grains consisting of, inter alia, proteins, and dietary fiber.

***Sugar Analysis***

For the determination of the soluble sugar content a method was developed by using Carrez clarification.

Carrez filtrations are often used in sample preparation and affect the precipitation of proteins. Due to the addition of the Carrez reagents to a sample, an insoluble precipitation is formed at which macromolecules such as proteins are adsorbed and hence also precipitate (Acker et al., 2013).

For the extraction of the soluble sugars from the sprouted flours, 2 g of the different flours were weighed in a 50 ml volumetric flask, and 20 ml distilled water was added. The flask was shaken and 2.5 ml Carrez I solution and 2.5 ml of Carrez II solution were added. 5 ml of 0.1 M sodium hydroxide was added to neutralize the solution. Afterward the flask was filled up with distilled water and the solution was filtered (*Machery-Nagel MN 631*).

***Starch Analysis***

In order to prevent the deposit of proteins, which are present in the sprouted flour, in the column of the SEC during the analysis of the molecular characteristics (see chapter *7*), a method was developed to isolate starch completely from other substances. The protein content in the starch sample after the separation as described below was determined using Dumas method (*Dumatherm N64+ DT 64+* by *C. Gerhardt GmbH & Co. KG*) and was found to be below 1%.

A solution containing 20% (w/w) sprouted flour or extrudate sample and 80% (w/w) 10 mM copper chloride solution was exposed to a boiling water bath for 30 min and the paste was high shear treated in an Ultra-Turrax *T25* (*IKA-Werke*) at 20 000 rpm for 2 min in order to prevent aggregation of starch molecules. Other concentrations were also tested but were found to have too high viscosity or the starch concentration was too low and the amount of separated starch was insufficient for analysis.

The paste was clarified using Carrez clarification (*see Separation of soluble sugars* above). The precipitated proteins were then separated by centrifugation (Thermo Scientific - MEGAFUGE 40R) at 3000 rpm for 5 min. The supernatant, which contains the starch, was filtered (*Machery-Nagel MN 631*). The starch was then precipitated using ethanol (40%v/v) and was separated from the liquid solution by centrifugation (*Thermo Scientific - MEGAFUGE 40R*) at 3000 rpm for 5 min. Finally, the starch was dried at room temperature for 1 day.

***Inactivation of enzymes***

Another issue was the boosted enzyme activity coming along with the sprouting process. Enzymes have to be inactivated before analyses in order to prevent further degradation of starch which could affect the starch properties such as gelatinization behavior. For this purpose, copper (II) chloride was

used in this work. On one hand, 10 mM copper (II) chloride reduces the relative activity of α-amylase to 4.5% (Aquino, Jorge, Terenzi, & Polizeli, M. L. T. M., 2003). On the other hand, copper (II) chloride is not so hazardous and environmentally unfriendly as mercury (II) chloride which is usually used to inactive enzymes.

### 3.2.2 Analytical methods

In this chapter, the analytical methods used in this thesis are listed.

#### 3.2.2.1 Determination of the α-amylase activity

The α-Amylase activity was determined by using the Megazyme (2015) Malt-Amylase assay procedure K-MALTA 05/15. Determinations were done in duplicate.

#### 3.2.2.2 Determination of the ascorbic acid content

The vitamin C content was determined according to the *Indophenol Method* (Nielsen, 2003). The indophenol solution was diluted 8 times. The solid material was dispersed in a mixture of 30 g/L metaphosphoric acid and 8% (v/v) acetic acid and centrifuged at 3000g. A volumetric sample of the resulting supernatant was diluted and used for titration according to the method. The procedure was executed in duplicate per specimen. The calibration curve is given in Figure 11.

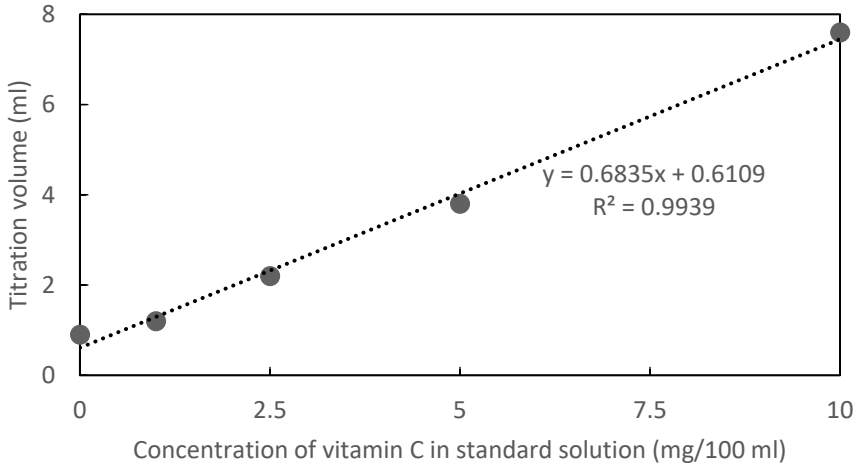



*Figure 11 Calibration of vitamin C titration method.*

#### 3.2.2.3 Viscous behavior of sprouted flour suspensions

The sprouted grain flour was characterized rheologically by studying an aqueous suspension of 10% (w/w) flour. The dosing was done explicitly on dry matter basis. Analyses were performed using an *Anton Paar* rheometer *MCR 302* equipped with a starch cell and a stirrer having a vane geometry.

Throughout the experiments, the stirrer speed was kept at 150 rpm. Copper(II) chloride at a concentration of 10 mM was added to the suspension in order to inactivate the enzymes.

The suspension was prepared at room temperature. Temperature scans from 30 °C to 95 °C were performed with a heating rate of 1.5 °C/min. Cooling from 95 °C to 30 °C was performed with a cooling rate of 4.33 °C/min. The temperature regime and heating rate were adopted from the method using an amylograph (DIN ISO 7973, 2016). The stirrer speed was chosen after preliminary tests.

These experiments give the so-called *peak viscosity* and the *final viscosity* once the system was cooled down to 30 °C.

#### 3.2.2.4 Reducing sugar content

The reducing sugar content was determined using DNS (3,5 dinitrosalicylic acid) and a colorimetric analysis. 1 g sprouted flour or 1 g milled extrudates was mixed with 5 ml of a mixture of 40% (v/v) ethanol and 60% (v/v) 10 mM copper chloride solution to extract the reducing sugars. After shaking the mixture for 10 min the suspension was centrifuged for 15 min at 3000 g (Heraues, Labofuge 200). This procedure was repeated twice. The collective supernatant, three subsequent extractions of the same material were diluted with the above-mentioned ethanol/copper chloride solution mixture by factor 1.5. 0.625 g polyvinylpolypyrrolidone was added to the solution to precipitate any phenols present. The precipitate was filtered off (Tian et al., 2010). Afterward, 1 ml of the remaining solution was mixed with 1 ml of an aqueous solution containing 10 g/l DNS, 16 g/l sodium bicarbonate, and 300 g/l potassium sodium tartrate. After shaking the mixture for 10 min at 100 °C the solution was diluted with 5 ml distilled water. The reducing sugar content was determined spectrophotometrically (*Spekol 1300 – Analytik jena*) at 545 nm. For the calibration different concentrations of maltose monohydrate were used. The calibration curve is given in Figure 12.

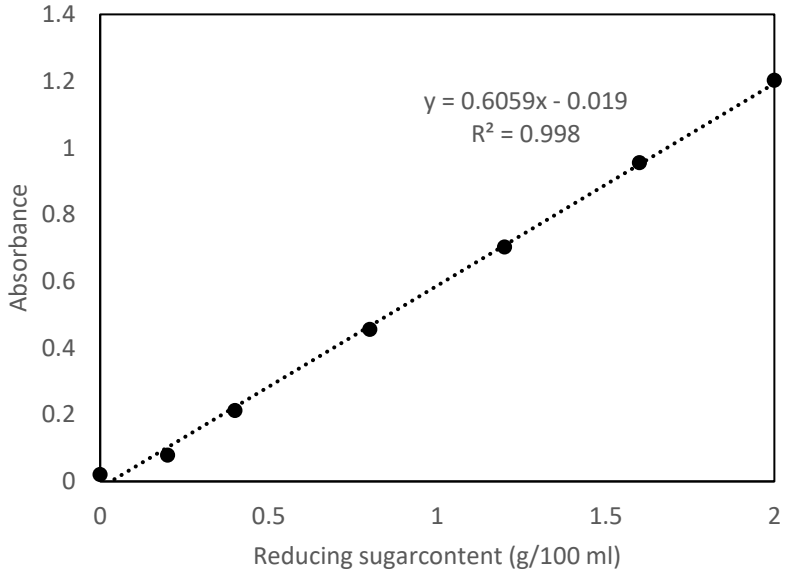



*Figure 12 Calibration curve of the reducing sugar determination.*

#### 3.2.2.5 Determination of β-glucan content

The β-glucan contents of the differently sprouted oat samples were determined using the Megazyme (2017) Mixed-Linkage Beta-Glucan assay procedure (McCleary Method – K-BGLU 02/17; AACC Method 32-23.01). Determinations were done in duplicate.

#### 3.2.2.6 Determination of the starch content

The starch content in the flours and extrudates was determined using the Megazyme Total Starch HK assay procedure (*AMG/α-Amylase/HK Method – K-TSHK 08/18*). The starch was separated from other components by extraction. Therefore, solids (flour or extrudate) were mixed with a 10 mM copper(II) chloride solution and centrifuged. The final sediment was dried, finely ground and used for the starch content determination. Determinations were done in duplicate.

#### 3.2.2.7 DSC measurements

DSC analysis was carried out with a *DSC204 F1 (NETZSCH)*. 10 mg of wheat flour or 7 mg of oat flour and 20 of mg water were weighed into stainless aluminum pans (*NETZSCH; 25 µl*) and then heated from 30 °C to 110 °C with a heating rate of 10 K/min. The thermograms were analyzed with regard to the onset temperature ($T_{on}$), the peak temperature ($T_{peak}$), the offset temperature ($T_{off}$), and the gelatinization enthalpy (ΔH). The determination was performed in duplicate.

Before the analysis with the *NETZSCH Proteus Thermal Analysis software* was performed, the baseline of the thermograms needs to be defined by the user. The enthalpy is then determined from the area between the signal curve and baseline.

#### 3.2.2.8 Molecular characterization using SEC-MALS

In order to analyze the molecular characteristics of the starch of the sprouted flours and extrudates, the starch was isolated from the samples. Therefore, a 20% (w/w) solution containing the sprouted flour or extrudate sample was exposed to a boiling water bath for 30 min and the paste was high shear treated using an Ultra-Turrax *T25* (*IKA-Werke*) at 20000 rpm for 2 min. 10 ml of the paste was clarified using Carrez clarification (*see sample preparation for HPLC*). The protein was then separated by centrifugation (Thermo Scientific- MEGAFUGE 40R) at 3000 rpm for 5 min. The supernatant, containing the starch, was filtered (*Machery-Nagel MN 631*). The starch was then precipitated using ethanol (40% v/v) and was separated from the liquid solution by centrifugation (*Thermo Scientific-MEGAFUGE 40R*) at 3000 rpm for 5 min. Finally, the starch was dried at room temperature for 1 day. For the analysis by SEC-MALS the starch was finely ground and mixed with DMSO to a final concentration of 2.5 mg/ml. The solution was stirred at 80 °C for 24 h before it was injected through a filter (*5 µm PTFE filter* of *Roth*) for analysis in SEC-MALS.

The size exclusion chromatography was conducted with a SEC-3010 module (*WGE Dr. Bures*) and a Bi-MwA detector from Brookhaven Instruments Corporation was used. The determination of the average molecular weight (Mw) was done using *ParSEC Enhanced V5.6 1* chromatography software.

#### 3.2.2.9 Analysis of soluble sugars using HPLC

The soluble sugars were extracted from the flours and a Carrez clarification was conducted. Therefore, 2 g of the different flours were weighed in a 50 ml volumetric flask, and 20 ml distilled water was added. The flask was shaken and 2.5 ml Carrez I solution, 2.5 ml of Carrez II solution and 5 ml of 0.1 M sodium hydroxide were added. Afterward the flask was filled up with distilled water and the solution was filtered (*Machery-Nagel MN 631*).

The solution was pressed through a 0.45 µm glass fiber filter (*CHROMAFIL GF/PET-45/25*) before analyzed in a *VWR Hitachi Chromaster* HPLC by using an ELSD (Evaporative Light Scattering Detector) detector (*VWR ELSD 90*) and a column from *AppliChrom* (*ABOA SugarSep-Ca*). The samples were immediately analyzed after clarification to avoid further changes. 0.02 ml of the sample was injected

and a flow rate of 0.5 ml/min was chosen. Distilled water was used as eluent and the oven temperature was 80 °C.

Calibration was done using glucose and maltose, because they were the main hydrolysis products (Bertoft & Kulp, 1986), and fructose. For the calibration of samples containing one of the mentioned substances of known concentration (0.25, 0.5, 1, 2, 5 g/100 g distilled water) were analyzed and from that, a calibration curve was calculated, see Figure 13.

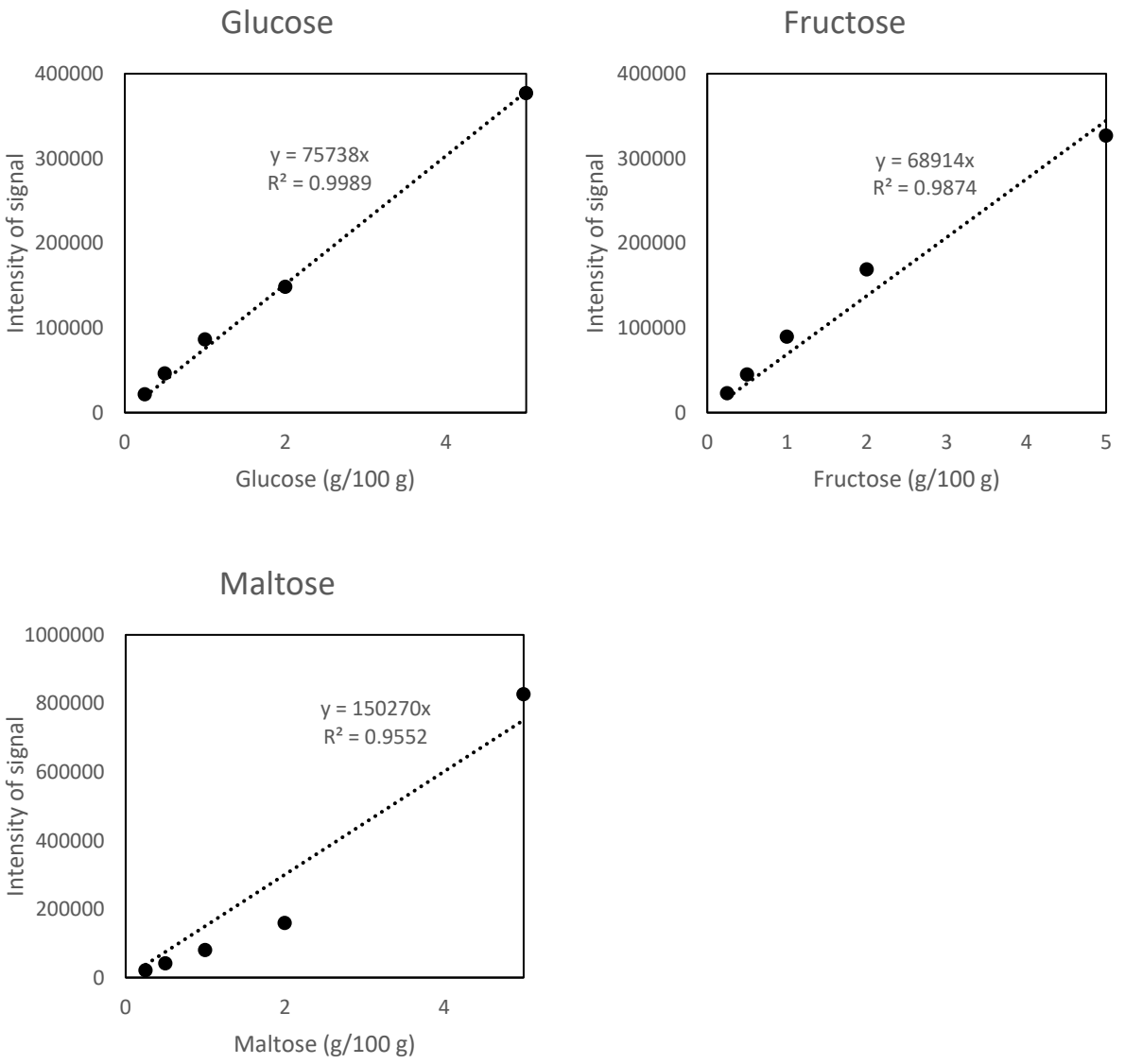



*Figure 13 Calibration of HPLC using glucose, fructose, and maltose.*

The chromatograms were analyzed using *Peakfit (Version 4.12)* giving peak areas and elution volumes of peak on-set, off-set, and maximum.

#### 3.2.2.10 Scanning electron microscopy

In order to analyze wheat and oat flours by use of SEM (scanning electron microscopy) the flours were directly stuck on a carrier for SEM and were covered with a 30 nm gold film. A *DSM 982* by *Zeiss* was used for this research at the *ZELMI* of the *Technical University Berlin*.

The pore size analysis of the extrudates was done using a SEM *ZEISS Gemini SEM500.* Sample preparation included cutting the extrudates to create an axial crosssection that was sputtered with a 100 nm gold layer. The size of the pores and wall thickness was determined using *Digital Imaging Processing System*.

#### 3.2.2.11 Expansion index of extrudates

The sectional expansion index was determined based on the diameter of extrudates. The values were averaged over five individual extrudate strands. Each strand itself was measured three times at various locations. The expansion index is defined as the ratio of the circular cross-section area of the extrudates and the cross-section area of the die. The relative longitudinal expansion index was derived from the measurement of the length of extrudates produced in 5 s. The results provided are averages of five individual determinations.

#### 3.2.2.12 Texture analysis of extrudates

The hardness of the extrudates was determined using a *Zwick testControl II* texture analyzer. A cylindrical probe, diameter 25 mm, was breaking and crunching a single extrudate strand. The constant speed of the probe was 15 mm/min and the peak force was recorded giving the hardness of the extrudate. Each data point represents the average of eight independent measurements.

#### 3.2.2.13 Sensory evaluation of selected samples

The sensory attributes of the different extrudates were evaluated by an untrained panel of 22 members. The participants tasted the four extrudates (native, sprouting time: 1, 3, and 7 days). Afterward, they were asked to sort these samples in their order of darkness, hardness (assessed by manual breaking), sweetness, taste of maltiness, crispiness (mouthfeel), and overall preference. The procedure followed the ranking test according to DIN ISO 8587 (2010). The results are the ranking sums subjected to the Friedman test. The questionnaire can be found in the appendix.

#### 3.2.2.14 Water solubility index

To determine the water solubility index of the extrudates, 0.5 g dry matter was suspended in 50 ml distilled water. The dosing of the milled extrudates was corrected for dry matter content. The suspension was stirred for 30 min. It was filtered (S&S 595) using a Büchner funnel and the solid residue was weighed after removing the water from the filter cake by drying at 130 °C for 1 h. The water solubility index in percent is the difference between the initial dry matter weight and the weight of the residue. Determinations were done in duplicate.

#### 3.2.2.15 Cold paste viscosity

The rheological characterization of the extrudates was performed by measuring aqueous suspensions of 10% (w/w) - corrected for dry matter content - of the milled extrudates. The viscosity of the paste emerging after 30 minutes of stirring was measured with an *Anton Paar* rheometer *MCR 302* using the starch cell - stirring rate of 150 rpm and a shear rate of 150 $s^{-1}$, temperature 20 °C. Data given are average paste viscosities from 60 data points taken per minute. Each experiment was performed at least in duplicate.

#### 3.2.2.16 Lab color

The color analysis of the extrudates was done using the *CR-400*, *Konica Minolta* attached to cylindrical glass containers. Therefore, milled extrudates were used. The color characterization of the milled extrudates was done in triplicate. The color was expressed in lightness (L), redness (a), and yellowness (b) values.

## 3.3 Repeatability, reproducibility & process stability of extrusion and sprouting tests

In this chapter, the repeatability and reproducibility of the sprouting and extrusion tests are discussed.

In this work, the term '*repeatability*' describes the variation that occurs analyzing the same sample under the same conditions (same operator, setup, device, environmental conditions) (Lean Six Sigma, 2016). In contrast, the term '*reproducibility*' is used in this thesis to describe the variation that occurs when analyzing several individually produced samples by use of the same conditions for the analysis for all samples. The term '*process stability*' describes the variation of a process or product properties that occurs during the process while running the same conditions for a certain time.

### 3.3.1 Extrusion tests

The repeatability and stability of the extrusion tests were studied and the results are presented in this chapter. For this purpose, native wheat flour was extruded for 1 h testing the process stability. The experiment was repeated in the same manner on two more days in order to study the repeatability. During the extrusion process, the back pressure in the extruder die, the product temperature in the die and the SME were recorded at least every 10 s. The data over time is depicted in Figure 14 - Figure 15. Furthermore, the product properties, longitudinal and sectional expansion index, were measured every 5 min. The results over time are shown in Figure 17 and Figure 18.

The back-pressure in the extruder die was measured for 1 h on 3 days and showed a variation between 47 and 56 bar (Figure 14). These fluctuations are always in the same range in the studied time period of 1 h. Also, on the three trial days, a similar mean value and relative standard deviation were found indicating a good repeatability of the pressure measurement in the extruder die.

Due to the variation of the pressure in the die, it is of special importance to record the pressure for a while and obtain the average from these data. That is why the pressure was recorded 10 times per second for around 5 min in chapters *5, 6* & *7*.

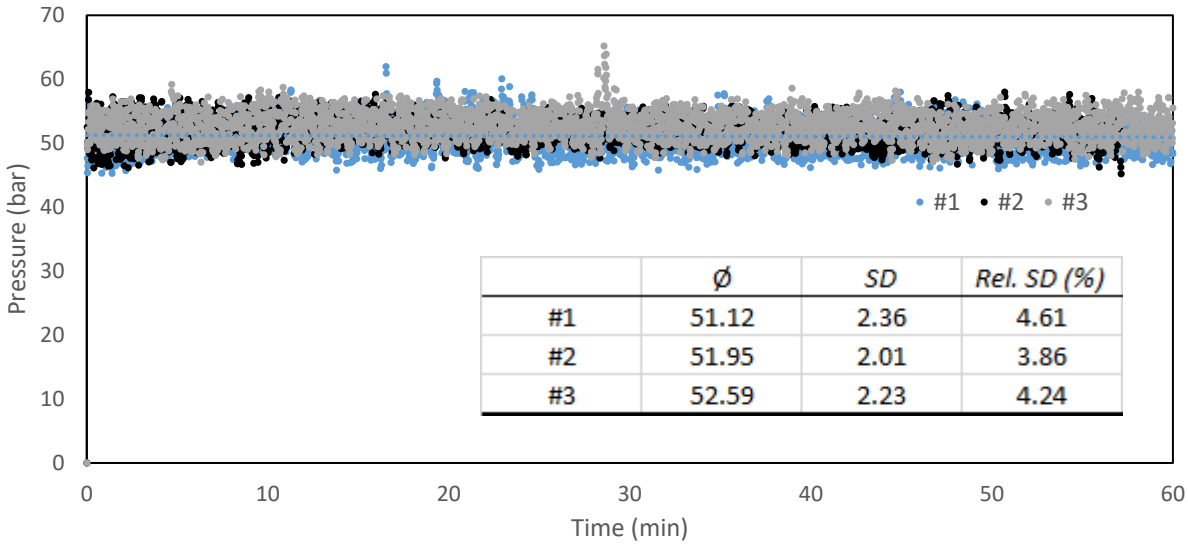
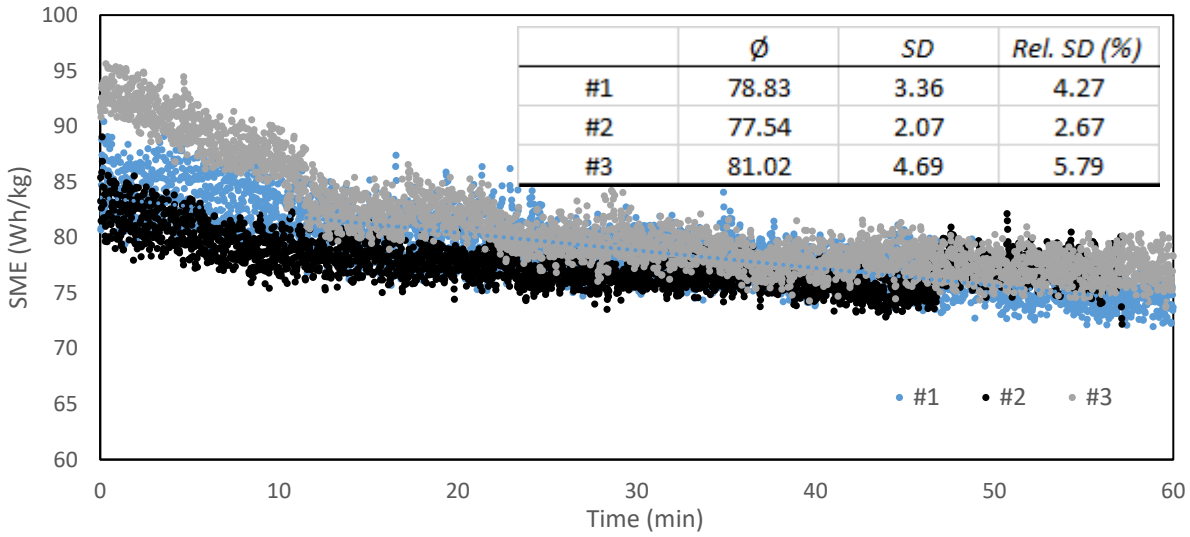



*Figure 14 Repeatability and process stability of the back pressure in the extruder die.*

The SME is a measure of the mechanical energy input into the extruded mass during the extrusion process. It was found that it needs around 10 to 15 min in order to ensure a constant mechanical energy input (Figure 15). After this setting time, an over the time constant mechanical energy input

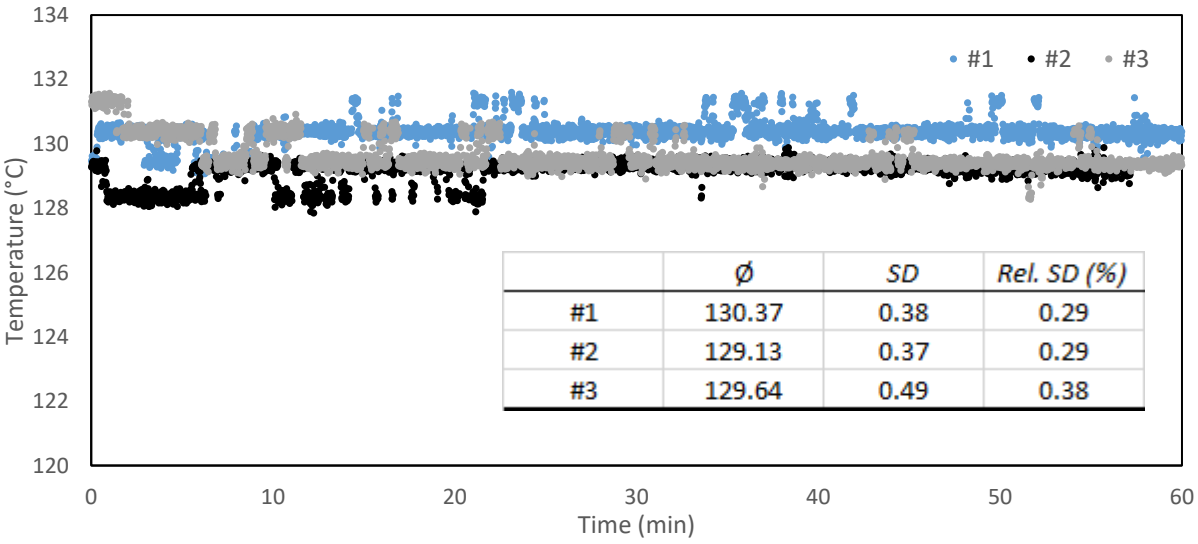
was found. This input was not affected by the trial day which hence marks good repeatability of the mechanical energy input into the extruded mass.




| | Ø | SD | Rel. SD (%) |
|---|---|---|---|
| #1 | 78.83 | 3.36 | 4.27 |
| #2 | 77.54 | 2.07 | 2.67 |
| #3 | 81.02 | 4.69 | 5.79 |

*Figure 15 Repeatability and process stability of the SME.*

The repeatability as indicated by similar mean values and relative standard deviations of the product temperature as measured in the extruder die was found to be good (Figure 16). This indicates that the process can be considered stable once the start-up period is finished. The stability of the product temperature over time and different trial days, with constant energy input into the extruded mass, ensures that the temperature does not influence the experiments in an uncontrolled way.

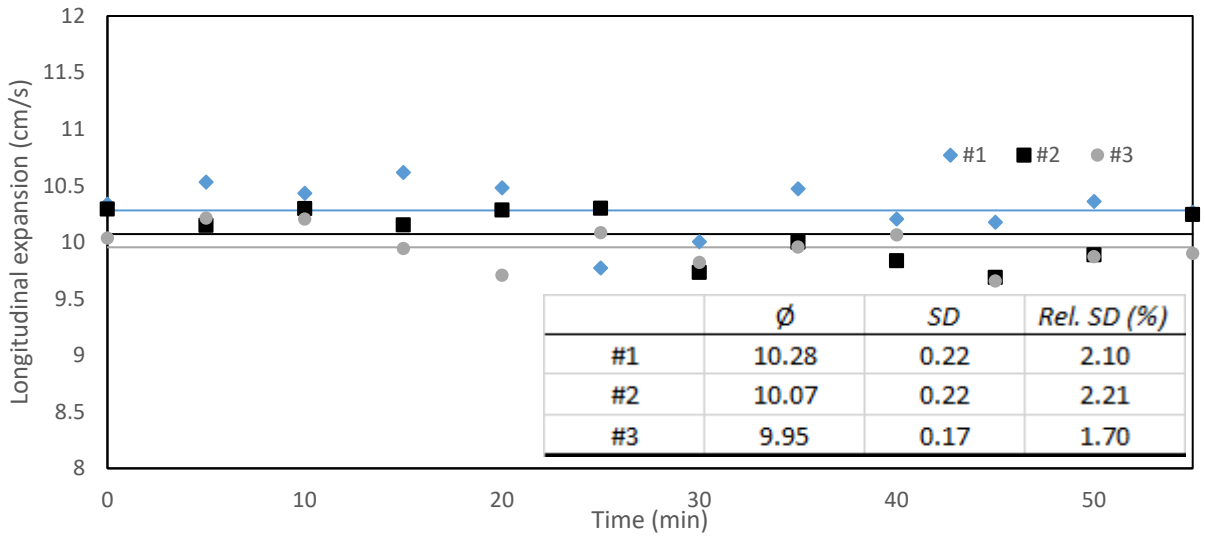



| | Ø | SD | Rel. SD (%) |
|---|---|---|---|
| #1 | 130.37 | 0.38 | 0.29 |
| #2 | 129.13 | 0.37 | 0.29 |
| #3 | 129.64 | 0.49 | 0.38 |

*Figure 16 Repeatability and process stability of the product temperature as measured in the extruder die.*

This thesis focuses is on constant product quality. This is to be tested here with the example of the longitudinal and sectional expansion index also over time and at different trail days.

The longitudinal expansion index of the extrudate strands was found to hardly vary for extrudates produced in the same manner on different trial days indicating good repeatability of the longitudinal expansion determination (Figure 17). In addition, the relative standard deviation of the longitudinal expansion over 1 h was found to be around 2%, also suggesting a constant longitudinal expansion over time.




| | Ø | SD | Rel. SD (%) |
|---|---|---|---|
| #1 | 10.28 | 0.22 | 2.10 |
| #2 | 10.07 | 0.22 | 2.21 |
| #3 | 9.95 | 0.17 | 1.70 |

*Figure 17 Repeatability and process stability of the longitudinal expansion.*

As it is the case for the longitudinal expansion index, also the sectional expansion index of the extrudates produced over a period of 1 h was found to be fairly constant. The relative standard deviation of less than 4% and good reproducibility over different experimental runs are striking (Figure 18).

In conclusion, the extrudate quality appears to suffer from very limited random variation by the choice of the described extrusion conditions.

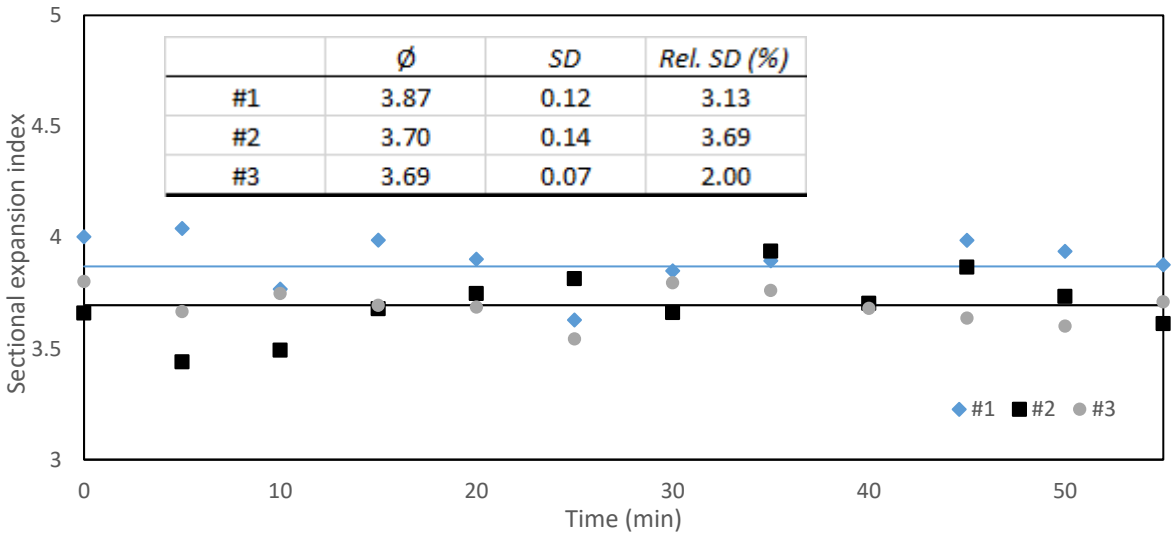



*Figure 18 Repeatability and process stability of the sectional expansion index.*

***Residence time in the extruder***

In order to evaluate the reproducibility of the experimental setup the average residence time of three different executions of the same experiment was determined. The distribution of the a-value over time depicts the residence time distribution within the process, see Figure 19. It is hence a measure for the deviation from a perfect plug-flow. The data are given in Table 8 and indicate an excellent reproducibility. The overall average time is 38.5 s. Combining this time with the fact that after another 40 s the color reading is back to its original value, this shows that sampling after any kind of change is meaningless at times shorter than one minute.

*Table 8 Determination of the residence time of three independent trials*

| #1 | #2 | #3 | **Ø** | **SD** |
|---|---|---|---|---|
| 38.4 | 38.7 | 38.4 | 38.5 | 0.1 |

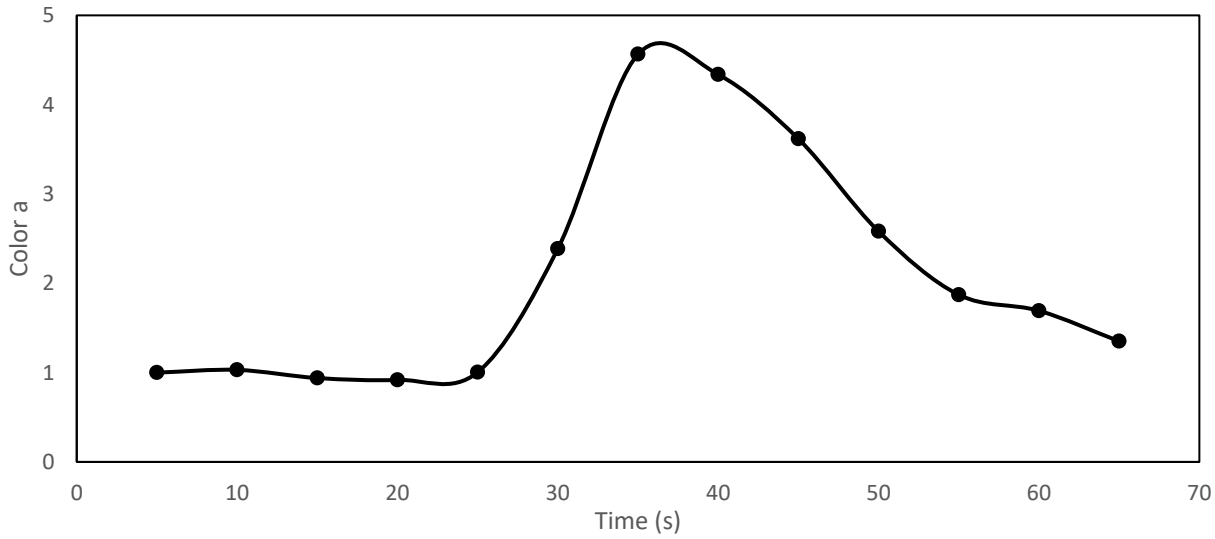



*Figure 19 Measurement of the residence time of flour in the extruder.*

On the basis of the determined residence time and setting time, the sampling and recording of characteristic process variables were started after running the extruder for 15 min for all experiments presented in this thesis (chapters *5, 6* & *7*).

### 3.3.2 Sprouting process

The reducing sugar content, α-amylase activity, peak viscosity, and vitamin C content were chosen to be the most important properties to assess the sprouting process.

In order to evaluate the repeatability and reproducibility of the sprouting process, oat was sprouted seven times at the same conditions (20 °C, 3 days). The small-scale sprouting method, see Table 5, was applied. After the sprouting process, the *degree of sprouting* of 300 grains was directly determined and the results are presented in Table 9. Afterward, the seven batches were freeze-dried and milled. The reducing sugar content, α-amylase activity, peak viscosity, and vitamin C content were determined in duplicate in all seven samples. In addition, one trial was picked in which the repeatability of the methods was tested and this trial was analyzed seven times for the characteristic properties.

The experimental design of the total error analysis of the standard sprouting process is depicted in Figure 20.

The total error of the sprouting process composes of the standard deviation of the reproducibility and the standard deviation of the repeatability.

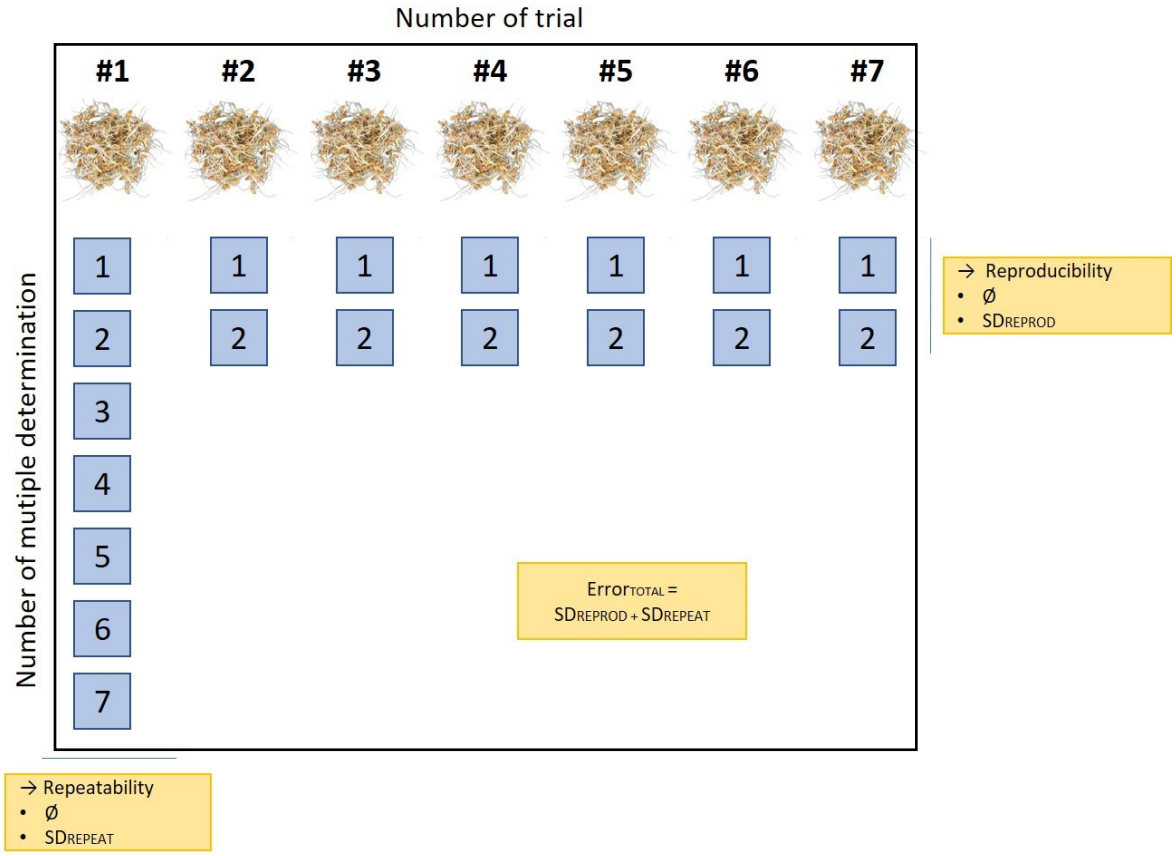



*Figure 20 Experimental design of the determination of the total error.*

In Table 9 the percentage of the different degrees of sprouting of seven independent sprouted trials is shown. The average *degree of sprouting* and the standard deviation were calculated for each trial.

However, the standard deviation of each trial does not give evidence of the repeatability of the *degree of sprouting* determination but rather describes the homogeneity of the sprouting process. A homogenous sprouting process comes along with a low standard deviation.

As can be seen in Table 9 the reproducibility of the determination of the *degree of sprouting* in independent sprouted trials is quite good. A relative standard deviation of 5.7% was found.

However, the sprouting process is a very sensible, biological process which is affected by many external factors such as water content in the grains, temperature, aeration of the grains, strain on the grains during washing and transferring, and layer thickness of the grains during sprouting (Narziß,

Back, & Gastl, 2012). Throughout the experiments, it was tried to keep the conditions as constant as possible. However, small deviations cannot be ruled out. Moreover, the used sample quantities for the determination of the *degree of sprouting* were quite small with regard to the development of a new method minimizing the time requirement.

*Table 9 Reproducibility of the degree of sprouting determination of 7 individual produced samples*

| | | **Degree of Sprouting** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **#** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **Ø** | **SD** |
| **Trial Number** | **1** | 11% | 9% | 10% | 16% | 22% | 30% | 2% | 0% | *3.28* | *1.72* |
| | **2** | 11% | 18% | 15% | 15% | 16% | 22% | 3% | 0% | *2.86* | *1.77* |
| | **3** | 10% | 17% | 14% | 21% | 19% | 18% | 1% | 0% | *2.81* | *1.63* |
| | **4** | 10% | 14% | 8% | 17% | 20% | 29% | 2% | 0% | *3.19* | *1.74* |
| | **5** | 11% | 16% | 10% | 17% | 14% | 29% | 3% | 0% | *3.08* | *1.82* |
| | **6** | 11% | 15% | 13% | 18% | 19% | 23% | 1% | 0% | *2.93* | *1.70* |
| | **7** | 12% | 17% | 13% | 16% | 16% | 23% | 2% | 0% | *2.85* | *1.77* |
| | **SD** | *0.0064* | *0.0280* | *0.0236* | *0.0181* | *0.0256* | *0.0419* | *0.0076* | *0* | | |
| | | | | | | | | | **Ø** | **3.00** | **1.74** |
| | | | | | | | | | **SD** | **0.17** | **0.06** |
| | | | | | | | | | **Rel. SD (%)** | **5.72** | |

In Table 10 the repeatability of the determinations of the four chosen characteristic properties is shown. It can be seen that the relative standard deviation of the four determination is quite low and good repeatability of the applied methods is ensured. The methods deliver reliable results.

In Table 11 the results of reproducibility of the determination in seven independently sprouted trials are given and in Table 12 the total error is presented. In contrast to the results of the repeatability

(Table 10), the reproducibility in independently sprouted trials is lower. Especially the reducing sugar content and α-amylase activity showed a comparably high standard deviation. For all properties, except for the peak viscosity, significant differences between the samples were found in the ANOVA.

On the one hand, the high standard deviation of the reducing sugar content and α-amylase activity determinations can be explained by the low sample size which was used for the determinations (reducing sugar determination: 1 g sample weigh-in, α-amylase activity: 0.5 g sample-weigh-in). In contrast, for the peak viscosity measurement 1.75 g flour were used and for the vitamin C determination 2 g flour were needed. By the use of a larger sample size, the sampling was more homogenous.

In connection with the sampling, one has to point out that the reducing sugar content and α-amylase activity are depending much more on the grain's *degree of sprouting* than it is the case for the vitamin C content and peak viscosity. For example, the reducing sugar content increases more than six-fold comparing grains from DoS 1 and DoS 4 (reducing sugar content in grains of DoS 1: 2.7 g/100 g; DoS 4: 16.5 g/100 g) and the α-amylase activity increases almost 18 times (α-amylase activity in grains of DoS 1: 1.3 U/g; DoS 4: 22.6 U/g), also see chapter *4*. Conversely, the vitamin C content only increases 3.5-fold for these grains. This means, that the sampling is particularly important in the case of reducing sugar content and α-amylase activity determination. The fact that the sprouting process is a biological process and as has been shown in Table 9 all trials exhibit a spread around the average *degree of sprouting* as marked by the standard deviation that needs to be taken into account. Hence, it depends on the sampling which grains of which *degree of sprouting* are analyzed. Moreover, it should be noted that while sprouting on metal sheets like it was the case here (see chapter *3.2 Experimental Procedure*), differences in the *degree of sprouting* between the grains which were sprouted in the middle of the sheet and at the edge were found. Therefore, grains were washed and mixed daily to compensate these differences.

However, when it comes to the production of extrudates, bigger amounts of flour were used (around 5 kg) and, therefore, a better reproducibility is ensured.

On the other hand, even though a high relative standard deviation was found in the reducing sugar content and α-amylase activity, the results shown in chapters *5* and 6 are providing systematic data and significant effects of the sprouting process on product properties were found.

Errors during the production of the sprouted grain material might occur due to a slight variation of the ambient temperatures and humidity. The grains were exposed to the ambient air during the washing process which was part of the steeping and which was also conducted as a daily routine to clean and moisten the grains during sprouting.

These errors were eliminated when sprouting in the self-constructed malting unit, see Table 5 (chapter *3.2 Experimental Procedure*). Here, the washing, steeping and sprouting processes were all conducted inside the temperature and moisture-controlled cabinet.

However, in the industrial production of sprouted grains these variations might also occur and should be considered.

*Table 10 Repeatability of the determination of characteristic properties of one sprouting trial (7-fold determination)*

| | **Unit** | **Ø** | **SD** | **Rel. SD (%)** |
|---|---|---|---|---|
| Reducing sugar content | g/100 g | 12.29452 | 0.1707 | 1.3188 |
| α-amylase activity | U/g | 25.0410 | 0.5761 | 2.3005 |
| Peak viscosity | Pa s | 0.0481 | 0.0004 | 0.7434 |
| Vitamin C | mg/100 g | 20.4669 | 0.1463 | 0.7148 |

*Table 11 Reproducibility of the determination of characteristic properties of 7 individual sprouted samples (2-fold determination for each trail)*

| | **Unit** | **Ø** | **SD** | **Rel. SD (%)** |
|---|---|---|---|---|
| Reducing sugar content | g/100 g | 11.0879 | 1.8865 | 17.0140 |
| α-amylase activity | U/g | 28.2375 | 4.1481 | 14.6900 |
| Peak viscosity | Pa s | 0.0501 | 0.0012 | 2.4121 |
| Vitamin C | mg/100 g | 18.9202 | 1.4176 | 7.4927 |

*Table 12 Total error of the reproducibility and repeatability of the determination of characteristic properties*

| | **Rel. SD (%)** |
|---|---|
| Reducing sugar content | 18.3328 |
| α-amylase activity | 16.9905 |

| | |
|---|---|
| Peak viscosity | 3.1555 |
| Vitamin C | 8.2075 |

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# 4 SPROUTING OF OATS: A NEW APPROACH TO QUANTIFY COMPOSITIONAL CHANGES

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The following chapter is a post-print version.

## Tweet

A new method which visually assigns sprouted oat grains into 6 degrees of sprouting was found to show a good correlation with other changes of grain properties during sprouting at different temperatures and for different durations.

## Abstract

**Background and objectives**

The aim of this research was to gain a deeper insight of the effect caused by the addition of sprouted oat to food products. The effect of temperature and duration of the sprouting process was systematically studied by sprouting oat grains between 10 and 30°C for up to 3 days.

**Findings**

Overall it was found that temperatures between 20 and 25°C yield the most dramatic changes in the properties of sprouted oats. Based on the data a simple system to characterize the sprouting progress by a visual inspection of the lengths of the coleoptile and radicles was developed. This degree of sprouting (DoS) was correlated with further grain properties.

**Conclusion**

It was found that an exponential relationship between the DoS and grain properties existed. Furthermore, the observed increase in the reducing sugar content (up to 14.6 g/100 g) with increasing DoS was closely related to the increase in α-amylase activity (up to 25 U/g).

**Significance and novelty**

The good predictive power found, indicates that the application of the concept *degree of sprouting* could develop into a reliable characterization method for sprouted grains usable for product development and specification.

## 4.1 Introduction

During the past years, many food products containing sprouted grains appeared on the market. Consequently, the use of sprouted grain to produce bread, pasta, breakfast cereals, biscuits, and porridge was studied and discussed (Richter, Christiansen, & Guo, 2014; Singkhornart, Gu, & Ryu,

2013). In order to achieve this desired incorporation in a controlled manner, it is important to understand how the raw material changes during the sprouting process. This is necessary to understand the possible consequences of the incorporation of sprouted grains for final product properties.

The term sprouted grains is used to refer to germinated grains with radicles and coleoptile with a length greater than that of the most important reference product, the green malt in malting for brewing purpose. The coleoptile of green malt is only allowed to grow to a maximum of two-thirds of the grain length under controlled conditions. In contrast, during sprouting of grains further growth of the seedling is tolerated up to the onset of the photosynthetic metabolic activity. Sprouting, hence further progressed germination, gives rise to significant metabolic changes. This offers the opportunity to produce sprouted grains according to desired objectives, such as improved nutritional profiles.

Basically, during sprouting the embryo generates a new plant by metabolizing carbohydrates, allowing the growth of the radicles and coleoptile (Kunze, 2011). Therefore, hydrolases, for example, amylases and proteinases, are secreted from the aleurone layer into the endosperm of the grain. As a consequence, starch and proteins are degraded in the endosperm into transportable sugars, for example, glucose, peptides, and amino acids. In the embryo these substances are transported into the growing regions for the synthesis of a first leaf and radicles (Bewley, 2001). Sprouting only takes place at a sufficiently high moisture content (>30%), at beneficial temperatures, and under aerobic conditions. Controlling these conditions, the biological processes in the grain can be directly affected (Narziß & Back, 2012).

It is well-established that the sprouting process positively affects the nutritional value of the grains. This fact is used in different technical applications, for example, the hydrothermal activation of grains for the bread production (DE3038463A1, 1980). The activation of the grain results in an enhancement of the vitamin and mineral content in the bread. Moreover, the taste of the bread is improved.

A significant increase in the vitamin content in grains due to sprouting was also reported in many other studies (Harmuth-Hoene, Bognar, Kornemann, & Diehl, 1987; Yang, Basu, & Ooraikul, 2001; Žilić et al., 2014). Tian et al. (2010) found that, next to vitamins, also the total polyphenol content in oat increased by 100% after 3 days of sprouting at 16°C. Xu et al. (2009) also studied the changes in the phenolic acids after different steeping and sprouting times of huskless oat. They found a 60% increase

in the total phenol content after 2 days of sprouting at 16°C. Due to physiological changes during sprouting, stress is exerted on the grain and the redox equilibrium is disturbed. This way, the formation of secondary metabolites like antioxidants such as phenolics and vitamins is stimulated to protect the seedling (Swieca & Dziki, 2015). It was stated that sprouted grains with increased levels of antioxidants can be applied in products to suppress rancidity and color changes (Xu et al., 2009).

An additional benefit of the sprouting process is the reduction of the phytic acid content (Tian et al., 2010). Since phytic acid hampers the bioavailability of minerals, its content in bread is typically reduced during the sourdough leavening process (Schuchmann & Schuchmann, 2012). In product concepts not suited for sourdough processing, the usage of flour from sprouted grains could be a means to reduce the level of phytic acid.

The sprouting process has, however, not only positive effects. During the sprouting process, cell wall material, especially β-glucan, a soluble fiber with health benefits, is degraded as well. β-glucan increases the viscosity in the intestine and causes a retarded absorption of glucose and hence reduced surges of the blood sugar level (Anttila, Sontag-Strohm, & Salovaara, 2004).

Wood et al. (1994) studied the acid hydrolysis of oat gum drinks for 15 and 60 min. The acid-hydrolyzed drinks and the reference had the same β-glucan concentration but differed in viscosities and showed different glucose responses. The strong correlation between glucose response and reduced viscosities found indicates that not only the total β-glucan concentration but also the molecular weight distribution of β-glucan is important.

Wilhelmson et al. (2001) studied degradation of β-glucan during sprouting of oat grains as a function of temperature and time depending. The β-glucan content was most reduced, by 75%, after 3 days of sprouting at 25°C. Under the same conditions, the average molecular weight reduced by 38% compared to the initial value.

Another problem arising during sprouting is microbiological activity. The usual sprouting conditions, such as long steeping stages, high moisture contents, and possible temperatures of 25 - 30°C, benefit the growth of microorganisms and can result in unwanted fermentation processes. Consequently, bacteria, mold, and yeast growths were observed during sprouting and dormant spores might be activated as well (Helland, Wicklund, & Narvhus, 2002; Wilhelmson et al., 2001).

Tian et al. (2010) studied the length of the coleoptile and radicles of 30 sprouted oat kernels by ruler and did not find any correlation with other grain characteristics except for color changes. The work presented here documents an attempt to systematically relate the changes in the composition of sprouted grains to the progression of the sprouting process. Grains were analyzed after sprouting for defined time-temperature combinations. In order to quantify the degree of sprouting, a new method based on the length of the coleoptile was developed and employed in an effort to correlate progress of the sprouting process and composition. The targeted simple approach to categorize the sprouted material would have the advantage to define and standardize specifications for sprouted material, which are directly verifiable and yet ensure a successful product application.

## 4.2 Material and Methods

The huskless oat "*Gehl*", cultivated in 2016 in Canada, was used throughout the study. The grains were stored in sealable containers at 10°C.

During preliminary studies, different methods to evaluate the sprouting progress were tested. Finally, the grains were sprouted at different temperatures (10, 14, 20, 25, and 30°C) and for different times (1, 2, and 3 days). A temperature of 14°C is applied because this setting is usually used in the malting step for brewery purposes (Jacob, 2016). During the sprouting process, the changes in the content of respectively vitamin C, β-glucan, and reducing sugar were monitored. Additionally, the α-amylase activity was studied as a marker for the total enzyme activity.

### 4.2.1 Standard sprouting process at laboratory scale

For the steeping and sprouting process, 500 g of oat grains was washed for 30 min under running tap water in order to clean the grain surface from microorganism to minimize microbiological growth. Afterward, the grains were steeped in closed containers filled with water (so that all grains were covered with water): 4.5 hr wet steeping, 19 hr air rest and 4 hr steeping, all at 20°C (Jacob, 2016). After the steeping step, the grains were drained and put on a metal sheet. The steeped grains were covered with cling film and put in a climate cabinet (*Lovibond 220P-02*) in the dark. During the sprouting step, the grains were washed once a day using a sieve. Due to the washing process the water content was kept constant and checked by using the moisture analyzer MA35 (*Sartorius*).

At the end of the different sprouting periods, the grains were deep-frozen (Siemens Comfort Plus, -20°C) and subsequently freeze-dried (*Beta 1-16 – Christ*) for approximately 60 hr until a final moisture content between 4% and 8% was reached.

Prior to analysis, the oat grains were ground in a speed rotor mill (*Pulverisette 14 – Fritsch*) with a sieve ring of 0.5 mm at 3200 g.

### 4.2.2 Study of different methods to characterize grain growth progress

By use of visual and gravimetric measurements using different sprouted oat material, an attempt was made to find an easy systematic characterization method for the quantification of the progression of the sprouting process.

For the different samples, the 1,000 kernel weight was determined gravimetrically by counting 100 kernels and multiplying the result by 10. The dry matter was analyzed using the moisture analyzer MA35 (*Sartorius*). Determinations were done in duplicate.

The mass percentage of the radicles and coleoptile of the total grain was determined by cutting off radicles and coleoptile with a dissecting needle and weighing the undried grain with and without the radicles and coleoptile. The determination was performed in duplicate.

The degree of sprouting was determined by visually classifying the length of the coleoptile and radicles of 100 kernels by dividing them into the six categories shown in Figure 21. The determination was done in triplicate. On each day of sprouting an average degree of sprouting was calculated as the sum of relative occurrence of the different classes ($DoS_i$) multiplied by its respective degree of sprouting ($i$):

$$\textbf{Average DoS} = \sum_{i=0}^{5} i \cdot relative\ occurrunce\ (DoS_i) \qquad \textbf{(Equation 2)}$$

### 4.2.3 Determination of the α- and β-amylase activity

The α- and β-amylase activities were determined by using the Megazyme (2012) Malt-Amylase assay procedure K-MALTA 05/15. Determinations were done in duplicate.

### 4.2.4 Determination of the ascorbic acid content

The vitamin C content was determined according to the *Indophenol Method* (Nielsen, 2003). For the determination of the vitamin C content in flour, a modified dilution factor of eight had to be applied. The sprouted flour sample was dispersed in a mixture of 30 g/L metaphosphoric acid and 8% (v/v)

acetic acid and centrifuged at 3,000 g. A volumetric sample of the resulting supernatant was diluted and used for titration according to the method. The procedure was executed in duplicate per specimen.

### 4.2.5 Determination of the reducing sugar content

The reducing sugar content was determined using DNS (3,5 dinitrosalicylic acid) in combination with a colorimetric analysis. Determinations were done in duplicate. In detail, the sprouted flour samples (1 g) were mixed with 5 ml of a mixture of 40% (v/v) ethanol and 60% (v/v) of a 10 mM copper chloride solution to extract the reducing sugars. After shaking the mixture for 10 min, the suspension was centrifuged for 15 min at 3,000 g (Heraues, Labofuge 200). This procedure was repeated twice. The collective supernatant, three subsequent extractions of the same material, were diluted and filled to 25 ml with the above-mentioned ethanol/copper chloride solution mixture. By addition of 0.625 g polyvinylpolypyrrolidone to the solution, all phenols present were precipitated. The precipitate was filtered off (Tian et al., 2010) and 1 ml of the filtrate was mixed with 1 ml of an aqueous solution containing 10 g/L DNS, 16 g/L sodium bicarbonate, and 300 g/L potassium sodium tartrate. After shaking this mixture for 10 min at 100°C, the solution was diluted with 5 ml distilled water. The reducing sugar content was determined spectrophotometrically (*Spekol 1300 – Analytik jena*) at 545 nm. For the calibration, different concentrations of maltose monohydrate were used. The reducing sugar content determination is thus related to the reducing potential of maltose. However, glucose exhibits a reducing potential, which is identical to maltose per molecule.

### 4.2.6 Determination of β-glucan content

The β-glucan contents of the differently sprouted oat samples were determined by using the Megazyme (2017) Mixed-Linkage Beta-Glucan assay procedure (McCleary Method – K-BGLU 02/17; AACC Method 32-23.01). Determinations were done in duplicate.

### 4.2.7 Statistical evaluation (ANOVA)

The statistical evaluation of the methods tested to evaluate the sprouting process, the temperature and time effect on selected grain properties, and the dependence of selected grain properties and the degree of sprouting was performed using Microsoft Excel 2016. The p-value (probability of error) was calculated by analyzing the experimental data by means of ANOVA (analysis of variance) using a single-factor variance analysis. A level of significance (α) of .05 was chosen.

The significance of the temperature effect was analyzed by using the data of the 3-day sprouted samples, and the significance of the temperature effect was analyzed by comparing the data of the samples sprouted at 20°C.

## 4.3 Results and Discussion

### 4.3.1 Statistical evaluation of experimental data

Table 13 presents the p-values of all properties considered. These were calculated as part of the ANOVA. Since all p-values are lower than the level of significance, the found difference can be considered significant.

*Table 13 p-values from statistical analysis (ANOVA) of all studied properties*

| | **β-glucan content (Figure 22)** | **Properties in dependence on the degree of sprouting** | **Time effect (1-3 d, 20°C)** | **Temperature effect (10-30°C, 3 d)** |
|---|---|---|---|---|
| **Degree of sprouting** | 8.46E-06 | | 4.64E-05 | 7.78E-10 |
| **Coleoptile and radicle percentage** | 3.52E-09 | | | |
| **1000 kernel weight** | 7.36E-03 | | | |
| **α-amylase** | | 5.26E-11 | 1.84E-05 | 1.27E-08 |
| **Reducing sugar content** | | 4.67E-10 | 4.31E-05 | 9.45E-08 |
| **Vitamin C content** | | 4.85E-04 | 5.00E-02 | 3.07E-04 |
| **β-glucan** | | 8.46E-06 | 3.27E-03 | 1.19E-03 |

### 4.3.2 Evaluation of different methods to characterize the sprouting progress

Different properties of the grains were evaluated for their usability to characterize the progress of the sprouting process. This evaluation of the different approaches was done based on the β-glucan content as an output property. This marker was chosen because of its importance due to health-promoting and functional property effects (Choi et al., 2012). A first approach to characterize the

progress of the sprouting process is based on the visual inspection of the lengths of the coleoptile and radicles.

In Figure 21, the definition of the degree of sprouting as used in this study to identify the sprouting progress is shown and the different development stages of the oat grains during the sprouting process can be seen. The length of the coleoptile was selected as a criterion of the categorization of the degree of sprouting.

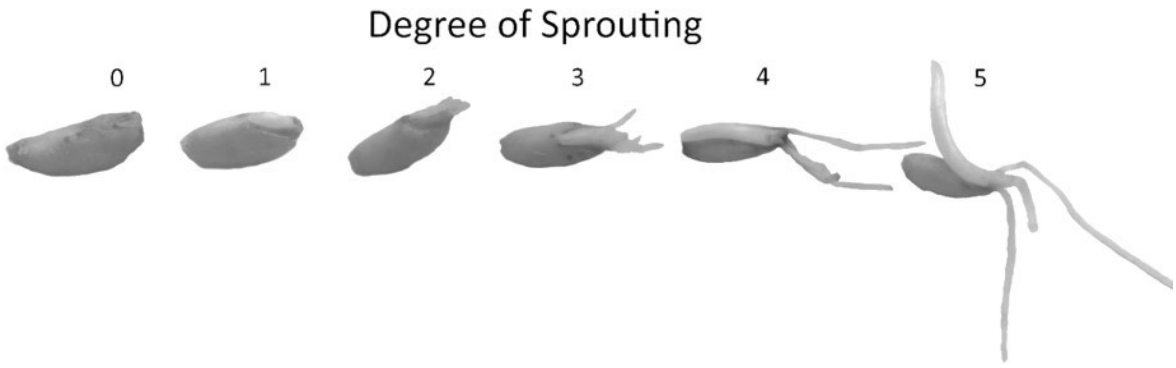


*Figure 21 Definition of the degree of sprouting of oat grains by the lengths of their coleoptile and radicles.*

Grains of degree 0 do not show any radicle or coleoptile growth. Degree 1 characterizes grains with visible embryos (small white point), while the radicles and coleoptile are not visible. Degree 2 describes grains already showing a developed embryo emerging from the seed coat. The grains of degree 3 reveal coleoptile lengths of at least half the oat grain length. Degree 4 represents coleoptile lengths between half and a full grain length. Under degree 5, grains with a coleoptile longer than a full grain length are summed up.

Alternatively, to the degree of sprouting it is conceivable to determine the 1,000 kernel weight (dry matter based) to characterize differently sprouted grains. Another alternative to describe the progress of the sprouting process is the determination of the weight fraction of both the radicle and coleoptile of the full grain.

In Figure 22, the β-glucan content is shown as a function of three possible properties to characterize the progression of the sprouting process, respectively.

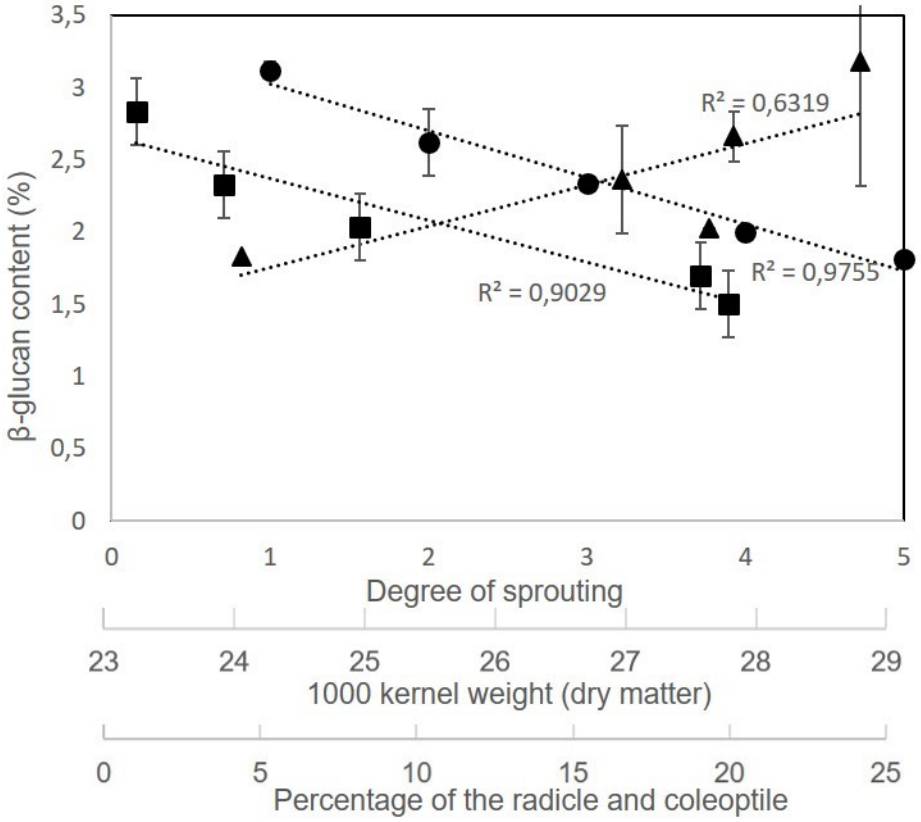


*Figure 22 Correlation of degree of sprouting (●), coleoptile and radicle percentage (■) and 1000 kernel weight (dry matter based) (▲) with the β-glucan content; oat was sprouted for 3 days at 20°C.*

From the properties, evaluated the 1,000 kernel weight corresponds to the least with the β-glucan content ($R^2$= .63). Both other methods show a high degree of correlation: degree of sprouting ($R^2$= .98) and weight fraction of coleoptile and radicles ($R^2$= .90). Even though both correlations with the β-glucan content were found to be very good, only the degree of sprouting was considered for further consideration. Reason to do so is the simplicity of the procedure, which renders it less prone to errors. An obvious downside is the integer nature of this parameter. However, the "degree of sprouting" method was used as lead parameter to correlate with changes in grain properties due to sprouting.

### 4.3.3 Influence of sprouting temperature and time

The degree of sprouting as defined above for subsets of oats in a single sprouting process was used to quantify the oat sprouting process at different temperatures and for different periods of time. For each time-temperature combination for the sprouting process the grain population exhibited a specific distribution of the degree of sprouting.

The number fractions of the grains with different degrees of sprouting are shown in Figure 23. Here, the results obtained for the five different sprouting temperatures after 3 days of sprouting are depicted. From the distribution of degrees of sprouting within a sample, the average degree of sprouting can be derived according to Equation 2. These values are represented in Figure 23 by diamonds. The error bars are based on counting three independent samples from one sprouting experiment. For the 3-day sprouting period, the longest coleoptile was observed for sprouting at 25°C. Sprouting at a temperature of 20°C resulted in less vigorous sprouting. At 30°C, the oat grains did practically not show any radicle growth.

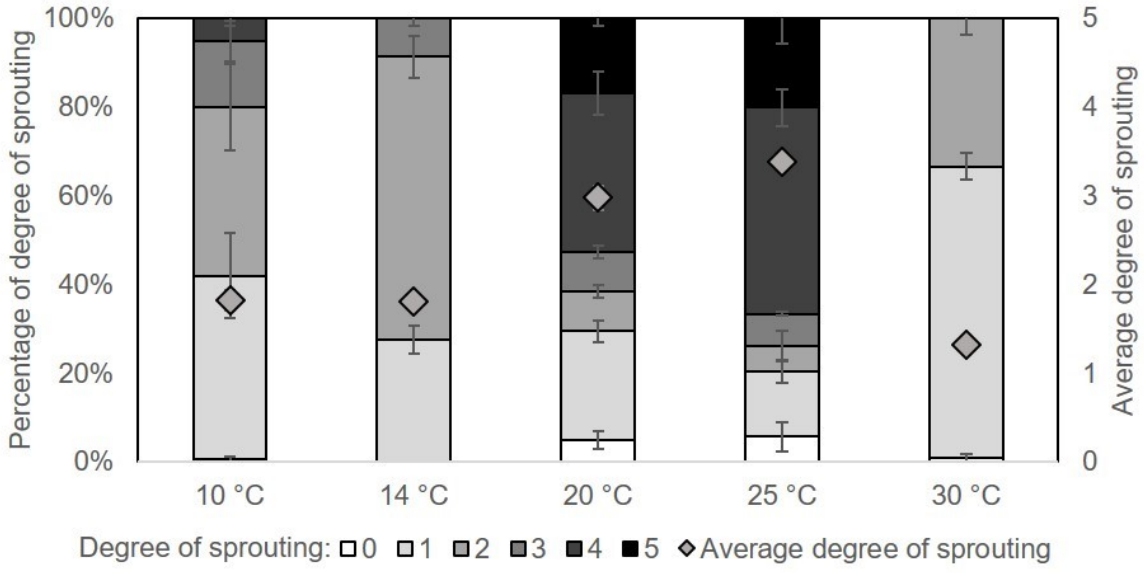


*Figure 23 Effect of the sprouting temperature on the degree of sprouting after 3 days.*

The data gathered also allowed to determine the germinability. The germinability is defined as the percentage of grains reaching a degree of sprouting above 0. For all temperatures investigated, the germinability after 3 days was about 99%.

Figure 23 also reveals that around 20% of the grains which were sprouted at 20 and 25°C have a coleoptile longer than a full grain length (degree of sprouting 5). Less long coleoptiles developed at the other sprouting temperatures studied.

No standard deviation of the average degree of sprouting is given in Figure 23, because the chart already illustrates the contribution of different degree of sprouting within the sample to the average degree of sprouting. This illustrates how homogeneously a sample had been sprouted. The consideration of the homogeneity is an important point with respect to a large-scale sprouting

operation. A narrow distribution of the degree of sprouting within a production run would allow for better control of product properties.

The data gathered reveal that a high average degree of sprouting (e.g., 20 & 25°C) corresponds to a high standard deviation and thus a low homogeneity. Vice versa, processes with a low average degree of sprouting (e.g., sprouting at 30°C – average degree of sprouting 1.4) do not show a high variation and are rather homogeneous. This was also found for the standard malting temperature of 14°C. The choice for this temperature is probably motivated by low risk for microbiological spoilage, homogeneous radicle growth and limited losses due to radicle and coleoptile growth.

In Figure 24, the effect of the sprouting time and temperature on the average degree of sprouting is shown. One can see that the sprouting started fastest for the oat which sprouted at 20°C. The data indicate linear increase in the degree of sprouting with time for the samples sprouted at 10, 20 and 25°C.

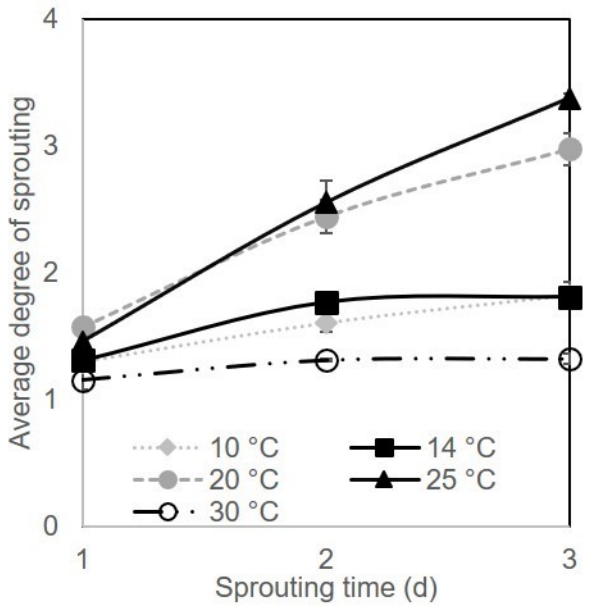


*Figure 24 Effect of sprouting time and temperature on the average degree of sprouting.*

#### 4.3.3.1 Effect of sprouting and temperature on oat properties

In Figure 25a-d, the effect of the sprouting temperature and time on the different quality parameters of the sprouted grains is illustrated.

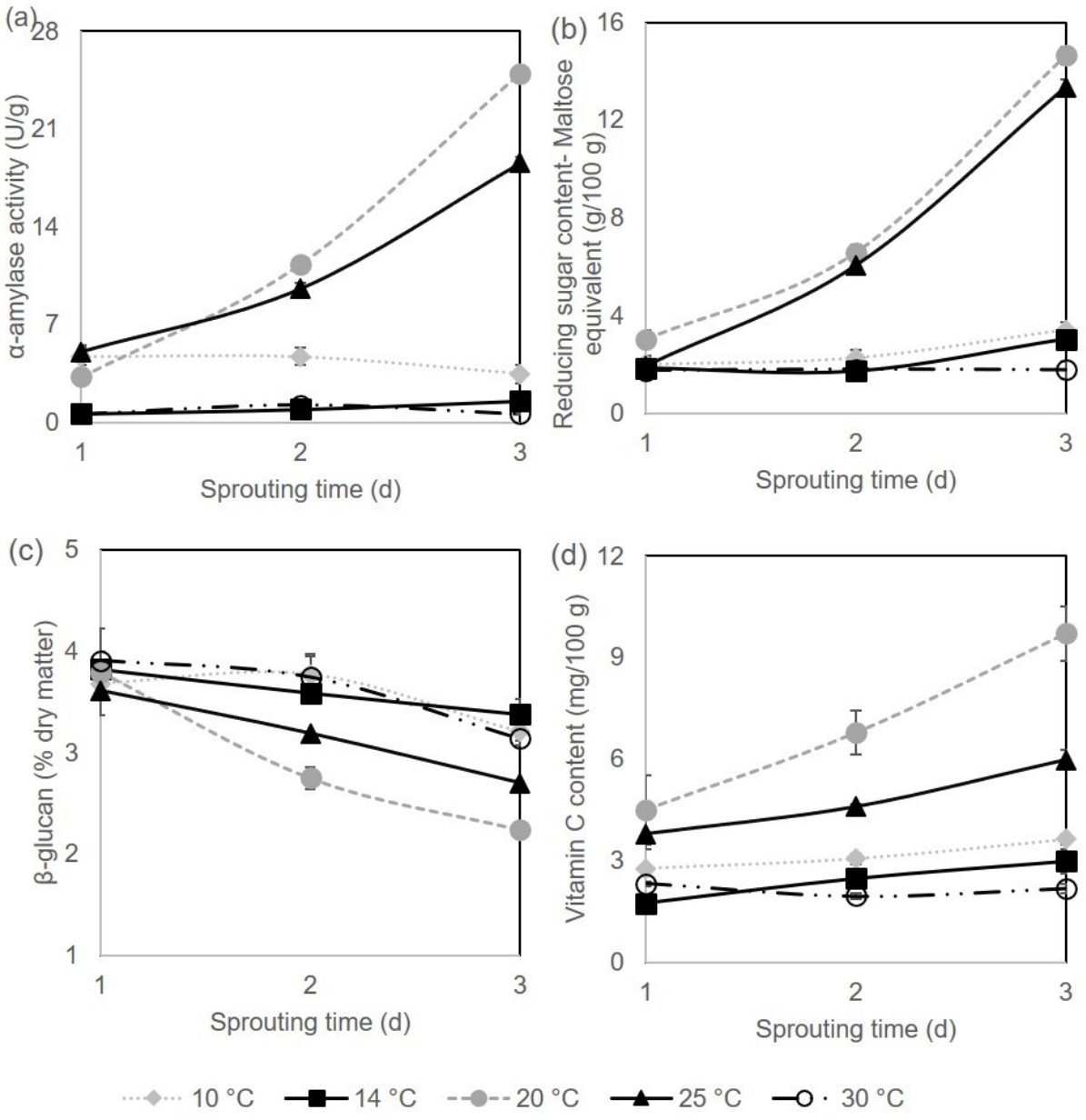


*Figure 25 Effect of time and temperature during oat sprouting on the changes in α-amylase activity (a), in reducing sugar content (b), in β-glucan content (c), and in vitamin C content (d).*

In Figure 25a, the results of the α-amylase analysis are shown. The data reveal that after 1 day, the α-amylase activities between the different temperatures did not differ too much. After 3 days however, the α-amylase activities at 20 and 25°C increased significantly to values one order of magnitude larger than those for the other temperatures (10, 14, and 30°C).

In contrast to the increase in α-amylase activity during the sprouting process, the analysis of β-amylase activity revealed no changes *(data not shown)* and the enzyme was apparently not synthesized de novo.

In line with the data for the α-amylase activities, the content of reducing sugars in oat increased most at sprouting temperatures of 20 and 25°C (Figure 25b). Expectedly, the limited α-amylase activity at 10, 14, and 30°C corresponded to only subtle increases in the reducing sugar contents. The limited data available suggest a linear relation between the levels of reducing sugars and the duration of sprouting.

As can be seen in Figure 25c the β-glucan content is also a function of the temperature and duration of the sprouting process. At all sprouting temperatures studied, the β-glucan content was decreased after 3 days of sprouting. For a sprouting temperature of 20°C, the degradation is most pronounced, almost halving the initial β-glucan content to 3.9%. At sprouting temperatures of 10 and 14°C, the β-glucan content only slightly decreased, confirming the findings by Wilhelmson et al. (2001) that the β-glucan content decreases less at low sprouting temperatures.

In line with Lintschinger et al. (1997), vitamin C was chosen as a marker for the general vitamin content because of its high reactivity. No ascorbic acid was present in the native grain. Upon sprouting, a significant increase in the ascorbic acid content was found (Figure 25d). The highest levels were found when sprouting at 20°C. Except for the sprouting temperature of 30°C, all other sprouting temperatures also resulted in increased levels of ascorbic acid.

#### 4.3.3.2 Discussion of the effect of sprouting time and temperature on property changes in oat grains

The results gathered reveal a rather consistent picture, revealing relations between the different grain properties. It was shown that the different properties changed systematically with temperature and duration of the sprouting process.

During the sprouting process a de novo synthesis of α-amylase in the oat grain was observed. This is most pronounced at a sprouting temperature of 20°C. Since the starch degradation relates to the α-amylase activity, the increase in the amount of reducing sugars followed a corresponding pattern. The relationship of certain properties appears more complicated considering that sugars are transported into the growth regions of the grain for further development of the coleoptile and radicles. This

suggests that the coleoptile and radicle growth (input parameters for the degree of sprouting) and the reducing sugars and α-amylase activity are interdependent.

The acceleration of the sprouting process on increasing sprouting temperatures was also found for barley by Müller (2015). Varying the sprouting temperature from 16 to 24°C resulted in a reduction of the sprouting time of about 24 hr per 4°C. It was further found that sprouting at 20°C yielded best results, which is in line with the results presented here.

Progression of the sprouting process also resulted in changes of the ascorbic acid levels. Ascorbic acid is needed as protective antioxidant in the growing grain. The increase in the vitamin C contents of oat during sprouting (see Figure 25) is a function of several processes. In order to terminate the dormancy and start the sprouting process, reactive oxygen species have to be released. However, the presence of these species results in an oxidative stress in the cells. Moreover, the exposure to light also causes stress in the grain inducing the synthesis of antioxidants, for example, ascorbic acid (Pitzschke, Fraundorfer, Guggemos, & Fuchs, 2015).

With respect to improve the nutritional value of sprouted oats, the increase in vitamins is desired while the degradation of beta-glucan is considered a downside (El Khoury, Cuda, Luhovyy, & Anderson, 2012). Consequently, optimizing the sprouting process for best nutritional values would involve balancing the levels of these two nutrients.

### 4.3.4 Study of oat having different degrees of sprouting

Oat samples of varying degrees of sprouting were studied. These grains were sprouted at 20°C for 3 days and sorted according to their degree of sprouting. In Figure 26, the data on reducing sugar content, ascorbic acid content, β-glucan content, and α-amylase activity in oat samples with each a homogeneous different degree of sprouting (1 - 5) are shown.

In this context, a homogeneous sample means that it compromises only grains with the respective degree of sprouting.

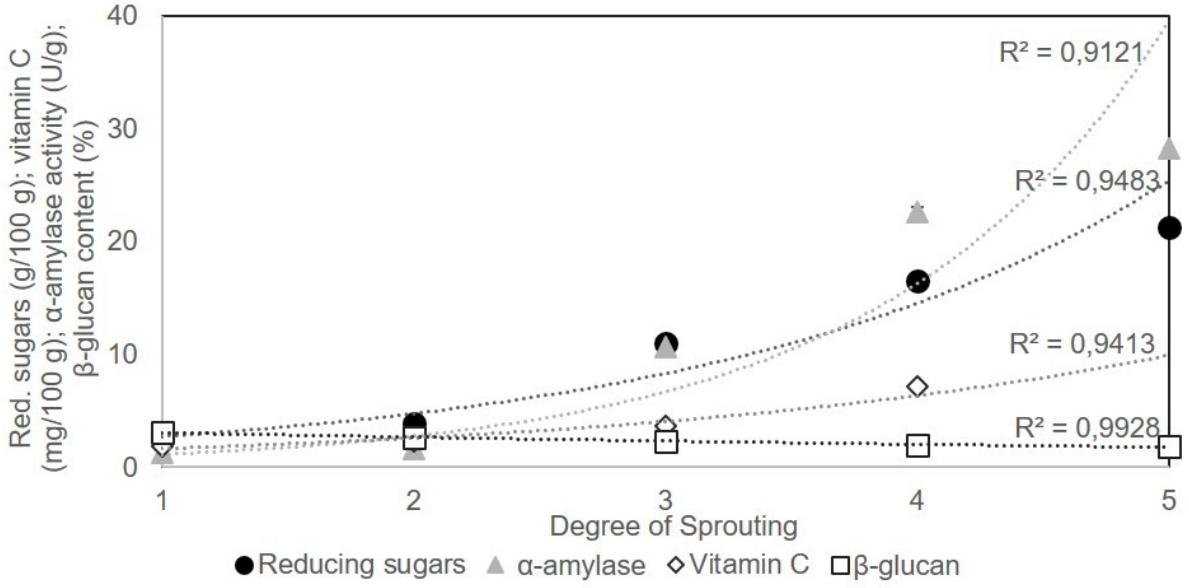


*Figure 26 Changes in oat kernel properties in dependence on the degree of sprouting: 3 days sprouting, 20°C.*

The data reveal a systematic evolution of the α-amylase activity, ascorbic acid, β-glucan, and reducing sugar contents with increasing degree of sprouting. One could argue about the correct choice of the mathematical function to be fitted to the experimental data. It appears to be beyond the scope of this work to formulate a model to describe the changes. The choice of the function to be fitted to the data remains thus arbitrary. Even though linear regressions would also allow to describe the data quite well, it was chosen to use simple exponential functions. This choice was motivated by the nature of the properties of the described reaction products. The exponential fits show a quite strong correlation between the response parameters as a function of the degree of sprouting. It has to be noted though that the degree sprouting is not a transformed reaction time.

Increased amounts of reducing sugars and ascorbic acid were found particularly in the radicles and coleoptile (data not shown). The ascorbic acid content in the radicles and coleoptile was four times higher than that in the grain without the radicles and coleoptile. Hence, for the production of oat flour having a high nutritional value it is of special interest to leave radicles and coleoptile at the grains. The oat grains which were sprouted for 3 days at 20°C had an average degree of sprouting of 3 (Figure 23); hence, the radicles and coleoptile contribute about 8% of mass.

These findings indicate that a fast visual determination of the degree of sprouting allows to estimate, for example, the ascorbic acid content without doing expensive experiments. Moreover, the

sweetness of the product can be estimated based on the correlation between the degree of sprouting and the reducing sugar content.

### 4.3.5 Evaluation of the suitability of the degree of sprouting

In order to evaluate whether or not the degree of sprouting could be of any practical value in estimating characteristic data of sprouted material, calculated data were compared to experimental data (see Figure 27).

A set of 15 samples (five different sprouting temperatures, three different sprouting durations) was used for the evaluation. It has to be pointed out that the correlations between properties of samples and degree of sprouting, as displayed in Figure 26, were based on homogeneous subsamples of the sprouting process at 20°C. The 15 samples differing due to variation of the process conditions are each inhomogeneous (see Figure 23 and Figure 24). Consequently, the training set for the correlations and the evaluation set are not only independent from one another, but also differ with respect to homogeneity. The content of ascorbic acid, β-glucan, and reducing sugars and α-amylase activity were calculated as function of the respective degree of sprouting based on the functions displayed in Figure 26. It goes without saying that for an exponential function averaging the argument of the function yields a different result than averaging the values. Hence, good predictions are only possible by averaging the properties over the different homogeneous fractions constituting an inhomogeneous sample. The use of the average degree of sprouting would systematically yield overpredictions as a function of the homogeneity of the samples.

The parity plots depicted in Figure 27 show that the functions derived from the data shown in Figure 26 yield reasonably good predictions for the different properties of the inhomogeneous samples. Each data point represents the grain population generated by a specific process setting. For each sample, the distribution of sprouted grains over the different degrees of sprouting was determined (Figure 23). Per degree of sprouting the respective property was computed. The property per sample was subsequently derived by pro rata contribution from the different degrees of sprouting within a sample.

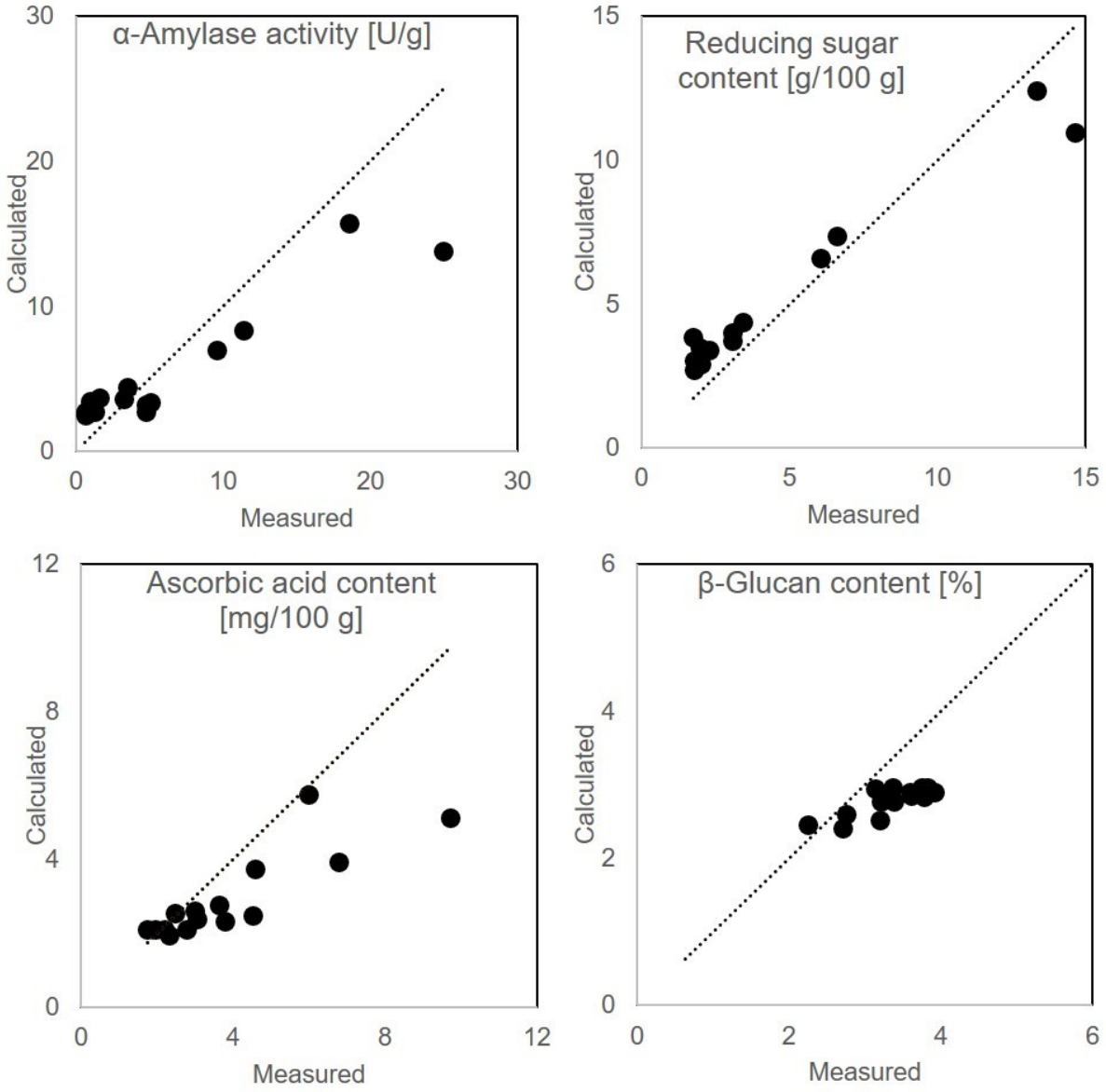


*Figure 27 Parity plots for the calculated and measured values of the contents of reducing sugars, β-glucan and ascorbic acid and α-amylase activity of samples of various sprouting conditions.*

In detail, the α-amylase activities and contents of reducing sugars were predicted quite well by the approach outlined. The level of ascorbic acid appears to be underpredicted systematically. This is also true for the prediction of the β-glucan levels. In this case, it has to be noted that the experimental values did only vary in a limited range between the different samples. However, it appears fair to summarize that Figure 27 documents that the concept of the degree of sprouting can be used to predict the properties of a sprouted sample, taken that averaging is done in an adequate way.

## 4.4 Conclusion

The effect of temperature and duration of the sprouting process was systematically studied for oats. Process temperatures between 10 and 30°C were studied for a duration of up to 3 days. The resulting samples of sprouted oats were studied for their concentrations of β-glucan, ascorbic acid, and reducing sugars. Additionally, the α-amylase activity was determined. It was found that the composition of oat changed in a rather systematic pattern. The obvious interdependency of reducing sugar content and α-amylase activity was verified. The degradation process of β-glucan seemed to correlate with the degree of sprouting as well. This is only true for a lesser extent for the presence of ascorbic acid. This might be due to complex processes in the growth process during which ascorbic acid is formed and later consumed. Overall, it is found that for a process duration of 3 days, temperatures between 20 and 25°C yield the most significant changes in the properties of sprouted grains.

In order to simplify the categorization of samples of sprouted materials, a correlation of the compositional properties mentioned above to an easy applicable descriptor of a sprouted grain sample was sought. Initial assessment revealed that the 1,000 grain weight is insufficiently linked to quality parameters. The mass fraction of radicle and coleoptile in the grain correlated very well with the β-glucan level. A similarly good correlation was found for the much easier applicable degree of sprouting. This DoS is derived based on the visual assessment of coleoptile length set into relation to the grain size.

The degree of sprouting was assessed for various samples, and it was found that for different process settings, a typical distribution of the degree of sprouting within a sample existed. Correlations between the measured compositional properties and the degree of sprouting were derived from subsets of grains for a single process condition (20°C, 3 days). The sample of grains was subdivided into homogeneous subsamples with identical degree of sprouting. Based on these homogeneous samples, functions to calculate the grain properties as a function of the DoS were derived. These functions were used to predict the properties of inhomogeneous samples originating from different sprouting process settings. The surprisingly good predictive power found indicates that the application of the concept of degree of sprouting could develop into a reliable characterization method for sprouted grains usable for predicting compositional and nutritional changes of oats during sprouting and ultimately leveraging this information for product development and specification.

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# 5 EFFECT OF SPROUTING CONDITIONS ON PROPERTIES OF EXTRUDED, DIRECT EXPANDED WHEAT

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## Abstract

The present study investigates the use of flour from sprouted wheat for the production of extruded, directly expanded products. Until now, studies on the use of sprouted material in extrusion have not been concerned with differences in the sprouted material. This contribution documents the first attempt to study the effect of the variation of the sprouting process on extrudate properties. Extrudates were produced at standard conditions using material produced by the variation of sprouting times (steeped, 1, 3, 5, 7, and 9 days sprouted).

It was found that an increasing duration of the sprouting process also increases the starch degradation and hence sugar generation. During this process, vitamin C levels showed an increase of up to 10-fold. The accompanying changes in viscosity propagate systematically into different observations. With increased starch degradation back pressures in the extrusion die were reduced, extrudates show less sectional expansion, but densities were decreased because longitudinal expansion was increased significantly.

The extrudates made with sprouted material were preferred during sensory assessment. The degree of sprouting, which was defined earlier proved to be a useful property to characterize the progress of the sprouting process and correlated well with changes observed for the sprouted flour and extrudate properties.


**Keywords:** sprouted grains, extrusion, directly expanded products, degree of sprouting

## Practical application

The use of sprouted wheat in the production of direct expanded extrudates is a promising opportunity to change and improve extrudate properties. On the one hand, the nutritional value of the extrudates is improved due to the use of sprouted wheat flour which contains newly synthesized vitamins, more essential amino acids, and a decreased phytic acid content. On the other hand, the texture of the extrudates is improved and less additives are needed in the production. Due to the starch degradation in the sprouting process short-chain sugars were formed, which naturally increase the sweetness of the extrudates.

## 5.1 Introduction

Sprouted grains are used in many food products like bread, pasta, breakfast cereals, biscuits, and bars. Currently, little is known about the changes of the product properties in foods caused by the incorporation of sprouted material. Especially the starch degradation during sprouting is expected to have a significant effect on the functionality of sprouted grain flour because many properties of the above-mentioned products are governed by the starch structure due to the high starch content in cereal grains. By adding flour from sprouted grains to products, their typical properties could be altered. For example, the high enzyme activity in the flour results in a reduction of the falling number and a deterioration of the baking quality (Richter, Christiansen, & Guo, 2014). To avoid quality losses in food products caused by the addition of flour from sprouted grains, one has first to study and understand changes of the flour as a result of the sprouting process.

Bewley (2001) defines germination as a process, which starts with the water uptake and ends with the radicle emergence. This event marks the beginning of the seedling's growth and is hence considered the start of the sprouting process. The overall process of grain sprouting and subsequent kiln-drying is called malting. However, the terms "sprouting", "malting", "germination" are often used interchangeably (Chavan & Kadam, 1989). The sprouting process is based on metabolizing carbohydrates, allowing the growth of the radicles and coleoptile (Kunze, 2011).

Sprouting only takes place at a sufficiently high moisture content (>30%), at beneficial temperatures, and under aerobic conditions. Obviously, the set of these conditions maintained during sprouting has a significant influence on the different processes taking place (Narziß & Back, 2012).

The application and use of sprouted cereal grains in the diet has become quite popular due to their positive image with consumers. The perception of sprouted grains as being health-promoting is supported by several studies. First of all, sprouting of grains was reported to positively affect the level of available vitamins, other nutrients, and anti-nutrients (Harmuth-Hoene, Bognar, Kornemann, & Diehl, 1987). Moreover, due to the starch degradation, reducing sugars are formed. These sugars give the product sweetness and Maillard reactions during cooking, baking, or drying allow the generation of pleasant flavors and colors (Rausch, 2009).

The starch degradation also matters with regard to the usage of flour from sprouted grains in direct expanded products, for example, breakfast cereals. Extrusion is a continuous process in which the

material is pressed through one or more dies. Prior to this, the mass is mixed and processed at specific pressure, shear, and temperature conditions. These process conditions typically result in a reduction of the vitamin content (Singh, Gamlath, & Wakeling, 2007). Using feed material with an improved initial nutritional profile, such as flour from sprouted wheat, could be an approach to compensate for the reduction of nutrient levels caused by the extrusion process and fortify foods with naturally and inherently formed nutrients from grain.

The use of sprouted material in extrusion processes was already studied by Singkhornart, Edou-ondo, and Ryu (2014). In their study wheat was used which was sprouted at 25°C for 3 days. Compared to native wheat an increased amount of reducing sugars was found in the extruded sprouted wheat products. According to this work the specific mechanical energy, expansion index, bulk density, breaking strength and water absorption index decreased for samples with sprouted material. An increase was found in the specific length and in the water solubility index of the extrudates. A similar study was presented by Zhu, Adedeji, and Alavi (2017). Their work, essentially confirmed the findings of Singkhornart et al. (2014) but is based on slightly different sprouting conditions.

The studies of Singkhornart et al. (2014) and Zhu et al. (2017) were both based on a specific single set of sprouting conditions and focused primarily on the variation of the parameters of the extrusion process (die temperature, screw speed, and $CO_2$ injection).

However, it is important to elucidate how different sprouting conditions propagate systematically into changes of extruded products. In this study, wheat was sprouted for different durations at 20°C and the effect of the sprouted wheat on directly expanded extrudates was investigated at constant extrusion conditions. In an earlier study (Krapf, Kandzia, Brühan, Walther, & Flöter, 2019) it was proposed to characterize the progress of the sprouting process systematically by a defined degree of sprouting. Building on this, this study hence aims to find a correlation between the degree of sprouting and the extrudate properties, for example, changes in texture, cell structure, and cold paste viscosity. Additionally, sensory evaluations of the extrudates were performed to identify possible accompanying changes of organoleptic properties.

## 5.2 Material and methods

In total, seven different wheat flours were extruded. All flours were based on the soft wheat variety *Runal.* Native wheat and steeped wheat were used as reference samples for the sprouted materials.

Preliminary experiments indicated that a sprouting temperature of 20°C results in a robust process. Sprouting was hence performed at this temperature and variation was only introduced by changes of the duration of the sprouting process. Sprouting of steeped wheat was allowed for periods of 1, 3, 5, 7, and 9 days. All other conditions were kept constant.

### 5.2.1 Sprouting process

The malting plant *A1-2008* from *Seeger* was used for the sprouting. About 600-800 g of grains was filled into each basket of the malting cabinet. The grains were washed once, steeped for 5 hr in water (20°C), dry steeped for 19 hr at 20°C and again wet steeped in water (20°C) for 4 hr (Jacob, 2016). During the sprouting process the grains were turned four times daily and sprayed with water twice a day. 30% (v/v) of the air was recirculated and the air was conditioned to be saturated at 20°C. The sprouting process was terminated after 1, 3, 5, 7, or 9 days of sprouting. Grains were dried in the kiln-drying plant *A1-2008* from *Seeger* at 65°C to 88-90% (w/w) dry matter content. These gentle process conditions minimize the loss of the newly synthesized vitamins and ensure a good millability of the sprouted grains. The parameters were determined in preliminary tests. Subsequent cooling of the sprouted grains to ambient temperature was achieved by exposure to ambient conditions. The dried, cooled sprouted grains were milled by using a hammer mill (*Siemens-Schuckertwerke AG*). A 0.5 mm mesh size was used during the milling process.

### 5.2.2 Extrusion process

Extruded products were manufactured utilizing a *Berstorff ZE 25* extruder of 870 mm screw length at standardized settings (200 rpm; throughput of 6 kg/h; feed moisture content of 27%; die of 3 mm). These settings resulted in a maximum sectional expansion index in preliminary tests. Mixtures of flour and water were used exclusively as feedstock in order to emphasize the differences between the different sprouted wheat flours.

The chosen temperature profile of the eight different barrels of the extruder is depicted in Figure 28. The final temperature of 140°C was derived from preliminary tests. The aim was on the one hand to protect the nutrients, and on the other to stimulate Maillard reactions in this specific temperature range (Rausch, 2009). The screw was assembled of standard forward elements with two different pitches. Once stable extrusion process conditions were established, the pressure in the die was measured 10 times per second. Mean pressure values and standard deviations per setting were calculated from datasets of at least 1,000 individual data points.

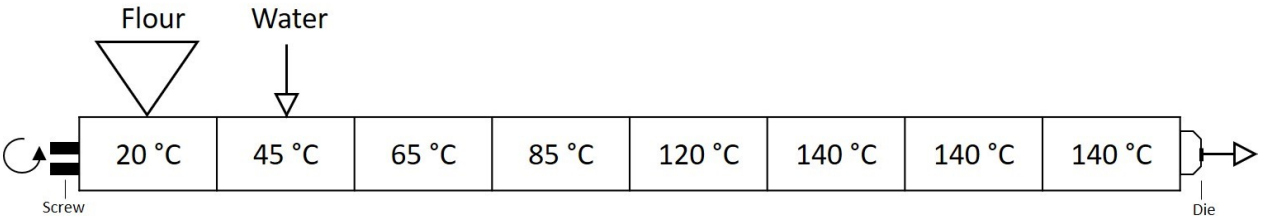


*Figure 28 Schematic representation of the extruder and its barrel temperatures.*

The extruded strands were cut into pieces of about 50 cm length after leaving the die. They were dried at 65°C for 1.5 hr in an oven (*Thermo Scientific T 6420*) to a water content of less than 10% (w/w) in order to receive a crunchy texture. Analyses were performed either directly (expansion index, texture measurement, pore structure, sensory evaluation) on the extrudates or by using the milled extrudates (cold paste viscosity, water solubility index, reducing sugar content, vitamin C content, color). Extrudates were crushed by exposing them for 30 s to a kitchen blender (*Petra MZ12.35*). Subsequently, the coarse flour was milled in a speed rotor mill (*Pulverisette 14, Fritsch*) with a sieve ring of 0.25 mm at 8000 rpm.

### 5.2.3 Analytical methods

#### 5.2.3.1 Degree of sprouting

The sprouting progress was characterized as degree of sprouting according to Krapf et al. (2019). The degree of sprouting was determined by visually classifying the length of the coleoptile and radicles of a kernel assigning it to one of the eight categories, DoS0 to DoS7. Grains of degree of sprouting 0 (DoS 0) do not show any radicle growth. Degree 1 characterizes grains with visible embryos (small white point) and not yet visible radicles or coleoptile. Degree 2 describes grains already showing a developed embryo emerging from the seed coat. The grains of degree 3 reveal radicles' lengths of less than half a grain length. Degree 4 represents radicle lengths between half and a full grain length. Under degree 5 grains with radicles longer than a full grain length but with miniscule coleoptile are summed up. In contrast, degree 6 counts grains with a coleoptile longer than a full grain. Finally, degree 7 covers grains with coleoptile at least twice the length of a grain.

After the different sprouting periods, the average degree of sprouting was calculated as the sum of relative occurrence of the different classes (i):

$$\textbf{Average DoS=} \sum_{i=0}^{7} \boldsymbol{i \cdot occurrunce}\ \%\ (\boldsymbol{DoS_i}) \qquad \textbf{(Equation 3)}$$

This was derived based on a set of 300 kernels. Each kernel is evaluated for its individual degree of sprouting (DoS).

#### 5.2.3.2 Viscous behavior of sprouted flour suspensions

The sprouted grain flour was characterized rheologically by studying an aqueous suspension of 10% (w/w) flour. The dosing was done explicitly on dry matter basis. Analyses were performed using an *Anton Paar* rheometer *MCR 302* equipped with a starch cell and a stirrer having a vane geometry. Throughout the experiment runs the stirrer speed was kept at 150 rpm. Copper(II) chloride at a concentration of 10 mM was added to the suspension in order to inactivate the enzymes, especially amylases according to Aquino, Jorge, Terenzi, and Polizeli, M. L. T. M. (2003). The enzymes had to be inactivated to prevent further starch degradation in the heating cycle during the rheological measurements. Preliminary test had shown that this precaution is necessary to determine the rheological properties reliable.

The suspension was prepared at room temperature. Temperature scans from 30°C to 95°C were performed with a heating rate of 1.5°C/min. Cooling from 95°C to 30°C was performed with a cooling rate of 4.33°C/min. The temperature regime and heating rate were adopted from the method using an amylograph (DIN EN ISO 7973, 2016). The stirrer speed was chosen based on preliminary tests.

These experiments revealed the so-called *peak viscosity* and the *final viscosity* once the system was cooled down to 30°C.

The error bars represent the variation determined by a sevenfold repetition of a standard analysis.

#### 5.2.3.3 Reducing sugar content of extrudates and sprouted flour

The content of reducing sugars is used as an indicator for the progress of the starch degradation during sprouting.

The reducing sugar content was determined using DNS (3,5 dinitrosalicylic acid) and a colorimetric analysis. The 1 g of sprouted flour or 1 g of milled extrudates was mixed with 5 mL of a mixture of 40% (v/v) ethanol and 60% (v/v) 10 mM copper chloride solution to extract the reducing sugars. After shaking the mixture for 10 min the suspension was centrifuged for 15 min at 3000g (Heraues, Labofuge 200). This procedure was repeated twice. The collective supernatant, three subsequent extractions of the same material were diluted with the above-mentioned ethanol/copper chloride solution mixture by factor 1.5. To this solution 0.625 g polyvinylpolypyrrolidone was added to

precipitate any phenols present. The precipitate was filtered off (Tian et al., 2010). Afterwards, 1 mL of the remaining solution was mixed with 1 ml of an aqueous solution containing 10 g/L DNS, 16 g/L sodium bicarbonate and 300 g/L potassium sodium tartrate. After shaking the mixture for 10 min at 100°C the solution was diluted with 5 ml distilled water. The reducing sugar content was determined spectrophotometrically (*Spekol 1300 – Analytik jena*) at 545 nm. For the calibration different concentrations of maltose monohydrate were used. The reducing sugar content determination is based on the reducing potential of maltose. However, glucose exhibits a reducing potential which is identical to maltose per molecule. Per weight the glucose reducing potential contribution is twice that of maltose. Consequently, it is difficult to determine the degree of starch degradation with certainty if it is not known whether glucose or maltose in generated as degradation product. This, because actually the concentration of the free aldehyde groups was determined. Converting the data gathered into mass fraction of decomposition products and finally starch degradation is thus a function of the type of the degradation products, in particular their molecular weight, themselves. To overcome this problem, it was postulated throughout this paper for data conversion that the degradation products are exclusively maltose. This results in a systematic error overestimating the starch degradation with increasing presence of glucose.

Again, the error bars represent results of a sevenfold repetition of the procedure outlined.

#### 5.2.3.4 Expansion index of extrudates

The sectional expansion index was determined based on the diameter of extrudates. The values were averaged over five individual extrudate strands. Each strand itself was measured three times at various locations. The expansion index is defined as the ratio of the circular crosssection area of the extrudates and the cross-section area of the die. The relative longitudinal expansion index was derived from the measurement of the length of extrudates produced in 5 s. The results provided here are averages of five individual determinations.

#### 5.2.3.5 Texture analysis of extrudates

The hardness of the extrudates was determined using a *Zwick testControl II* texture analyzer. A cylindrical probe, diameter 25 mm, was breaking and crunching a single extrudate strand. The constant speed of the probe was 15 mm/min and the peak force was recorded giving the hardness of the extrudate. Each data point represents the average of eight independent measurements.

#### 5.2.3.6 Extrudate pore size analysis

The pore size analysis of the extrudates was done using a scanning electron microcopy *SEM ZEISS Gemini SEM500.* Sample preparation included cutting the extrudates to create an axial cross section that was sputtered with a 100 nm gold layer. The size of the pores and wall thickness was determined using *Digital Imaging Processing System*.

#### 5.2.3.7 Sensory evaluation of selected samples

The sensory attributes of the different extrudates were evaluated by an untrained panel of 22 members. The participants tasted the four extrudates (native, sprouting time: 1, 3, and 7 days). In the following, they were asked to sort these samples in their order of darkness, hardness (assessed by manual breaking), sweetness, taste of maltiness, crispiness (mouthfeel), and overall preference (*ANNEX 1*). The procedure followed the ranking test according to DIN ISO 8587 (2010). The results are the ranking sums subjected to the Friedman test.

#### 5.2.3.8 Water solubility index of extrudates

To determine the water solubility index, 0.5 g dry matter was suspended in 50 ml distilled water. The dosing of the milled extrudates was corrected for dry matter content. The suspension was stirred for 30 min. It was filtered (S&S 595) using a Büchner funnel and the solid residue was weighed after removing the water from the filter cake by drying at 130°C for 1 hr. The water solubility index in percent is the difference of the initial dry matter weight and the weight of the residue. Determinations were done in duplicate.

#### 5.2.3.9 Cold paste viscosity of extrudate suspensions

The rheological characterization of the extrudates was performed by measuring aqueous suspensions of 10% (w/w) - corrected for dry matter content - of the milled extrudates. The viscosity of the paste emerging after 30 minutes of stirring was measured with an *Anton Paar* rheometer *MCR 302* using the starch cell - stirring rate 150 rpm and shear rate of 150 $s^{-1}$, temperature 20°C, according to Meuser, van Lengerich, and Reimers (1984). Data given are average paste viscosities from 60 data points taken per minute. Each experiment was performed at least in duplicate.

#### 5.2.3.10 Lab color of extrudates

The color analysis was done using the *CR-400*, *Konica Minolta* attached to cylindrical glass containers. The color characterization of the milled extrudates was done in triplicate. The color was expressed in lightness (L), redness (a), and yellowness (b) values.

#### 5.2.3.11 Vitamin C content of sprouted flour and extrudates

Vitamin C was chosen as a marker for general vitamin content because of its high reactivity as suggested by Lintschinger et al. (1997). The vitamin C content was determined according to the indophenol method (Nielsen, 2003). The indophenol solution was diluted 8 times. The solid material (milled extrudates or flour) was dispersed in a mixture of 30 g/L metaphosphoric acid and 8% (v/v) acetic acid and centrifuged at 3000g. A volumetric sample of the resulting supernatant was diluted and used for titration according to the method. The procedure was executed in duplicate per specimen.

#### 5.2.3.12 Statistical evaluation (ANOVA)

The statistical evaluation of all properties studied was performed using Microsoft Excel 2016. The p-value (probability of error) was calculated by analyzing the experimental data by means of ANOVA (analysis of variance). A level of significance (α) of .05 was chosen.

## 5.3 Results & Discussion

### 5.3.1 Statistical evaluation of experimental data

Table 14 presents the p-values of the properties considered in this study which were calculated as part of the ANOVA. The analysis reveals, that the calculated p-value is lower than the level of significance, indicating that stated differences are significant.

*Table 14 p-values from statistical analysis (ANOVA) of studied properties of sprouted wheat flour and extrudates containing sprouted wheat flour*

| | **Data from flour** | | **Data from extrudates** | |
|---|---|---|---|---|
| **Impact** | **Dependent variable** | **p-value** | **Dependent variable** | **p-value** |
| Sprouting time | Vitamin C content | 6.11E-10 | Vitamin C content | 1.76E-08 |
| | Peak viscosity | 4.41E-06 | Peak force | 6.77E-38 |
| | Reducing sugar content | 1.41E-17 | Reducing sugar content | 1.18E-17 |
| | | | Water solubility index | 4.68E-08 |
| | | | Cold paste viscosity | 1.52E-08 |
| | | | L color | 7.27E-11 |
| | | | a color | 1.47E-07 |

| | | |
|---|---|---|
| | b color | 2.13E-15 |

### 5.3.2 Characterization of the sprouting process

In Figure 29, the effect of the sprouting time on the percentage of the different degrees of sprouting and the average degree of sprouting (Krapf et al., 2019) are shown. The average degree of sprouting increased with longer sprouting times indicating radicles and coleoptile growth over time. The increased average degree of sprouting with longer sprouting times was also reported for oat (Krapf et al., 2019). 43% of the grains started to sprout after the steeping period was completed, which was indicated by visible embryos. After the longest sprouting period (9 days), 80% of the grains had a coleoptile which was at least twice as long as the grain itself. The data further revealed that the degree of sprouting evolves rather systematically over time. However, it must be noted that a wide spread of different degrees of sprouting at similar sized proportions was observed in some samples. For instance, after 3 days of sprouting the distribution over different degrees of sprouting was most spread as four categories were found at almost similar proportions (DoS 2: 16%, DoS 3: 24%, DoS 4: 20%, DoS 5: 30%).

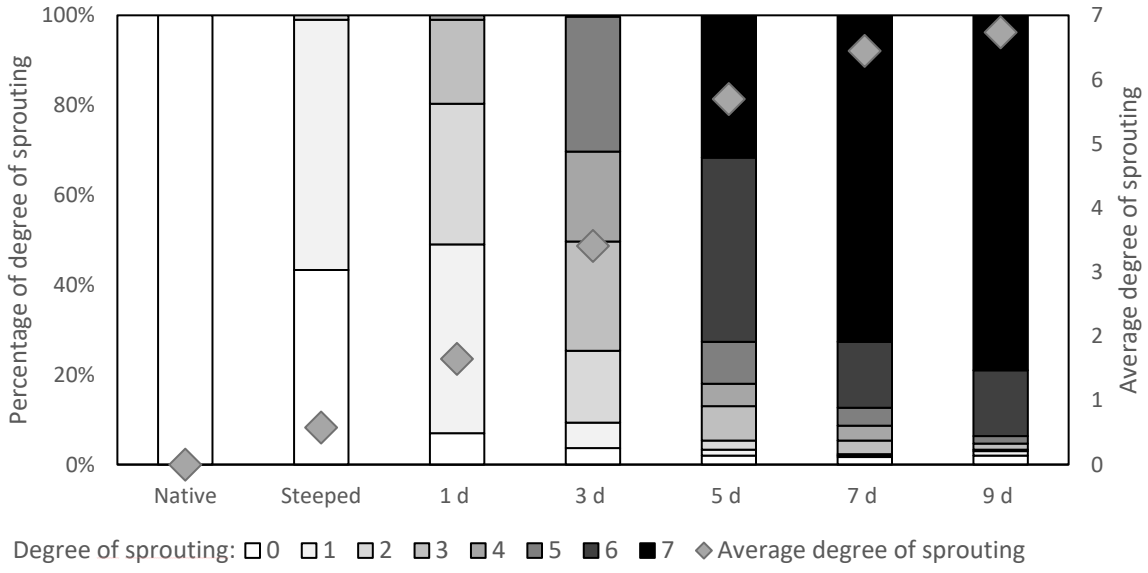


*Figure 29 Effect of different sprouting durations on the proportions of the grains from different degrees of sprouting and their average degree of sprouting, indicating the visible length of the coleoptile and radicles.*

### 5.3.3 Rheological behavior of flour suspensions

The sprouting process mainly affects the starch in the grains. As already pointed out, the changes in the reducing sugar contents in the flours and extrudates are used as indicator of the starch degradation. However, the presence and nature of the starch is also clearly manifested in the rheological behavior of flour and extrudate suspensions. This is of importance because the rheological behavior of the flour suspension directly corresponds to the processability of materials in the extruder and the expansion properties of the extrudates (Moraru & Kokini, 2003). In Figure 30, the reducing sugar contents are illustrated as a function of the average degree of sprouting for both, flours and extrudates. The lines are drawn to guide the eye and do not express any model beyond simple mathematical functions. Longer sprouting times and thereby higher degrees of sprouting correspond systematically with the observed increases in reducing sugar content. For the flour and extrudates relating to 9 days sprouting, a 20 times higher quantity of reducing sugars compared to the reference was found. During the sprouting process starch is degraded to reducing sugars by enzyme action and transported into the growing regions of the grain (Bewley, 2001). It was found that the extrudates have a lower reducing sugar content than the respective flour. This corresponds well with the data concerning color formation due to extrusion expressed as decreased L-values (Figure 35). Maillard reactions involving reducing sugars and amino acids taking place during the extrusion process consuming some reducing sugars. It is known that the resulting Maillard reaction products do not have reducing potential anymore (Hodge, 1953). Any interpretation of the data must consider that the calibration to determine the reducing sugar content is exclusively based on maltose, and is hence prone to overestimate the starch degradation.

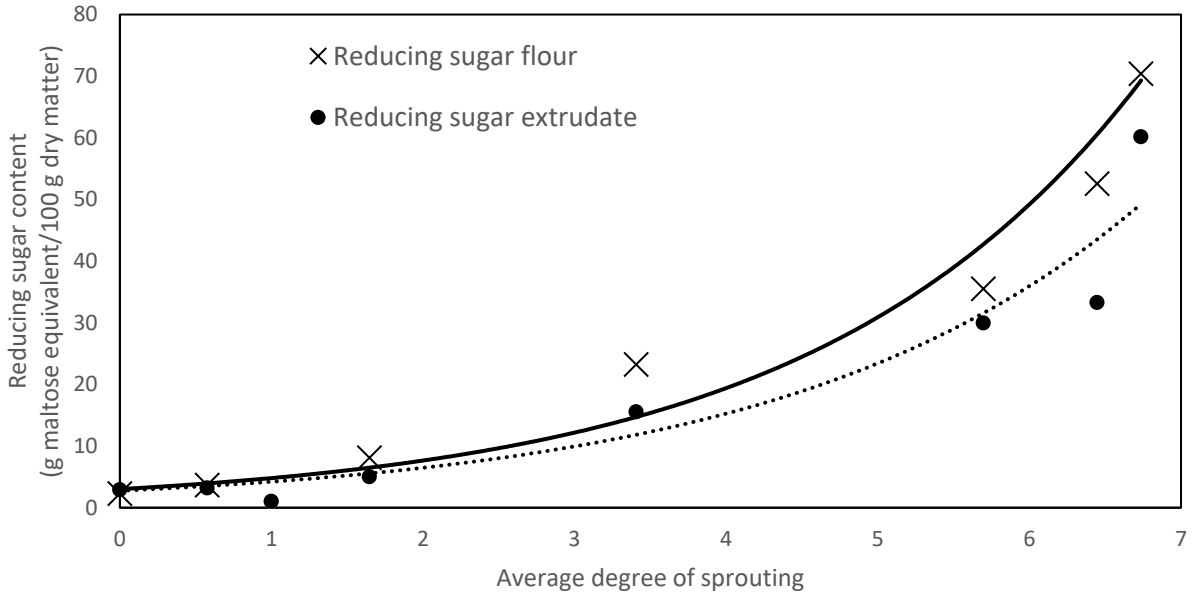


*Figure 30 Correlation of average degree of sprouting of differently sprouted wheat grains and reducing sugar content in sprouted wheat flours and corresponding extrudates.*

Measurements of the rheological behavior of flour suspensions reveal that the sprouting conditions affect the rheological behavior systematically (Figure 31). It was found that increased sprouting times and hence increased degrees of sprouting correspond to reduced peak viscosities. The data suggest a clear relationship, which is not surprising because increased sprouting times imply progressive starch degradation into short-chain sugars. These contribute much less to increased viscosities of solutions and suspensions. In this context, one could be tempted to correlate the viscosity of the suspension to the actual starch content. This is, however, beyond the scope of this work because the determination of reducing sugar levels as maltose equivalents does not allow to determine the remaining starch levels by mass balances.

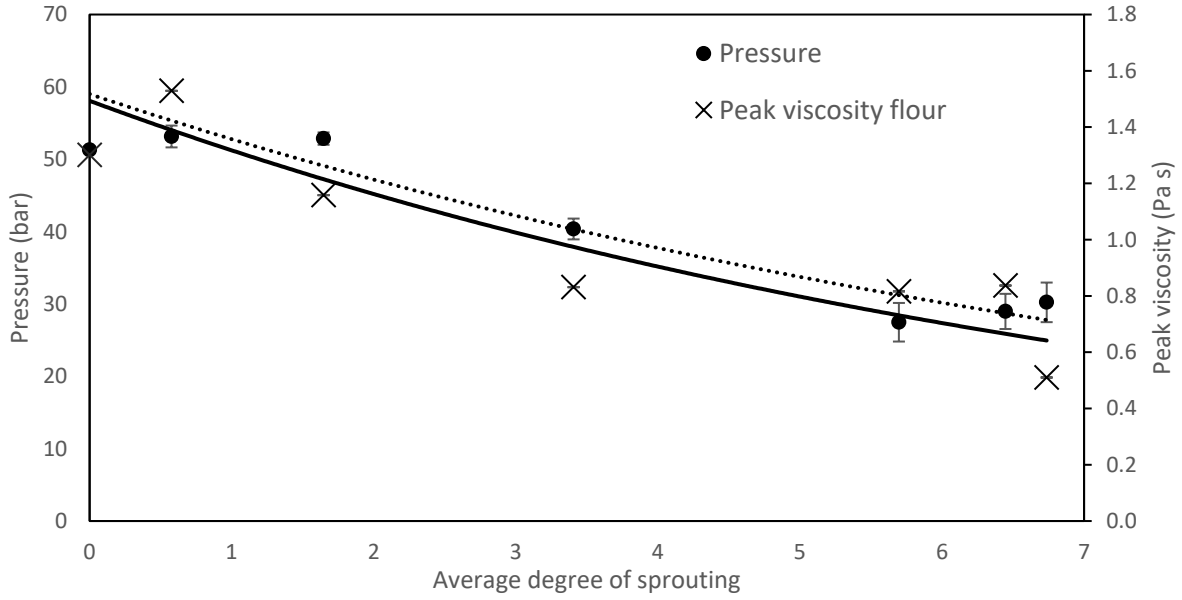


*Figure 31 Correlation of average degree of sprouting of differently sprouted wheat grains and peak viscosity of corresponding flour suspensions and die pressure measured while extruding these differently sprouted wheat flours.*

### 5.3.4 Pressure in the extruder die

Next to the data of the development of viscosity with the average degree of sprouting, Figure 31 also displays the respective pressure difference over the extruder die when processing the different samples. One can see that the back pressure generated by the extruder die is decreased with increasing average degree of sprouting and hence longer sprouting times. The increased degree of sprouting corresponded with a drop in peak viscosity as well.

Starch polymers are the most important viscosity-building and structure-forming components in directly expanded products. They are responsible for generating viscosity in the melt and allow forming stable cell walls of gas bubbles in cooled-down extruded products. It is established that starches have to have a minimum molecular weight in order to cause high enough die back pressures at moisture levels typically used in extrusion (Guy, 2001). The starch polymers are reduced during sprouting and low molecular mass sugars are generated as demonstrated earlier with the increase in reducing sugar levels. The results found corroborate this because the use of sprouted material, accompanied by depolymerization of starch, resulted under standardized extrusion conditions in lower die back pressures. The reduction of more than 20 bars in back pressure is significant (see Table 14). In line with Guy (2001), the reduced pressure over the die had a substantial impact on puffing and structure-forming properties. Also, Barrett, Kaletunç, Rosenburg, and Breslauer (1995) showed in

their study that the die pressure in the extruder decreased due to the presence of disaccharides in the extruded mass. The explanation offered is that the interaction between the starch molecules is suppressed due to the presence of sugars. This is in line with the findings presented here, where reduced melt viscosity due to an increase of sugars, is accompanied by the reduced die pressures. Understanding these relationships is of prime importance for the design of directly expanded products using sprouted grains.

### 5.3.5 Extrudate properties

***Expansion and structure***

The starch degradation during sprouting affected not only the rheological properties of the feedstock of the extrusion process and consequently the die pressure (Figure 31). Varying the sprouted wheat flour used also caused changes in the properties of the extrudates. Figure 32 illustrates that product properties correlated to peak viscosity of the feedstock of extrusion. In detail the sectional expansion index corresponds very well with the peak viscosity ($R^2$= .88). Alternatively correlating the expansion index to the degree of sprouting yields an equally good fit (data not shown). The quality of the different correlations illustrates that degree of sprouting, degradation of polymers, changes of viscosity, and expansion properties are interrelated. The findings presented are in line with those by Singkhornart et al. (2014) who also stated that the application of sprouted wheat corresponds to reduced expansion indexes of extrudates. Additional to the sectional expansion index also the longitudinal expansion was investigated. It was found that the length of extrudate strands produced in 5 s varied from 0.46 to 0.74 m using flour from native wheat and material sprouted for up to 7 days (data not shown). Also, for the data on relative longitudinal expansion of the extrudates a systematic relation to the peak viscosities and degrees of sprouting was found.

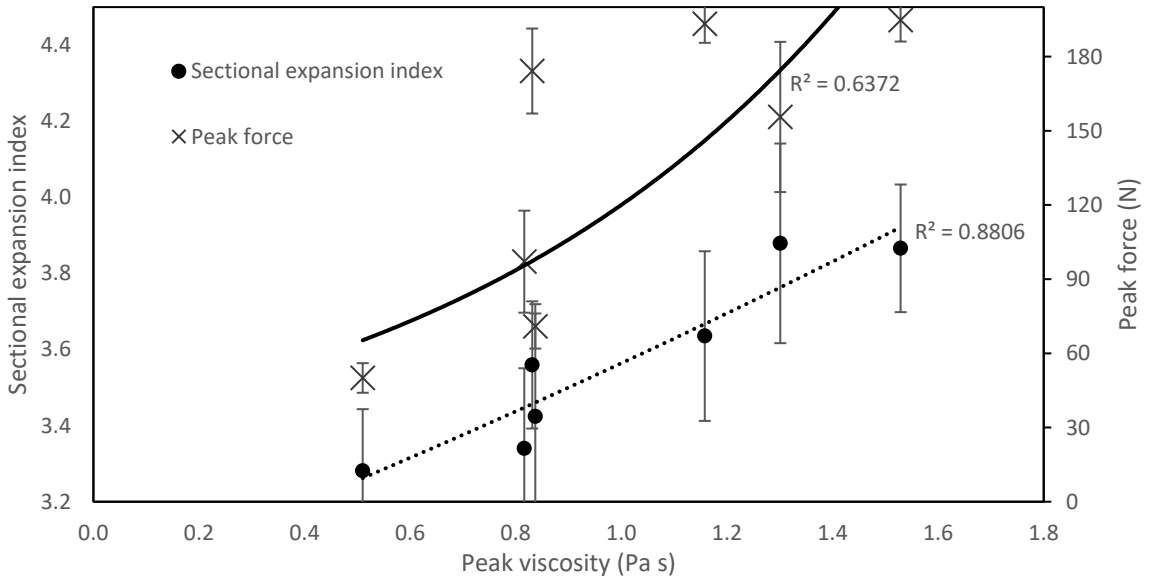


*Figure 32 Correlation of peak viscosity of differently sprouted wheat flour suspensions to expansion index of corresponding extrudates and peak force needed to break extrudates containing flour from sprouted wheat.*

The strong correlation found for sectional expansion is not surprising because it is generally acknowledged that the extrudate expansion is a result of several steps involving the so-called die swell caused by order-disorder transformation, the nucleation of steam bubbles, the extrudate swell, the bubble growth, and bubble collapse (Moraru & Kokini, 2003). The expansion index resulting from the interplay of these processes is also subject to the kinetics of the glass transition temperature that accounts for the fixation of the extrudate matrix (Moraru & Kokini, 2003). As shown, due to the starch degradation during sprouting into short-chain sugars the viscosity of the dough was reduced and consequently the internal die pressure was lower (Figure 31). Hence, more shrinkage and collapse of the bubble cell walls after the initial expansion occurred prior to fixation of the matrix resulting in a decreased sectional expansion index of the final extrudates. Furthermore, it is known that sugars, which are generated during sprouting, have a plastifying effect on the dough, and also lower the glass transition temperature, melting viscosity and elasticity of the melting mass (Fan, Mitchell, & Blanshard, 1996; Launay & Lisch, 1983). In addition, the type and content of the different sugars have an effect on the expansion, shrinkage and specific length of the extrudates. Fan et al. (1996) found that maltose has a greater expansion, lower specific length and lower rate of shrinkage compared to glucose. Maltose and glucose are the reducing sugars primarily generated during the sprouting process (Suhasini, Muralikrishna, & Malleshi, 1997).

There are some interesting questions in this context which are beyond the scope of the work reported here, but should be subject to future research. Relevant subjects are, for example, additional starch degradation during extrusion, degree of starch gelatinization, energy dissipation in the extruder and the die as function of viscosity, and the specific role of the different sugars.

Figure 32 further reveals that the peak force to break the extrudates also correlates reasonably well with the peak viscosity of the flour suspensions/solutions and is reduced with lower viscosity. Other publications link crispiness and acoustics of expanded cereals to the microstructure of the extrudates, in particular pore size and wall strength, which again is a result of several overlapping complex processes (Chanvrier, Jakubczyk, Gondek, & Gumy, 2014).

It was found that after extended sprouting times, 5 days or longer corresponding to average degree of sprouting of 5.5 or higher, the force needed to penetrate the sample decreases. Hence, extrudates from sprouted material were softer, as already pointed out by Singkhornart et al. (2014).

In Figure 33, the SEM micrographs of the different extrudates are shown to illustrate the microstructure. The images show and image analysis confirms that extrudates based on increasingly sprouted material were characterized by regular pores of larger sizes, 1.7 mm, compared to extrudates made with native wheat flour which had more irregular structures and pore sizes below 1.0 mm. The differences in regularity of the cavities were accompanied by differences in the wall thickness. The pore wall thickness of the extrudates made with material with progressing degrees of sprouting was less than 100 µm compared to those with native and shortly sprouted wheat which were around 150-200 µm (data not shown).

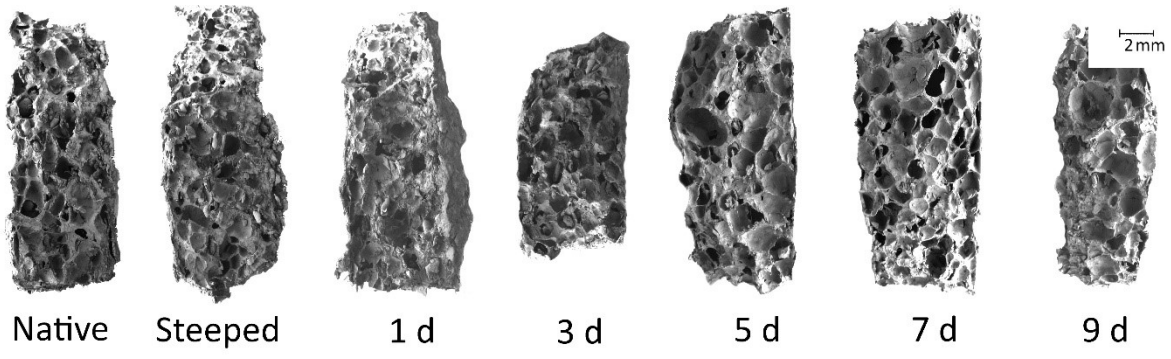


*Figure 33 SEM pictures of sliced wheat extrudates containing flour from wheat grains which sprouted for different times.*

These differences in wall thickness were most pronounced in the outer layer of the extrudates. The thickness varied from 55-120 µm for usage of highly sprouted material to 260-1,100 µm in case of samples with native wheat flour (data not shown in detail). For comprehensive interpretation of these observations it is important to know that the density of the extrudates is decreasing with increased degree of sprouting of the feedstock. Simple combination of the sectional and relative longitudinal expansion reveals that the density of extrudates based on native wheat is about 20% higher than that of extrudes based on highly sprouted material. This corresponds well to the conclusions from the data on peak force given in Figure 32, namely softer products emerging from material with increased degrees of sprouting. The results in this study imply that the generated microstructures were directly related to the peak viscosity of flour suspensions implying that less viscous material is easier expandable. It is known that the most abundant polymer amylopectin in starch has a high molecular weight which contributes to high viscosity and elasticity of the pore cell walls of the extruded dough and consequently poor expansion (Guy, 2001). It seems that starch polymer degradation, which was indicated by the increase in the reducing sugar content in this study, as a result of sprouting could improve the puffing properties and allow for the production of lighter and crispier products. However, given the complexity of the pore formation processes in extruded directly expanded products this observation must be verified, and more research is needed to fully understand the effect of sprouting on expansion.

***Properties relevant for application***

To further evaluate the changes of the properties of the extrudates made with sprouted raw materials, additional characterization was performed. Both, water solubility index and cold paste viscosity of the extrudates are properties that relate to the behavior of cereals in contact with fluids, hence the final application. Figure 34 shows the changes in these properties as a function of the degree of sprouting. The level of dissolvable material in flour and milled extrudates is defined by the molecular compositions. For material that has been sprouted for longer times and hence has a higher average degree of sprouting as well as a higher level of short-chain sugars, the water solubility of the milled extrudates is increased.

The data reveals that the increases were relatively small compared to the expected changes due to starch degradation (see Figure 30*)*. In this context, it must be considered that during the actual

extrusion process starch degradation also generates smaller, more soluble molecules (Guy, 2001; Meuser, Lengerich, & Köhler, 1982).

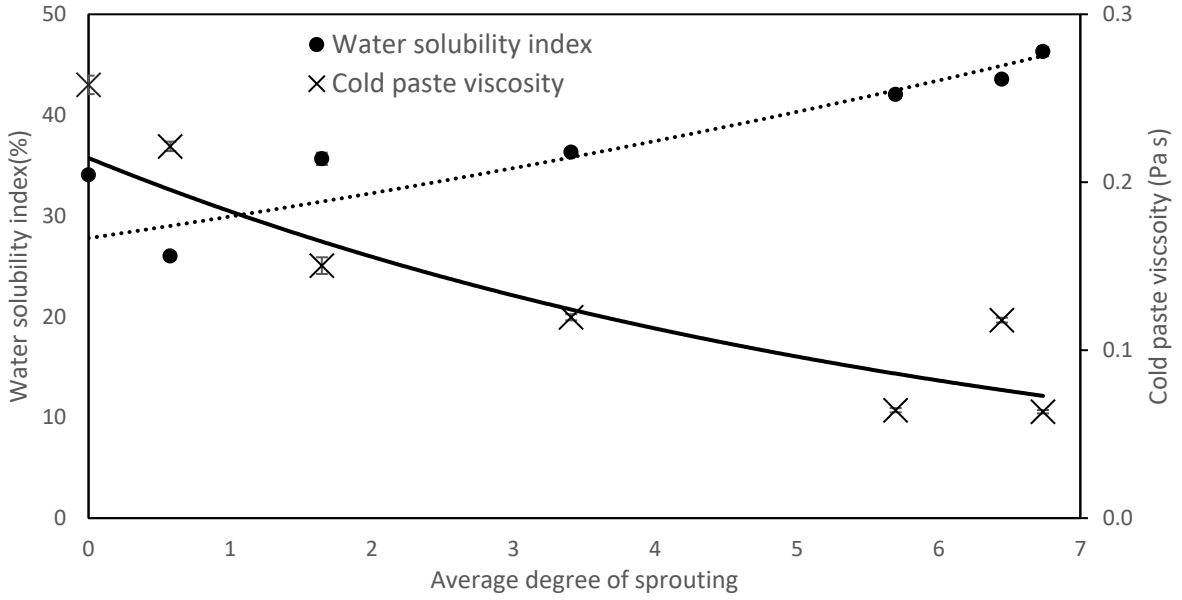


*Figure 34 Correlation of average degree of sprouting of differently sprouted wheat grains and water solubility index and cold paste viscosity of corresponding milled extrudates containing flour from sprouted wheat.*

This process, however, is related to primarily debranching of amylopectin (Brümmer, Meuser, van Lengerich, & Niemann, 2002) and does thus not yield increased levels of reducing sugars, see also Figure 30. The data generated for the water solubility index of the different flours give an indication of the extend of this effect. The differences in the water solubility index between flours and respective extrudates were found to be approximately 12% (w/w) for the native grains and about 6% (w/w) for highly sprouted materials.

After extrusion, the solubility of the highly sprouted material changes only marginally because already after sprouting, a large portion of the starch was converted into cold-soluble material. The data presented by Flamme, Kurpjun, Sedding, Jansen, and Jürgens (2003) also indicate that after a sprouting period of 7 days a substantial fraction of the starch has been degraded. The starch content according to this research dropped from 75% to 25% during sprouting at 25°C for 6 days. These findings are in line with the results given in Figure 30 on the increase of the reducing sugars content.

Figure 34 also shows the evolution of the cold paste viscosity of suspensions containing milled extrudates. For extrudates containing flour from increasingly sprouted wheat, the cold paste viscosity is decreased.

The cold paste viscosity is interpreted as an indication for the changes in the chemical bonds in the starch molecules (Meuser et al., 1982). The lower molecular weight of the starch molecules mainly contributed to the decreased cold paste viscosity. Again, these measurements confirmed that during the sprouting process the molecular weight of the starch decreased. The data on cold paste viscosity as much as the other data further suggest that already during the steeping process, which takes in total 28 hr, a change in the material occurred. Further research is necessary to identify in detail what type of molecular changes occurs during this process.

As pointed out above, the presence of reducing sugars and proteins allows for Maillard reactions to take place during extrusion. Color can additionally be generated by caramelization (Rausch, 2009). How the color of the extrudates changes systematically with increasing degree of sprouting is depicted in Figure 35. Colors of the milled extrudates are expressed as L, a, b values representing lightness, yellowness, and redness. Longer sprouting times correspond to darker, more yellowish and more reddish extrudates.

Similar results were reported by Singkhornart et al. (2014). This study found an increase in the redness and yellowness when extruding flour from sprouted material at 130°C.

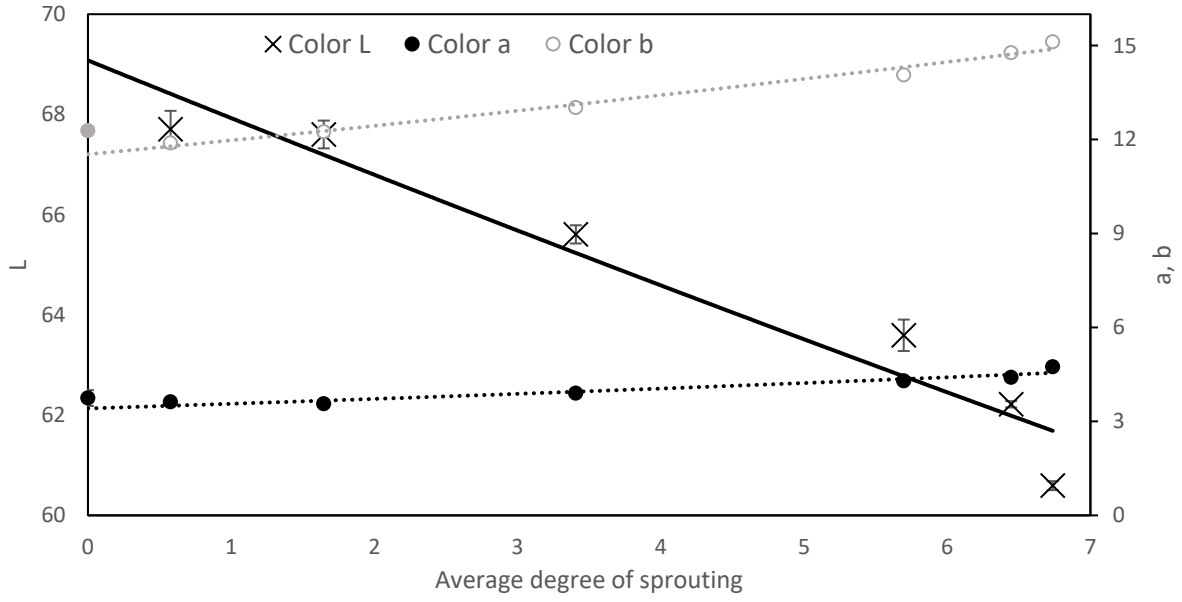


*Figure 35 Correlation of average degree of sprouting of differently sprouted wheat grains and Lab color of corresponding extrudates containing flour from sprouted wheat.*

### 5.3.6 Sensory evaluation of selected extruded samples

The results of the sensory evaluation by an untrained panel are presented in Table 15. Significant differences between the extruded samples containing flour from non-sprouted and sprouted wheat (duration 1, 3, 7 days) were found with regard to color, texture, maltiness, crispiness and preference. The extrudates containing long time sprouted wheat (7 days) were the darkest. Moreover, the extrudates containing flour from grains, which were sprouted for longer periods, were evaluated to be easier to break. This outcome is surprising considering the data on peak force of extrudates (Figure 32) which indicated clear differences between differently sprouted materials. Extrudates produced from material sprouted for longer periods (3 and 7 days) were evaluated to be sweeter and maltier. The increase in the sweetness is in accordance with the enhanced levels of reducing sugars (Figure 30). The extrudates from flour based on material sprouted for 7 days were found to have the highest crispiness. Finally, the panelists favored the extrudates from sprouted material over those from the native reference material. The data gathered indicate how product properties change with inclusion of sprouted grains. However, it remains open how this result should be used in product development when different inclusion levels of sprouted grains are considered.

*Table 15 Ranking sums of sensory test - testing the color, texture, sweetness, maltiness, crispiness and preferred sample of the extrudates containing flour which was sprouted for 0, 1, 3 and 7 days; highest rank sum indicates darkest, hardest, sweetest, maltiest, crispiest and least favorite extrudate sample; Means not sharing a same letter are significantly different $P \leq 0.05$*

| **Property** | **Native** | **1 d** | **3 d** | **7 d** | **Significant differences (α=0,01) Friedman test** |
|---|---|---|---|---|---|
| **Color** | $34^{a}$ | $45^{ab}$ | $53^{b}$ | $88^{c}$ | ✓ |
| **Force to break** | $75^{a}$ | $68^{a}$ | $36^{b}$ | $41^{b}$ | ✓ |
| **Taste: Sweetness** | 42 | 59 | 54 | 65 | - |
| **Taste: Maltiness** | $45^{a}$ | $40^{a}$ | $62^{b}$ | $73^{b}$ | ✓ |
| **Crispiness** | $44^{a}$ | $42^{a}$ | $60^{ab}$ | $74^{b}$ | ✓ |
| **Least Favorite Sample** | $66^{a}$ | $66^{a}$ | $42^{b}$ | $46^{b}$ | ✓ |

### 5.3.7 Nutritional value of extrudates containing sprouted flour

As pointed out in the introduction increased consumption of sprouted material is in part motivated by a known improved nutritional profile of sprouted grains (Harmuth-Hoene et al., 1987). To address this, the vitamin C content evolution during the sprouting process was monitored in this study. In Figure 36 the vitamin C content of the different flours and extrudates is illustrated. The vitamin C content increased significantly with increasing degree of sprouting (Table 14). The vitamin C level on dry matter basis increased up to a factor 10 from native grains to 9 days sprouted grains. It deserves to be pointed out, that this increase was certainly not a result of the reduction of the grain mass due to sprouting.

Some data points suggest a reduction of the vitamin C content of up to 15% due to the extrusion process. Regarding the entire data set, however, only small losses of vitamin C during the extrusion process were found.

Considering vitamin C as a marker for the generation of nutrients the data show that significant increases (Table 14) in the vitamin content accompany the sprouting process rendering sprouted grains a means to improve the nutritional profile of extruded products.

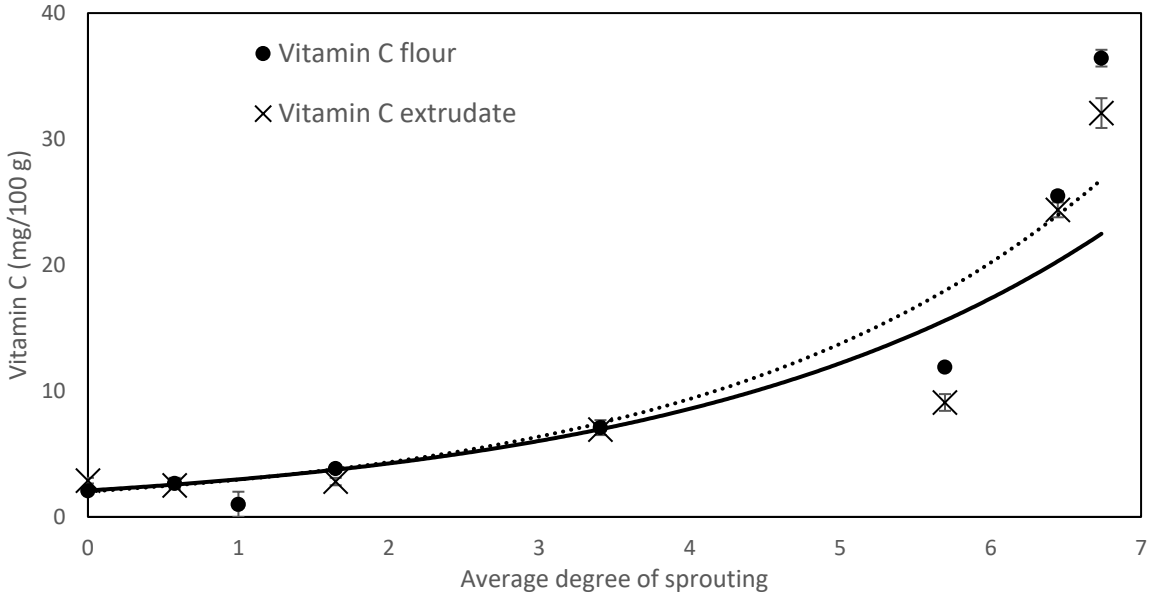


*Figure 36 Correlation of average degree of sprouting of differently sprouted wheat grains and vitamin C content in corresponding flour and extrudates containing sprouted wheat flour.*

## 5.4 Conclusion

The results of the investigation of different sprouting times of wheat on the characteristics of resulting wheat flours and extrudates produced show a remarkable system and provided new insights. It was found that during sprouting for up to 9 days at 20°C the starch was substantially degraded so that high levels of sugars were generated. This confirms data given elsewhere. The level of degradation corresponds well to the characteristics of the flour, such as water solubility and viscosity, which are increased and decreased, respectively. To evaluate changes in nutritional profile the vitamin C levels in the flours were monitored and found to increase 10-fold on 9 days of sprouting. More surprisingly, it was found that the formed vitamin C was fairly stable during extrusion under the chosen process conditions. With increasing starch degradation due to the sprouting process the properties of the flour and resulting paste translated into reduced extrusion pressures. The different viscosities of the pastes, possibly also further influenced by starch degradation due to the extrusion process and related shear, were found to influence the expansion of the different extrudates. In detail, it was found that the density of the extrudates made from sprouted material is reduced. This is, however, not due to increased sectional expansion, but due to an increased longitudinal expansion. The different observations regarding density, microstructure, and peak force of the extrudates appear to be consistent. Sensory assessment of the extrudates by an untrained panel had as primary outcome that extrudates made from sprouted material were clearly preferred. The evolution of changes of properties was correlated with the degree of sprouting defined elsewhere and proved to be a useful characterization of the progress of the sprouting process. To what extent this characterization is suited to generally correlate flour characteristics independent of the sprouting conditions used to achieve a certain degree of sprouting, that is, the development stage of the grain, remains subject to further studies. Some of the observations made can tentatively be linked to molecular changes due to the sprouting or to the extrusion process. But this could not be verified in detail within the scope of this study. Ongoing work is focusing on the elucidation of these underlying molecular changes.

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# 6 EFFECT OF SPROUTING TEMPERATURE ON SELECTED PROPERTIES OF WHEAT FLOUR AND DIRECT EXPANDED EXTRUDATES

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The following chapter is a post-print version.

## Abstract

The effect of the sprouting temperature on wheat sprouting and on the properties of expanded extrudates produced from sprouted wheat flour was studied. Therefore, wheat was sprouted at five different temperatures and subsequently dried, milled and the resulting flour was used to produce extrudates using a twin-screw extruder. In order to understand the sprouting temperature effect, the degree of sprouting (DoS) of the differently sprouted samples and characteristic properties of flour and extrudates were studied and compared.

During sprouting of wheat with increasing temperature and time an increase of the α-amylase activity, the vitamin C and reducing sugar content, and a decrease of the peak viscosity was observed. The greatest effect was found at 20°C.

Furthermore, the lowering of the viscosity of the flour suspension results in a reduction of the pressure and temperature in the extruder die. The extrudates of sprouted wheat flour were found to be easier to break, had a lower density, an increased longitudinal expansion index and an improved cold-water solubility.

A good correlation between the DoS and other properties of flour and extrudates was found, indicating a good predictive power and applicability of the DoS concept for wheat samples and their product development and specification.


**Keywords:** Sprouting, wheat, sprouting temperature, extrusion

## Practical Application

The use of sprouted wheat flour for the production of extruded, direct expanded breakfast cereals is a promising opportunity to alter extrudate properties.

Thereby, the sprouting temperature can be used as a means affecting the sprouted grain and extrudate properties intentionally and developing products being crunchier, and having an improved cold-water solubility, a lower density and a changed expansion behavior. Moreover, due to an increased amount of reducing sugars in sprouted flour, which is a result of an intense starch degradation during sprouting, less additional sugar is needed to produce sweet breakfast extrudates.

## 6.1 Introduction

Sprouting processes have been widely used in order to improve the nutritional quality and flavor of food products as well as to optimize the food production process. Sprouted grains have already been used for years in the malt production because of its boosted enzyme activity, which is crucial to produce beer. Especially barley is steeped and sprouted for around 6 days at 14°C and subsequently kiln-dried (Jacob, 2016).

During the sprouting process α-amylase is one of the most important enzymes because of its starch degrading activity. Thereby, α-amylase hydrolyses the α-1,4-glycosidic bonds degrading starch into dextrins, maltose and glucose. The metabolites except glucose are further degraded by β-amylase and limit dextrinase to glucose (Bewley, 2001).

According to various studies sprouted grains have an increased amount of essential amino acids (van Hung, Maeda, Yamamoto, & Morita, 2012), more vitamins (Harmuth-Hoene, Bognar, Kornemann, & Diehl, 1987) and free phenolics (van Hung et al., 2012), an increased bio-accessibility of minerals (Platel, Eipeson, & Srinivasan, 2010) and a decreased level of phytic acids (Harmuth-Hoene et al., 1987).

Because of these advantages sprouted grains could be a means to produce superior extruded breakfast cereals. Usually breakfast cereals are produced on the basis of grain flours, water, sugar and additives like cacao or baking agents. Typical properties of extruded, direct expanded breakfast cereals are their puffed and crunchy texture, a low density and a long stability in milk or water, which has been correlated to a low cold-water solubility index.

Sprouting occurs at a moisture content of at least 30%, at adequate temperatures in the range of 10 - 30°C and under aerobic conditions. Thus, the biological processes in the grain during sprouting can be controlled by varying these conditions (Narziß & Back, 2012).

An adequate sprouting temperature is discussed to improve the nutritional value of the grains and to increase the enzyme activity, and, therefore, also the starch degradation. But sprouting will also increase respiration losses. Temperature and time are two of the main factors also determining the growth of microorganisms. For example, microorganism growth is doubled at a temperature increase of 10 K until the maximum temperature is reached at which microorganisms can survive. Dziki, Gawlik-Dziki, Kordowska-Wiater, and Doman-Pytka (2015) showed that the microorganism level was within

an acceptable limit by sprouting processes lasting up to 4 days at 15 or 20°C. At higher temperatures or longer sprouting times the aerobic bacteria and mold growth were enhanced to unacceptable levels. Molds were found after 4 days when sprouting took place at 25°C and after 8 days when samples were sprouted at 15°C.

Finding an optimal sprouting temperature in regards to different objectives is of great importance and was part of various research studies. The alteration of the typical sprouting temperature of 14°C in the barley malt production can result in a faster sprouting process accompanying with lower production costs. For example, Müller and Methner (2015) developed an optimized barley malting process. By increasing the sprouting temperature from 16 to 24°C a reduction of the sprouting time by 48 hr (24 hr per 4°C) could be established. In addition, the increase of the sprouting temperature also results in a reduction of the energy consumption because of a lower cooling demand (Müller, 2015).

In earlier work, the degree of sprouting (DoS) was introduced and verified to be a simple characterization of the sprouting progress (Krapf, Kandzia, Brühan, Walther, & Flöter, 2019). A fast, visual evaluation of the sprouted grains allows the classification into different degrees of sprouting and the subsequent prediction of sprouted grain and extrudate properties such as vitamin C and reducing sugar contents as well as hardness and expansion of extrudates produced from these sprouted grains. Thereby, the applicability of the DoS was already shown for oat grains (Krapf, Kandzia et al., 2019). Moreover, the influence of sprouting time on the properties of extrudates produced from flour of sprouted wheat grains was studied by sprouting wheat for different sprouting durations at 20°C. These sprouted wheat grains were milled and the resulting flours were used for the production of extrudates. Studying the properties of the extrudates allowed their correlation with the DoS (Krapf, Arysanto, Walther, & Flöter, 2019).

In this work, the previous study was further extended and continued. Thereby the aim of this work was to study the variation of the sprouting temperature and its effect on flour and extrudate properties. The so-called DoS was evaluated for various sprouting temperatures and the results were analyzed for the prediction of the properties of extrudates from sprouted wheat grains. Therefore, wheat was sprouted at five different temperatures up to 3 days and the DoS of the samples was determined. Additionally, the DoS was correlated with other characteristic grain properties such as α-amylase activity, peak viscosity of the flour suspension, reducing sugar and ascorbic acid (vitamin C)

content. Finally, flours from the differently sprouted grains were used to produce extrudates. The effect of the sprouting temperature on the extrudate properties and the relation between DoS and extrudate properties was studied. Extrudate properties considered in this study are expansion, density, hardness and the cold-water solubility index. Additionally, the effect of the sprouting time that was studied previously (Krapf, Arysanto et al., 2019) and the temperature effect on extrudate properties were compared to be presented in a more comprehensive way.

## 6.2 Material & methods

The soft wheat variety *Runal w*as used throughout the study (59.3% total starch, 14.1% protein, 13.4% fiber). The grains were stored in sealable containers at around 10°C prior to the study.

### 6.2.1 Sprouting process

For the first part of the study investigating the temperature and time effects on wheat sprouting, 500 g of wheat grains were washed for 30 min under running tap water to clean the grain surface from microorganism and to avoid their later growth. Then, the grains were steeped in a closed container and covered with water: 4.5 hr wet steeping, 19 hr dry rest and 4 hr wet steeping, all at 20°C. This steeping time was determined in preliminary tests and is in accordance with the recommendations of the Central European Commission for Brewing Analysis (MEBAK) (Jacob, 2016). After the steeping step the grains were dripped and put on a metal sheet which was part of the sprouting step. The steeped grains were covered with cling film to prevent the loss of water and were put in a climate cabinet (*Lovibond 220 P-02*) in the dark. During this sprouting step, the grains were washed once a day using a sieve to clean the grains and prevent drying. This experimental procedure was performed at five different temperatures (10, 14, 20, 25, and 30°C) for three different periods (1, 2, and 3 days). A temperature of 14°C is usually used in the malting step for brewery purposes (Jacob, 2016). At the end of the different sprouting periods, the grains were directly deep-frozen and subsequently freeze-dried (*Beta 1-16 – Christ*) at -40°C and 0.05 mbar for around 60 hr until a final moisture content in the range of 4 - 8% (w/w) was reached. Before analysis the wheat was ground in a speed rotor mill (*Pulverisette 14 – Fritsch*) with a sieve ring of 0.5 mm at 8000 rpm.

For the extrusion experiments a large amount (3 kg) of sprouted grains was needed. For this purpose, the wheat grains were again sprouted at five different temperatures (10, 14, 20, 25, and 30°C) for 3 days. For these samples, the steeping and sprouting process was conducted in a self-constructed sprouting box using the climate cabinet of *Lovibond 220 P-02* which was aerated permanently with

conditioned air, humidified at a constant temperature of 20°C. The same steeping regime as explained above was applied. After the sprouting process of 3 days the grains were dried in the malting plant *A1-2008* from *Seeger* at 65°C until a final dry matter content of 88-90% (w/w) was reached. This level of drying was chosen such that microbiological stability and millability were ensured. The parameters applied were determined in preliminary tests. The dried, cooled sprouted grains were milled using a hammer mill (*Siemens-Schuckertwerke AG*) with a mesh size of 500 µm.

### 6.2.2 Extrusion process

Extruded products were manufactured utilizing a *Berstorff ZE 25* extruder with a screw length of 870 mm at standard settings (screw speed 200 rpm, throughput 6 kg/hr, die hole diameter 3 mm). Flour with a known moisture content was mixed with tap water to achieve a water content of 27%. These settings resulted in a maximum SEI in preliminary tests. Mixtures of sprouted flour and water were used exclusively as feedstock in order to emphasize on the differences between the different sprouted wheat flours.

The chosen temperature profile over the different barrel elements of the extruder was 20 – 45 – 65 – 85 – 120 – 140 – 140 – 140°C. The screw was assembled of standard forward elements with two different pitches. Once the extrusion process was stabilized the pressure and temperature in the die were experimentally determined 10 times per second. The mean pressure and die temperature per setting were calculated from datasets of at least 1,000 individual data points.

The produced extruded strands were cut into pieces of about 0.5 m length after leaving the die. They were dried at 65°C for 1.5 hr in an oven (*Thermo Scientific T 6420*). The expansion index, the texture measurement and the density were determined directly on the extrudates. The cold-water solubility index, the total starch and the sugar content were determined using milled extrudates. Extrudates were milled by exposing them first for 30 s to a kitchen blender (*Petra MZ12.35*) before milling the coarse flour in a speed rotor mill (*Pulverisette 14, Fritsch*).

### 6.2.3 Analytical methods

#### 6.2.3.1 Degree of sprouting

There are eight degrees of sprouting (see Figure 37) which were determined by visually classifying the length of the coleoptile and radicles of 300 kernels. During sprouting every day, the average DoS was calculated as the sum of relative occurrence of the different classes (i):

**Average DoS=** $\sum_{i=0}^{7} \boldsymbol{i \cdot occurrunce}\ \%\ (\boldsymbol{DoS_i})$ **(Equation 4)**

This was derived based on a set of 300 kernels. Each kernel is evaluated for its individual DoS.

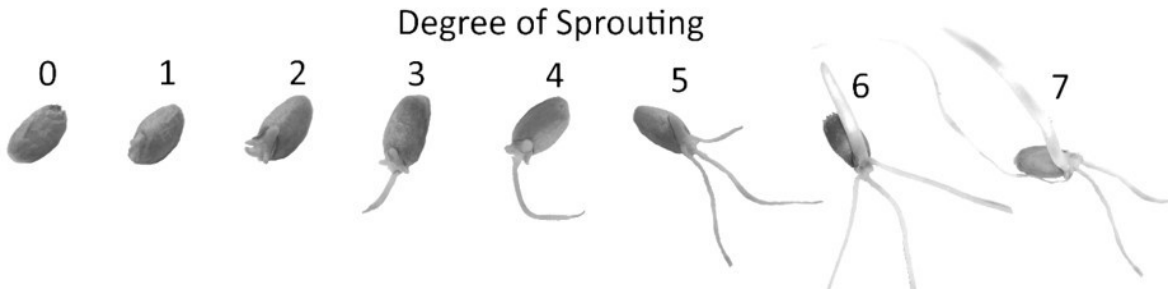


*Figure 37 Definition of the degree of sprouting of wheat grains by the lengths of their coleoptile and radicles.*

In Figure 37 the DoS is given. Grains of Degree 0 do not show any radicle growth. Degree 1 characterizes grains with visible embryos (small white point) and not yet visible radicles or coleoptile. Degree 2 indicates grains already showing a developed embryo emerging from the seed coat. The grains of Degree 3 have radicles with a length less than half a grain length. Degree 4 is represented by grains with a radicle length between half and a full grain length. Degree 5 grains are characterized by radicles longer than a full grain and having a miniscule coleoptile. Grains of Degree 6 have coleoptiles longer than a full grain length. Degree 7 describes grains with coleoptiles which are at least twice as long as the grain.

#### 6.2.3.2 Sprouting losses

The weight and the moisture content of the sprouted grains were determined by using the moisture analyzer *MA 35* (*Sartorius*). Based on these data the losses during the sprouting process were calculated:

$$\mathbf{Losses} = \mathbf{1} - \frac{\boldsymbol{m\ (dry\ matter\ based)_{after\ sprouting}}}{\mathbf{m\ (dry\ matter\ based)_{native\ grains}}}$$ **(Equation 5)**

#### 6.2.3.3 Reducing sugar content

The determination of the reducing sugar content was described elsewhere (Krapf, Arysanto et al., 2019). Average values and the SD were calculated from a twofold determination.

#### 6.2.3.4 α-amylase activity

The α-amylase activity was determined by using the Malt-amylase assay procedure (Megazyme International Ireland, 2015). Average values and the SD were calculated from a twofold determination.

#### 6.2.3.5 Viscous behavior of flour suspensions

The viscosity of an 10% (w/w) aqueous solution containing sprouted grain flour was determined using an *Anton Paar* rheometer *MCR 302* equipped with a starch cell and a stirrer having a vane geometry. Throughout the experiments the stirrer speed was kept at 150 rpm. Copper(II) chloride at a concentration of 10 mM was added to the suspension in order to inactivate the enzymes, especially amylases according to Aquino, Jorge, Terenzi, and Polizeli, M. L. T. M. (2003). The enzymes were inactivated to prevent further starch degradation during the heating cycle of the rheological measurements. Preliminary tests had shown that this precaution is necessary to determine the rheological properties reliably.

A 35 ml suspension was prepared at room temperature and stirred during the complete viscosity determination. During the determination the temperature of the sample was increased from 30 to 95°C with a heating rate of 1.5°C/min. Then, the suspension was stirred at a constant temperature of 95°C for 10 min before cooling the sample from 95 to 30°C with a cooling rate of 4.33°C/min. The temperature regime and heating rate were adopted from the method using an amylograph (DIN EN ISO 7973, 2016). The stirrer speed was chosen based on preliminary tests.

From these analyses the so-called *peak viscosity* (PV) was obtained. The average values and the SD were calculated from a twofold determination.

#### 6.2.3.6 Ascorbic acid content

The ascorbic acid (vitamin C) content was determined according to the *Indophenol Method* (Nielsen, 2003). The indophenol solution was diluted eight times. The flour was dispersed in a mixture of 30 g/L metaphosphoric acid and 8% (v/v) acetic acid and centrifuged at 3,000g. A volumetric sample of the resulting supernatant was diluted and used for titration according to the method. The procedure was executed in duplicate per specimen.

#### 6.2.3.7 Extrudate properties

The cut extrudate strands were characterized using the so-called sectional expansion index (SEI), longitudinal expansion index (LEI) and hardness ($F_{max}$). The sectional expansion rate is defined as the ratio of the circular cross section area of the extrudates and the cross-section area of the die. It was determined based on the diameter of the extrudates. The given values are averages of six different extrudate strands from the same experimental run. The dimensions of each strand were taken five times. The relative longitudinal expansion rate was derived from measuring the length of extrudates produced in 5 s.

The hardness of the extrudates was determined using a *Zwick testControl II* texture analyzer. A cylindrical probe with a diameter of 1 in. was used to break and crunch a single extrudate strand. The speed of the probe was 15 mm/min and the peak force to break an extrudate strand was defined as hardness of the extrudate. The given data points are the respective average of eight independent determinations.

The density was calculated assuming a cylindrical shape with an average diameter, average length and mass of the extrudate strand. The average density was obtained from six different data sets of one extrudate.

The cold-water solubility index was determined by suspending 0.5 g of the milled extrudates (dry matter based) in 50 ml distilled water. The suspension was stirred for 30 min before the not dissolved material was filtered from the suspension (*S&S 595*) using a Büchner funnel. The solid residue was determined by weighing after removing the water from the filter cake by drying at 130°C for 1 hr. The cold-water solubility index in percent, which actually uses the ratio of the mass of dissolved extrudates and the mass of the solution multiplied by 100, equals the difference of the initial dry matter weight and the weight of the residue. Determinations were done in duplicate.

The total starch and total sugar content of the extrudates were analyzed by Medallion Labs (Minneapolis, MN) by use of the methods AOAC 977.20, AOAC 979.10 and AACC 76-11.

### 6.2.4 Statistical evaluation (ANOVA)

The statistical evaluation of the properties studied after 3 days of sprouting was performed using Microsoft Excel 2016. The p-value (probability of error) was calculated by analyzing the experimental data by means of ANOVA (analysis of variance). A level of significance (α) of .05 was chosen.

## 6.3 Results and Discussion

### 6.3.1 Effect of sprouting time and temperature on sprouted wheat flour properties

In a first step the effect of the five tested sprouting temperatures and three different sprouting periods was tested. In Figure 38, the average DoS and the underlying distribution within a sample are shown as a function of temperature after 3 days of sprouting. Furthermore, Figure 39 illustrates the development of the average degree over time for different sprouting temperatures.

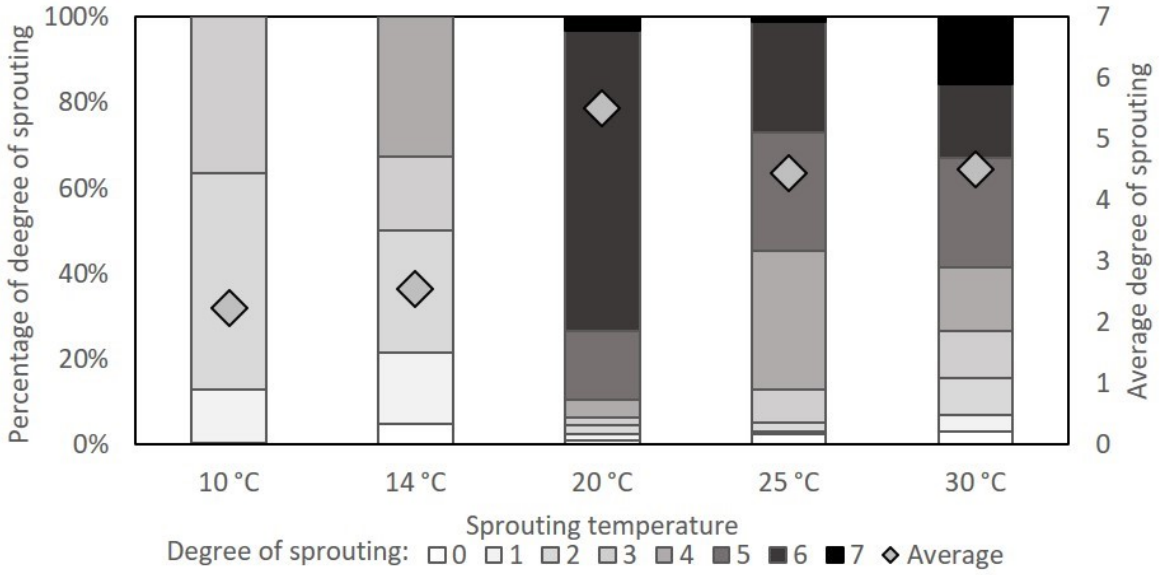


*Figure 38 Effect of the temperature on the degree of sprouting after 3 days.*

One can clearly see an increase of the DoS over the sprouting period irrespective of temperature. Already after 1 day differences in sprouting progress between different temperatures were visible. Initially, the sprouting process at 25°C was most prominent. After 2 days the average DoS is practically indistinguishable for the temperatures of 20, 25, and 30°C. However, more detailed analysis revealed that the sample at 20°C showed a more homogeneous sprouting progression indicated by a lower SD of the DoS. In contrast to oat which showed almost no sprouting activity at 30°C (Krapf, Kandzia et al., 2019), wheat sprouted really good at 20, 25, and 30°C. The highest average DoS was reached at 20°C after 3 days of sprouting. 70% of the grains were found to have a DoS of 6, characterizing coleoptiles longer than a full grain length. The homogeneity of the grains DoS at 20°C was particularly high, indicating that large-scale production would be most robust at this temperature. In comparison, the sprouting process at 30°C exhibited a slightly lower average DoS after 3 days. This corresponds to the much lower homogeneity at 30°C, for example, 15% of the grains had DoS 7, 15% DoS 6, 25% DoS 5.

After 3 days the average DoS was the lowest for the sprouting taking place at 10°C. Here about 50% of the grains had a DoS of 2 which indicates an already developed embryo emerging from the seed coat.

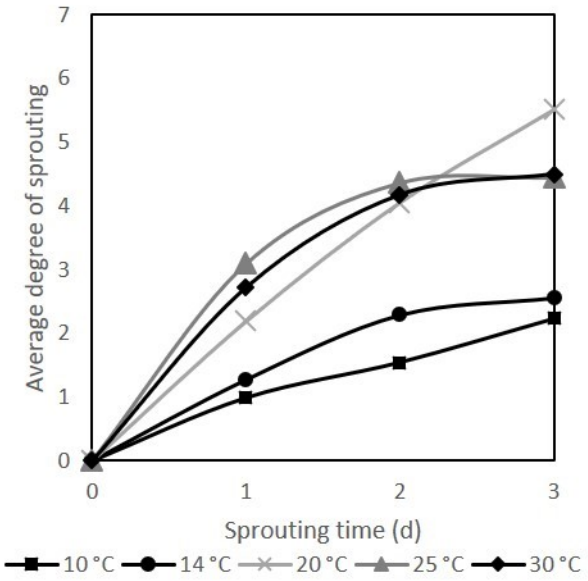


*Figure 39 Average degree of sprouting as function of the sprouting time at various temperatures.*

Growth and respiration processes are part of the sprouting process and affect the losses in dry matter. In Table 17 the losses during sprouting at different temperatures are listed. One can see that the losses due to sprouting were quite low after 3 days. Maximum losses found were 5% (w/w). These were found after 3 days at sprouting temperatures of 20 and 25°C. In general, the highest losses were detected for samples with the highest DoS and hence the biggest visual changes. Suhasini and Malleshi (1995) found malting losses of up to 15% for wheat sprouted for 3 days at temperatures between 20 and 30°C. However, these losses also involved the separation of the vegetative growth (coleoptile and radicles). In our study, the coleoptile and radicles were not separated and were part of the sprouted grain flour.

*Table 16 p-values from statistical analysis (ANOVA) of studied properties of sprouted wheat flour after 3 days of sprouting*

| | **α-Amylase activity** | **Reducing sugar content** | **Peak viscsoity** | **Vitamin C content** |
|---|---|---|---|---|
| **p-value** | 1.3146E-11 | 3.448E-17 | 2.9042E-12 | 0.02247834 |

In Table 17, the respective values of these parameters are given after 3 days of sprouting. In addition, Table 16 presents the p-values of the properties considered in this study which were calculated as part of the ANOVA. The analysis reveals, that the calculated p-value is lower than the level of significance, indicating that stated differences are significant.

*Table 17 Losses, α-amylase activity, peak viscosity, reducing sugar and ascorbic acid content in grains sprouted for 3 days at different temperatures*

| **Sprouting temperature** | **Losses** | **α-Amylase activity** | **Reducing sugar content** | **Peak viscosity** | **Ascorbic acid content** |
|---|---|---|---|---|---|
| | % (w/w) | U/g | g/100 g | Pa s | mg/100 g |
| **Native** | - | 1 ± 0.00 | 2.91 ± 0.01 | 1.22 ± 0.00 | 0.97 ± 0.15 |
| **10°C** | 0.97 | 29 ± 0.51 | 6.03 ± 0.01 | 0.97 ± 0.00 | 4.95 ± 0.22 |
| **14°C** | 0.99 | 65 ± 0.58 | 10.56 ± 0.00 | 0.94 ± 0.00 | 6.72 ± 0.22 |
| **20°C** | 4.72 | 140 ± 0.16 | 16.84 ± 0.00 | 0.65 ± 0.00 | 8.35 ± 0.51 |
| **25°C** | 5.17 | 85 ± 0.00 | 15.91 ± 0.01 | 0.73 ± 0.00 | 7.24 ± 0.74 |
| **30°C** | 4.31 | 43 ± 0.44 | 24.99 ± 0.00 | 0.77 ± 0.00 | 7.61 ± 0.37 |
| **p-value** | | 1.3E-11 | 3.5-17 | 2.9E-12 | 0.22 |

From the results in Table 17, it becomes clear that the chosen sprouting temperature has a substantial effect on the α-amylase activity. In native grains a very low α-amylase activity was found which indicates a new synthesis of α-amylase throughout the sprouting process (Bewley, 2001). In Figure 40a, the alpha-amylase activity is shown as function of the DoS. For low degrees of sprouting, also a low α-amylase activity was found irrespective of the temperature. In Figure 39, it can be seen that the average DoS is the highest after 3 days at 20°C where it reached about 5.5. As seen in Figure 40a, this corresponds with the highest α-amylase activity (140 U/g). This is in line with the findings of Reddy,

Ching, and Metzger (1984) and Müller (2015). Correlating the average degree of sprouting (Figure 39) with the data for the α-amylase activity (Figure 40a), it can be seen that after 3 days of sprouting at either 25 or 30°C an average degree of sprouting of only 4.5 was obtained with α-amylase activities of 85 U/g and 43 U/g respectively. This clearly shows a lower α-amylase activity than at 20°C (140 U/g). After 3 days of wheat sprouting an average degree of sprouting of around 2.3 was found for temperatures of 10 and 14°C. One would hence expect for these lower degrees of sprouting an even lower α-amylase activity than for the other investigated temperatures. That is true, a sprouting temperature of 10°C resulted in an α-amylase activity of 29 U/g while 14°C resulted in 65 U/g. The temperature of 14°C is often used for malting in brewery applications (Jacob, 2016). The process is essentially designed to degrade the starch contained in malt with existing amylolytic enzymes in a wort to fermentable sugars. The α-amylase activity found in this work is exceptionally high, 65 U/g. However, the conversion of starch to reducing sugars is more prominent at higher temperatures despite the α-amylase activity.

Furthermore, it has to be pointed out that it is known that the extent of the enzyme synthesis differs for wheat cultivars (Reddy et al., 1984).

Due to the presence and, thus, the activity of α-amylase during the sprouting process, starch is degraded into dextrins and short-chain sugars (Bewley, 2001). The so produced short-chain sugars exhibit a reducing potential and can be determined as part of the reducing sugar content which is used as a marker of the starch degradation in this study. In the given study the reducing sugar concentration is related to the reducing content of maltose because maltose was used for the calibration. In Figure 40b, the content of the reducing sugars with respect to a maltose equivalent as function of the DoS is presented.

Combining the data in Figure 40b and Figure 39, it can be deduced that the reducing sugar content increases with increasing DoS, thus if considering the data from Figure 39, with increasing sprouting time. An exponential relationship was found and is also presented in Figure 40b where the results for reducing sugar is plotted as a function of DoS disregarding the temperature the data was obtained at.

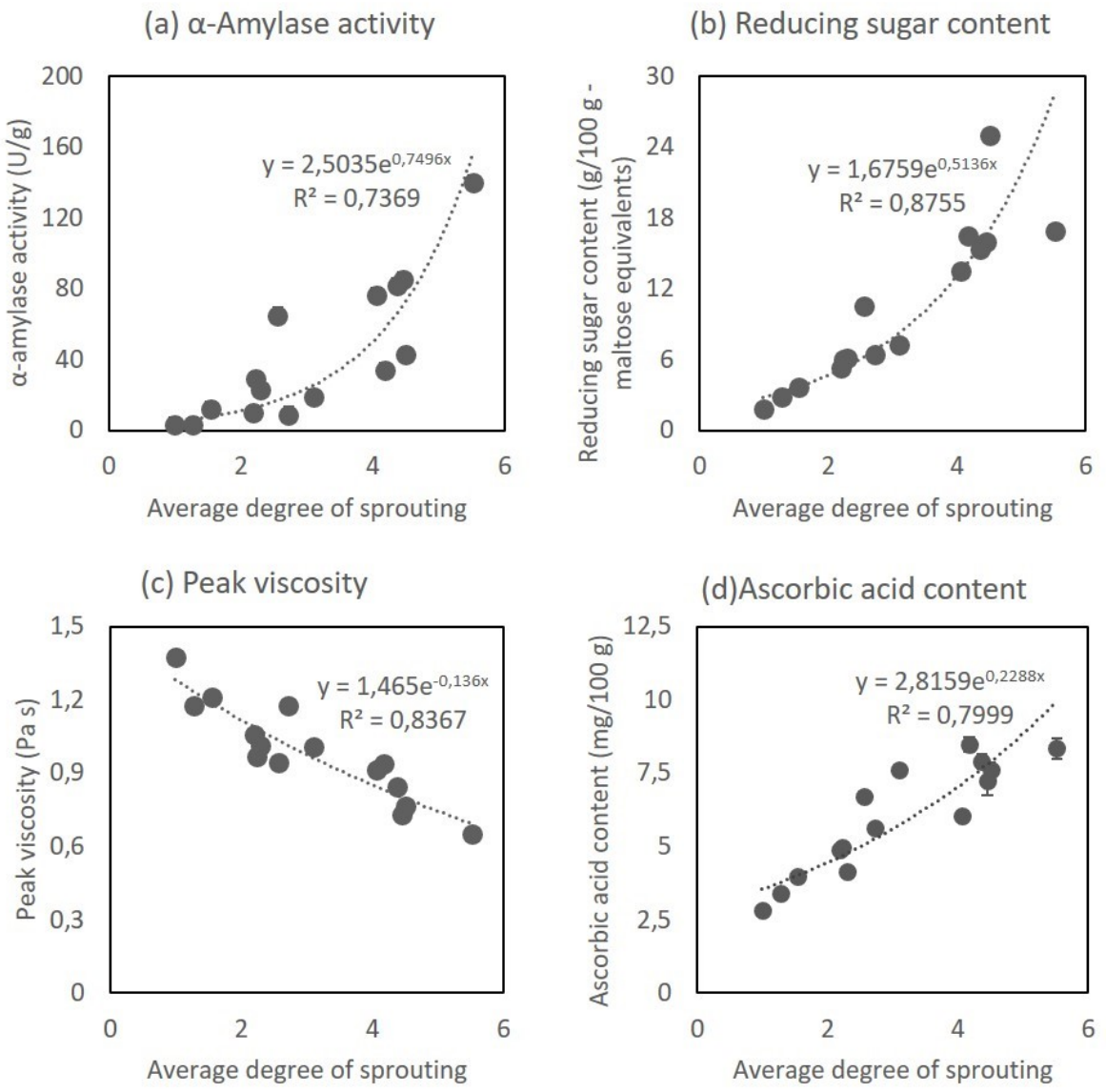


*Figure 40 α-amylase activity (a), reducing sugar content (b), peak viscosity (c), and ascorbic acid content (d) as function of the average degree of sprouting of all temperatures and sprouting times. Error bars of peak viscosity and reducing sugar content are too small to be identifiable. An exponential trend line was added and the function and coefficient of determination are given.*

However, if only considering the data after 3 days of sprouting, the reducing sugar content increases with increasing sprouting temperature. The highest reducing sugar content was found after 3 days of sprouting at 30°C. The α-amylase activity seems to be the highest at this temperature. According to Mohamed, Al-Malki, and Kumosani (2009), the temperature optimum of wheat malt α-amylase is 50°C. This is no contradiction to the above studied α-amylase activities which were measured at a specific temperature (40°C (Megazyme International Ireland, 2015)) and consequently rather provide

information about α-amylase concentration during sprouting. It is conceivable that at 30°C a lower concentration of α-amylase was synthesized, however, due to enhanced temperatures the enzymes were more active at this sprouting temperature in the grain and the starch was more efficiently mobilized and more reducing sugar were formed.

Furthermore, the reducing sugar content was described by an exponential trend line as function of the DoS. The correlation is rather good with $R^2$= .88 which indicates that the DoS concept can be used to estimate the sweetness of sprouted wheat products.

In Figure 40c, the peak viscosity is given as function of the average DoS. The peak viscosity decreases with increasing DoS. In light of the data depicted in Figure 39, it can be seen that as one would expect the lowest peak viscosity is found after 3 days of sprouting at 20°C, the highest after 1 day of sprouting at 10°C. The low peak viscosity after the sprouting process indicates a lower starch content and a higher degree of starch degradation whereby the swelling capacity of the starch granules was probably affected.

These results are in accordance with the decreased cooked paste viscosity due to increasing sprouting times found by Suhasini and Malleshi (1995).

In a previous study the dependence of the peak viscosity on the average molecular size of starch molecules was demonstrated (Krapf, Majocco, Walther, & Flöter, 2019). Due to a smaller average molecular size of starch, flour suspensions were found to have a lower viscosity and, thus, a higher flowability.

Moreover, the nutritional improvement of the wheat grains due to the variation of the sprouting conditions were studied. Because of its high reactivity (Lintschinger et al., 1997), ascorbic acid was chosen as a marker vitamin and its concentration was determined (see Figure 40d). In native wheat grains hardly any ascorbic acid was found. After 3 days of sprouting, the average DoS varied between 2.1 and 5.5. At all these varying degrees of sprouting a notable ascorbic acid content was detected. In general, the ascorbic acid content increases with increasing DoS. The data were described by an exponential trend with a respectable correlation between ascorbic acid content and degree of sprouting ($R^2$= .8).

The results are in accordance with Swieca and Dziki (2015) who found the highest antiradical activity at 20°C in comparison those found at 25°C.

Significant amounts of ascorbic acid are present to protect the growing grain from oxidation processes.

However, embryos of wheat were found to be free of ascorbic acid. In the first hours of germination the embryos are protected by ascorbic acid which has been formed by the reduction of already present low amounts of dehydroascorbic acid. With progressing sprouting, reducing sugars were formed, and converted into ascorbic acid (see also Figure 40b) (Gara, Pinto, & Arrigoni, 1997).

Besides, ascorbic acid is of technological importance. It is used in bread and flour industry as a redox agent. Addition of only 10 ppm ascorbic acid improves the rheological properties of doughs. Further, it was found that ascorbic acid increases the gas retention capacity, the dough strength, and the volume of biscuits (Grosch, 1986). The amounts of ascorbic acid formed during sprouting seem to be sufficient to achieve similar positive effects which would make further addition of ascorbic acid redundant.

Concluding from the results described above a sprouting temperature of 20°C in combination with a duration of 3 days should be applied for the sprouting of wheat grains to obtain the most homogenous sprouting, at the highest DoS, and with the formation of the highest amylase activity and vitamin C. At this temperature a high amount of reducing sugars is formed and the lowest peak viscosity was analyzed. These findings can leverage and used for extrudate production.

### 6.3.2 Sprouting temperature effect on extrudate properties

In Figure 41, key characteristics of extrudates as affected by the sprouting material used for their production are depicted.

Due to the use of sprouted grain flour for the production of extrudates, typical properties of the extrudates such as breaking force ($F_{max}$), cold-water solubility (CWS), expansion (LEI and SEI), and density are affected.

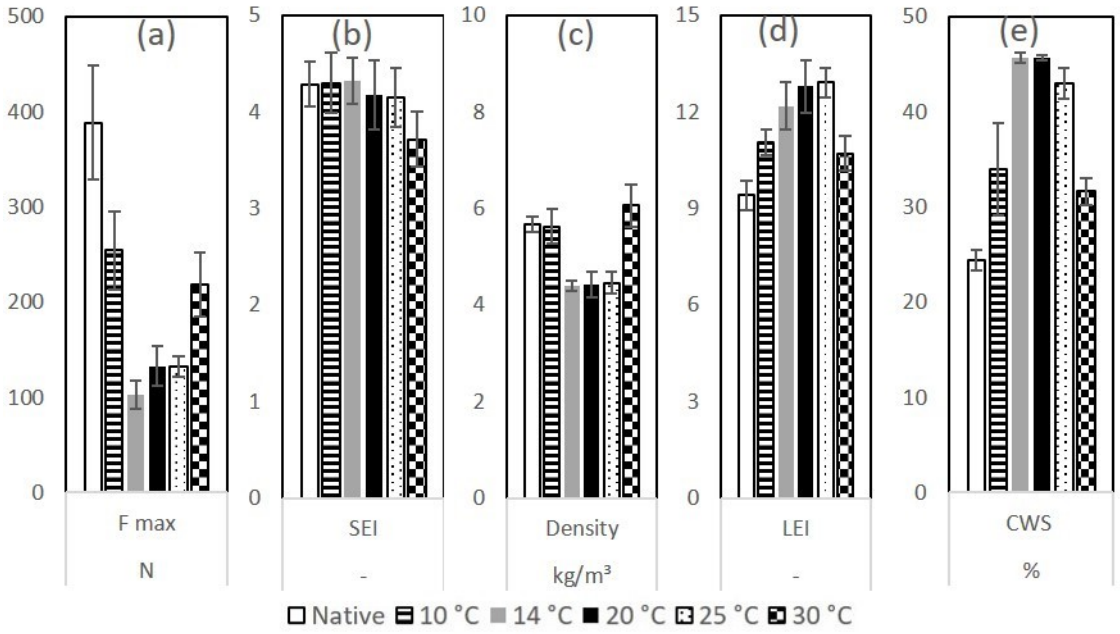


*Figure 41 Effect of sprouting on wheat flour and their extrudate properties: hardness Fmax (a), sectional expansion index SEI (b), density (c), longitudinal expansion index LEI (d), cold-water solubility index CWS (e). The grains were sprouted at 5 different temperatures.*

As shown in Figure 41a, the hardness of the extrudates decreases when using sprouted grain flour. The samples which contain sprouted grain flour from grains which were sprouted at 14, 20, and 25°C were the easiest to break extrudates while the extrudates produced from grains sprouted at 10 and 30°C or from native grains show the highest hardness. The SEI, indicating radial expansion of the extruded mass after leaving the die and drying, is only slightly affected by the sprouting process and temperature. However, a slight decrease with sprouting temperature is observed (Figure 41b). The density of the extrudates produced from grains sprouted at 14, 20, and 25°C is minimally lower than that of extrudates produced from native grains or grains sprouted at 10 and 30°C (Figure 41c). This agrees with the findings concerning the hardness of the extrudates (Figure 41a). Extrudates with a higher density are harder to break.

In Figure 41d, the longitudinal expansion is depicted. Here an increase is observed for the extrudates produced from sprouted grains sprouted at 14, 20, and 25°C. The LEI of the extrudates produced from native grains is clearly smaller.

It is interesting to see that the three samples sprouted at 14, 20, and 25°C are the hardest, have lowest density and have the highest LEI. This can be used and leveraged in product development and producing extruded puffs.

Similar behavior is found for the cold-water solubility index of the milled extrudates made from the native or sprouted grains (Figure 41e). The extrudates based on the grains sprouted at 14, 20, and 25°C have the highest DoS and the highest α-amylase activities, which has an impact on starch degradation and hence cold-water solubility. Higher level of short-chain sugars increases the water solubility of the milled extrudates. These samples might have the shortest bowl life (stability in milk), which should also be considered in developing products from sprouted grains.

The increase in the CWS and LEI and the decrease of the breaking force of extrudates based on sprouted wheat compared to native was also found by Singkhornart, Edou-ondo, and Ryu (2014). In their study wheat was used which was sprouted at 25°C for 3 days and was compared to native wheat samples.

In Table 18, the correlation matrix for simple linear relation between the determined system parameters and extrudate properties is given. Very interesting correlations were detected which will be highly valuable for product development, process scale up, and production of products made with sprouted wheat. All correlations greater ±.85 are highlighted bold and will be discussed.

*Table 18 Correlation of extrusion system parameters and product properties of wheat which was sprouted for 3 days at different temperatures; correlations greater ±0.85 are highlighted bold*

| | **DoS** | **$F_{max}$** | **SEI** | **Den-sity** | **LEI** | **PV Flour** | **CWS Extru-date** | **Die Pres-sure** | **Die Temp** | **Total Sugar Con-tent** | **Total Starch Con-tent** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **DoS** | 1.00 | **-0.88** | -0.40 | -0.41 | 0.80 | **-0.99** | 0.75 | **-0.96** | **-0.92** | **0.94** | **-0.90** |
| **$F_{max}$** | | 1.00 | 0.06 | 0.76 | **-0.93** | **0.90** | **-0.96** | **0.86** | **0.96** | **-0.91** | 0.81 |
| **SEI** | | | 1.00 | -0.46 | 0.09 | 0.28 | 0.18 | 0.52 | 0.19 | -0.38 | 0.58 |
| **Density** | | | | 1.00 | **-0.86** | 0.46 | **-0.90** | 0.43 | 0.72 | -0.53 | 0.40 |
| **LEI** | | | | | 1.00 | -0.82 | **0.96** | -0.79 | **-0.96** | 0.82 | -0.72 |
| **PV Flour** | | | | | | 1.00 | -0.79 | **0.92** | **0.91** | **-0.92** | 0.84 |
| **CWS Extrudate** | | | | | | | 1.00 | -0.72 | **-0.91** | 0.78 | -0.65 |
| **Die Pressure** | | | | | | | | 1.00 | **0.93** | -0.82 | **0.86** |
| **Die Temp.** | | | | | | | | | 1.00 | **-0.93** | **0.88** |
| **Total Sugar Content** | | | | | | | | | | 1.00 | **-0.97** |
| **Total Starch Content** | | | | | | | | | | | 1.00 |

It was found that the peak viscosity of flour suspensions from sprouted grains decreases with increasing average DoS (R= -.99). Furthermore, the starch content decreases with increasing average DoS, so that the pressure in the extruder die decreases.

The pressure difference in the extruder die is the driving force for expansion (Fan, Mitchell, & Blanshard, 1996). Due to the decreased pressure and the changes in the expansion behavior, the hardness of the extrudates is affected and a strong correlation between the average DoS and hardness

of extrudates is found. Furthermore, a good correlation between the longitudinal expansion of the extrudates and the hardness is determined. Extrudates with a high DoS and hence a high LEI show a lower hardness (R= -.93). In addition, the density and LEI of the extrudates correlated well (R= -.86).

The average DoS correlates well with the die temperature of the extruder (R= -.92). As explained above, sprouted grains suspensions with a high DoS and hence a greater starch degradation are lower-viscous and less frictional heat is produced during the extrusion process (Fan et al., 1996). Consequently, the extruded mass is subjected to less energy dissipation and the mass is heated up less. Die temperatures remain hence lower when flour relating to higher DoS is processed.

Furthermore, it was found that the total sugar content correlates well with the average DoS, which was already explained above for the reducing sugar content (Figure 40b). The increase in the total sugar content lowers the glass transition temperature (Fan et al., 1996), and hence contributes to the reduction of the viscosity (R= -.92) and the hardness of the extrudates (R= -.91). Similar results were found by Barrett, Kaletunç, Rosenburg, and Breslauer (1995), who studied the effect of sucrose on extrusion characteristics.

The lowered viscosity in the extruder coming along with the increase in the total sugar level can also explain the good correlation between the die temperature and the total sugar content (R= -.93).

Reasonably a good correlation between the total starch and sugar content was found (R= -.97). The starch content is reduced at specific sprouting temperatures, at the same time the total sugar content increases.

Compared to another study of ours (Krapf, Arysanto et al., 2019), where the effect of the sprouting time was investigated, the results presented here reveal a similar effect of advantageous sprouting temperatures and long sprouting times.

## 6.4 Conclusion

The study of the effect of the sprouting temperature on wheat flour characteristics offered systematic results and new insights. Significant effects of the sprouting time and sprouting temperatures on flour and extrudate characteristics were identified.

This study suggests that the DoS concept which was presented in an earlier study (Krapf, Kandzia et al., 2019), yields good correlation with the sprouted wheat flour and extrudate properties as affected

by the sprouting temperature. Building on this, it is conceivable that just by visual inspection and evaluation of the sprouted grains to predict respective product properties.

Generally, a sprouting temperature of approximately 20°C was found to result in the highest sprouting activity.

The highest sprouting activity corresponded well with the results of the studied extrudate property changes, whereby flour from sprouted wheat was used. Using wheat flour from grains sprouted at 20°C showed the greatest changes compared to extrudates based on native wheat. These extrudates were found to have the lowest hardness and density but also the highest longitudinal expansion and solubility.

Combining the results presented here with earlier work of ours (Krapf, Arysanto et al., 2019) it appears that optimization of sprouting time at a given temperature or optimization of the sprouting temperature at a given time yield similar extrudate properties. The methodical investigation of this and previous studies on the sprouting process have resulted and very valuable insights that can be useful in developing products from sprouted grain. The results of this work will help making it easier to optimize product and process development.

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# 7 EFFECT OF SPROUTING CONDITIONS ON MOLECULAR CHANGES OF WHEAT AND OAT STARCH AND ON PRODUCT PROPERTIES OF EXTRUDED PRODUCTS FROM WHEAT

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# Abstract

The aim of this study was to gain a deeper insight of the changes in the starch granules as affected by the sprouting process. Therefore, oat and wheat grains were steeped and sprouted for different periods (1, 3, 5, 7, 9 days) at 20 °C. Furthermore, the sprouted wheat samples were extruded in a twin screw extruder. For the first time, the average molecular weight of the starch isolated form sprouted grain flour and extrudates was determined and compared with other characteristic properties of the flour and extrudates containing exclusively sprouted grain flour.

The study showed that longer sprouting times decreased the starch content and the average molecular weight of the starch, increased the concentration of short-chain sugars and partly attacks the granular structure of the starch from sprouted grain flour. These changes of the starch molecules were found to affect the gelatinization and pasting properties of sprouted grain flour and, furthermore, alter extrudate properties.

This work demonstrated that the degree of sprouting is a helpful visual tool to quickly estimate the average molecular weight of the starch isolated from sprouted grain flour and hence predict extrudate properties of extrudates produced based on sprouted grain flour.


**Keywords:** Sprouting, wheat, oat, sprouting time, extrusion, starch from sprouted grains

## 7.1 Introduction

In recent years adding sprouted grains to food products such as pizza, flakes, and crackers is trending. The sales of sprouted grain products were predicted to reach 250 million $ by 2018 (Crawford, 2015). Consumers are interested in the improved bioavailability of nutrients provided by the addition of sprouted grains to food. According to studies the use of sprouted grains results in nutritional improvements such as an increased vitamin content (Harmuth-Hoene, Bognar, Kornemann, & Diehl, 1987), a reduction of phytic acid (Tian et al., 2010), an increase in GABA (gamma-aminobutyric acid) (Ohtsubo, Suzuki, Yasui, & Kasumi, 2005), and an increase in limiting amino acids (Harmuth-Hoene et al., 1987). For food developers the use of sprouting grains presents an interesting opportunity because the addition of sugar to products can be reduced and replaced with natural occurring short-chain sugars in sprouted grains, possibly allowing ‘cleaner labels’. The sprouting process and subsequently

the drying process also significantly change the sensory profile and texture of the products, e.g. sprouting can help to mask the intense flavor of some grains (Heiniö, Oksman-Caldentey, Latva-Kala, Lehtinen, & Poutanen, 2001). So far, the development of novel products using sprouted grains is complicated because the changes in functional properties of resulting flours are not well understood. Additionally, currently only few sprouted grain ingredients have been commercially available in large enough quantities. In an earlier work, a predictive tool was developed by directly relating changes in grain composition such as vitamin C content, β-glucan content, α-amylase activity and reducing sugar content to the recently introduced degree of sprouting (Krapf, Kandzia, Brühan, Walther, & Flöter, 2019). Furthermore, it was shown that the degree of sprouting of grains used for the production of direct expanded extrudates correlates well with the extrudate properties (Krapf, Arysanto, Walther, & Flöter, 2019). While these studies and the obtained positive results already support and promote the use of sprouted grains in food products, gaining a deeper insight of the effect of the molecular changes of the starch on product properties is crucially important for developing high quality food products.

Currently there is only limited information available about starch degradation due to sprouting. Therefore, the aim of the present study was to characterize the starch changes during sprouting for different process durations (only steeped; 1, 3, 5, 7, and 9 days sprouted). A comparative study of the behavior of oat and wheat during sprouting was conducted and is presented here.

Basically, during sprouting the embryo generates a new plant by metabolizing carbohydrates, inducing the growth of the radicles and the coleoptile (Kunze, 2011). Due to the addition of water the hormone gibberellic acid is released and activates or synthesizes a series of enzymes located in the aleurone layer which are then secreted into the starchy endosperm. These hydrolases start to degrade starch and proteins. Thereby β-amylase breaks the starch down from the non-reducing end and α-amylase randomly degrades the large starch molecules. Limit dextrins and maltose are formed, which are then further degraded by maltase into their constituent glucose molecules (Bewley, 2001). The sprouting process mostly affects large starch granules. After a specific sprouting time, starch granules isolated from sprouted flour showed successive enzymatic erosion of the surface and hole formations (Lineback & Ponpipom, 1977).

Typical functional properties of starch such as water solubility, viscous behavior, swelling behavior and flow characteristics (Meuser, Lengerich, & Köhler, 1982), which are essential for bread baking,

extrusion processes or pudding preparation, are affected by the molecular weight of the starch (Yoo & Jane, 2002) and, therefore, altered due to the sprouting process. It is, therefore, of special interest to study the starch molecular size evolution during sprouting. Building on the work of Meuser et al. (1982) characterizing functionality analyzing the molecular size and functional properties will allow to characterize the effect of sprouting on the processing properties and performance. This corresponds well with the approach of the above-mentioned degree of sprouting which was introduced to correlate functionality and progression of the sprouting process in a practical manner.

In order to get a better understanding of the application of sprouted grain flour in direct expanded extrudates (see (Krapf, Arysanto et al., 2019)), the differently sprouted wheat flours were extruded using a twin-screw extruder. For each extruder test a mixture of 100% sprouted wheat flour and water was used.

It is believed that in this study, for the first time the molecular changes in the starch molecular weight due to the sprouting process were shown. The starch was isolated from other grain substances and a subsequent analysis by the use of the SEC-MALS (size-exclusion chromatography-multiangle laser light scattering) was developed. Changes in the starch molecules were further studied using DSC (differential scanning calorimetry) and rheological measurements. Moreover, SEM (scanning electron microscope) was applied to observe structural changes of the starch granules.

## 7.2 Material & Methods

### 7.2.1 Experimental Design

Wheat and oat grains were sprouted for different periods at the same sprouting conditions. The grains were dried, milled and analyzed. Using sprouted wheat flour direct expanded wheat extrudates were produced. These extrudates were then milled and further analyzed.

### 7.2.2 Sprouting Process

The huskless oat variety *Gehl* and the wheat variety *Runal* were used for all experiments. The grains were each sprouted for 5 different durations (1, 3, 5, 7, 9 days) at 20 °C. Additionally, native and steeped grains were used for comparison. The steeping and sprouting process was conducted in a self-constructed sprouting box which was aerated permanently with conditioned, humidified air at constant temperature of 20 °C using the climate cabinet of *Lovibond 220 P-02*. First, the grains were steeped for 5 h in water, followed by air rest for 19 h at 20 °C and a second steeping step for 4 h. This

steeping regime was determined in preliminary tests and is in accordance with Central European Commission for Brewing Analysis (MEBAK) (Jacob, 2016). The moisture content after steeping was about 42 % for wheat and 38 % for oat. After the different sprouting times the grains were dried in a drying cabinet of Heraeus Instruments (*UT 6120*) at a temperature of 65 °C until a final moisture content of 12 % was reached to ensure a proper milling of the sprouted grains.

The dried, cooled sprouted grains were milled using a hammer mill and a mesh size of 500 µm (*Siemens-Schuckertwerke AG*).

#### 7.2.2.1 Losses during Sprouting

The weight and the moisture content of the sprouted grains were determined daily. From this the mass losses during the sprouting process were calculated.

#### 7.2.2.2 Determination of the degree of sprouting

The degree of sprouting (DoS) of the undried oat and wheat kernels was determined using the earlier defined degrees of sprouting method of Krapf, Arysanto et al. (2019) and Krapf, Kandzia et al. (2019). Due to a longer sprouting process which took up to 9 days, two more degrees of sprouting were defined for oat. Degree 6 grains have a coleoptile longer than a full grain. Degree 7 covers grains with a minimum length of coleoptile which is twice the length of the grain.

### 7.2.3 Extrusion Process

Extruded products were manufactured utilizing a *Berstorff ZE 25* extruder with a screw length of 870 mm at standardized settings (screw speed 200 rpm, throughput 6 kg/h, moisture content 27 %, die 3 mm). These setting resulted in a maximum sectional expansion index in preliminary tests. Flour and water were used exclusively as feedstock in order to emphasize the differences between the sprouted wheat flours.

The chosen temperature profile over the eight different barrel elements of the extruder was 20 – 45 – 65 – 85 – 120 – 140 – 140 – 140 °C. The screw was assembled of standard forward elements with two different pitches. Once stable extrusion process conditions were established the pressure in the die, the current and the voltage was measured 10 times per second. The mean pressure, voltage and current were calculated from at least 1000 individual data points.

The extruded strands were cut into pieces of about 50 cm length after leaving the die. They were dried at 65 °C for 1.5 h in an oven (*Thermo Scientific T 6420*) in order to dry the extrudate strands to a water

content below 10 % (w/w) and to achieve a crunchy appearance. Expansion indices and texture measurements were either performed on the extrudates. Milled extrudates were used for all other analyses. Extrudates were milled by exposing them for 30 s to a kitchen blender (*Petra MZ12.35*) and afterwards milling the coarse flour in a speed rotor mill (*Pulverisette 14, Fritsch*).

### 7.2.4 Analytical Methods

#### 7.2.4.1 Viscous properties of sprouted flour suspensions

The sprouted grain flour was characterized by rheological measurements of a 10 % (w/w) aqueous suspension containing the sprouted grain flour. Analyses were performed using an *Anton Paar* rheometer *MCR 302* equipped with a starch cell and a stirrer with a vane geometry. Throughout the experiments the stirrer speed was kept at 150 rpm. Copper(II) chloride at a concentration of 10 mM was added to the suspension in order to inactivate the enzymes, especially amylases according to Aquino, Jorge, Terenzi, and Polizeli, M. L. T. M. (2003). The enzymes were inactivated to prevent further degradation in the heating cycle during the rheological measurement which otherwise would have affected the rheological properties reducing the peak viscosity.

The suspension was prepared at room temperature. For the rheological temperature scans from 30 °C to 95 °C were performed with a heating rate of 1.5 °C/min. Then, the suspensions were stirred at 95 °C for 10 min before cooling down from 95 °C to 30 °C with a cooling rate of 4.33 °C/min. The temperature regime and heating rate were adopted from the method using an Amylograph (DIN, 2016). The stirrer speed was adapted according to preliminary tests.

These experiments yield data on the so-called *gelatinization temperature (GT)*, *peak viscosity (PV)* and the *final viscosity* (FV). The latter was recorded once the system was cooled down to 30 °C.

#### 7.2.4.2 Determination of the starch content

The starch content of the differently sprouted oat flours, wheat flours and wheat extrudate samples were determined using the Megazyme Total Starch HK assay procedure (*AMG/α-Amylase/HK Method – K-TSHK 08/18*). The starch was separated from other components by extraction. Therefore, solids (flour or extrudate) were mixed with a 10 mM copper(II) chloride solution and centrifuged. The final sediment was dried, finely ground and used for the starch content determination. Determinations were done in duplicate.

#### 7.2.4.3 DSC measurements

DSC analysis was carried out with a *DSC204 F1 (NETZSCH)*. 10 mg of wheat flour or 7 mg of oat flour and 20 mg water were weighed into stainless aluminum pans (*NETZSCH; 25 µl*) and then heated from 30 °C to 110 °C with a heating rate of 10 K/min. The thermograms were analyzed with regard to the onset temperature ($T_{on}$), the peak temperature ($T_{peak}$), the offset temperature ($T_{off}$) and the gelatinization enthalpy (ΔH). The determination was performed in duplicate.

Before the analysis with the *NETZSCH Proteus Thermal Analysis software* was performed, the baseline of the thermograms needed to be defined by the user. The enthalpy was then determined from the area between signal curve and baseline.

#### 7.2.4.4 Molecular characterization using SEC-MALS

In order to analyze the molecular characteristics of the starch of the sprouted flours and extrudates, the starch was isolated from the samples. Therefore, a solution containing 20 % (w/w) sprouted flour or extrudate sample and 80 % (w/w) 10 mM copper chloride solution was prepared. Exposure to 100 °C for 30 min was accompanied by high shear treatment of the paste using an Ultra-Turrax *T25* (*IKA-Werke*) at 20000 rpm for 2 min. 10 ml of the paste was clarified using Carrez clarification (*see sample preparation for HPLC*). The protein was then separated by centrifugation (Thermo Scientific-MEGAFUGE 40R) at 3000 rpm for 5 min. The supernatant, containing the starch, was filtered (*Machery-Nagel MN 631*). The starch was subsequently precipitated using ethanol (40 % v/v) and separated from the liquid solution by centrifugation (*Thermo Scientific- MEGAFUGE 40R,* 3000 rpm, 5 min). Finally, the starch was dried at room temperature for 1 day. For the analysis by SEC-MALS the starch was finely ground and mixed with DMSO to a final concentration of 2.5 mg/ml. The solution was stirred at 80 °C for 24 h before it was injected through a filter (*5 µm PTFE filter* of *Roth*) for analysis in SEC-MALS.

The size exclusion chromatography was conducted with a SEC-3010 module (*WGE Dr. Bures*) and a Bi-MwA detector from Brookhaven Instruments Corporation was used. The determination of the average molecular weight (Mw) was done using *ParSEC Enhanced V5.6 1* chromatography software.

Further details on the molecular characterization procedure by the use of SEC-MALS are described by Ulbrich, Wiesner, and Flöter (2015).

#### 7.2.4.5 Analysis of soluble sugars using HPLC

The soluble sugars were extracted from the flours and a Carrez clarification was conducted. Therefore, 2 g of the different flours were weighed in a 50 ml volumetric flask and 20 ml distilled water was added. The flask was shaken and 2.5 ml Carrez I solution, 2.5 ml of Carrez II solution and 5 ml of 0.1 M sodium hydroxide were added. Afterwards the flask was filled up with distilled water and the solution was filtered (*Machery-Nagel MN 631*).

The solution was pressed through a 0.45 µm glass fiber filter (*CHROMAFIL GF/PET-45/25*) before analyzed in a *VWR Hitachi Chromaster* HPLC by using an ELSD (Evaporative Light Scattering Detector) detector (*VWR ELSD 90*) and a column from *AppliChrom* (*ABOA SugarSep-Ca*). The samples were immediately analyzed after clarification to avoid further changes. 0.02 ml of the sample was injected and a flow rate of 0.5 ml/min was chosen. Distilled water was used as eluent and the oven temperature was 80 °C.

A calibration was done using glucose and maltose, because they were the main hydrolysis products (Bertoft & Kulp, 1986), and fructose. For the calibration of samples containing one of the mentioned substances of known concentration (0.25, 0.5, 1, 2, 5 g/100 g distilled water) were analyzed and from that a calibration curve was established.

The chromatograms were analyzed using *Peakfit (Version 4.12)* giving peak areas and elution volumes of peak on-set, off-set and maximum.

#### 7.2.4.6 β-glucan determination

The β-glucan contents of the differently sprouted oat samples were determined by using the Megazyme (2017) Mixed-Linkage Beta-Glucan assay procedure (*McCleary Method – K-BGLU 02/17*). Determinations were done in duplicate.

#### 7.2.4.7 SEM

The pictures of the wheat and oat flours were recorded using a scanning electron microscope of *ZEISS* (*DSM 982*) at the *ZELMI* of the *Technical University Berlin.* Flours were fixed on a carrier for the analysis and samples were sputtered with a 100 nm gold layer.

#### 7.2.4.8 Properties of wheat extrudates

The sectional expansion index (SEI) was determined by measuring the diameter of extrudates. The values were averaged over five individual extrudate strands. Each strand itself was measured three

times at various locations. The expansion index is defined as the ratio of the circular cross section area of the extrudates and the cross-section area of the die. The relative longitudinal expansion index (LEI) was derived from measuring the length of extrudates produced per second. The results provided here are averages of five individual determinations.

Hardness of the extrudates was determined using a *Zwick testControl II* texture analyzer. A cylindrical probe (diameter 2.54 mm) was breaking and crunching a single extrudate strand. The constant speed of the probe was 15 mm/min and the peak force was recorded giving the hardness of the extrudate ($F_{max}$). Each data point represents the average of eight independent measurements.

## 7.3 Results & Discussion

### 7.3.1 Characterization of the sprouted flour

Figure 42 shows the effects of the different sprouting durations on the distribution of the degrees of sprouting amongst samples and the average degree of sprouting. As shown before (Krapf, Arysanto et al., 2019; Krapf, Kandzia et al., 2019) longer sprouting durations resulted in a higher average degree of sprouting. For wheat a considerable increase in the degree of sprouting was found after 1 day of sprouting. In contrary to the assumption, the highest average degree of sprouting for wheat was found after 7 days of sprouting and not after 9 days. It is likely, that some very long roots of the samples sprouted for the longest time (9 days) got broken off a bit during the daily spraying with water and the movement during stirring. Hence, the roots were shorter and were assigned to a lower degree of sprouting due to the experimental execution. Therefore, the amount of grains which had the highest degree of sprouting of 7 after 9 days of sprouting was smaller than the amount of these grains after 7 days of sprouting. For oat grains, an approximate linear increase of the average degree of sprouting over the sprouting time was found.

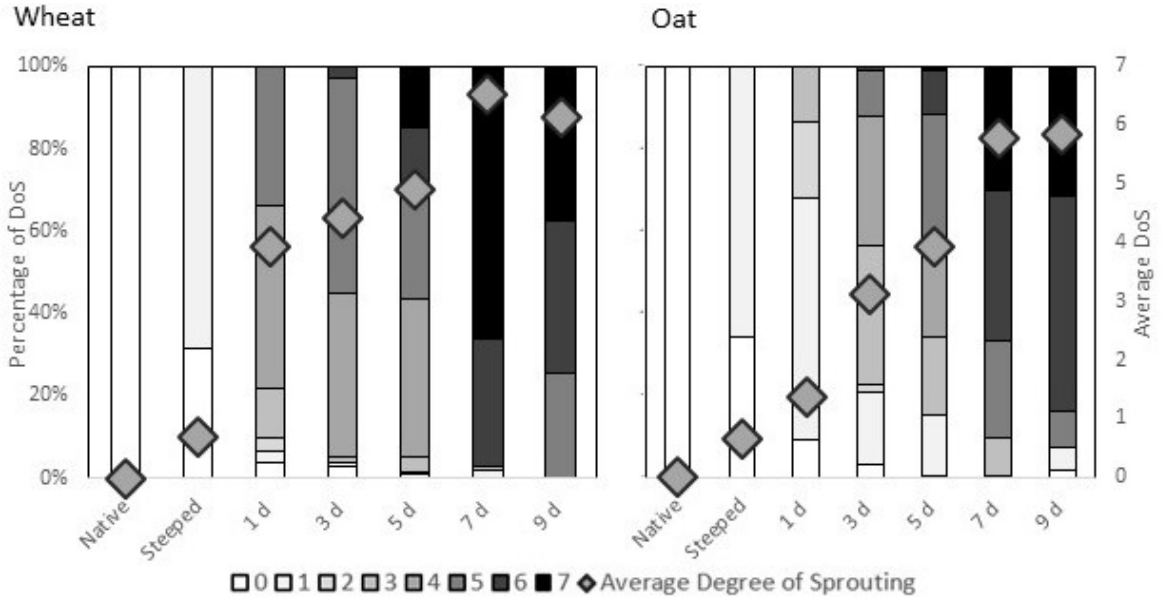


*Figure 42 Effect of different sprouting times on degree of sprouting of wheat and oat.*

To understand the changes in composition of the grains during sprouting the dry matter weight losses in connection with the sprouting process were studied. In Table 19 the dry matter weight losses for wheat and oat for the different sprouting durations are given. Our results suggest that the losses due to respiration on the first days of sprouting were quite low. After 9 days of sprouting the dry matter weight losses was 11 % for wheat and 13 % for oat. These values were in an acceptable range considering such a long sprouting process. After a few days of sprouting the grains started to change their color to green which indicated the beginning of the photosynthetic process. Different from our study, Tian et al. (2010) found the highest percentage respiration losses between 2 and 3 days of sprouting and after 4 days of sprouting the losses due to respiration dropped significantly.

In malting for brewery purposes dry matter losses of 6-10 % during long-term sprouting processes of 5 days at 14-18 °C are typical (Müller & Methner, 2015). These losses are mainly attributed to mechanical removal of roots and coleoptiles. In our case, the coleoptile and roots were left at the grains and were not counted as losses. The losses can, therefore, only be explained by consumption of dry matter due to respiration.

Furthermore, Table 19 depicts the total starch content in the grains depending on the sprouting duration. As the values in Table 19 show, the starch content of wheat decreased by 12.5 % after 9 days of sprouting and those of oat by 6 %. The reduction in the starch content was very low compared to the results of other researchers. E.g., Tian et al. (2010) found a 31 % decrease in oat starch content

after 6 days of sprouting. The starch content in the wheat extrudates is similar to the starch content in wheat flour.

In addition to the starch degradation, the changes in β-glucan content of oat grains due to the variation of the sprouting time are also shown in Table 19. The cell structures of the grains which are in large parts made of β-glucan are degraded by enzymes in order to start the degradation of the starchy endosperm to generate energy. In the native oat a β-glucan content of 3.95 % was found which was reduced to 2.4 % after 9 days of sprouting (Table 19). Together with the starch reduction, this was a weight reduction of around 8 % after 9 days of oat sprouting.

As shown the degradation of carbohydrate polymers was much higher in wheat than in oat. This is, because the starch degradation was found to be closely correlated with the α-amylase activity (Sutcliffe & Baset, 1973), due to the fact that sprouted wheat exhibits a higher amylase activity compared to sprouted oat (Lineback & Ponpipom, 1977).

*Table 19 Effect of sprouting time on dry matter losses of initial weight of oat and wheat grains, starch content in wheat and oat flour and wheat extrudates and β-glucan content in oat flour.*

| | | Native | Steeped | 1 d | 3 d | 5 d | 7 d | 9 d |
|---|---|---|---|---|---|---|---|---|
| **Dry matter losses (%)** | Wheat | | 0 | +1 | 0 | -9 | -8 | -11 |
| | Oat | | +2 | +1 | -2 | -2 | -2 | -13 |
| **Starch content (g/100 g)** | Wheat flour | 60.65 | 59.00 | 58.13 | 58.10 | 55.37 | 52.55 | 48.08 |
| | Wheat extrudate | 62.79 | 65.62 | 60.95 | 60.11 | 56.46 | 53.38 | 47.26 |
| | Oat flour | 58.02 | 53.34 | 57.75 | 58.40 | 59.73 | 55.11 | 52.03 |
| **β-glucan content (g/100 g)** | Oat flour | 3.95 | 5.52 | 4.15 | 2.42 | 2.01 | 2.29 | 2.42 |

#### 7.3.1.1 Determination of short-chain sugars

The short-chain sugars which contribute to sweetness of the sprouted product and support the growth of the sprouted grain were analyzed using HPLC. Figure 43 depicts the results of the HPLC analysis of the sprouted grain flours in comparison to their native flours and in the extrudates based on sprouted wheat flour.

For wheat considerable changes in the content of short-chain sugars analyzed by HPLC were found. With increasing sprouting duration, the content of short-chain sugars increased due to the starch degradation. After 9 days of sprouting the amount of maltose increased by a factor 20 compared to the amount in native wheat flour. Maltose was produced by the β-amylase catalyzed hydrolysis of starch fragments. Further degradation of the maltose into glucose was enhanced by the enzyme maltase. The fructose was produced during the hydrolysis of sucrose invertase (Narziß & Back, 2012). In the native wheat flour no glucose and fructose was found, though after 9 days of sprouting 6.7 g/100 g glucose and 1.1 g/100 g fructose were found.

Different than for the wheat flour, in the sprouted oat flour no significant changes in the maltose content were found (data not shown). It seems that either starch does not degrade via maltose into short-chain sugars or that these sugars were directly degraded by enzymes into monosaccharides. In native oat the monosaccharides, glucose and fructose, were not detected. However, in the oat which was sprouted for 9 days 2.0 g of glucose/100 g flour and of 0.7 g fructose/100 g flour were found. As mentioned before the oat sprouting process and thus the formation of short-chain sugars was much slower than in wheat. This is due to the reduced enzyme activity of oat compared to wheat (see Krapf, Kandzia et al. (2019): α-amylase activity in oat: 26 U/g, see Krapf, Ding, Brühan, Lorimer, and Flöter (2020): α-amylase activity in wheat: 140 U/g (both sprouted for 3 days at 20 °C).

The total amount of starch and short-chain sugars was not changed during the sprouting process. Simply, the share of short-chain sugars of the total carbohydrates amount in the grain was increased (see Table 19 and Figure 43). In the native wheat flour the total amount of starch was 61 % and that of short-chain sugars in sum 1 % (62 % carbohydrates). After 9 days of sprouting the amount of starch was 48 % and the amount of short-chain sugars 14 % which also adds up to a total of 62 % carbohydrates. However, considering respiration losses and the coleoptile and radicle growth the total amount must be reduced. The high amount of short-chain sugars can probably be explained by measurement inaccuracies.

During the extrusion process and, the corresponding high energy input into the extruded product, mid-chain sugar molecules can further degrade into small-chain sugars (van Lengerich, Meuser, & Ng, 2007). This was confirmed by this study. The concentration of short-chain sugars in wheat extrudates was slightly higher than in wheat flour (Figure 43).

Any changes occurring during drying are negligible in comparison with the effect of the sprouting and extrusion process on the starch molecules.

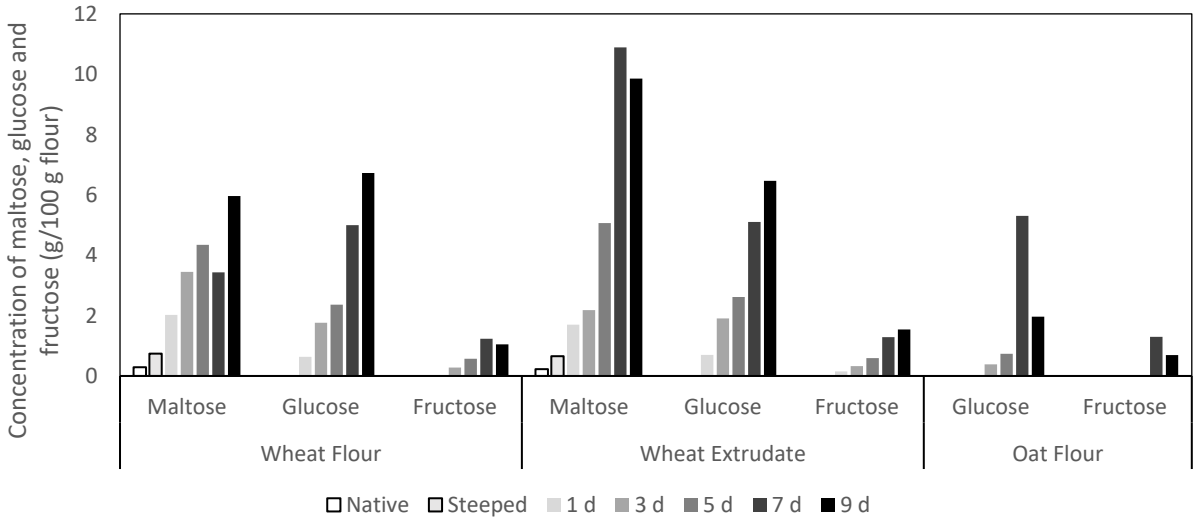


*Figure 43 Concentration of maltose, glucose and fructose in wheat and oat flour and in extruded wheat flour as analyzed by use of HPLC and detection by ELSD.*

#### 7.3.1.2 Characterization of starch

The average molecular weight of the starch separated from the different sprouted grains was determined by means of SEC-MALS and is presented in Table 20. As expected, the average molecular weight of the starch was decreased with increasing sprouting times.

*Table 20 Comparison of average molecular weight of starches separated from differently sprouted wheat and oat and wheat extrudates*

| | **Native** | **Steeped** | **1 d** | **3 d** | **5 d** | **7 d** | **9 d** |
|---|---|---|---|---|---|---|---|
| **Wheat flour** ($\cdot 10^7 g \cdot mol^{-1}$) | 3.3 | 4.5 | 2.2 | 2.0 | 1.6 | 2.2 | 0.8 |
| **Wheat extrudate** ($\cdot 10^7 g \cdot mol^{-1}$) | 4.0 | 3.4 | 2.6 | 2.4 | 2.0 | 1.1 | 1.4 |
| **Oat flour** ($\cdot 10^7 g \cdot mol^{-1}$) | 3.8 | 3.9 | 3.1 | 3.1 | 3.0 | 3.9 | 3.1 |

It should be noted that the average molecular weight of the starch molecule is higher than anticipated. This is due to the fact that short-chain sugars were not detected by the use of SEC-MALS and the determined average molecular weight did hence not include the these falsifying the result. During the preparation step, during which starch is isolated using the fact that it is insoluble in ethanol, for the SEC-MALS analysis short-chain sugars such as maltose and glucose were leached out and were not considered in this analysis.

In this context, one could be tempted to combine the HPLC and SEC-MALS results in order to have a more comprehensive approach to determine the average molecular weight. However, this attempt does not yield meaningful results because it still ignores intermediate oligosaccharides.

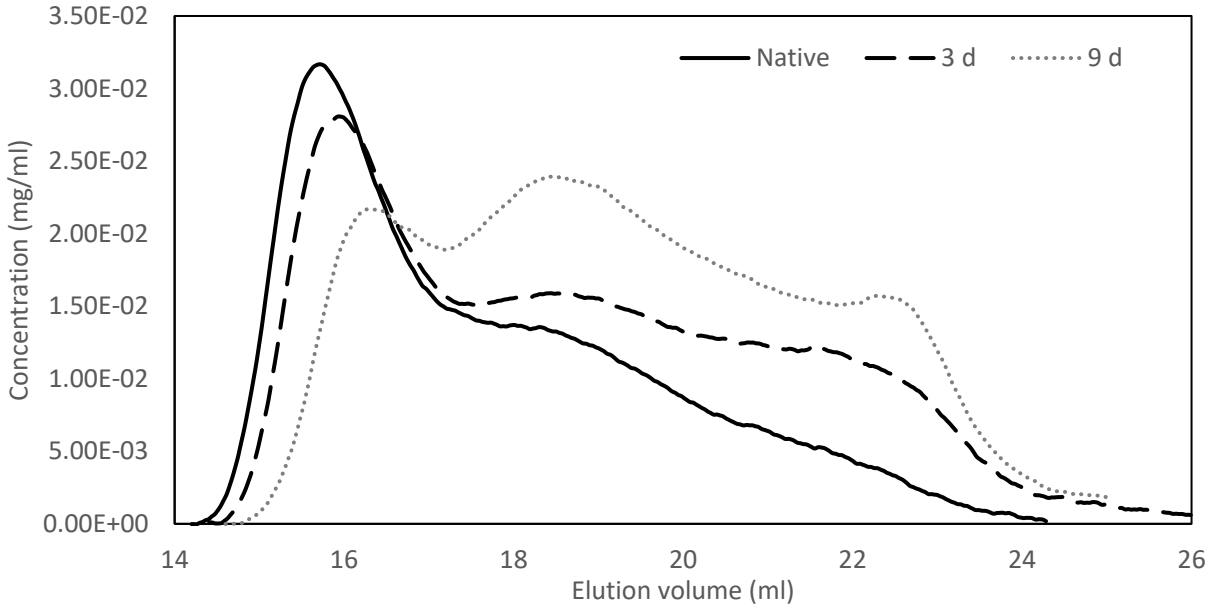


*Figure 44 Chromatogram of starches isolated from native, 3 and 9 days sprouted wheat grains.*

The average molecular weight of starch is obtained from chromatograms of the SEC-MALS analysis. In Figure 44 the chromatograms of the starches from native wheat grains and 3 and 9 days sprouted grains are shown. Here higher the elution volumes correspond to smaller molecular weights. At an elution of about 15.5 ml the peak of the amylopectin fraction can be found (Ulbrich et al., 2015). The chromatographs of the SEC-MALS analyses indicate the primary degradation of the amylopectin into smaller molecules such as limit dextrins indicated by the decreased peaks at low elution volumes (15.5 ml) for long time sprouted samples compared to native wheat starch. This is in line with the degradation pattern reported elsewhere (Ulbrich et al., 2015) due to accessibility in starch kernel.

The higher average molecular weight of the extruded wheat flour compared to wheat flour (see Table 20) can, therefore, be explained by the fact that in these average molecular weight volumes are relative volumes based on the obtained isolated long-chain starch molecules after the short-chain sugars were washed out during the preparation.

For wheat and oat grains which were only steeped, dried and milled the average molecular weights were higher than of the native wheat or oat flour (see Table 20). Taken the information given by Tian et al. (2010) this could be related to the initial phase of metabolism, primarily consuming small and medium chain oligosaccharides. These are considered for the analysis of the native grain, but their decomposition products are not subject to the SEC-MALS analysis and possibly leached out during the preparation step. Hence, the percentage share of long-chain starch molecules is enhanced and the average molecular weight is higher than in native grains. This interpretation is supported by the considerably increased concentration of the short-chain sugars, e.g. maltose and glucose, shown in Figure 43, in flour from steeped grains compared to flours from native grains.

The calculated average molecular weight for all samples behaves according to expectation up to a sprouting period of 5 days. A systematic reduction of the average molecular weight accompanies the increased starch degradation indicated by the data in Table 20. However, after a sprouting period of 7 days higher average molecular weights of starches in wheat and oat flour were found. Currently no convincing explanation for this observation can be given, beyond a possible change of metabolism. Surprisingly, the data concerning the average molecular weight of the starch after extrusion are exceptionally low. This indicates that the starches after 7 days of sprouting are in a different configuration and hence more prone to decomposition on extrusion.

To gain a deeper understanding of the visual changes of the starch granule changes, SEM micrographs were taken for the native flours and flours produced from grains sprouted for 3 or 9 days sprouted grains and are shown in Figure 45.

As can be seen in Figure 45 wheat starch granules isolated from sprouted wheat flour show an enzymatic erosion of the surface and hole formations. It can be seen that after long sprouting times the surface of the high molecular weight starch molecules showed enzymatic attack in certain areas of the starch or attacked starch granules were completely degraded and thus disappeared. At the parts of the starch granules where no enzyme attack was detected, there was probably also no starch degradation and reduction of the molecular weight.

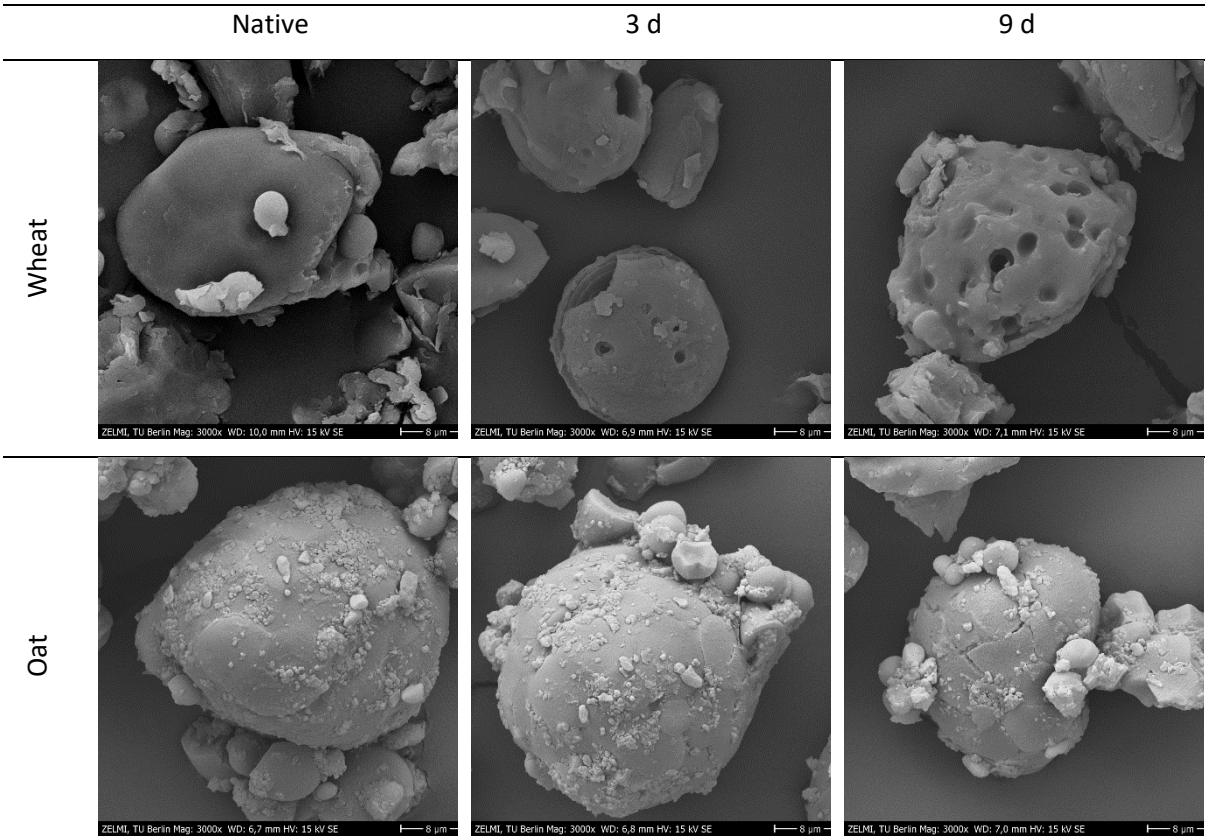


*Figure 45 SEM micrographs of different long sprouted oat and wheat flour, magnification bars indicating 8 µm.*

The starch granules of wheat flours sprouted for 9 days appeared to be more affected by the enzyme attack than the granules of the oat flours sprouted for 9 days. For the oat, practically no impact of the enzyme action was visible at the starch granule level. This is accordance with the findings of Asiri, Ulbrich, and Flöter (2018). They showed significant molecular changes of the starch isolated from potato but no enzymic attack on the starch granule surface was found. Hence, the oat and potato starch graunles are probabaly more attacked from the inside of the granules.

After 9 days of sprouting the number of small wheat starch granules decreased. A possible explanation is that these small granules were fully degraded so that they disappeared. The total surface area per gram sample which was exposed to the hydrolases was bigger in the small granules and, therefore, they were more prone to be attacked. Our results are in agreement with Bertoft and Kulp (1986). Small granules were hydrolyzed by eroding the whole starch granule surface. Whereas, in big granules channels were formed allowing the enzymes to ingress into the starch granule interior.

Lineback and Ponpipom (1977) also showed in their work that oat starch is less susceptible to enzymic attacks and in addition to this, the enzyme concentration in oat was found to be lower compared to wheat. The samples and corresponding pictures of this study confirm those results.

The above-described molecular changes also affected the gelatinization and pasting properties and the behavior during heating in the extruder. This was confirmed by the rheological analyses. The results of these analyses are expressed as gelatinization temperature, peak viscosity (PV) and final viscosity (FV). They are summarized in Table 21. Addition of copper (II) chloride ensured that the amylase was inactivated and no further degradation of the starch during the heating cycle took place.

The data showed a decrease in the peak viscosity with extended sprouting times. A reduction in the peak viscosity indicates a lower degree of starch swelling. How substantial the relative contributions of the enzymatic degradation of the starch during sprouting (see Table 19 and Table 20) and of the disappearance of smaller granules (Figure 45) to the reduced swelling are remains unsolved here.

Comparing the viscosities of wheat and oat samples one can see that oat exhibited higher peak and final viscosities. This was due to the presence of β-glucan in oat which also contributes to the viscosity and was degraded much slower in the sprouting processes (Table 19). It is noteworthy that the decomposition of β-glucan is also relevant for the nutritional value because the health benefit of β-glucans is mainly due to the increased viscosity they also generate in the human intestine resulting in a delayed adsorption of glucose (Anttila, Sontag-Strohm, & Salovaara, 2004).

The final viscosity was determined after the samples were cooled down to 30 °C after the heating up to 95 °C. During cooling the starch molecules re-associate and form gel-like structures which cause the increase of the final viscosity. The samples sprouted for longer periods were found to have a lower FV indicating a lower ability to form a viscous paste. This again to the reduced level of starch per se and a change, reduced molecular weight and debranching, of the remaining starch molecules.

Moreover, the gelatinization temperature was found to increase on longer sprouting times. This can be interpreted as higher resistances towards swelling and is counterintuitive in the first place. Since during the gelatinization and pasting process water moves into the amorphous regions of the starch granules one could assume that for porous structures as shown Figure 45, this would appear easier. However, due to the sprouting process probably the amorphous regions were reduced initially so that more crystalline regions remain and hence dominate and determine the properties. This would

explain why relative more energy is needed in order to cause swelling in starch granules from longer sprouted grains. The results presented here are in agreement with the findings of Suhasini and Malleshi (1995) who studied wheat sprouted for 3 and 5 days. In addition, the amount of small starch granules was reduced due to the long sprouting process (see Figure 45), which also supports retardation of the gelatinization process due to mass transfer limitations.

As already described for the data of the average molecular weight the 7 days sprouted oat sample showed an exceptional behavior. Compared to the other samples a very low PV and FV were found. A possible explanation is the formation of higher molecular components such as fibers. As has been shown in Figure 42 the DoS of oat grains is higher after 9 days compared to 7 days sprouted oat grains. This comes along with longer roots and hence, a higher fiber content, because the newly developed roots are basically composed of fibers and sugars. Fibers are known to increase the peak and final viscosity.

*Table 21 Comparison of gelatinization temperature (GT), peak (PV) and final (FV) viscosity of flour suspensions containing differently sprouted oat and wheat determined from rheological analysis*

| | | Native | Steeped | 1 d | 3 d | 5 d | 7 d | 9 d |
|---|---|---|---|---|---|---|---|---|
| **Wheat** | **GT (°C)** | 81.33 | 82.06 | 82.77 | 83.01 | 84.93 | 85.90 | 86.13 |
| | **PV (Pa·s)** | 1.49 | 1.47 | 1.27 | 0.89 | 0.71 | 0.39 | 0.28 |
| | **FV (Pa·s)** | 1.37 | 1.40 | 1.18 | 0.89 | 0.62 | 0.49 | 0.37 |
| **Oat** | **GT (°C)** | 83.90 | 89.50 | 90.70 | 91.40 | 92.60 | 94.49 | 94.49 |
| | **PV (Pa·s)** | 2.22 | 1.90 | 1.68 | 0.87 | 0.85 | 0.28 | 0.66 |
| | **FV (Pa·s)** | 2.22 | 1.75 | 1.72 | 0.98 | 0.89 | 0.37 | 0.78 |

In Table 22 the results from the gelatinization analysis of the differently sprouted flours studied by DSC are presented. The sprouting process considerably affected the gelatinization properties of the flours. Our data indicate an increase in the onset, offset and peak temperature for longer sprouted oat and wheat flours. The onset temperature was enhanced for about 5 K and the peak temperature for about 5 K for wheat and for 4 K for oat compared to their native grains after 9 days of sprouting respectively.

The onset temperature characterizes the beginning of the melting of the areas of the amylopectin which are in a suboptimal crystalline state. With increasing temperature, the imperfection of the chain packing is increasingly healed and the perfection of amylopectin crystalline structure proceeds. This is indicated by the peak temperature (Ulbrich, Beresnewa-Seekamp, Walther, & Flöter, 2016). An

increase of both temperatures as found in case of longer sprouted flours indicated thus a more stable crystalline configuration of the amylopectin chains. In addition, the molecular degradation in the starch molecule enhanced the mobility and hence, the rearrangement of the molecular chains. As described above, the relative share of amorphous regions was reduced and the share of crystalline regions was increased according to these processes due to the changes induced by the sprouting process. More solid structures as a consequence hamper the accessibility for water so that the gelatinization temperatures are increased for increasing sprouting times (Ulbrich et al., 2016). Izydorczyk and MacGregor (2001) explained the increase of the peak temperature in malt with progressing degradation of starch with the competition of small chain sugars and starch for water. Moreover, they indicated annealing effects of the starch during malting as another factor supporting the increase of the gelatinization temperature. Generally, it can be stated that an increase of the gelatinization temperature mirrors the advancing stage of degradation of starch due to enzymatic attack.

For the decrease in the peak area, and thus of the gelatinization enthalpy one could be tempted to use the explanation that the decrease in total starch content (Table 19) despite the increase of the crystallinity of the remaining material is responsible. However, reduction of the gelatinization enthalpy after 9 days of sprouting by 68 % for wheat and by 48 % for oat cannot be explained by a 20 % and 10 % starch decomposition, respectively.

However, the observations made are in line with the results of Keßler (2002) comparing the thermal behavior of barley flour and malt.

For the milled extrudates no gelatinization enthalpies were found by DSC analysis indicating a fully gelatinized starch. This was already reported earlier (Liu et al., 2010). Due to the massive mechanical energy input and temperature profile during the extrusion process the hydrogen bonds and granule structure of the starch were already disintegrated.

*Table 22 Results from thermal analysis by use of DSC of differently sprouted wheat and oat flour*

| | | **Native** | **Steeped** | **1 d** | **3 d** | **5 d** | **7 d** | **9 d** |
|---|---|---|---|---|---|---|---|---|
| **Wheat** | **Peak Area (J/g)** | 1.59 | 1.50 | 1.35 | 0.92 | 0.79 | 0.71 | 0.51 |
| | **$T_{on}$ (°C)** | 61.90 | 62.10 | 63.75 | 63.90 | 65.90 | 67.25 | 66.25 |
| | **$T_{peak}$ (°C)** | 67.50 | 67.60 | 68.75 | 69.25 | 70.10 | 72.45 | 72.10 |
| | **$T_{off}$ (°C)** | 73.85 | 73.00 | 70.15 | 73.40 | 73.60 | 75.05 | 75.25 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Oat** | **Peak Area (J/g)** | 1.83 | 1.91 | 1.91 | 1.86 | 1.44 | 1.27 | 0.96 |
| | **$T_{on}$ (°C)** | 58.95 | 59.90 | 61.30 | 61.55 | 62.35 | 62.95 | 64.25 |
| | **$T_{peak}$ (°C)** | 64.05 | 64.55 | 65.75 | 66.10 | 67.05 | 67.35 | 68.70 |
| | **$T_{off}$ (°C)** | 69.50 | 69.45 | 70.30 | 70.45 | 71.35 | 70.95 | 72.50 |

### 7.3.2 Extrusion process and extrudate properties

Extrusion is a frequently used process to change starch properties and/or to produce expanded products such as breakfast cereals. When applying extrusion, additional to the molecular changes induced by the sprouting process, the starch is further modified by the thermal and mechanical energy input during the extrusion process.

Despite the changes in starch content, average molecular weight, and relating properties described above, the flour from sprouted grain could be extruded and expanded.

In this work, the effect of changes of molecular weight as well as the lower starch and higher short-chain sugar content on the extrudate properties, e.g. hardness of extrudates, longitudinal and sectional expansion index, and pressure over the extruder die were correlated. The correlation matrix for simple linear relation between the properties studied is given in Table 23.

The aim of this research was to evaluate in how far a correlation between the degree of sprouting (Figure 42) and the functionality of flour from sprouted grains exists.

The measured properties of the extrudates are expected to correlate systematically with the starch content and the average molecular weight of the starch isolated from the sprouted flour.

The reduction of the starch content and the increase in short-chain sugars was studied by Fan, Mitchell, and Blanshard (1996) and was found to affect the extrudate properties. Thereby, a reduction of the sectional expansion, and an increase in the density, longitudinal expansion and ratio of shrinkage due to the addition of sugars and the reduction in the total starch content was investigated.

Brümmer, Meuser, van Lengerich, and Niemann (2002) studied the relation between expansion and functional properties of corn starch extrudates and the molecular weight. They found that a decreased molecular weight of the corn starch used resulted in a decreased cold paste viscosity, cold water solubility and sectional expansion and in an increase in the longitudinal expansion.

In line with these earlier works, our results showed, that not surprisingly the average molecular weight of the starch isolated from the sprouted flour correlated well with process and product properties. It is known that the average molecular weight of the starch significantly affects the behavior of the flour in the extruder, see Table 23. Only the sectional expansion yields an R of less than 0.85.

As described above the average molecular weight of the starch was reduced during sprouting and the share of middle and short-chain sugars was increased. In spite of this, some parts of the starch remained unattacked. Both together result in changes in the behavior of the starch during extrusion and other applications such as baking.

Furthermore, the degree of sprouting was correlated with extrudate properties. Thereby, a good correlation of the DoS with the hardness of the extrudate was determined. Extrudates produced from longer sprouted grains and having a higher DoS were easier to break and less hard. The peak force also showed a good correlation with the average molecular size of the starch isolated from the sprouted grain flour. The changes in the hardness can be explained by the changes in porosity. Due to the lower average molecular weight of the starch in long-time sprouted grains and their lower viscosity, the pores which were formed during the expansion process after leaving the extruder die collapsed into bigger pores. These pores were easier to break and the extrudates appeared to be less hard. Results are in accordance with our earlier findings and were further discussed in the publication of Krapf, Arysanto et al. (2019).

In addition, the DoS also correlated well with the longitudinal expansion index which can further be explained by a good correlation of the peak viscosity of the flour and the longitudinal expansion index. The pressure difference in the extruder die is the driving force for expansion (Fan et al., 1996) and is affected by the viscosity. In Table 23 the good correlation of the peak viscosity and extrusion pressure is also depicted (R=0.94). The flow properties of the melt have a great influence on expansion mechanism in the extruder. As shown above, suspensions of long-time sprouted grains had a lower viscosity mainly attributed to the reduced average molecular weight of the starch (Table 20) and the reduced starch content (Table 19). Additionally, it was found that for the flour from grains sprouted for 9 days, the material coming out of the extruder die collapsed as a result of thinner pore walls, a lower viscosity of the melt, as well as the lower glass transition temperature of the melt (Fan et al., 1996). The lower the glass transition temperature, the longer it takes during flash off and cool down to reach the lower glass transition temperature and during this time the bubbles have time to collapse

(Bindzus, 1997). Hence, the sectional expansion was reduced. The lower viscosity and the better flowability of the sprouted flour suspension resulted in an increased longitudinal expansion index (R= -1.00).

This study has shown that there are strong correlations between DoS and the molecular changes in starch as well as correlations between the changes in molecular structure and product properties. Hence, the DoS can be used to predict outcome provided the correlations are well understood for different grains and validated over a range of grain varieties.

*Table 23 Correlation matrix of extrudate properties ($F_{max}$ – peak force needed to break extrudates, SEI – sectional expansion index, LEI – longitudinal expansion index), DoS of sprouted grains and starch characteristics (Mw - average molecular mass, PV – peak viscosity)*

| | DoS | Starch content flour | $M_w$ | Glucose content flour | PV Flour | Pres-sure in die | $F_{max}$ | SEI | LEI |
|---|---|---|---|---|---|---|---|---|---|
| DoS | 1.00 | -0.84 | **-0.98** | **0.93** | **-0.93** | **-0.97** | **-0.90** | -0.44 | **0.93** |
| Starch content flour | | 1.00 | **0.85** | **-0.98** | **0.94** | **0.85** | 0.71 | 0.81 | **-0.96** |
| $M_w$ | | | 1.00 | **-0.89** | **0.95** | **0.95** | **0.88** | 0.47 | **-0.95** |
| Glucose content flour | | | | 1.00 | **-0.95** | **-0.86** | -0.43 | **-0.85** | **0.99** |
| PV Flour | | | | | 1.00 | **0.94** | **0.87** | 0.60 | **-1.00** |
| Pressure in die | | | | | | 1.00 | **0.95** | 0.41 | **-0.93** |
| $F_{max}$ | | | | | | | 1.00 | 0.17 | -0.84 |
| SEI | | | | | | | | 1.00 | -0.66 |
| LEI | | | | | | | | | 1.00 |

## 7.4 Conclusions

In this study the effect of the sprouting time on characteristic starch properties of wheat and oat grains was investigated. The degree of sprouting was found to show strong correlations with both properties of the sprouted grains and properties of extruded products based on the respective flour.

The variation of sprouting time was found to affect grain properties in a natural way. Furthermore, differences between the sprouting behavior of wheat and oat were shown in this study.

DoS was found to increase for wheat and oat with longer sprouting times. Moreover, the total starch content significantly decreased, the average molecular size of the starch isolated from the sprouted grain flour decreased, and the amount of short-chain sugars increased as a result of starch degradation. It is shown additionally that the granular structure of the starch was partly destroyed during sprouting. These considerable changes of the starch and the starch granules also affected the gelatinization and pasting characteristics of the flours. Thereby, suspensions of longer sprouted flours were found to have lower peak and final viscosities, and a decreased enthalpy. Onset, peak and offset temperature during gelatinization were increased as a result of the sprouting process. These changes in the characteristic properties of the flours propagate into the extrudate properties when extruding the differently sprouted wheat flours. Extrudates produced using longer sprouted wheat flour were found to be easier to break and to have a higher longitudinal and lower sectional expansion index.

Again, the degree of sprouting proved to be a good method to characterize changes in the properties of both grains/flours and extrusion products. Strong correlations between the degree of sprouting and the average molecular weight of the starch, paste and extrudate properties were found. The established correlations reflecting a better understand of ingredient functionality and sprouting effects can ultimately benefit targeted development of products containing sprouted grains.

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# 8 FINAL CONCLUSION AND OUTLOOK

The use of sprouted grains in breakfast cereal production is a promising approach to reduce the addition of sugar, to generate new colors and flavors, to improve the nutritional value of the final product, to satisfy the consumer interest in sprouted grain products, and to develop a whole grain breakfast cereal products.

It was the purpose of the work documented in this thesis to gain a better understanding of the use of sprouted grain flour in the production of directly expanded breakfast cereals. Special focus was put on the effect of different sprouting conditions on flour and resulting extrudate properties and on the evaluation of the sprouting process by use of a newly defined characteristic, the *degree of sprouting*.

This study presented systematic and comparable results that provide a comprehensive framework for manufacturers and researchers to apply sprouted grains.

The analytical methods used throughout this work were verified and show very good repeatability (relative standard deviation ≤ 1.3%). A standard sprouting process and extrusion process were developed as part of this work and proved to have good repeatability and reproducibility which is within the scope of the biological variance. Within a short setting time, the extrusion process proved to be stable over time. Thus, the differences found in the properties of flours and extrudates by varying the sprouting conditions can actually be attributed to the changes in composition.

***Degree of Sprouting***

The *degree of sprouting* concept was tested as part of this study. Thereby, the *degree of sprouting* was determined based on the visual assessment of the radicle and coleoptile length in relation to the individual grain size.

The determination of the *degree of sprouting* was identified to be a fast and cost-effective method without the need for time-consuming analytical methods. The *degree of sprouting* showed a good correlation with many characteristic properties of the sprouted flours and extrudates, e.g. β-glucan content and sectional expansion index of extrudates. Therefore, it is believed to be a helpful tool in product development and raw material specification. By knowing the *degree of sprouting* of a sample, it is possible to assure the sprouted material of the same *degree of sprouting* will cause no change of the product properties.

To get more insight into the *degree of sprouting* concept, sprouted grain samples were subdivided into homogenous samples of the same *degree of sprouting* based on sprouted grain samples sprouted

at one single set of sprouting conditions (20 °C, 3 days). Based on this, a function was found to calculate characteristic sprouted grain properties based on the *degree of sprouting* of homogenous samples ($R^2$= 0.91 - 0.99). By a linear combination of the different degrees of sprouting after the sprouting, it is possible to calculate an arithmetic average *degree of sprouting* and hence by use of the determined function, to estimate the properties evolving.

However, the *degree of sprouting* concept is limited for the application on single samples originating from a sprouting process. These samples might be inhomogeneous, as a result of the naturally occurring distribution after sprouting for a certain period at a specific temperature. The limitation depends on the homogeneity of the *degree of sprouting* within the distribution of the *degree of sprouting* of the grains. Therefore, it might be useful to state the standard deviation, which is a measure for the homogeneity in a sprouted sample, in order to improve the validity. The concept appears to be less applicable for grain populations that are mixtures of differently sprouted subsets. Furthermore, the correlation between the *degree of sprouting* and different grain properties was identified to be of exponential nature. Calculated values are, therefore, found to be predicted too conservative so far as a function of inhomogeneity.

Furthermore, the *degree of sprouting* concept allows the transfer of the results of this thesis to other food applications of sprouted grain flour such as cookies and bread.

However, the *degree of sprouting* cannot explain complex synthesis and metabolism processes during sprouting. This is beyond the scope of this thesis but certainly deserves attention in future research activities.

***Effect of sprouting conditions***

The effect of the sprouting conditions on flour and extrudate properties was demonstrated in this thesis by varying sprouting temperature (10, 14, 20, 25, 30 °C) and sprouting period (1 to 9 days). Significant effects of the sprouting period and sprouting temperature on flour properties were identified.

As part of the sprouting process, the α-amylase activity was proven to be increased during longer sprouting times. A progressing starch degradation as indicated by a lower starch content, a reduction of the average molecular weight of the starch molecules isolated from sprouted flour, and an increase in short-chain sugars, such as glucose and maltose, was one result of it. Along with the enhanced

metabolic activity and the degradation of polysaccharides, a reduction of the dry matter content was identified. Furthermore, the main cell wall component and quality determining substance in oat, β-glucan, was found to be progressively degraded during the ongoing sprouting process. In addition, a positive effect of the sprouting on the nutritional value as indicated by the enhanced vitamin C content was found for longer sprouting times.

A sprouting temperature of 20 °C was found to yield the most significant changes in the properties of sprouted oat and wheat. These changes were more pronounced by sprouting grains for longer periods.

In malting for brewery purposes, efficient sprouting conditions are long-established and a sprouting temperature of 14 °C is typically used. On that score, this temperature was also used as a reference temperature in this study. The results of this study confirmed a high homogenous sprouting process at 14 °C. However, the conversion of starch to reducing sugars and the α-amylase activity was more prominent at higher temperatures. The choice for this temperature in malting was probably motivated by low risk for microbiological spoilage, homogenous radicle growth, and limited dry matter losses due to radicle and coleoptile growth.

Thus, depending on the desired changes to be achieved through the addition of sprouted grains, it is possible to use sprouted grains that were sprouted for long times to maximize the vitamin content and sweetness. However, this also leads to a higher degradation rate of β-glucan, enhanced dry matter losses, and increased process costs accompanied by higher energy demand and lower production capacities. However, even after 9 days of sprouting the dry matter losses are still in an acceptable range.

In order to combine low process costs and maximized vitamin and sugar levels, the optimal sprouting temperature of 20 °C should be used. However, at this sprouting temperature, the β-glucan is also degraded the most, and dry matter losses show the highest values.

To the best of our knowledge, these opposing effects cannot be separated from each other.

***Effect of sprouting on starch molecular changes***

In a particularly interesting part of this study, the average molecular weight of the starch isolated from either flour of oat and wheat or the respective extrudates was assessed. Doing this after different

sprouting periods was reported for the first time. For this purpose, a special procedure to isolate the starch from other flour substances was developed and the starch was analyzed using SEC-MALS.

The chromatographs of the SEC-MALS analyses showed the degradation of the amylopectin into smaller molecules such as dextrins, indicated by the decreased peaks at low elution volumes for starch isolated from sprouted flour. The average molecular weight of the starch was found to be reduced systematically due to the sprouting process.

SEM micrographs provided a deeper insight into starch granule changes as a result of the sprouting process. Starch granules were found to show partial enzymic erosion on the surface and hole formation. Some starch granules were degraded completely.

Our results revealed that the changes in the starch molecular weight, starch and β-glucan content, and starch granule structure affect the thermal and rheological characteristics of the flours. As an indicator of this, the onset, peak, and offset temperatures during gelatinization were found to be increased as a result of the sprouting process. These outlined changes especially affect functional properties of the sprouted grain flour, which are inter alia essential in the production of directly expanded breakfast cereals.

***Effect of sprouting on extrudate properties***

As part of this thesis, a model product was developed. In doing so, solely the effect of 100% sprouted grain flour mixed with water during twin-screw extrusion using standardized extrusion conditions was studied. Thereby, the sprouted material varied by means of changes in the sprouting conditions.

The effect of the feedstock variation, initiated by different sprouting periods and temperatures, on the extrusion system parameters and extrudate properties was found to be substantial. Thereby, the rheological determination of the peak viscosity during heating of the sprouted grain flour suspensions was found to be a good indicator of the flow behavior of the sprouted grain flour in the extruder. As described, starch is degraded during the sprouting process. Consequently, the viscosity of the sprouted grain flour suspensions was reduced and, hence, the pressure difference in the extruder was reduced. These changes were also affecting the expansion behavior. After the initial expansion, the pore cell walls shrunk and the structure collapsed markedly. This resulted in a decreased sectional expansion index of the final extrudates. The collapse and coalescence of several pores led to the formation of bigger pores. The pore wall thickness and the thickness of the outer layer was reduced

due to the fact that the generated microstructure was found to correlate to the peak viscosity of the sprouted flour suspensions. The changes in the structure of the extrudates based on sprouted grain material were also reflected in the textural properties of the extrudates. The peak force necessary to break the extrudates was determined to be reduced in extrudates containing sprouted flour due to the bigger pores and thinner cell walls.

Furthermore, the changes in the starch molecules and in the viscous behavior of the sprouted grain flour suspensions were found to improve the water-solubility of the extrudates which is important for the bowl life of breakfast cereals.

Contrary to the expectations the use of 9 days sprouted flour in the production of breakfast cereals in this study showed good results with regard to the expansion behavior. The low starch content, the significant reduction of the average molecular weight of the starch, and the high enzyme activity in sprouted grains fed the expectation that these flours would not be expandable very well. However, the good performance can be explained by an average molecular weight of the starch isolated from 9 days sprouted wheat of at least $8 \cdot 10^6$ g·mol$^{-1}$ and the fast enzyme inactivation in the extruder at high barrel temperatures. In addition, the dry matter losses were found to be within an acceptable range. Due to this surprising finding, the elongated sprouting processes resulting in the synthesis of high amounts of nutritional substances such as vitamins, appear less critical to functionality than expected.

Furthermore, the high-temperature-short-time extrusion process was found to be suitable to protect high amounts of vitamins. This study revealed that one of the most unstable vitamins, vitamin C, was only reduced by 15% due to the extrusion process at standard extrusion conditions.

A sensory panel confirmed significant changes in the sensory profile of the extrudates based on sprouted flour. These extrudates were identified to have a darker color, a maltier and sweeter taste, and a crispier texture. The results are attributed to the increased amount of reducing sugars and accompanying Maillard reactions. The panel clearly preferred the newly produced extrudates over the reference produced manufactured the same way.

The results presented represent a good base for a broad application and acceptance of the extrudates from sprouted grains for breakfast cereal production.

***Differences between oat and wheat***

In this work, oat and wheat, being the common raw material for breakfast cereals in Europe and the US respectively, were used as model grains. However, the use of the *degree of sprouting* concept makes a transfer of the results to other grains conceivable.

Although the same optimal sprouting temperature of 20 °C was found for both grains, oat and wheat, this study showed significant differences in the sprouting behavior of oat and wheat. Wheat was found to have a higher enzyme activity compared to oat, e.g. the α-amylase activity was almost six times higher after 3 days of sprouting at 20 °C in wheat compared to oat. Associated with this, a higher degradation rate of polysaccharides, indicated by a lower starch content and lower average molecular weight of the starch in wheat, was found. SEM micrographs of sprouted wheat and oat starch granules correspond well with these findings. Already after short sprouting times, significant visual differences, indicated by the *degree of sprouting*, between the sprouting progress in wheat and oat grains were observed. Sprouted wheat grains showed much longer radicles compared to sprouted oat grains. This trend also holds after longer sprouting periods.

Depending on the product application, wheat could be chosen if more dramatic product changes are desired. Thereby, wheat has to be sprouted by the use of shorter sprouting times to achieve similar changes compared to sprouted oat, where longer sprouting times are necessary. Hence, the process costs are expected to be lower for wheat.

***Outlook***

The work at hand has confirmed many of the positive expectations concerning the production of directly expanded breakfast cereals based on sprouted grains. The promising results of this work are in general suitable as a basis for future innovations. For industrial production, it is still necessary to upscale the process to industrial size.

The effects presented in this thesis are based on the results of a laboratory size extruder. Therefore, industrial size extruders and original recipes including more additives such as sugar, salt, and tripotassium phosphate have to be tested. These additives are believed to additionally change the product quality and properties. Therefore, the use of sprouted grains must be tested in interaction with these additives.

Our results can be the basis for industrial manufacturers to develop new recipes and products depending on the desired claims. This study exclusively studied the effect of 100% sprouted grain flour mixed with water as feedstock which yields good results. It is still conceivable to mix lower amounts of sprouted grain flour with native flour in order to increase profitability and to vary product properties. By use of the *degree of sprouting*, it is possible to calculate the needed amount of sprouted grain to obtain a certain content of a substance, e.g. sugar, β-glucan, or vitamin content. For example, it could be the producers wish to design a new product based on sprouted oat which has a reducing sugar content of 10 g/100 g. It is possible to obtain this by the use of 40% of oat grains having a DoS of 5 mixed with 60% native oat (reducing sugar content in DoS 5: 21.2 g/100 g) or 87% of oat grains having a DoS 3 mixed with 13% native oat (reducing sugar content in DoS 3: 10.9 g/100 g). Consequently, choices for mixing native and sprouted grains could be made balancing on product cost and product properties considering different levels of differently sprouted grains.

Also, further product improvement is conceivable by varying the extrusion conditions. With regard to higher barrel temperatures of the extruder, the color and flavor formation could be further boosted, because, as described, extrudate properties can be affected by many parameters.

The application of sprouted grain is also possible in other food products, such as cookies, as described before. With regard to these applications, it would be of great value to further investigate and understand the effects of sprouting conditions on the product properties. The new *degree of sprouting* concept could certainly help in this respect to characterize the raw material and develop sound correlations. The first results indicated that the properties of cookies are also altered by the addition of differently sprouted grains. Thereby, the property changes depend on the tested cereal grain (wheat, oat, buckwheat, millet, spelt). In general, it was found that cookies based on longer sprouted grains become darker, have a significantly smaller diameter, increase in thickness and hardness, and have a lower spread ratio. The results can be explained by the Maillard reaction and the increased concentration of more water-absorbing substances such as dietary fiber and proteins. Again, a good correlation between the *degree of sprouting* of the sprouted grains used in the cookies produced and cookie characteristics was found.

Future studies could focus on the synthesis of vitamins, antioxidants, and phytic acid in detail, to gain a better understanding of the metabolic pathways of the sprouting process. Furthermore, for a better understanding of the application of sprouted flours in other food products such as cookies, it would

be beneficial to investigate the degradation of the proteins during sprouting at different conditions and the effect on the gluten in more detail.

Moreover, further research is needed in regard to the microflora of the sprouted and subsequently extruded flour, because sprouts are vulnerable to microbiological spoilage. This important aspect has to be considered in future product development. Anyhow, it is fair to assume that the extrudates produced are of low risk due to the thermal treatment during drying and extrusion.

In summary, the concept of the *degree of sprouting* is believed to be a very useful tool to characterize sprouted grains in a simple yet relevant way. The experimental data gathered covers a wide range of sprouting conditions. It was found that the resulting changes in the flours obtained propagate systematically into the properties of extruded model breakfast cereals. Consequently, there is good hope that the work presented here can be instrumental in the development of products based on sprouted grains.

# 9 ANNEX

# ANNEX 1: Sensory evaluation test of wheat extrudates

Welcome to our Sensory Evaluation of Wheat Extrudates. Please enter your information in the box below:

| | |
|---|---|
| **Name** | |
| **Age** | |
| **Where do you come from?** | |
| **Did you study food technology or a related subject?** | ☐ Yes<br>☐ No |
| **How often do you eat breakfast cereals?** | ☐ Never (0 times per week)<br>☐ Rarely (1-2 times per week)<br>☐ Sometimes (3-4 times per week)<br>☐ Often (5-6 times per week)<br>☐ Every day |

### Sensory Evaluation of Wheat Extrudates – Part I

You will see a tray with four different wheat extrudates coded by numbers.

In this part you need to rank the four samples you have from the lowest to the highest for each characteristic asked for.

### Please read the whole instruction before starting the test!

**A. Color**

- Rank the **darkness** of the wheat extrudates from lowest to highest using the list below (**1** represents the **least dark** one and **4** represents the **darkest** one).
- Please still rank the wheat extrudates even if you only find a very slight difference between them and tick the box.

| No. | Sample Code Number | Slight Difference |
|---|---|---|
| 1. | | ☐ |

| | | |
|---|---|---|
| 2. | | ☐ |
| 3. | | ☐ |
| 4. | | ☐ |

**Comment:**

**B. Texture**

- Take one wheat extrudate strand (choose randomly within one code number).
- Break the strand into two parts. The harder the wheat extrudate, the higher the force you need to break it.
- Rank the **hardness** of the wheat extrudates from lowest to highest using the list below (**1** represents the **least hard** one and **4** represents the **hardest** one).
- Please still rank the wheat extrudates even if you only find a very slight difference between them and tick the box.

| No. | Sample Code Number | Slight Difference |
|---|---|---|
| 1. | | ☐ |
| 2. | | ☐ |
| 3. | | ☐ |
| 4. | | ☐ |

**Comment:**

**C. Flavor**

- Rinse your mouth with a sip of water before trying the sample.
- Start by tasting the sample on the left and move to the right.

- Take a bite of the wheat extrudate and let it stay in your mouth for a few seconds to get the taste.
- Rank the wheat extrudates from lowest to highest using the list below for each characteristic (**1** represents the **least hard** one and **4** represents the **hardest** one).
- Repeat the steps for each characteristic asked below.
- Please still rank the wheat extrudates even if you only find a very slight difference between them and tick the box.
-

| **Characteristics** | **Ranking** | **Slight Difference** |
|---|---|---|
| Rank the sample with regard to the **sweetness** (**1** represents the **least sweet** one and **4** represents the **sweetest** one) | **1.** | ☐ |
| | **2.** | ☐ |
| | **3.** | ☐ |
| | **4.** | ☐ |
| Rank the sample with regard to the **maltiness** (**1** represents the **least malty** one and **4** represents the **most malty** one) | **1.** | ☐ |
| | **2.** | ☐ |
| | **3.** | ☐ |
| | **4.** | ☐ |
| Rank the sample with regard to the **crispiness** (**1** represents the **least crispy** one and **4** represents the **crispiest** one) | **1.** | ☐ |
| | **2.** | ☐ |
| | **3.** | ☐ |
| | **4.** | ☐ |

**Comment:**

## Sensorics Evaluation of Wheat Extrudate – Part II

Imagine yourself as a customer and answer these questions. Don't think too long before you answer the questions, answer the questions based on your first thought of the sample.

1. From four samples you tried earlier, please rank the samples with regard to the overall evaluation starting with your favorite one. (**1** represents your **most favorite** and **4** represents your **least favorite**)

| | |
|---|---|
| 1. | |
| 2. | |
| 3. | |
| 4. | |

2. If you could find this product in the market, how likely are you to buy it?
   - ☐ Never
   - ☐ I am not sure
   - ☐ Of course!
   - ☐ It depends on ... (Please note your comment below)

3. What are the reasons you prefer the chosen sample (rank 1)?

4. What are the reasons for not preferring the other samples?