**TECHNISCHE UNIVERSITÄT MÜNCHEN**

TUM School of Life Sciences

# Bran as a protein source for gluten-free cereal products – Insights in hydration, gelatinization and processing performance

**Maike Föste**

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| Vorsitzende: | apl. Prof. Dr.-Ing. Petra Först |
| Prüfer der Dissertation: | 1. Prof. Dr.-Ing. Thomas Becker |
| | 2. Prof. Dr. Bernd Hitzmann |

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*„Die **Wissenschaft** besteht nur aus Irrtümern.*

*Aber diese muss man begehen. Es sind die Schritte zur **Wahrheit**.“*

*[Jules Gabriel Verne]*

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# Preface

Maike Föste

M. Sc.

The results and publications of this thesis were developed at the Technical University of Munich, Institute of Brewing and Beverage Technology, Research Group Cereal Technology & Process Engineering from January 2011 to Mai 2017.

## Publications

The following peer reviewed publications were generated in the period of this work. Publications, which are part of this thesis are marked in bold.

1 Föste, M., Elgeti, D., Brunner, A.K., Jekle, M., Becker, T.: **Isolation of quinoa protein by milling fractionation and solvent extraction.** Food and Bioproducts Processing 96 (2015), 20-26.

2 Föste, M., Verheyen, C., Jekle, M., Becker, T.: **Fibers of milling and fruit processing by-products in gluten-free bread making: A review of hydration properties, dough formation and quality-improving strategies.** Food Chemistry 306 (2020), 125451.

3 Föste, M., Nordlohne, S.D., Elgeti, D., Linden, M.H., Heinz, V., Jekle, M., Becker, T.: **Impact of quinoa bran on gluten-free dough and bread characteristics.** European Food Research and Technology 239 (2014), 767-775.

4 Föste, M., Jekle, M., Becker, T.: **Structure stabilization in starch-quinoa bran doughs: The role of water availability and gelatinization.** Carbohydrate Polymers 174 (2017), 1018-1025.

5 Elgeti, D., Nordlohne, S.D., Föste, M., Besl, M., Linden, M.H., Heinz, V., Jekle, M., Becker, T.: Volume and texture improvement of gluten-free bread using quinoa white flour. Journal of Cereal Science 59 (2014), 41-47.

6 D'Amico, S., Jungkunz, S., Balasz, G., Föste, M., Jekle, M., Tömösköszi, S., & Schoenlechner, R.: Abrasive milling of quinoa: Study on the distribution of selected nutrients and proteins within the quinoa seed kernel. Journal of Cereal Science. 86 (2019), 132-138.

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# Abbreviations

| | |
|---|---|
| $a_w$ | Water activity |
| BV | Biological value |
| CD | Coeliac disease |
| DACH | Deutschland, Österreich, Schweiz |
| db | Dry base |
| $G^*$ | Complex shear modulus |
| GF | Gluten-free |
| GI | Glycemic index |
| GL | Glycemic load |
| HPMC | Hydroxy-propyl-methyl-cellulose |
| IDF | Insoluble dietary fiber |
| IEP | Isoelectric point |
| Pa | Pascal |
| pH | Decimal logarithm of the reciprocal of the hydrogen ion activity |
| rel. | Relative |
| SD | Standard deviation |
| SDF | Soluble dietary fiber |
| S:L ratio | Solid-to-liquid ratio |
| tan (δ) | Loss factor |
| TDF | Total dietary fiber |
| TM | Trockenmasse |
| $T_{Onset}$ | Start of gelatinization |
| $T_X$ | Time of dough porosity |
| US | United States of Amerika |
| WHC | Water holding capacity |
| WRC | Water retention capacity |
| WBC | Water binding capacity |
| $\Delta$ H | Enthalpy of gelatinization |

## Summary

The processing of plant-based raw materials annually leads to huge amounts of side streams. As these by-products contain valuable macronutrients, valorization of press cake or bran is of growing interest, however, is not fully explored. Several studies pointed out that in particular gluten-free (GF) bakery products require fortification with proteins, minerals and dietary fibers. Unlike gluten-containing bread, the impact of bran on GF bread is seldom investigated and therefore not completely enlightened. Especially soluble or insoluble protein fractions or dietary fibers cause a shift in water absorption and availability in GF dough. Even these hydration properties affect dough functional properties (rheology, gelatinization gas retention) and thus final bread quality. The objective of this thesis was to investigate the potential of quinoa bran for its application in GF bread, considering hydration properties and furthermore to identify mechanisms, that are responsible for stabilizing/ destabilizing GF dough. In regard to processing adaptions prior grinding, optimization of tempering settings revealed protein enrichment many times over up to 27% dry base (db). Subsequent solvent extraction enabled further protein concentration up to > 65% db.

Rheological investigations on GF dough without a material-based water adaption (hydration level of 80%) showed an increase in dough firmness. In addition, gas retention was decreased and final loaf volume impaired by using quinoa bran amounts higher than 20%. The texture of GF bread was significantly improved when 10% quinoa bran was incorporated in the formulation. Determination of gelatinization characteristics provided knowledge on water availability in GF dough. By elevating the bran amount und maintaining the hydration level, the start of gelatinization ($T_{Onset}$) significantly increased, meaning that gelatinization was delayed. To assess quality characteristics of GF bread independently of dough functional properties, a material-based standardization of dough firmness ($G^*$) and $T_{Onset}$ by means of an adapted water addition, in the range of 80 to 120 g water per 100 parts of flour, was conducted. Considering the practical-oriented range for a hydration level in GF dough, neither $G^*$ nor $T_{Onset}$ could be adjusted by a definite water amount. However, by elevating the water content, a decrease in gelatinization temperature was achieved, consequently improving final loaf volume and crumb texture.

In summary, the results enlightened that quinoa bran has high potential to improve GF bread quality. In addition, it confirms that recipe formulation and water addition have to be carefully coordinated and require specific adaptions depending on the hydrophilic macronutrients that influence availability of water in GF dough. Finally, it is recommended to aim at a temperature at which gelatinization begins as soon as possible (indicated by $T_{Onset}$) in order to receive a high gas holding capacity. Consequently, gas bubbles can be stabilized in the GF dough matrix and final quality of bran-enriched GF bread can be improved.

## Zusammenfassung

Durch die Verarbeitung pflanzlicher Rohstoffe entstehen jährlich große Mengen an Kleie oder Presskuchen mit zahlreichen wertvollen Inhaltsstoffe. Deren Potenzial für die Weiternutzung in Lebensmitteln ist oftmals nicht vollständig bzw. nur teilweise erschlossen. Gleichzeitig lässt sich einer Vielzahl von Studien entnehmen, dass glutenfreie Backwaren aus ernährungsphysiologischer Sicht einer Anreicherung mit Ballaststoffen, Proteinen oder Mineralstoffen bedürfen. Im Gegensatz zu glutenhaltigen Backwaren sind die bisherigen Erkenntnisse zum Einfluss von Kleie in glutenfreien Produkten selten. Insbesondere lösliche/unlösliche Proteinfraktionen und Ballaststoffe haben Einfluss auf die Absorption von Wasser und somit dessen Verfügbarkeit im Teig. Aufgrund dieser Hydratisierungseigenschaften werden die funktionellen Teigeigenschaften (Rheologie, Verkleisterung, Gashaltefähigkeit) und folglich die finale Brotqualität maßgeblich beeinflusst. Das Ziel dieser Arbeit bestand darin, das Potenzial von Quinoakleie für die Anwendung in glutenfreien Backwaren unter Berücksichtigung der Hydratisierungseigenschaften zu analysieren und mögliche Mechanismen zu identifizieren, die zur Stabilisierung/Destabilisierung der Teigstruktur beitragen können. Im Rahmen einer angepassten Konditionierung vor der trockentechnischen Fraktionierung wurde der Proteingehalt in Quinoakleie bezogen auf die Trockenmasse (TM) um ein Vielfaches auf 27 % erhöht. Durch anschließende Extraktionsversuche gelang zudem die Aufkonzentrierung des Proteins auf > 65 % TM.

Im Rahmen von rheologischen Untersuchungen glutenfreier Teige mit variierendem Kleieanteil und konstantem Wassergehalt zeigte sich eine zunehmende Teigfestigkeit. Ebenso waren die Gashaltefähigkeit und folglich das Gebäckvolumen mit steigendem Kleieanteil reduziert. Zur Verbesserung des Brotvolumens, der Krumenhärte und der sensorischen Attribute wurde eine Quinoakleiemenge von 10 % identifiziert. Die Bestimmung der Verkleisterungseigenschaften lieferte zudem Erkenntnisse zur Verfügbarkeit des Wassers im Teig. So nahm der Verkleisterungsbeginn ($T_{Onsett}$) mit steigendem Kleieanteil bei gleichbleibender Wassermenge signifikant zu. Um eine Beurteilung unabhängig von der Teigfunktionalität vornehmen zu können, wurde nachfolgend die Teigfestigkeit bzw. der Verkleisterungszeitpunkt justiert. Dazu wurde der Wasseranteil sukzessive von 80 g auf bis zu 110 g pro 100 Teile Mehl erhöht. Weder die Teigfestigkeit noch die Verkleisterungseigenschaften ließen sich durch eine definierte Wasserzugabe einstellen. Allerdings bewirkte die Erhöhung der Wassermenge einen früheren $T_{Onset}$, wodurch sich zugleich auch das Brotvolumen und die Krumentextur verbesserten.

Zusammenfassend kann in dieser Thesis festgehalten werden, dass der Einsatz von Quinoakleie die Qualitätsmerkmale glutenfreier Brote entscheidend verbessern kann, dies jedoch ein hohes Maß an Abstimmung der Rezepturkomponenten und des Wasseranteils

erforderlich macht. Basierend auf den Erkenntnissen dieser Arbeit wird das Justieren eines möglichst frühen $T_{Onset}$ empfohlen, um die Gasblasen in glutenfreien Teigen zu stabilisieren und somit die Brotqualität zu verbessern.

# 1 Introduction

Recently, plant protein sources heavily came to the focus of food industry and research, to meet the rising demand for meat or dairy alternatives and the development of innovative life style products. The amount of plant proteins used for food applications is steadily increasing, achieving about 20 thousand metric tons in the European Union (Mulder, van der Peet-Schwering, Hua, & van Ree, 2016). Thus, more attention has also been paid to sustainable concepts, such as circular bioeconomy that maximizes efficient use of resources and minimizes waste (Overturf et al., 2020). The growing population is only one of the driving forces to valorize agricultural by-products into worthwhile food additives, such as proteins or dietary fibers, to complement human nutrition. Soy, wheat or peanut are good protein sources, however, they are responsible for more than 90% of allergic reactions to food (Hefle, Nordlee, & Taylor, 1996). Therefore, also alternative protein sources from for e.g. rice, legumes or pseudocereals are gaining attention. Rice is one of the most essential cereals for the human diet with annual global production amounting to 495.78 million metric tons for milled rice (Shahbandeh, 2020, November 01.). Since the majority of rice is consumed as white rice, over 60 million metric tons of bran emerge as by-product of the milling process (Childs, 2003; J. W. Lee et al., 2005; Simpson, Aryee, & Toldrá, 2019). Even if in lesser quantities, also other gluten-free (GF) raw materials result in huge amounts of by-products that are directly discarded or used as animal feed (Orthoefer & Eastman, 2004) (see Fig. 1).

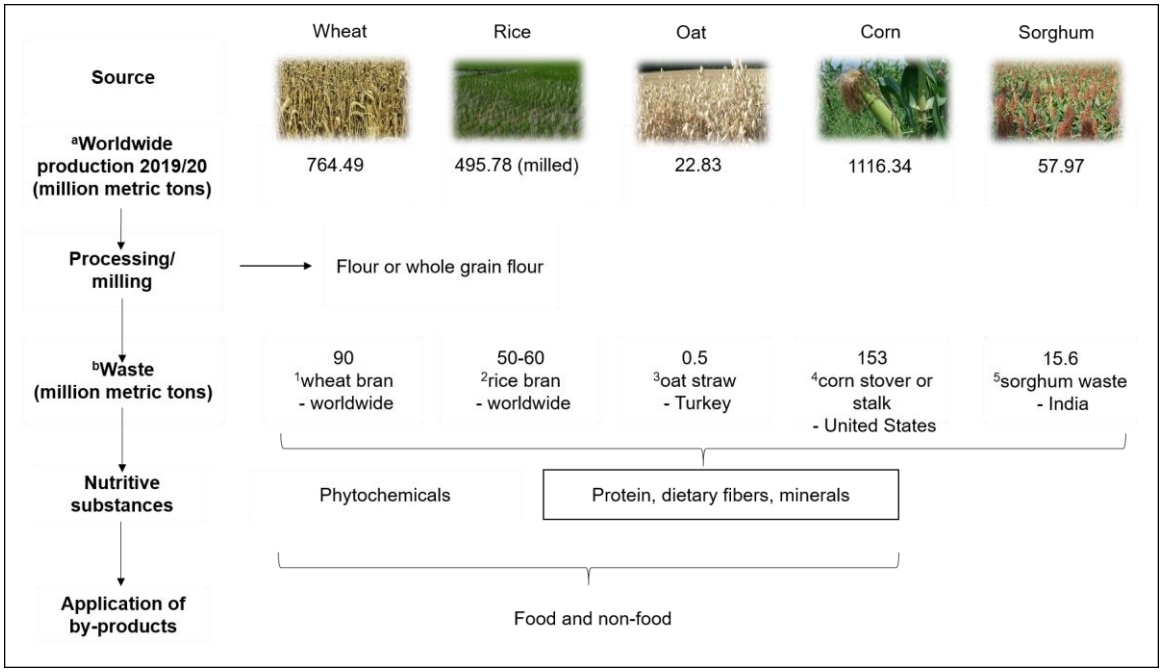


**Figure 1: Processing of wheat and selected gluten-free raw materials in the scope of arising by-products, its nutritive substances and potential applications.** (a) Shahbandeh (2020, November 01.); (b) Simpson et al. (2019). Data are adapted from 1) Onipe, Jideani, and Beswa (2015), 2) J. W. Lee et al. (2005), 3) Balat (2005), 4) Kadam and McMillan (2003) and 5) Jekayinfa and Scholz (2009).

Bran is rich in protein, dietary fibers and minerals and therefore presents a suitable raw material to enrich foods of inferior nutritional composition such as GF bakery products (Matos Segura & Rosell, 2011; Thompson, 2000). The incorporation of bran into dough is related with technological challenges during processing, as bran is known to have a high water retention capacity (WRC), which leads to an uncontrolled shift in dough hydration. However, the knowledge of hydration properties in GF dough is not fully understood yet. Against this background, the following introduction is divided into four topics. First: highlighting the necessity to enrich GF foods, second: showing the potential of quinoa as protein and dietary fiber source, third: revealing procedures for protein and fiber enrichment and fourth: discussing the technologically relevant key values (viscosity, gelatinization, gas bubble stabilization) during the processing (dough formation, proofing, baking) of bran-enriched GF bread.

## 1.1 Nutrient deficiencies - the necessity to enrich gluten-free foods

Coeliac disease (CD) is an autoimmune disorder of the small intestine that relates to the ingestion of gluten or related prolamines (Burger et al., 2017; Miranda, Lasa, Bustamante, Churruca, & Simon, 2014). To achieve complete remission of appropriate symptoms, susceptible individuals have to eliminate gluten and therefore strictly have to follow a GF diet (Kelly, Feighery, Gallagher, & Weir, 1990; Pantaleoni et al., 2014). As GF bakery products are commonly based on refined flours and/or starches, they also contain lower amounts of the valuable micro and macro substances compared to their gluten-containing counterparts such as wheat breads (Thompson, 2000; Wild, Robins, Burley, & Howdle, 2010). A study on the nutritional pattern of GF bread representatives in the Spanish market revealed strongly differing amounts of protein (0.9-15.5 g/100 g), fat (2.0-26.1 g/100 g), minerals (1.1-5.4 g/100 g) and dietary fiber (1.3-7.2 g/100 g) (Arranz, Fernández-Bañares, Rosell, Rodrigo, & Peña, 2015; Matos Segura & Rosell, 2011). While protein contents in GF bread were lower than recommended, especially rapidly digestible starch ranged from 75.6 to 92.5 g/100 g and thus significantly contributed to the carbohydrate reference intake (Matos Segura & Rosell, 2011). For packaged GF foods a high density of fat and sugar has been observed by Kulai and Rashid (2014). In addition, low levels of folate, thiamine, riboflavin, niacin and iron have been reported (Thompson, 1999). To sum up, GF bread strongly varies in nutrient composition, with a generally high glycaemic index (GI). The interrelation between a GF diet and deficiencies in alimentary intake of dietary fibers, minerals and vitamins (B, D, folate) has been observed by Vici, Belli, Biondi, and Polzonetti (2016) and Shepherd and Gibson (2013). Similarly, Martin, Geisel, Maresch, Krieger, and Stein (2013) reported a lower fiber content in the diet of coeliac patients and significantly lower levels of folic acid, vitamin C and B12 in comparison to the DACH reference value. A dietary survey in the US revealed inadequate intakes of fiber, ferrum and calcium, respectively, in female coeliacs on a strict GF diet (Thompson, Dennis, Higgins,

Lee, & Sharrett, 2005). A ten-year study showed signs of vitamin deficiency in half of coeliac patients following a GF diet (Hallert et al., 2002), being attributed to their dietary habit and food choice. In terms of an unbalanced vitamin D and calcium supply (Caruso, Pallone, Stasi, Romeo, & Monteleone, 2013) coeliacs have a 40% higher risk for bone fractures than non-affected patients (Grace-Farfaglia, 2015; Pantaleoni et al., 2014). Next to CD, further health issues may be present in those patients leading to allergic reactions or reduced nutrient uptake as a result of the damaged intestinal mucosa.

Lactose intolerance

As CD leads to the inflammation of the small intestine, there is often a link to secondary lactose intolerance. Patients with this diagnosis suffer from a villous atrophy because of the absence of a specific enzyme. Normally, lactase is located in the mucosa of the small intestine however, when absent lactose cannot be metabolized (Ortolani & Pastorello, 2006). Therefore, the application of high lactose whey powders in GF dough processing is not suitable for coeliac patients, as these consumers do not posses lactase (Ortolani & Pastorello, 2006). In consideration of technological aspects, improved water absorption and enhanced handling of the dough and/or batter were reported after the application of dairy additives for the processing of GF products (Gallagher, Gormley, & Arendt, 2004). The impact of different dairy powders on GF bread quality was investigated by Gallagher, Kunkel, Gormley, and Arendt (2003). They found that dairy powders with a high protein and low lactose content improved the appearance and volume and moreover led to a firmer crumb texture (Gallagher et al., 2003). For coeliac patients this means that the ingredients list has to be checked in detail, before the purchase decision can be made.

Diabetes Type 1 and glycemic response

CD is associated with an increased prevalence of insulin-dependent type 1 diabetes mellitus (Cronin & Shanahan, 1997). Besides a strict GF diet, also the maintenance of a good glycemic control is of great importance for individuals with concomitant diseases (Giuberti & Gallo, 2018). According to Capriles and Arêas (2014), foods are classified and compared regarding to their postprandial glycemic response. Therefore two different key values are used, namely the GI and the resulting glycemic load (GL). To calculate the GI, a fix amount of carbohydrates available in a test food is divided by the same amount of available carbohydrates from a reference food (normally glucose) multiplied with 100, showing the increase in glycemic response (Jenkins et al., 1981). By multiplying the GI of each food with the amount of carbohydrates in the food portion, the GL is achieved (Capriles & Arêas, 2014; Salmerón et al., 1997). Reliable data of the GI and GL can be taken from the “International Tables of Glycemic Index and Glycemic Load Values” (Atkinson, Foster-Powell, & Brand-Miller, 2008).

Due to their starchy composition, GF bread oftentimes cause high glycemic response, ranging from 83.3 to 96.1 in comparison to 71 for white wheat flour bread (Capriles & Arêas, 2013; Matos Segura & Rosell, 2011; Penagini et al., 2013). Several authors reported about different clinical health issues for e.g. over-weight, new-onset insulin resistance, obesity and metabolic syndrome in connection with a GF diet (Kabbani et al., 2012; Reilly et al., 2011; Rewers, 2005; Tortora et al., 2015; Tucker, Rostami, Prabhakaran, & Dulaimi, 2012). To overcome these nutritional disadvantages, especially plant sources rich in soluble fiber, inulin-type fructans and resistant starch can decrease the glycemic response. This is of high relevance for individuals, who simultaneously suffer from CD and type 1 diabetes (Capriles & Arêas, 2013; Murray, 2005). In conclusion, it is necessary to identify alternative plant proteins and to develop new processing strategies to nutritionally and qualitatively improve GF bread.

## 1.2 Quinoa - micronutrients, macronutrients and phytochemicals

The disadvantageous composition of GF bread requires the selection of alternative raw materials that match nutritional expectations and fit technological requirements. Several studies clearly suggested the application of pseudocereals, as they meet the requirements to enrich GF foods (Alvarez-Jubete, Arendt, & Gallagher, 2009, 2010; Kupper, 2005; Saturni, Ferretti, & Bacchetti, 2010). Due to its worthwhile composition and important role in combating starvation, malnutrition and poverty, the year 2013 was avowed as “International Year of Quinoa” by the United Nations General Assembly (FAO, 2013; Nascimento et al., 2014). The reference work by Souci, Fachmann, and Kraut (2008) showed that quinoa has a particularly high mineral, protein and dietary fiber content in comparison to conventional cereals and common GF flours/ starches (see Tab. 1).

**Table 1: Summary of the analytical key components in gluten-free cereals and pseudocereals.**

| | Minerals (g/100 g) | Protein (g/100 g) | Carbohydrates (g/100 g) | Dietary fiber (g/100 g) | Energy (Kcal/ 100g) |
|---|---|---|---|---|---|
| **Flours** | | | | | |
| Rice | 0.6 | 7.2 | [a)]79.6 | | 351 |
| Corn | 1.2 | 8.7 | [a)]66.3 | [b)]9.4 | 323 |
| **Starches** | | | | | |
| Rice | 0.4 | 0.8 | 85.0 | | 343 |
| Corn | 0.2 | 0.4 | 85.9 | | 346 |
| Potato | 0.4 | 0.6 | 83.1 | | 336 |
| Tapioca | 0.3 | 0.6 | 84.9 | | 344 |
| **Pseudocereals** | | | | | |
| Amaranth | 3.3 | 15.8 | 56.8 | [c)]8.0 | 365 |
| Quinoa | 3.3 | 14.8 | 58.5 | [c)]11.0 | 334 |
| Buckwheat | 1.7 | 9.8 | 71.0 | [c)]4.0 | 336 |

Values are adapted from Souci et al., (2008). [a)]Estimated by the difference method: 100-(water + protein (N x 5.8) + fat + minerals), [b)]Modified AOAC method. [c)]Retrieved from J. H. Lee, Kim, and Aufhammer (1995) and Glowienke, (1997).

Quinoa (*Chenopodium quinoa* Willd.) is a dicotyledonous plant, which has its origin in the Andean highlands of South America (Lamothe, Srichuwong, Reuhs, & Hamaker, 2015).

Because of its tolerance against cold and drought, the cultivation of quinoa is possible in higher altitudes. Classified as a gluten-free pseudocereal, the containing seeds of quinoa can be milled either into whole grain flour or bran and flour. These small-sized seeds consist of micronutrients, macronutrients and phytochemicals, which are located in different seed tissues and can be classified according to their solubility in hydrophilic and lipophilic components (see Fig. 2).

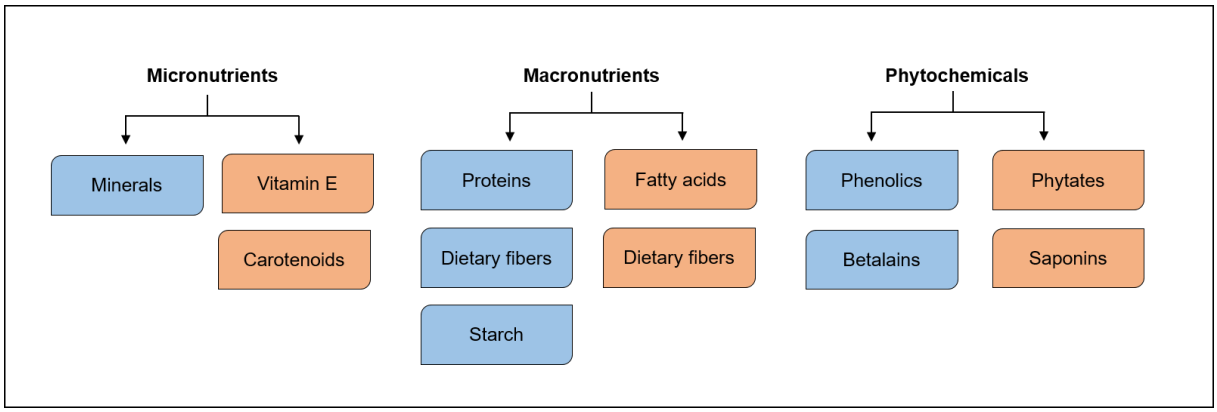


**Figure 2: Overview on micronutrients, macronutrients and phytochemicals in quinoa.** Presented are hydrophilic (■) and lipophilic (■) components.

Micronutrients, including **minerals or vitamins,** are primarily contained in quinoa's seed coat and the embryo. Contents of ferrum (11-33 mg/ 100 g) and calcium (100-150 mg/100 g) are higher in quinoa than in wheat, barley, sorghum or rice (Alvarez-Jubete et al., 2009; Kozioł, 1992; Penagini et al., 2013). Also contents of magnesium, copper, manganese and chloride are higher than in common cereals (Kozioł, 1992). In addition to minerals, quinoa seeds are rich in vitamin B, C and E (Kozioł, 1992; Repo-Carrasco, Espinoza, & Jacobsen, 2003).

Macronutrients, include protein, soluble and insoluble dietary fiber, starch and fat. Starting with quinoa **protein**, this is localized in the embryo (57%), the perisperm (39%) and seed tissues (4%) as stated by Ando et al. (2002). The structural form is constituted of protein bodies with globoid crystals (Prego, Maldonado, & Otegui, 1998). According to their solubility in different media, proteins are classified into albumins (soluble in water), globulins (soluble in saline), prolamins (soluble in alcohol) and glutelins (insoluble), so called "Osborne fractionation" (Osborne & Harris, 1905). Unlike common cereals, quinoa is rich in the water-soluble albumin and salt-soluble globulin fraction. The major protein fractions are the 11S globulins, which account for about 37 to 38% of the total quinoa protein and the 2S albumins with a proportion of 25 to 31% (Capraro et al., 2020). The 11S storage protein also known as „chenopodin" was characterized by Brinegar and Goundan (1993). These authors showed that chenopodin consists of two subunit groups linked by a disulfide bond. The molecular weight of the acidic A subunit group varies between 32 to 39 kDa and the basic B subunit group reveals

a size of 22 to 23 kDa (Brinegar & Goundan, 1993). In contrast, the 2S cystein-rich albumin fraction has a molecular weight of 8 to 10 kDa (Brinegar & Goundan, 1993; Brinegar, Sine, & Nwokocha, 1996; Dakhili, Abdolalizadeh, Hosseini, Shojaee-Aliabadi, & Mirmoghtadaie, 2019). Contents of albumin, globulin or glutelin for quinoa were reported to be (33.1%, 28.9% and 31.6%), whereas these values greatly differ from rice (10.8%, 9.7% and 77.3%) and corn (4.0%, 2.8% and 45.3%) (Ando et al., 2002; Belitz, Grosch, & Schieberle, 2009). According to their physicochemical composition, protein fractions are ascribed different functional properties for e.g. foaming, gelling or emulsifying characteristics. Thus, for quinoa albumins with a low molecular weight and high amount of acidic amino acids, good foaming properties were reported (Elsohaimy, Refaay, & Zaytoun, 2015). Moreover, from the technological point of view, the sulphur containing amino acids are of great interest for gel formation. The presence of sulphur containing amino acids may favor non-covalent interactions (hydrophobic, hydrogen and electrostatic interactions) or covalent interactions (disulfide bonds) between sulfhydryl-groups (Chen, Swaisgood, & Foegeding, 1994). Consequently, these interactions may lead possibly to disulfide bond formation when denatured and thus contribute in structure formation during heating. The nutritional uptake of proteins and its metabolization is important with regard to different physiological functions and the immune system. For each protein source a biological value (BV) is assigned. This value indicates how close the composition of amino acids in a protein match with the protein requirements for a human body. Compared to the high BV of animal source proteins (cow milk: 84.5 and whole egg: 93.7), plant-based proteins are generally lacking in one or more of the essential amino acids achieving therefore lower BVs (wheat: 64; rice: 64; corn: 60) (J. A. Gonzalez, Konishi, Bruno, Valoy, & Prado, 2012). Standing out among them, quinoa protein achieves high contents of lysine (twice that of wheat and three times that of corn), methionine, arginine, tryptophan and sulphur containing amino acids, reaching a BV of 83 (Ruales & Nair, 1992). Lysine is the first limiting essential amino acid, meaning that it first becomes deficient in the diet. This amino acid is primarily present in animal-based protein sources, however, because of the high lysine amount, quinoa can be seen as a nutritionally adequate vegetarian/ vegan protein source.

Carbohydrates are the major component in quinoa varying from 58 to 74% db. In comparison to corn or rice, the carbohydrate content in quinoa is lower (see Tab. 1). This might contribute to a lower glycemic response as underpinned by Priyanka, Suneetha, Maheswari, Suneetha, and Kumari (2018). Quinoa **starch**, located in the perisperm, achieves contents of 52.6 % db and is constituted by an amylose to amylopectin ratio of 11:89. The starch granules are characterized by a small size of 2 µm and a gelatinization temperature varying from 57 to 64 °C (Atwell, Patrick, Johnson, & Glass, 1983; Qian & Kuhn, 1999). In quinoa, mono- and disaccharides each represent about 2% db, crude fibers vary from 2.5 to 3.9% db and for pentosans 2.9 to 3.6% db have been reported (Valencia-Chamorro, 2003). Saturni et al. (2010)

observed higher contents of **total dietary fiber** in the range of 7 to 10 g/100 g in respect to other cereals, fruits and nuts. Although quinoa is consumed in the same way as cereals, one of the major difference is that insoluble dietary fibers (IDF) of quinoa do not contain xylans. Unlike fruits and vegetables, in which the dietary fibers are hydrated, dietary fibers from in pseudocereals appear in a more dehydrated form. A detailed investigation of dietary fiber fractions from quinoa was conducted by Lamothe et al. (2015). They identified 22% of the total dietary fiber (TDF) to be soluble dietary fiber (SDF) and 78% to be IDF as presented in Table 2.

**Table 2: Composition of dietary fibers in quinoa seeds according to their solubility.**

| | Fiber components | Main chain | Branch chain | Amount (%) |
|---|---|---|---|---|
| TDF | | | | 9-12 |
| IDF | | | | 78 |
| | Lignin | | | ~ 9 |
| | Xyloglycan | β-D-(1,4) glucose | α-D-xylose | ~ 30 |
| | Pectic polysaccharides | | | ~ 55 |
| | Homogalacturonan | α-(1,4) galacturonic acid, (carboxyl groups are partly methyl esterified) | | |
| | Rhamnogalacturonan I | (1,4) galacturonic acid, (1,2) rhamnose and 1-, 2-, 4- rhamnose | Galactose, arabinose, xylose, rhamnose, galacturonic acid | |
| | Arabinanes | α-(1,5)-L arabinofuranose | α-arabinose | |
| | Galactanes | β-(1,4)-D-galactopyranose | | |
| SDF | | | | 22 |
| | Xyloglycan | β-D-(1,4) glucose | α-D-xylose | ~ 40-60 |
| | Pectic polysaccharides | | | ~ 55 |
| | Homogalacturonan | α-(1,4) galacturonic acid, (carboxyl groups are partly methyl esterified) | | |
| | Arabinanes | α-(1,5)-L arabinofuranose | α-arabinose | |

Distribution of all shares for fiber components belonging to the appropriate insoluble or soluble fiber fraction. These values refer to 100% either for IDF or as well for SDF. Abbreviations: TDF: Total dietary fiber; SDF: Soluble dietary fiber; IDF: Insoluble dietary fiber. Modified from the data of Lamothe et al. (2015) and Elleuch et al. (2011).

Lamothe et al. (2015) clearly pointed out that IDF contained lignin, xyloglycans and pectic polysaccharides. The latter are divided into homogalacturonans, rhamnogalacturonan I, arabinanes and galactanes. In contrast, SDF contained xyloglycans and as well pectic polysaccharides, classified into homogalacturonans and arabinanes. In particular, the xylose content in these SDFs is lower than in common cereals. Because of their advantageous effects on health and gut viscosity, SDFs are ascribed to decrease the cholesterol level and glycemic response (Mann & Cummings, 2009), and therefore are of special interest for further research. Besides their impact on health issues, these SDFs will also change the functional properties of a GF formulation.

Phytochemicals such as phenolics, phytates, saponins and small amounts of trypsin inhibitors were found in quinoa (Ando, Tang-Hanjun, Mitsunaga, & Tang, 1999; Chauhan, Eskin, & Tkachuk, 1992; Coulter & Lorenz, 1990; J. Gonzalez, Roldan, Gallardo, Escudero, & Prado, 1989; Ruales & Nair, 1993).

**Phenolics** may include hydrophilic substances such as phenolic acids, flavonoids and tannins. As stated by Y. Tang and Tsao (2017) they account for the main proportion of plant secondary metabolites and show huge differences in their physiological effects. Phenolic acids can be predominantly found in quinoa's seed coat (Y. Tang & Tsao, 2017). Recently, numerous phenolic compounds (amongst others: vanillic acid, ferulic acid) have been identified in black, red and white quinoa seeds (Pereira et al., 2020). Also flavonoids were found in the outer seed tissues of quinoa, which are ascribed valuable antioxidative effects on consumer's health (Pereira et al., 2020). For instance kaempferol or quercetin as well as catechin, epicatechin, or epigallocatechin were found in quinoa seeds (Y. Tang & Tsao, 2017). In addition, also betalains are present in the pericarp and have been divided into two subgroups: betacyanins, of red-violet color and betaxanthins of yellow-orange color (Imamura et al., 2018). Based on UV/Vis absorbance data, a total betanin content of 0.6 to 9.7 mg/kg was determined in quinoa (Escribano et al., 2017).

In general, the **phytic acid** (myoinositol hexaphosphoric acid) content in quinoa varies from 10.5 to 13.5 g/kg (Kozioł, 1992) and is lower than in soy or rice (unpolished and cooked) (Kumar, Sinha, Makkar, & Becker, 2010). Because of its molecular structure, phytic acid strongly chelate with cations. The formation of insoluble salts may occur with calcium, magnesium, ferrum, potassium, zinc or copper, so called phytates (Kumar et al., 2010). Consequently, phytates negatively affect the absorption, digestion and availability of these minerals (Kumar et al., 2010). In addition, a decrease in protein solubility, enzymatic activity or proteolytic digestibility may result from the complex formation with proteins in a wide pH range.

The bitter tasting **saponins** are primarily located in quinoa's pericarp (Ruales & Nair, 1993) and may favor complex formation with proteins or lipoids (e.g. cholesterol). This may result in a hemolytic effect (hemolysis of red blood cells) as well as complex formation with zinc or ferrum, lowering minerals' bioavailability (Chauhan et al., 1992). Depending on their saponine content, quinoa is classified into sweet and bitter varieties (Koziol, 1991; Valcárcel-Yamani & Lannes, 2012). Whereas bitter genotypes contain 0.14 to 2.3% saponins, sweeter varieties comprise 0.02 to 0.04% saponins (Chauhan et al., 1992; Cuadrado et al., 1995; Güçlü-Üstündağ, Balsevich, & Mazza, 2007). To reduce saponine contents in quinoa, abrasive dehulling, a combination of abrasion and washing or extrusion can be applied. Reichert, Tatarynovich, and Tyler (1986) found that 1.2 to 14.8% of the seed had to be removed by means of tangential abrasive dehulling to obtain an acceptable saponine level. Two different

approaches for saponine reduction were tested by Chauhan et al. (1992). While whole quinoa seeds contained 2.05% saponin, this value decreased to 1.39% after manually dehulling and to 0.70% after water-dehulling in the analyzed whole grain flours. In contrast, the bran fraction achieved higher saponin contents than the whole grain flour after appropriate treatments (Chauhan et al., 1992). To reduce the bitter taste of the seeds, Zhu et al. recommended to use slightly alkaline water (2002). Ridout, Price, Dupont, Parker, and Fenwick (1991) observed a comparable saponine reduction level after either washing or abrasion. These combined technologies enabled to physically remove the saponine-rich pericarp and concomitantly minimize the losses of nutrients.

## 1.3 Methods of protein and dietary fiber enrichment

Over the years, methods to generate fractions of GF raw materials with different technological applications gained numerous attraction. Before the mid 1980s, quinoa was mostly used as whole seeds or ground into whole grain flours. Newer approaches focus as well on efficient ways to extract individual components contained in the seeds. The processing of protein concentrates from quinoa and evaluation of their functional properties has been the objective of a US patent No. 7,563,473 (Scanlin & Stone, 2009). As quinoa seeds contain many secondary phytochemicals and shelf-life reducing enzymes, first processing steps should consider the reduction of for e.g. saponins by appropriate pre-treatment. Especially endogenous enzymes, such as lipase favor autoxidation during storage and may contribute to a bitter, grassy flavor (Rackis, Sessa, & Honig, 1979). One approach to isolate the most important macronutrients (fat, protein, carbohydrates) is outlined in Figure 3.

The first step is to fractionate the seed tissues. In general, milling has been conducted by rollers or grinding discs in order to split the kernels of amaranth, oat, rice, buckwheat or quinoa into functional seed tissues such as a protein and a starch-rich fraction. Several studies have been conducted on the milling of quinoa (Becker & Hanners, 1990; Chauhan et al., 1992; Reichert et al., 1986). The adjustment of processing parameters is, however, a neglected issue. Especially, tempering is a decisive key factor to separate the bran from starch as this approach toughens the kernel. In addition, tempering prevents the formation of bran powder during grinding and consequently simplifies physical separation. In wheat milling, the tempering approach is one of the most important steps prior to milling as it affects breakage of endosperm particles and thus determines the extent of mechanical starch damage during milling. Hard wheats are tempered between 15 to 19%, soft wheats require lower moisture content between 14.5 to 17% using a resting time in the range of 18 to 72 h (Hibbs & Posner, 1997). The amount of water, which is required to elevate the actual moisture content ($M_0$) to a targeted moisture content ($M_1$) of a definite seed weight ($m_{seed}$), is calculated according to Equation 1.

$$Water\ addition\ (g) = m_{seed} \times \frac{M_1 - M_0}{100 - M_1} \qquad \textbf{Equation 1}$$

This approach targets kernel moisture elevation by adding water for a defined period of time (resting time). However, knowledge on tempering of niche raw materials is scarce in contrast to wheat. In particular, roller milling of amaranth or quinoa might be a technological challenge because of the small kernel size. For amaranth, various milling gap settings were analyzed by means of a modified stone mill, which uniformly removed all of the germ and most of the bran from the kernel (Becker, Irving, & Saunders, 1986). As pointed out by Becker et al. (1986) moisture content being higher than 17.8% led to sticking of the ground amaranth at the mill faces and the mill chamber. In comparison to wheat or corn, removal of the pericarp, seed coat and germ increased the fiber and fat content in the bran fraction of pseudocereals (Belitz et al., 2009). Rimbach, Möhring, and Erbersdobler (2010) stated that the bran fraction in wheat accounted for 17% of the dehulled kernel, whereas, after laboratory milling of quinoa, the bran fraction accounted for some 40% of the dehulled seed (Chauhan et al., 1992). Whether it is possible to shift the milling yield of flour to bran by tempering of quinoa is therefore one of the gaps in this thesis, that has to be elucidated by fundamental analysis.

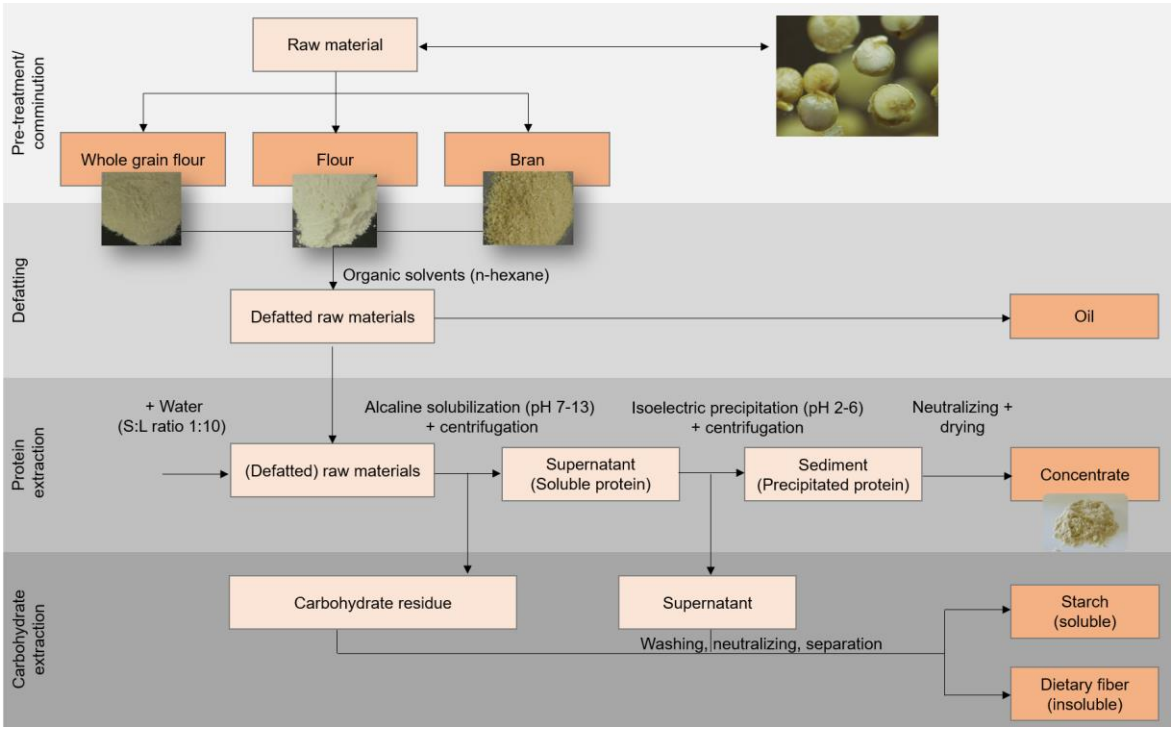


**Figure 3: Flow chart for dry fractionation and further extraction of oil, protein, starch and carbohydrates.** First row: Pre-treatment (including cleaning, hulling, tempering, comminution) of raw material to achieve whole grain flour, bran or flour. Second row: Defatting to achieve oil. Third row: Protein extraction by alkaline solubilization and isoelectric precipitation to obtain a concentrate. Fourth row: Carbohydrate extraction to obtain starch or dietary fiber. Modified from Scanlin and Stone (2009).

By defatting the flour or bran with organic solvents (e.g. hexan, ethanol) or alternative methods such as carbon dioxide extraction, oil can be obtained. How much of the solvent may be left in the sample after extraction is regulated in the “Extraktionslösungsmittelverordnung” (ElmV) (BMJV, 1991, November 08.).

For the extraction of proteins, native or defatted whole grain flour, flour or bran can be used as initial substrate. Thereby, flour’s or bran’s constitution (particle size, surface, disturbing components) as well as the extraction settings determine final purity and recovery. In the last years, protein isolation from cereal by-products has been intensively analyzed for rice bran (J. S. Hamada, 2000; S. Tang, Hettiarachchy, & Shellhammer, 2002), oat bran (Guan & Yao, 2008) and wheat bran (Prückler et al., 2014). However, thinning out investigations on protein isolation for quinoa or amaranth (defatted or non-defatted) were conducted (Abugoch, Romero, Tapia, Silva, & Rivera, 2008). To solubilize proteins, hydrophobic side chains must orientate to the inner molecule part, whereas polar and charged residues orientate to the molecular surface close to the water phase (Baltes & Matissek, 2011). According to their solubility, for quinoa primarily albumin fractions are dissolved in aqueous media considering specific alkaline pH-values. Then, solubility of proteins can be determined according to Equation 2 and is expressed as percentage of crude protein (N x 5.54) present in the supernatant ($m_{p,\ sup.}$) in relation to bran’s total protein content ($m_{p,\ bran}$) (Paredes-López, Guzman-Maldonado, & Ordorica-Falomir, 1994).

$$Solubility\ (\%) = \frac{m_{p\ sup.}}{m_{p\ bran}} \times 100 \qquad \textbf{Equation 2}$$

To facilitate protein dissolution, aqueous salt solutions (e.g. sodium (bi)-carbonate, sodium chloride or sodium sulphite, at various concentrations or buffer solutions (e.g. Tris-HCl) can be applied. Depending on the extraction focus, a preliminary defatting of raw materials may prevent later formation of lipid-protein complexes.

Following the dissolution step, proteins have to be precipitated which can be performed by means of isoelectric precipitation, precipitation with ammonium sulphate as well as concentration procedures such as dialysis, ultrafiltration or ion chromatography. By the addition of an acidic solution, changes in charge distribution of the amino acid and carboxyl-groups are induced, leading to protein flocculation. For protein precipitation, pH-value at the isoelectric point (IEP) has to be chosen, where proteins are simultaneously characterized by highest stability (Baltes & Matissek, 2011). Positive and negative charges are balanced at the IEP, so that protein-protein interaction increase, whereby net charge is at its minimum, leading to decreased interaction of water with proteins (Aceituno-Medina, Mendoza, Lagaron, & López-Rubio, 2013). Finally, precipitation yield can be determined according to Equation 3 and is expressed as percentage of the mass of the precipitate ($m_{prec.}$) multiplied with the protein

content of the precipitate ($p_{prec.}$) in relation to the mass of the bran ($m_{bran.}$) multiplied with the protein content of the bran ($p_{bran}$).

$$Yield\ (\%) = \frac{m_{\,prec.} \times p_{prec.}}{m_{bran} \times p_{bran}} \times 100 \qquad \textbf{Equation 3}$$

According to the protein content achieved after fractionation, extraction products can be classified as follows: Flours reveal a protein content up to 65% db, concentrates have a protein content of 65 to 90% db and isolates achieve a protein content of more than 90% db (Oreopoulou & Tzia, 2007). Consequently, concentrates or isolates vary in their functional properties (gelling, foaming and emulsifying characteristics) and therefore can be applied in appropriate food systems. From an economic point of view, most of the plant-based proteins are cheaper than animal-based protein sources, varying in the range of 2 to 3 Euro/ kg for soy and pea concentrates in contrast to whey concentrate with a protein content of 80% (5.5 Euro/ kg) or casein (6.5 Euro/ kg) (Mulder et al., 2016).

The major part of carbohydrates remains in the residue after protein extraction. Thus, carbohydrates can be exploited from the following two steps: 1) After solubilization with subsequent centrifugation and 2) After precipitation with subsequent centrifugation (see Fig. 3). Depending on the processing step, either starch or dietary fibers can be obtained from the polysaccharides. In the last years, the extraction of plant proteins from side streams gained numerous attention, because of its importance to enrich foods with desirable functional properties. Thus gelling, foaming or emulsifying characteristics were used to improve for e.g. meat substitutes, dairy alternatives and the development of “free-from” products. How the addition of these components is accompanied by changes in functional properties, also in respect of the baking performance in GF dough, will be introduced in the following section.

## 1.4 Baking performance of bran-enriched gluten-free dough

The processing of GF bread is influenced by dough viscosity, starch gelatinization and gas bubble stabilization. In general, the absence of network forming proteins in GF dough promote a settling of yeast and starch granules (Schober, 2009). Consequently, a loss of gas bubbles may result in a foamy structure. As the hydration of GF flour components is less effective and the proteins do not posses network forming characteristics comparable to wheat dough, this results in a liquid dough, resembling more a batter-like structure. Hydrocolloids are often added, to increase dough viscosity and to form a gel-like structure that mimic somehow the gluten-network (see Fig. 4) (Toufeili et al., 1994). Regarding to gas bubble stabilization, dough viscosity should neither be too liquid nor too solid, otherwise phenomena such as coalescence may occur. Increased dough viscosity and strengthened boundaries between expanding gas cells, may lead to improved gas retention and final bread volume as stated by Lazaridou, Duta, Papageorgiou, Belc, and Biliaderis (2007).

During baking, thermal transition from the liquid to the solid state occurs. Within this process, starch granules swell and finally gelatinize, giving the characteristic crumb texture of GF bread (see Fig. 4).

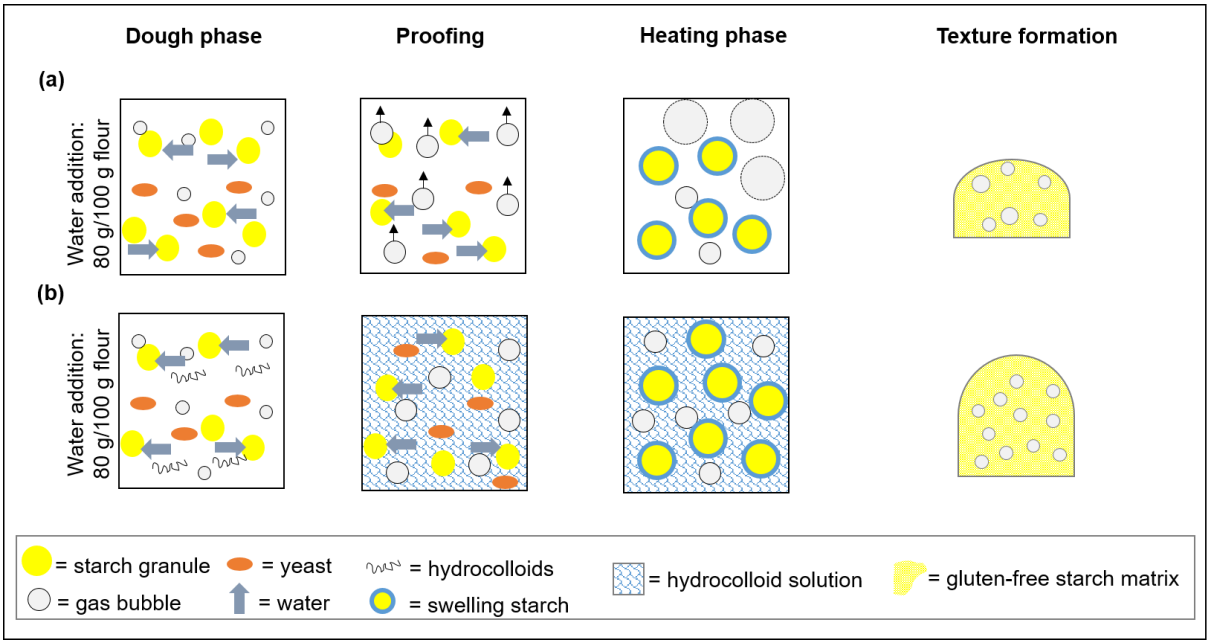


**Figure 4: Schematic visualization of the changes in starch granules and gas bubbles during the processing of gluten-free bread.** Presented are processing stages for (a) Starch-based GF dough and (b) Starch-based GF dough with hydrocolloids.

Unlike GF dough, in wheat dough a viscoelastic network is formed after hydration of flour particles during kneading, enclosing gas bubbles so that dough rises as fermentation proceeds. The enrichment of wheat dough by bran or dietary fibers in order to increase the dietetic value or for technological purposes was studied by lots of researchers (Noort, van Haaster, Hemery, Schols, & Hamer, 2010; Pomeranz, Shogren, Finney, & Bechtel, 1977; Xu, Luo, Yang, Xiao, & Lu, 2019). The drawbacks of fiber addition on wheat dough were reported to include the increase in water absorption and dough strength as well as impaired mixing and firming tolerances (Curti, Carini, Bonacini, Tribuzio, & Vittadini, 2013; Özkaya, Turksoy, Özkaya, & Duman, 2017; Rosell, Santos, & Collar, 2006; Zhang & Moore, 1997). In most cases, quality attributes of wheat bread were impeded after incorporation of cereal bran, which was expressed by decreased loaf volume, higher crumb firmness and a coarser crumb texture (de Kock, Taylor, & Taylor, 1999; Gan, Ellis, Vaughan, & Galliard, 1989; Gan, Galliard, Ellis, Angold, & Vaughan, 1992; Haridas Rao & Malini Rao, 1991; Lai, Hoseney, & Davis, 1989; Özkaya et al., 2017). Explanatory approaches referred to the disruption of gluten films (physical effect) (Gan et al., 1989; Gan et al., 1992; Pomeranz et al., 1977) or weakening of the gluten network and agglomeration changes in gluten by glutathione as reducing agent (chemical effect) (Noort et al., 2010; Rosell, Santos, & Collar, 2010). A possible explanation was that wheat bran leads to changes in the gluten conformation as bran promotes redistribution of

water in the dough, leading to lower hydration of the gluten network (Bock, Connelly, & Damodaran, 2013). Unlike wheat dough, this might not be the case in GF dough because of the absence in the gluten network.

Recently, Xu et al. (2019) analyzed the impact of increasing amounts of quinoa flour in a wheat dough formulation and observed a decrease in bread loaf volume. However, direct comparability with the formulation used in this thesis is not possible, because of the following two aspects. First, the investigated microstructures were based on wheat flour and thus succumb the formation of a gluten network. Second, wheat flour basis was replaced with quinoa flour instead of bran. Nevertheless, from these results it can be assumed that hydrophilic macronutrients and phytochemicals impaired the gluten network formation of the investigated wheat dough (Xu et al., 2019). Regarding to GF dough systems, precisely investigations to declare the impact of bran on the relevant key values for structure formation (viscosity, gelatinization and gas bubble stabilization) are limited. Furthermore, the elucidation of mechanisms during structure formation depending on dough hydration level are a neglected issue. Because of the absence in the gluten network, especially GF dough would represent an ideal matrix to study the impact of bran on dough functionalities.

As initially pointed out in the former chapters, bran is composed of dietary fibers and protein. Depending on their botanical origin, different types of polysaccharides, e.g. cellulose, hemicelluloses or pectines are contained. Consequently, water absorption may vary for pure bran as well as in combination with other recipe components in the GF dough formulation, thus changing dough functional properties. For instance, Rocha Parra, Ribotta, and Ferrero (2015) showed that apple fiber, consisting primarily of IDFs showed huge water absorption and therefore it was assumed that these fibers were in competition with the disposable water amount in the GF dough formulation. On the contrary, for the application of SDF from rice bran, beneficial effects on GF bread color, loaf volume and crumb firmness were reported (Phimolsiripol, Mukprasirt, & Schoenlechner, 2012).

Plant proteins are applied amongst others to increase the viscosity of GF dough, which improves dough formation in terms of gelatinization and as well to support foam stabilization (Arendt, Morrissey, Moore, & Bello, 2008; Damodaran, 1994; Kato, Ibrahim, Watanabe, Honma, & Kobayashi, 1990). Especially plant proteins for e.g. from soy are larger than high-molecular-weight gluten subunits or dairy proteins (Day, 2013). This is the reason why they form a thicker interfacial layer at the interface between oil and water (Wong et al., 2012). In their native form, none of the plant proteins are able to form a viscoelastic network during typical dough formation (Day, 2013). Although it was stated by Taylor, Taylor, Campanella, and Hamaker (2016) that a viscoelastic network with α-zein and co-proteins is not enough to mimic gluten-network, promising results for viscoelastic dough formation by using proteins

from amaranth, maize, sorghum or millet were mentioned by Avanza, Puppo, and Añón (2005) as well as by Lawton (1992). Particularly, the addition of soy protein isolate may increase the formation of disulphide linkages and thus provide dough elasticity (Hettiarachchy & Kalapathy, 1998). By using a formulation based on cassava starch and rice flour Sanchez, Osella, and de la Torre (2002) observed large holes in the crumb of GF bread. However, the application of soy flour significantly improved the crumb structure (Crockett, Ie, & Vodovotz, 2011). Enhanced gas retention and improved water binding of the loaves within the addition of soy protein isolate were observed by Arendt et al. (2008). From these findings, it was concluded that dough rheological characteristics were positively influenced by the addition of suitable protein-rich flours or concentrates. More viscous batter systems, either by the use of soy protein isolate or by the incorporation of hydrocolloids, were reported to be advantageous for GF loaf volume development (Crockett et al., 2011; Lazaridou et al., 2007). In this context, a reference value for targeted adjustment of dough viscosity would be helpful for the processing of high quality GF bread.

The distribution of protein fractions is one of the driving force, influencing the functional properties of proteins (gel formation, foaming, emulsification and solubility). Therefore, the presence of e.g. albumins or globulins might also result in changes of the structure formation in GF dough. In literature it is stated, that proteins with a high albumin and globulin content such as from quinoa or legumes (soy, pea, lupine) show good water solubility in contrast to cereals with a high prolamin/ glutelin fraction (zein). This has been attributed to their lower contents in charged amino acid residues (Day, 2013). In particular, globular proteins tend to form their gels through protein denaturation after heat treatment, due to balanced protein-protein and protein-solvent interactions (Day, 2013). As foaming properties are essential to stabilize gas bubbles in GF batter (Elgeti, Jekle, & Becker, 2015), it has been pointed out by several co-workers that albumin-rich plant proteins from pea, lupines, or quinoa (36% in embryo) showed good foam stabilizing properties similar to egg white (Alamanou & Doxastakis, 1997; Elsohaimy et al., 2015). Increased loaf volume in GF bread was observed when albumin-rich protein fractions such as from lupine (Ziobro, Witczak, Juszczak, & Korus, 2013) or other legumes (Miñarro, Albanell, Aguilar, Guamis, & Capellas, 2012) were incorporated. Ziobro et al. (2013) assumed that albumin fractions possibly stabilize the crumb structure in early stages of baking, due to a lower denaturation temperature.

Whereas dough functional properties can be easily determined, the impact of selected recipe components considering hydration characteristics of raw materials is more difficult to transfer to the GF formulation. A fundamental knowledge on the estimation of water uptake in raw materials might be helpful to control final product quality. In general, the composition of a traditional wheat dough formulation is calculated based on a standardized procedure to

guarantee better comparability. Therefore, the calculation of Baker's percentage is used, which refers to the flour of the formula as 100 parts of flour (= 100%). To determine Baker's percentage, the ingredient weight is divided by the total flour weight and multiplied with 100% (Healea, 2007, April). By means of measuring the dough development in a Farinograph, differences in water absorption resulting from flour's origin, can be adjusted. Thus, the missing water to attain a moisture content of 14% is automatically balanced by water addition. Following the determination of Baker's percentage, also the hydration level of a formulation can be determined. The hydration level is defined by the mass of water ($m_{water}$) in relation to 100 parts of flour ($m_{flour}$) and can be calculated according to Equation 4.

$$Hydration\ level\ (\%) = \frac{m_{water}}{m_{flour}} \times 100 \qquad \textbf{Equation 4}$$

With regard to final product quality, differentiation in traditional wheat products is made between a low hydration level (50-57%) leading to stiff dough to be used in the processing of bagels, a moderate hydration level (58-65%) for the processing of sandwich or European bread and high hydration level (65-80%) resulting in wet and sticky dough for the processing of ciabatta or focaccia. Because of the poor hydration capacity in GF flours and starches, the hydration level in these formulations is innately higher. Moreover, investigations of water absorption by means of a Farinograph is restricted to the application for bran-enriched GF dough. The Farinograph measurement does not reveal the mechanism of the process at molecular scale, limiting its application to improve dough quality through the adjustment of processing settings (Schiraldi & Fessas, 2012). Thus, neither the improvement of dough quality through recipe modifications or baking conditions nor the interactions between recipe components can be displayed. Therefore, the determination of the required hydration level in a bran-enriched GF dough is more complicated and in most cases is performed arbitrarily.

Certainly, the water content in a formulation plays a major key role regarding to dough functional properties. The water content is highly depending on the initial hydration level and the competition between recipe components. Because of its polarity, water interacts with hydrophilic macromolecules from either starch or protein by forming hydrogen bonds, resulting in hydration. Consequently, also the proportion of free or bound water may change in the formulation. To get information on the proportion of free available water in a formulation, water activity ($a_w$) can be determined. In accordance with Syamaladevi et al. (2016) this key value is defined as measurand of water's energy status in a food system. The $a_w$ is calculated as the ratio of water vapor pressure ($P_v$) to the saturation vapor pressure ($P_{vs}$) considering food system's temperature presented in Equation 5 (Syamaladevi et al., 2016).

$$a_w = \frac{P_v}{Pvs} \qquad \textbf{Equation 5}$$

Whereas the $a_w$-value may vary from 0.287 for corn starch to 0.350 for wheat flour (Schmidt & Fontana, 2020), this value is significant higher in GF bread > 0.920 (Matos & Rosell, 2013). It is assumed that the application of bran, containing hydrophilic protein fractions or dietary fibers, may change the disposable water amount in a GF formulation and consequently must be properly synchronized. Therefore, a critical consideration of a polymer-based water absorption either by polysaccharides (starch, dietary fibers) or proteins would help to better understand the mechanisms that are responsible for stabilizing/ destabilizing bran-enriched GF dough.

With regard to the constitution of brans' dietary fiber, it could be hypothesized that increasing the amount of bran will increase starch gelatinization due to a limited amount of water in the dough. At this point, it would be helpful to develop a material-based characteristic that enables targeted control of GF bread quality, which does actually not exist. Due to the scarce knowledge and the elucidation of mechanisms leading to loaf volume decrease in bran-enriched GF dough, fundamental research is necessary to understand the mechanisms that influence the structure-function relationships in bran-enriched GF formulations.

## 1.5 Thesis outline

The importance to nutritionally enrich GF bakery products has been an elementary pillar in research over the last years. In wheat dough, the detrimental effects by bran from wheat, rye, oat, rice, corn on increased dough firmness, decreased final loaf volume, elevated crumb hardness and oftentimes altered sensory profile has been the objective of lots of research studies (Noort et al., 2010; Xu et al., 2019). However, if those reported negative side effects are transferable to quinoa bran throughout the processing of GF bread are largely unknown. In the light of a different dough matrix, also the hydration level in gluten-free and gluten-containing dough greatly vary. Unfortunately, the use of bran as additive for GF bakery products remains challenging, because dough functional properties are not completely enlightened up to now. Depending on the bran type, detailed scientific knowledge is missing on hydration properties and its transferability to GF dough formulations. No fundamental study on hydration properties in GF dough has been performed, although different concentrations of fibers and bran have been applied (Sabanis, Lebesi, & Tzia, 2009) and the use of additional water amounts has been recommended (Aprodu, Badiu, & Banu, 2016). However, the application of quinoa bran, as a niche by-product, to improve GF bread quality was not discussed in a study before. In addition, also a detailed study on changes in viscosity and gelatinization of bran-enriched GF dough considering the hydration level of the formulation is missing so far.

This thesis aims to show the relevance in enriching GF bread with nutrients from bran, focussing on bran's effect on hydration properties and disclosing consequences on structure formation in GF dough. According to the current state of knowledge, the following working hypotheses must be clarified:

- Dry fractionation of quinoa as a very gentle approach enables protein enrichment in the bran fraction and subsequent aqueous extraction of proteins from quinoa bran enables to achieve a concentrate with a protein content ≥ 65% db.
- A critical review on milling and fruit processing by-products reveals the differences in hydration properties, the impact of fibers and bran on dough formation and shows strategies for modifying bran-enriched GF bread quality.
- Incorporation of excessive bran in GF dough favors a weaker dough structure because of changes in dough functionality or destabilization by bran particles.
- Adjustment of the dough firmness $G^*$ and/or the start of gelatinization $T_{Onset}$ enables standardizing of the dough functional properties and bread quality characteristics.
- An optimized hydration level counteracts a structure-weakening effect by quinoa bran particles and stabilizes the GF dough.

The following points are taking up to confirm or decline the hypotheses listed above. To close the corresponding research gaps subsequent facts will be addressed. First, studies on fractionation performance to increase protein content in separated seed tissues of quinoa and the resulting concentrate will be performed. Therefore, tempering settings (e.g. moisture content, resting time) prior milling are optimized and settings for aqueous extraction (e.g. pH-value, temperature, duration, centrifugation speed) are developed. Within this approach, first insights into the extraction of quinoa proteins and their solubility are achieved.

The identification of challenges associated with brans' water absorption, its possible shift in water availability in GF dough and the resulting impact on dough functionality, has to be critically reviewed. Different approaches to quantify hydration properties in GF raw materials and its transferability to GF dough are addressed. To provide an in-depth look on the impact of bran on structure-function relationship throughout GF dough processing, batter rheology, stabilization of gas bubbles, gelatinization and structure stabilization are pointed out. Furthermore, strategies to modify hydration properties throughout GF bread processing are presented.

To close the knowledge gap between bran and its mode of action on GF dough, firmness $G^*$ and gelatinization ($T_{Onset}$ and $\Delta H$) will be fundamentally investigated considering the hydration level of the formulation. Initially, to achieve a base line, a fixed dough hydration level of 80% is settled. Characterization of GF dough is performed in a mixture of rice and corn flour combined with corn starch. $G^*$ and gas retention kinetics are examined to identify the maximum bran amount before a structure loss is promoted.

Moreover, to demonstrate that bran changes water availability in GF dough, the formulation is simplified hence to a corn-starch-based system, and the enthalpy changes are calculated considering the appropriate starch content of each recipe. To gain a tool for standardizing final bread quality (specific loaf volume, crumb hardness and baking loss), the property-based adjustment of $G^*$ and $T_{Onset}$ through elevation of the dough hydration level, in a preferably practicable orientation, is investigated. Lastly, possible mechanisms that stabilize or destabilize GF dough are elucidated. Figure 5 summarizes the addressed chapters in this thesis including the procedures that are performed to evaluate the hypotheses.

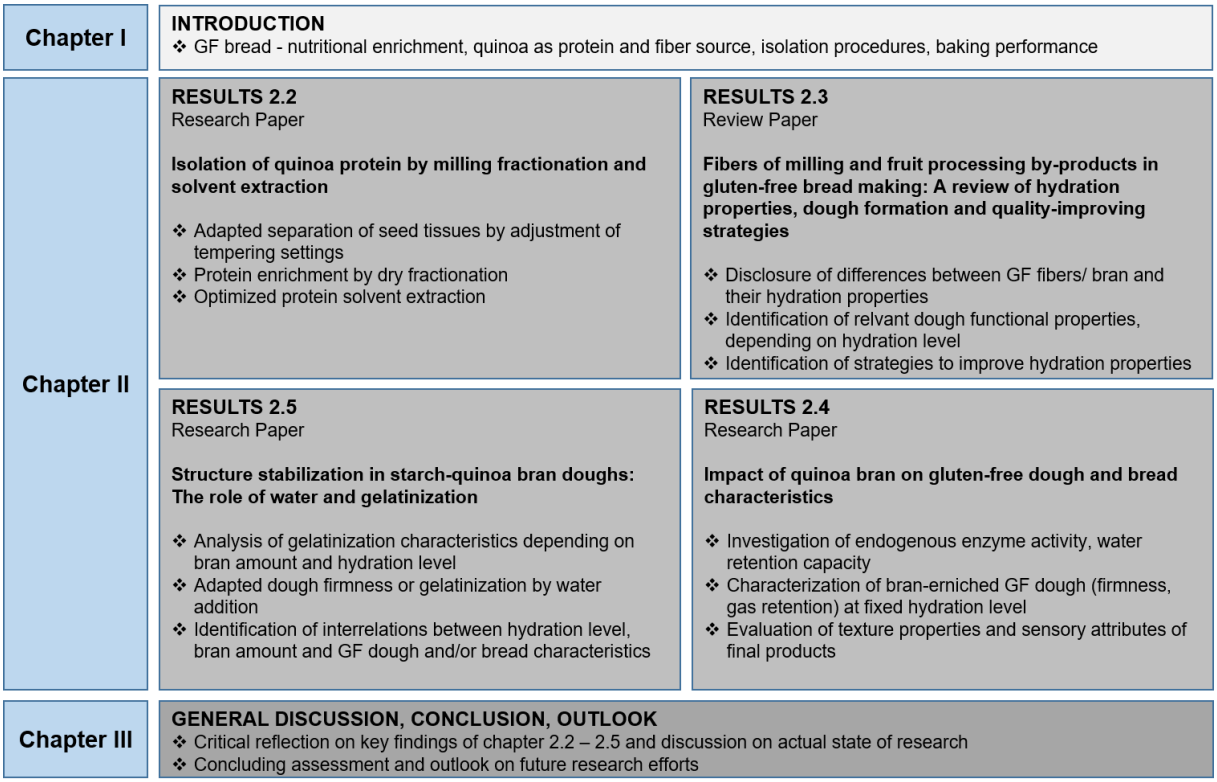


**Figure 5: Thesis outline.**

In conclusion, this knowledge is fundamental to increase the awareness of the need to adjust dough functional properties (dough firmness, gelatinization) of bran enriched GF dough. From an economic point of view, the adjustment of dough hydration level is of huge importance to minimalize processing losses because of inferior final bread quality. The successful incorporation of quinoa bran in GF bakery products could provide an immense chance to use a dry fractionated side stream product to enrich GF bread with protein and dietary fibers.

## 1.6 Methods

This section contains the main methods used for gluten-free dough and bread analysis.

**Investigations on analytical composition and functional properties**

The methods used for determining the analytical composition and functional properties are presented in Table 3.

**Table 3: Applied methods for analytical and technological investigations of quinoa bran.**

| Analysis | Method | Source | For detailed description see |
|---|---|---|---|
| **Moisture content** | AACC Method 44-01 | AACC International. Approved Methods of Analysis 2002 | Sections 2.2; 2.4; 2.5 |
| **Protein content** | AACC Method 46-10; N x 5.54 | AACC International. Approved Methods of Analysis 2002 | Sections 2.2; 2.4; 2.5 |
| **Ash content** | AACCI Method 08-12.01 | AACC International. Approved Methods of Analysis 2002 | Sections 2.2; 2.4; 2.5 |
| **Lipid content** | AACC Method 30-25 | AACC International. Approved Methods of Analysis 2002 | Section 2.5 |
| **Solvent retention capacity** | AACCI Method 56-11.02 | AACC International. Approved Methods of Analysis 2002 | Section 2.4 |
| **Particle size distribution** | ICC Method 207 | ICC – International Association for Cereal Science and Technology, 1998 | Section 2.2 |

**Dry fractionation**

Quinoa seeds were ground in an ultra-centrifugal mill ZM 200 (Retsch, Haan, Germany) using a mesh screen of 500 µm to obtain whole grain flour (see section 2.2). To fractionate the seeds, first the moisture content of whole grain flour was determined according to AACC standard method 44-01. Tempering was done in a hermetic sealed box and the initial moisture content was increased by spraying water onto quinoa seeds, followed by manual stirring. Tempered quinoa seeds were allowed to rest at room temperature (20 °C) for 16 h or 20 h, respectively. Different milling approaches were conducted in a Quadrumat Junior mill (Brabender GmbH &

Co. KG, Duisburg, Germany). This laboratory mill separated the seeds into quinoa white flour and bran through a rotating sifter having a mesh size of 200 µm. Detailed information on tempering settings can be found in the first publication of this thesis (Föste, Elgeti, Brunner, Jekle, & Becker, 2015).

**Extraction and purification of proteins**

After dry fractionation, quinoa bran was taken to further extract and concentrate the proteins (see section 2.2). In a first step, extraction settings (e.g. pH-value, temperature, particle size) were varied to solubilize maximum of proteins. In a second step, purification of protein was performed by the adjustment of processing settings such as pH-value or centrifugation speed. Re-suspension and neutralization to pH 7 was performed for precipitates before freeze-drying for a minimum of 48 h. This process was conducted by means of a Beta 2-8 freeze-dryer with LMC-1 control, (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). The settings and procedure of these two protein processing steps are described in the first publication of this thesis (Föste, Elgeti, et al., 2015).

**Determination of endogenous enzyme activity**

Endogenous enzymes have a decisive impact on storage, autoxidation, delivery of substrates or other dough related properties. Proteolytic activity may favor the liberation of amino groups and/ or acids, affecting dough rheology and as well liberation of precursors for the formation of aroma compounds and maillard reaction. Amylolytic enzymes such as α-glucosidase enable substrate delivery of glucose, which is relevant for yeast metabolization and the formation of carbon dioxide during dough proofing. Both enzymes were characterized and a detailed description of the assay, its settings and its definition are presented in the third publication of this thesis (see section 2.4).

According to Föste et al. (2014) proteolytic activity was investigated on GF model dough containing bran and distilled water in a ratio 1:1. To avoid undesirable growth of microorganisms, chloramphenicol and cycloheximide were added, each 0.02 % based on overall dough composition, before incubation (30 °C; 26 h). Monitoring of lactobacilli strains, yeast development and pH-values was performed in the beginning and in the end of incubation. To determine proteolytic activity, dough samples were collected in two-hour intervals, centrifuged and the supernatant was diluted (1:100) with distilled water. Determination of free amino groups in diluted model dough samples was conducted according to an adapted ninhydrin assay (Krauss, 1967). In order to perform photometric measurements, either a sample (containing 300 µL of incubated sample) or a blank (containing distilled water) was needed. These were mixed with cadmium-ninhydrin reagent, which consisted of 0.8 g ninhydrin, 80 ml ethanol combined with 10 ml acetic acid and a cadmium chloride-

hemipentahydrate solution, following a second incubation step (84 °C; 5 min) and cooling of samples on ice prior analysis. The reaction between free amino acids and/or amino groups with cadmium-ninhydrin reagent led to complex formation, which was photometrically determined using a wavelength of 507 nm in a Specord 210 plus photometer (Analytik Jena GmbH, Jena, Germany). To establish the calibration curve, glycin (neoLab Migge Laborbedarf GmbH, Heidelberg, Germany) was used.

To determine α-glucosidase activity, 1.0 g of quinoa bran was extracted in a 200 mM NaAc buffer solution (pH 4.5). After centrifugation, the supernatant was analyzed by means of the amyloglucosidase assay (Megazyme, Wicklow, Ireland). Therefore, thermostable β-glucosidase in combination with p-nitrophenyl-β-D-maltoside was used in accordance with Föste et al. (2014).

**Rheological investigations**

To determine the rheological characteristics of GF dough, an AR-G2 controlled stress rheometer (TA Instruments, West Sussex, UK) was used (see section 2.4 and 2.5). Dough sample analysis was performed with a parallel plate geometry of 4 cm diameter and a gap of 3 mm between the plates. The procedure and its settings as well as the definition of the most relevant key variables are precisely described in the third and in the fourth publication of this thesis (Föste, Jekle, & Becker, 2017; Föste et al., 2014).

**Proofing characteristics**

To investigate the development of GF dough and its gaseous release during proofing a Rheofermentometer (F3) (Chopin, Villeneuve-La-Garenne, France) was used (see section 2.4). Therefore, GF dough (315 g) was prepared, positioned in the measuring vessel and fermented at 30 °C for 3 h without using a cylindrical weight. The third publication of this thesis gives detailed description of the procedure, its settings and its key values (Föste et al., 2014).

**Investigations of gelatinization profile**

Thermal properties of GF dough samples were monitored by means of differential scanning calorimetry (DSC) (see section 2.5). Investigations were conducted in a DSC 6 (Perkin Elmer Inc., Wellesley, USA) and data were analyzed with the Pyris Manager Thermal Analysis Software. An empty pan was used as an inert reference and differential heat flow between sample and reference was measured at atmospheric pressure. Calibration of the equipment was performed with n-Octadecane (MP 8.5 °C) and Indium (MP 196 °C). Conditions of analysis and target figures to evaluate thermal properties, start of gelatinization $T_{Onset}$ (°C) and gelatinization enthalpy $\Delta H$ (J/g), are precisely explained in the fourth publication of this thesis (Föste et al., 2017).

**Dough preparation and characterization of gluten-free bread**

Two different formulations were used to study the impact of quinoa bran in GF dough. Detailed information regarding the composition of the appropriate formulation and its preparation process are described in the third and the fourth publication of this thesis (Föste et al., 2017; Föste et al., 2014)

- GF dough was prepared in a KitchenAid 5KSM150 (KitchenAid, St. Joseph, USA). Therefore, all ingredients were kneaded before weighing the GF dough (250 ± 0.05 g) into baking tins. Dough samples were tempered for 30 min in a proofing cabinet. Following, dough samples were positioned in a Matador MD 120 deck oven (Werner & Pfleiderer, Dinkelsbühl, Germany) and baked for 35 min at 220 °C. Initial steam level was set to 3.6 l/m³.
- Determination of GF loaf volume was conducted in accordance with the AACC method 10-05.01 in a BVM-L370 laser-based volumeter (TexVol Instruments AB, Viken, Sweden). To calculate the specific bread volume (mL/g), loaves were divided by their weight. Therefore, bread weight was recorded by means of a Kern QKE 8K005 laboratory balance (Kern & Sohn, Balingen-Frommern, Germany). Bake loss was calculated according to the following equation: ([weight of loaf before baking – weight of loaf after baking]/ [weight of loaf before baking] x 100) (Capriles & Arêas, 2013).
- Analysis of crumb characteristics was performed by means of a TVT-300 XP texture analyzer (Perten Instruments, Hägersten, Sweden). An aluminium-based cylindrical plug of 20 mm diameter was used as measuring device. The measurements followed the AACC method 74-09.
- Sensory evaluation of GF bread was performed on a scale from zero to ten by a trained panel (n > 10). The following attributes were addressed: Crust color, crumb porosity, firmness, juiciness, odor intensity, bitterness, off-flavor and overall acceptability.

**Characterization of color**

Milling fractions were evaluated regarding their color characteristics by using a spectrophotometer. This device was fitted with an optical sensor (BYK Gardner GmbH, Geretsried, Germany) (see section 2.2). For calibration of the instrument, a white standard color calibration plate was used. Based on the coordinates of the CIE L*, a*, b* color space, L* indicates brightness, a* the red-green axis and b* stands for the yellow-blue axis (Edney, Rossnagel, Endo, Ozawa, & Brophy, 2002). Detailed description including key variables are presented in the first publication of this thesis (Föste, Elgeti, et al., 2015).

# 2 Results (Thesis publications)

## 2.1 Summary of thesis publication

This chapter includes a summary of each thesis publication and additionally lists the full copies of their published version.

**Isolation of quinoa protein by milling fractionation and solvent extraction** **Pages 32-38**

The increasing demand for plant protein and concepts of sustainability require the adjustment of processing settings and fractionation technologies to receive worthwhile macronutrients. This study demonstrates that prior grinding of quinoa seed, the tempering is an important step to better separate functional seed tissues and to concentrate the protein in the bran fraction. While dry fractionation of unconditioned seeds revealed a protein content of 23.9% db in the bran, tempering (15% moisture; 16 h) significantly increased the protein content by 16% rel. In general, bran accounts as a by-product from the milling industries, however, in view of sustainable concepts can be seen as potential source to further extract proteins, dietary fibers or oil. Unlike the gentle fractionation procedure, in a second step, feasibility of protein isolation from quinoa bran by means of solvent extraction is investigated. Settings for aqueous extraction, considering pH-values for solubilization and precipitation, temperature and time, are analysed. Optimized settings for aqueous extraction of quinoa bran (S:L ratio 1:10; pH 10, 20 °C, 1 h) revealed 60% protein solubility. Further purification yielded a concentrate of 68% db protein, in comparison to the use of comparable extraction settings for whole grain flour (52% db) (see Fig. 6).

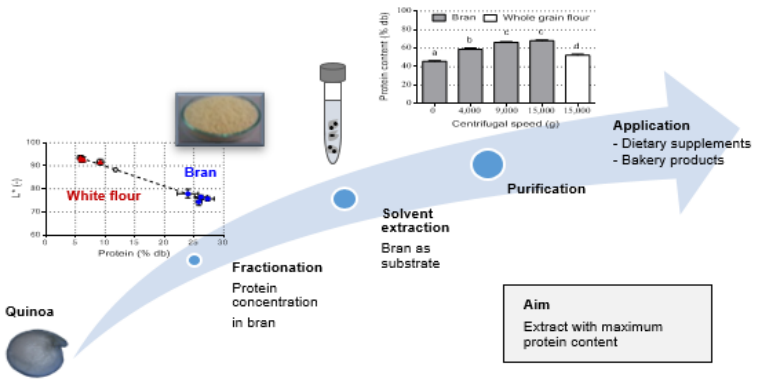


**Figure 6: Graphical abstract of the first publication.**

Authorship contributions: The doctoral candidate created the study concept, conducted literature search, data creation, analysis and its interpretation as well as drafted and revised the manuscript. The co-authors supported data creation and discussion as well as critically revised and approved the manuscript.

**Fibers of milling and fruit processing by-products in gluten-free bread making: A review of hydration properties, dough formation and quality-improving strategies** **Pages 39- 49**

Low nutritive value, high glycemic index and a short shelf-life are some of the major drawbacks of GF bread. Concurrent, food processing industries provide huge amounts of side streams, for e.g. bran, which contain worthwhile components (proteins, dietary fibers, minerals). The application of bran and/ or dietary fibers to counteract these deficits is a potential approach, however, is also faced with technological challenges. This review points out the differences in analytical constitution of bran and dietary fibers. At the same time, it highlights the huge variations in hydration properties of the analyzed raw materials, which were determined by using different methods for instance the swelling capacity, water binding capacity or water absorption. However, these values only give an indication of what the required water amounts are in GF dough and batter. While in wheat dough a standard device (Farinograph, Mixolab) is used to estimate water absorption, an appropriate transfer to bran-enriched GF dough and batter is questionable, because of the absence of network forming gluten proteins. In addition, the impact of bran and/or dietary fibers throughout the processing of GF bread, considering batter hydration level, is discussed. The findings of this review revealed firmer GF dough and simultaneously loaf volume decrease (1-2 ml/g) when using a low hydration level of ~80%. One of the major outcome of this review is that only few of these studies addressed a delay in thermal transformation and recommend to adjust the hydration level in order to stabilize the GF dough matrix. Furthermore, SDF are identified to significantly improve aging kinetics in GF bread. Finally, strategies for modifying or improving water absorption through pre-fermentation, enzymatic cross-linking or thermal treatment in fiber-enriched GF foods are addressed.

Authorship contributions: The doctoral candidate designed the study concept, conducted the literature search, performed data creation, analysis and its interpretation as well as drafted and revised the manuscript. The co-authors supported data analysis and discussion as well as critically revised and approved the manuscript.

**Impact of quinoa bran on gluten-free dough and bread characteristics** **Pages 50-58**

The processing of bran-enriched GF bread requires specific tuning of recipe components and the hydration level of the formulation. Quinoa bran and its impact on GF dough and bread characteristics has not been investigated yet. To obtain initial insight in how quinoa bran changes dough firmness, gas retention properties and final bread quality, in this study a rice/corn flour based control formulation with a constant water dosage (80 g/100 g flour) is used. By replacing the flour mixture with up to 40% quinoa bran, a significant increase in the complex shear modulus ($G^*$) by means of oscillatory measurements was observed. Additional investigations on gas holding properties revealed that GF dough containing 40% quinoa bran was more porous, starting its gaseous release earlier than the control formulation, which was indicated by the time of dough porosity ($T_x$). Unlike the control formulation, specific loaf volume in GF bread with 40% quinoa bran was reduced by 14.8% rel. Surprisingly, by replacing the flour mixture with 10% quinoa bran, specific loaf volume was improved by 7.4% rel. and also the crumb firmness was reduced by 22.3% rel. in comparison to the control formulation without bran. Sensory analysis highlighted lower firmness, higher juiciness and despite a slight off-flavor, an increase in overall acceptability.

Authorship contributions: The doctoral candidate designed the study concept, conducted the literature search, performed data creation, analysis and its interpretation as well as drafted and revised the manuscript. The co-authors supported in data creation, analysis and discussion as well as critically revised and approved the manuscript.

**Structure stabilization in starch-quinoa bran doughs: The role of water availability and gelatinization** **Pages 59-66**

Taking up the findings of the review and the previous study, high amounts of quinoa bran increased dough firmness (*G**) and reduced gas retention, destabilizing GF dough, so called "structure weakening". As dough functional properties are depending on the availability of water, the present study persecutes two approaches. First, the impact of bran on water availability in GF dough is demonstrated by quantification of the start of gelatinization ($T_{Onset}$) and the resulting enthalpy (∆ H), being determined in a simplified model formulation based on corn starch with constant water content (80 g water per 100 g flour). When 50% of corn starch was replaced by quinoa bran, an increase in $T_{Onset}$ and a decrease in ∆ H has been observed. In addition, for ∆ H, also the theoretically needed enthalpy, resulting from starch replacement with bran, is calculated to demonstrate bran's water uptake. Second, the effect of bran, independent of *G** and $T_{Onset}$ is considered. For this purpose, these key variables are adjusted by targeted water addition in a practical oriented range (90 to 110 g water per 100 g starch). Findings of this approach disclose that higher water amounts, far above from the practical range would have been required for standardization of *G** and $T_{Onset}$. However, the results clearly showed a connection between an increase in water addition and a decrease in $T_{Onset}$. Furthermore, it is highlighted that $T_{Onset}$ correlated well with the specific volume of GF loaves (r = -0.9042). In conclusion, a shift in $T_{Onset}$ by water addition is recommended to stabilize the structure of GF bread enriched with quinoa bran and to counteract the structure-weakening effect by bran.

Authorship contributions: The doctoral candidate designed the study concept, conducted the literature search, performed data creation, analysis and its interpretation as well as drafted and revised the manuscript. The co-authors supported data analysis and discussion as well as critically revised and approved the manuscript.

## 2.2 Isolation of quinoa protein by milling fractionation and solvent extraction

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# Isolation of quinoa protein by milling fractionation and solvent extraction

*Maike Föste, Dana Elgeti, Anna-Katharina Brunner, Mario Jekle*, Thomas Becker*

*Technische Universität München, Institute of Brewing and Beverage Technology, Research Group Cereal Process Engineering, Weihenstephaner Steig 20, 85354 Freising, Bavaria, Germany*

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ABSTRACT

Quinoa is an attractive non-animal protein source, because of its absence of gluten and the favorable amino-acid profile. The objective of this study was the extraction of quinoa protein by two consecutive approaches. Initially, the protein-rich bran was isolated by optimizing the conditioning parameters of a milling fractionation procedure. In contrast to the protein content of the whole grain, 11.75% in dry base (db), the bran fraction contained 27.78% (db) protein when conditioned with 15% moisture at 20 °C for 20 h. Subsequently, an aqueous extraction was developed, resulting in a protein solubility of 60% at pH 10 and 20 °C for 1 h. For purification at the isoelectric point, the pH-value and the separation method were varied. After drying, an extraction of proteins from bran yielded 68% (db) as compared to 52% (db) protein from quinoa whole grain flour.


## 1. Introduction

The uptake of proteins is one of the major factors for maintaining human health. Because of the higher costs for animal protein production and the huge amount of resources which are required, plant-based protein sources are of growing interest. In addition, the incidence of allergies and intolerances concerning proteins from egg or milk has been rising. Currently, cereals and legumes represent the main alternatives, for both human and animal nutrition. However, the alcohol soluble gliadins and glutenins in wheat are attributed to the celiac disease (Thompson, 2001). Thus, the identification of novel and sustainable protein sources is of great relevance. The pseudocereal quinoa might be a suitable candidate, since it features high protein and mineral content with a well balanced amino acid profile and lacks gluten (Aufhammer et al., 1995).

Protein from these sources can be isolated by solvent extraction to serve as an ingredient in dietary supplements. Additionally, for gluten-free products the application of protein isolates can be beneficial for processing and quality improvement of bread, pasta, dietary supplements, baby foods and beverages. According to Segura-Nieto et al. (1999) and Gorinstein et al. (2002) proteins of amaranth, soybean, buckwheat, and quinoa are highly soluble and applicable in functional foods.

The type of milling product, processing parameters and suitable solvents have to be taken into consideration to achieve a maximum yield. Previously, a lot of studies have focused on the extraction of proteins from whole grain flours for e.g. amaranth (Salcedo-Chávez et al., 2002), while only few reported extraction from byproducts such as rice bran (Jiamyangyuen et al., 2005). The separation of different grain tissues enables the selection of a fraction which is naturally

*Abbreviations:* AACC, American Association of Cereal Chemists; db, dry base; ICC, International Association for Cereal Science and Technology; CIE, Commission Internationale d'Eclairage.

* *Corresponding author*. Tel.: +49 8161 71 5327; fax: +49 8161 3883.
E-mail addresses: maike.foeste@tum.de (M. Föste) , mjekle@tum.de (M. Jekle).

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rich in target substances. The germ of quinoa, for example, contains most of the protein (Valencia-Chamorro, 2003), while starch is present in negligible levels. Additionally, protein can be solubilized more easily when less starch is available, because especially small starch granula are difficult to separate. Since amaranth and quinoa starch granula are particularly small (Aufhammer et al., 1995) typical separation methods based on gravimetric or size differences are difficult to realize.

Nevertheless, in the case of quinoa and amaranth, milling presents a technological challenge because of their small kernel sizes: for quinoa 1.0–2.5 mm (Taylor and Parker, 2002) and for amaranth 1.0–1.5 mm (Bressani, 2003), respectively, depending on the plant variety. Thus, only few studies have focused on the production and application of milling fractions (Chauhan et al., 1992; Ando et al., 2002). Chauhan et al. (1992) compared two methods for reducing the saponine content by a pretreatment before milling the grains into whole grain flour, bran and white flour. However, there are no available data, focusing on the duration and the water content during a conditioning step. Similarly US patent 20100184963 from 2010 suggests the use of protein-rich fractions for the production of quinoa protein concentrate (Scanlin et al., 2010). Scientific data and validation of the proposed methods are not available. In the case of wheat and other typical cereals, conditioning is said to be one of the most important steps prior to grinding, which renders the outer cell tissues more elastic, resulting in a better separation from the endosperm. Depending on the morphology of wheat kernels, the target moisture content during conditioning varies between 15% and 16% with a resting time between 6 and 36 h (Cauvain, 2003; Fang and Campbell, 2003).

After choosing a suitable milling fraction for protein concentration, an extraction procedure has to be developed. Salcedo-Chávez et al. (2002) varied processing parameters for alkaline extraction from amaranth whole grain flour, determining solubilization at pH 8 to 11 and acidic precipitation at pH 4.5 to 5.0 as optimum conditions. Depending on the extraction conditions, physicochemical changes of the proteins may affect their intended functionality and bioavailability. Scilingo et al. (2002) analyzed the effect of extraction and precipitation conditions on the electrophoretic and calorimetric behavior of amaranth proteins. Results from this study indicated that pH-values >9 and <5 reduced thermal stability and increased protein denaturation. Similarities regarding protein solubility of amaranth and quinoa can be expected, because of the same family (Amaranthaceae) and a related seed structure.

The aim of this study was to optimize a fractionation process in order to separate quinoa flour from the bran fraction. Second, proteins of the bran fraction were further concentrated by optimizing an extraction process, aiming to reach a protein content between 50% and 90%. Different pH conditions for extraction were analyzed and the resulting solution was further purified and dried to obtain a stable product which can be used as dietary supplements and functional food ingredients.

## 2. Materials and methods

### 2.1. Origin and fractionation of quinoa

Organic Royal Quinoa grains (*Chenopodium quinoa*) from Bolivia were purchased from Ziegler & Co. GmbH (Wunsiedel, Switzerland). Due to the mechanical pretreatment (involving washing and friction) by the manufacturer, the quinoa bran fraction in this work was lacking the pericarp. Quinoa seeds were ground to whole grain flour in an ultra-centrifugal mill Retsch ZM 200 (Haan, Germany) with a mesh screen of 500 μm. Prior to fractionation, the moisture content of whole grain flour was determined thermo-gravimetrically according to AACC standard method 44-01 (American Association of Cereal Chemists (AACC), 2002). Conditioning was carried out in an airtight box and the initial moisture content of 12.3% was elevated to 14%, 15% and 16% by spraying water onto the seeds, followed by manual stirring. Resting time lasted 20 h and was shortened to 16 h for 15% seed moisture samples at room temperature (20 °C). Milling trials were performed in a Brabender Quadrumat Junior mill (Duisburg, Germany), which is a laboratory roller mill, separating the quinoa seeds into bran and white flour by sieving in a rotating sifter (mesh of 200 μm). The milling yield as percentage was determined by dividing the mass of the respective fraction by the total mass of milling products.

### 2.2. Quantitation of protein, ash and moisture

Milling fractions were analyzed for their moisture and ash content by employing standard methods of analysis, AACC 44-01 and 08-12, respectively (American Association of Cereal Chemists (AACC), 2002). The crude protein content was determined by the Kjeldahl procedure according to standard method AACC 46-10 using a conversion factor of $N \times 5.54$ (American Association of Cereal Chemists (AACC), 2002; Fujihara et al., 2008). Each test was performed in quadruplicate and expressed on dry weight basis (db).

Color characterization of milling fractions was measured using a Spectrophotometer fitted with an optical sensor from BYK Gardner GmbH (Geretsried, Germany) on the basis of the CIE $L^*$, $a^*$, $b^*$ color system as describe by Edney et al. (2002) The instrument was calibrated using a white standard color calibration plate. The brightness, indicated by $L^*$ (0 = black, 100 = white) was recorded. Three measurements from three different samples were performed.

### 2.3. Particle size distribution of milling fractions

The particle size distribution of flour was measured according to ICC standard method No 207 (International Association for Cereal Science and Technology (ICC), 1998). Milling products (each 100 g) were sifted in a sieving chamber from Bühler GmbH (Braunschweig, Germany) with a set of graded standard sieves (45 μm, 90 μm, 125 μm, 180 μm, 250 μm, 355 μm, 500 μm, 1000 μm, 1250 μm) for 10 min in the case of flours and for 5 min when sieving bran. The retained product on each sieve was weighed and expressed as percentage retention. Three measurements from three different samples were conducted.

### 2.4. Extraction of proteins from quinoa bran

After enriching the proteins in the bran fraction through conditioning, milling and sieving, further concentration was conducted by solvent extraction followed by an optional purification procedure as described in Fig. 1. Proteins were solubilized (see Fig. 1a) by preparing a suspension of quinoa bran and water in a ratio of 1:10 (w:v). The impact of the pH-value on protein solubility was determined by adding 1N NaOH to obtain a pH-range from 7 to 12 according to the

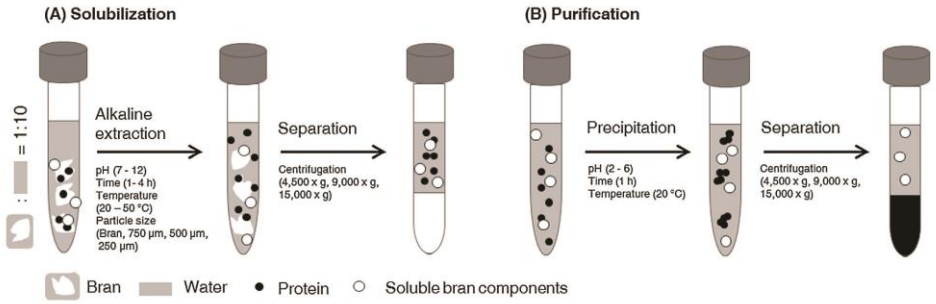


**Fig. 1 – Solubilization (a) and purification (b) of quinoa bran proteins. Varied parameters are listed below the arrows with the variation range given in brackets.**

method of Bera and Mukherjee (1989). Furthermore, the duration of the extraction process, the temperature as well as the particle size of the raw material (quinoa bran) were varied in order to determine the conditions yielding in the maximum protein solubility. The suspensions were stirred on a shaker for 1 h, 2 h, 3 h or 4 h at 20 °C, 30 °C, 40 °C, or 50 °C. In order to vary the particle size, the bran was milled in an ultracentrifugal mill Retsch ZM 200 (Haan, Germany) with different mesh sizes (750 mm, 500 µm, 250 µm). Separation of soluble proteins from the insoluble residue was performed by centrifugation at 4500 × *g* for 20 min at 4 °C. Solubility of proteins from quinoa bran was calculated according to Formula (1) and expressed as the percentage of crude protein (*N* × 5.54) present in the supernatant ($m_{p,sup.}$) in relation to the total protein content of bran ($m_{p,bran}$) (Paredes-López et al., 1994). Determination of protein nitrogen was performed as for the milling fractions in Section 2.2.

$$\text{Solubility}\ (\%) = \frac{m_{p,sup.}}{m_{p,bran}} \times 100 \quad (1)$$

### 2.5. *Purification and drying of protein extracts*

The preparation of quinoa bran protein isolates was conducted by using different pH values for extraction and precipitation according to Paredes-López et al. (1994). For protein purification (see Fig. 1b), supernatants from the solubilization step were adjusted in the pH-value range of 2 to 6 with 1 N HCl. Precipitated proteins were centrifuged at 4500 × *g*, 9000 × *g* or 15,000 × *g* at 4 °C for 20 min. Precipitates were re-suspended in water, neutralized to pH 7 and freeze-dried (Beta 2–8 with LMC-1, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany) for a minimum of 48 h reaching a moisture content below 5% before storage at −20 °C until further analyses.

### 2.6. *Statistical analysis*

Statistical analysis was performed with the software Prism 5 (Version 5.03, GraphPad Software, Inc.) on all data. To detect significant differences between means, one-way analysis of variance (ANOVA) with separation of means by the Tukey–Kramer test was applied with a significance level of $p < 0.05$.

## 3. Results and discussion

### 3.1. *Impact of conditioning on yield and protein content of milling fractions*

The results of varied conditioning settings are presented in Table 1. Separation of unconditioned seeds resulted in a yield of nearly 71% quinoa white flour and 28% bran. With regard to 14% moisture, the yield of quinoa white flour was elevated to a maximum of 68%. The higher moisture content led to better separation of seed tissues, resulting in significantly higher protein content in bran (26.18% db). By elevating the seed moisture up to 15%, the protein content reached 27.78% db, which represents an enrichment of over 50% (rel.) in comparison to whole grain flour. Moreover, the ash content of the white flour was 0.63% db, indicating that most of the bran

**Table 1 – Impact of seed conditioning on milling yield, moisture, ash and protein content of quinoa milling fractions.**

| Milling fraction | Conditioned moisture (%)[a] | Resting time (h) | Yield (%) | Moisture (%) | Ash (% db) | Protein (% db)[b] |
|---|---|---|---|---|---|---|
| Whole grain flour | – | – | – | 12.25 ± 0.03b | 2.33 ± 0.03d | 11.75 ± 0.12c |
| White flour | – | 0 | 71.27 ± 1.19f | 12.26 ± 0.10b | 1.47 ± 0.08c | 9.18 ± 0.54b |
| Bran | – | 0 | 28.18 ± 1.16a | 10.24 ± 0.20a | 4.70 ± 0.17e | 23.97 ± 1.78d |
| White flour | 14.0 | 20 | 68.19 ± 0.06e | 14.06 ± 0.03d | 0.91 ± 0.03b | 6.33 ± 0.11a |
| Bran | 14.0 | 20 | 31.55 ± 0.08b | 12.04 ± 0.05b | 5.22 ± 0.06f | 26.18 ± 1.04e |
| White flour | 15.0 | 20 | 64.43 ± 0.55d | 14.71 ± 0.08e | 0.63 ± 0.01a | 5.86 ± 0.53a |
| Bran | **15.0** | **20** | 35.29 ± 0.57c | 12.93 ± 0.40c | 5.44 ± 0.08g | **27.78 ± 1.10f** |
| White flour | 15.0 | 16 | 62.78 ± 0.38d | 14.91 ± 0.07e | 0.85 ± 0.01 | 5.96 ± 0.25a |
| Bran | 15.0 | 16 | 35.97 ± 0.01c | 13.24 ± 0.26c | 5.24 ± 0.07f | 25.82 ± 0.49e |

Mean values of two independent milling trials with standard deviation: yield ($n = 2$) moisture, ash and protein content ($n = 4$). Different letters denote statistically significant differences (ANOVA, $p < 0.05$). Protein and ash content calculated on dry base.

[a] Conditioned moisture amount added before milling.

[b] Determined with Kjeldahl (*N* × 5.54).

particles were separated. Higher amounts of conditioning water (16%) were not applicable since the high moisture content caused an obstruction of particles between the milling rolls. In comparison, Chauhan et al. (1992) obtained bran with a protein content of 20.4% and 24.3% (db) after using different dehulling procedures, respectively. The authors chose a conversion factor of 5.7 and the native whole grain had a higher protein content (13.7% db). Generally, literature uses different conversion factors for calculating the amount of crude protein from the total nitrogen determined via Kjeldahl or Dumas compromising the comparability. To sum up, conditioning to 15% moisture for 20 h improved the separation of the bran tissues from the starch rich perisperm significantly. Whereas the protein content in whole grain flour was 11.75% db, this was concentrated up to 2.3 fold in the bran fraction.

### 3.2. *Correlation of ash and protein content with the coloring of fractions*

The coloring of flour is often used to evaluate its quality and pureness. Especially the whiteness, which is composed of brightness and yellowness, is of interest for characterizing milling products. However, for untypical grains like quinoa the predictability of the pureness by these values is not guaranteed because of considerable differences regarding the seed structure. According to Oliver et al. (1993) the CIE LAB color space co-ordinates $L^*$ and $b^*$ are useful for measuring brightness and yellowness of wheat flour. The resulting brightness is influenced largely by the particle size and bran inclusion effects. In Fig. 2 the brightness of milling fractions is plotted against ash (see Fig. 2a) and protein content (see Fig. 2b) to determine correlations. Indeed, a correlation between the $L^*$ value and the ash content ($R^2 = 0.9909$, $p < 0.0001$) was observed. By elevating the seed moisture content, flour got purer as indicated by lower ash content and higher $L^*$ values. Similarly, the protein content correlated with the $L^*$ value ($R^2 = 0.9898$, $p < 0.000.1$). Thus, it can be concluded that the brightness measured with an LAB system can be used to determine the pureness of quinoa milling fractions.

### 3.3. *Particle size distribution of quinoa milling products*

The particle size distribution of milling fractions can be of interest for technological applications, because of differences regarding the reactive surface of particles, which is of particular importance for the extractability of components or other applications. As an example, de la Hera et al. (2013) observed that coarser maize flour particles resulted in higher loaf volume and Raghavendra et al. (2006) found the hydration properties of coconut residue were affected by their particle size.

**Table 2 – Particle size distribution and protein content of quinoa milling fractions in comparison to gluten-free flours.**

| Size[a] (μm) | Whole grain flour (%) | White flour (%) | Bran (%) |
|---|---|---|---|
| >0 | 3.93 ± 1.43 | 8.40 ± 3.44 | 5.06 ± 0.11 |
| >45 | 28.19 ± 0.68 | 43.27 ± 2.90 | – |
| >90 | 11.16 ± 0.38 | 11.13 ± 0.96 | – |
| >125 | 13.36 ± 0.62 | 27.27 ± 0.29 | 10.34 ± 0.28 |
| >180 | 15.83 ± 1.44 | 6.33 ± 0.23 | 15.95 ± 0.83 |
| >250 | 15.83 ± 0.11 | 2.50 ± 0.20 | 20.03 ± 0.46 |
| >355 | 12.46 ± 0.59 | 0.00 ± 0.00 | 23.24 ± 0.12 |
| >500 | 0.00 ± 0.00 | – | 24.47 ± 0.27 |
| >1000 | – | – | 2.73 ± 0.10 |

[a] Particles with different sizes were classified by weight after sieving. Means are presented with standard deviation ($n = 3$).

The particle size distributions of the quinoa milling fractions are presented in Table 2. Due to the milling of quinoa whole grain flour in an ultra-centrifugal mill with a sieve of 500 μm mesh size, flour particles varied between 45 to 355 μm. As expected quinoa white flour consisted of considerably smaller particles (>50% below 90 μm) than bran (>50% above 355 μm). In comparison to wheat bran, which can achieve a mean size of 1239 μm (Stewart and Slavin, 2009), the particle size of quinoa bran was smaller. This is not surprising, considering the different grain sizes. Since the quinoa germ is surrounding the starch-rich perisperm, a maximum length of 3 mm can be expected if the germ is pulled straight (for ~1.5 mm grain diameter).

### 3.4. *Protein extraction by solubilization*

The solubility of proteins depends on the physicochemical state of the molecules, which can be favorably or adversely affected by processing treatments such as heating or drying during processing and storage. Further factors are the ratio of polar to non-polar groups as well as the primary, secondary, tertiary and quaternary structure of the protein. In alkaline solutions the conformation alters, exposing more

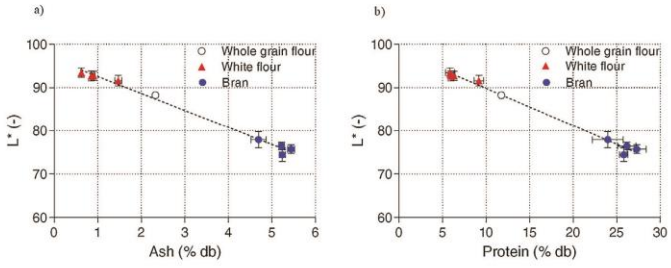


**Fig. 2 – *Correlation between ash (a) and protein content (b) with the brightness of quinoa milling fractions*. Deviations regarding ash and protein content of quinoa bran and white flour derive from different conditioning procedures before milling. Whole grain flour was produced in an ultracentrifugal mill. $L^*$ values represent the brightness. Db: dry base. Plotted are means with standard deviation ($n = 6$). Dotted lines represent linear correlations of the data with $R^2 = 0.9909$ ($p < 0.0001$) for the ash content (a) and $R^2 = 0.9898$ ($p < 0.0001$) for the protein content (b).**

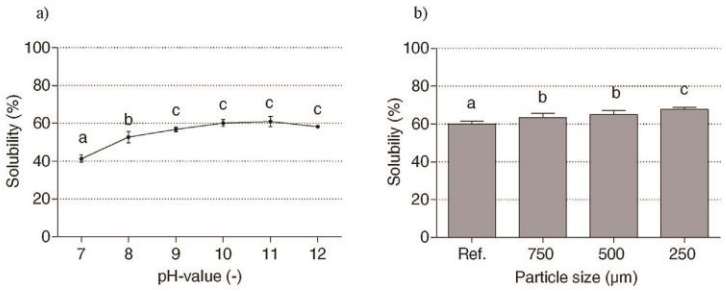


**Fig. 3 – Impact of pH-value and particle size on the protein solubility of quinoa bran. (a) Solubility: g proteins in solvent per 100 g proteins in bran. (b) Solubility of proteins at pH 10 depending on the particle size, as obtained after centrifugal milling. The protein content of the supernatant was measured after centrifugation at 4500 × *g* (*N* = 5.54). Ref: Bran without milling. Means are presented with standard deviation (*n* = 4). Different letters denote statistically significant differences (ANOVA, *p* < 0.05).**

hydrophobic groups, which promotes higher protein solubility by increasing the interactions with water (Aceituno-Medina et al., 2013; Salcedo-Chávez et al., 2002).

Alkaline solubilization of proteins was performed in the range of pH 7 to 12 revealing that solubility increased by elevating the amount of sodium hydroxide (see Fig. 3a). Similarly, plant-based proteins from soybean, rice, pea and amaranth become more soluble in alkaline medium (Maruyama et al., 1999; Wang et al., 1999; Anon et al., 2001; Tömösközi et al., 2001; Salcedo-Chávez et al., 2002). A pH-value of 10 was chosen for further extraction trials. Higher alkaline values were avoided in order to reduce a possible negative impact on essential amino acids such as lysine. These are said to generate lysinoalanine, resulting in a loss of protein digestibility and biological value (Finot, 1997).

The impact of the particle size (250 µm, 500 µm, 750 µm) on the protein solubility of quinoa bran is presented in Fig. 3b. The objective was to study the protein accessibility depending on the degree of comminution. The reduction in particle size up to 250 µm resulted in significantly higher protein solubility (67.7%). In comparison to the non-milled bran this was an increase in protein content of 13% (rel.). A possible explanation might derive from the accessibility of proteins through larger particle surfaces.

Another critical factor for extraction processes is the duration, since diffusion and enzymatic degradation processes are time-dependent. Therefore, the extraction was elongated up to four hours to test the effect on protein solubility. As a result, there was no significant variation regarding the protein solubility after varying the duration of the extraction (see Fig. 4a). Fortunately, an elongation of the extraction over 1 h, which would have provoked additional manufacturing costs and favored enzymatic degradation processes, was not necessary.

As a final factor, temperature is known to influence conformation and hydrophilic behavior of proteins. In order to avoid irreversible protein denaturation during extraction, temperature was kept below 60 °C. As can be observed in Fig. 4b the heating to 25 °C increased solubility by 6% (absl.) which was not significantly elevated by further heating. As for the duration, lower temperatures are advantageous to limit manufacturing costs and chemical modifications. Thus, further extractions were performed at room temperature (20 °C) because these factors were considered superior to the slightly elevated protein yield.

### 3.5. *Protein purification by precipitation*

Protein–protein interactions increase in the area of the isoelectric point as a consequence of minimum net electrostatic charges of the molecules. This isoelectric point of the quinoa proteins (extracted at pH 10) was evaluated in a pH-range between 2 to 6. After acidification, the solubility of proteins was determined in proportion to the amount of previously extracted proteins. Fig. 5a reveals that proteins which were initially extracted at pH 10, had the lowest solubility at pH

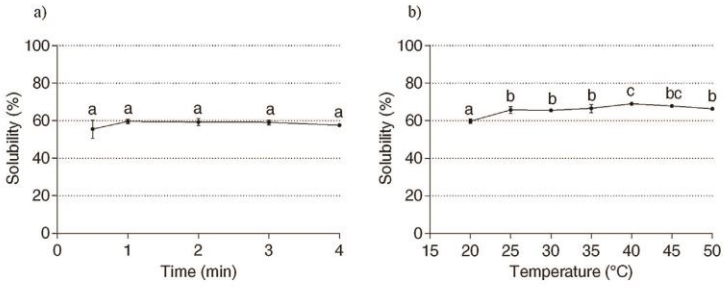


**Fig. 4 – *Impact of extraction time and temperature on the protein solubility of quinoa bran.* (a) Extraction in water over time (30 min to 4 h). (b) Extraction in water with varying temperature (20 °C to 50 °C). The protein content of the supernatant was measured after centrifugation at 4500 × *g* (*N* = 5.54). Means are presented with standard deviation (*n* = 4). Different letters denote statistically significant differences (ANOVA, *p* < 0.05).**

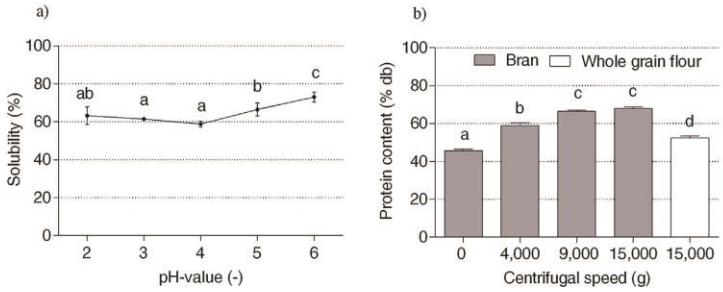


**Fig. 5 – Impact of purification by isoelectric precipitation and centrifugation on protein solubility and content. (a) Protein solubility (g protein per 100 g protein in bran) depending on the pH-value after aqueous extraction at pH 10. $N = 5.54$. (b) Protein content after freeze drying when using different centrifugal forces for the isolation of the precipitate. db = dry base. Means are presented with standard deviation ($n \geq 4$). Different letters denote statistically significant differences (ANOVA, $p < 0.05$).**

4, indicating the isoelectric point. Thus, precipitation at pH 4 can be used to exclude non-protein solutes but only 41.2% of the extracted bran proteins were yielded after this purification step, while the remaining proteins remained in solution.

Both, the separation of insoluble products from the alkaline protein solution after the initial extraction stage, as well as the separation of the precipitated proteins from soluble residues after acidification, were performed by centrifugation. Focusing on maximizing the protein yield, these centrifugation steps were performed with three different centrifugal forces: $4500 \times g$, $9000 \times g$ and $15{,}000 \times g$. The protein content (% db) of quinoa bran extracts is presented in Fig. 5b. Without purification (=reference) the extract contained 45.83% db protein, while precipitation stage for removing soluble non-protein compounds increased the protein content up to 58.92% db, as expected. By elevating the centrifugal speed, protein yield was further improved, reaching its maximum at 67.93% db at $15{,}000 \times g$. Moreover as a comparison to the use of quinoa bran as raw material the same extraction process was performed with quinoa whole grain flour (at maximum centrifugal speed) resulting in a lower protein content (−22.89% rel.).

To sum up, quinoa bran is a suitable byproduct of the milling process, which facilitates the concentration of the proteins. The results indicate that conditioning of quinoa is the first step to separate the outer parts of the seed from the starch-rich perisperm by choosing the right pre-conditioning settings (15% seed moisture and 20 h resting time). Compared to whole grain flour, in particular protein and minerals were enriched in the bran fraction up to 136% (rel.) and 133% (rel.), respectively. The protein content of the extract obtained in this study is comparable to the results of Jiamyangyuen et al. (2005) who produced extracts from non-defatted and defatted rice bran ending up with 72% protein. The presented combination of fractionation and solvent extraction provides an efficient method for the concentration of proteins from quinoa.

## 4. Conclusion

Depending on the industrial purpose, milling fractions feature their own technological characteristics, which can be individually applied for modifying gluten-free foods, bakery products or dietary supplements. More recently observed by Elgeti et al. (2014), quinoa white flour exhibits considerably higher substrate availability than rice and corn flour, resulting in elevated carbon dioxide formation and increased bread volume. In this case, the separation of quinoa bran resulted in beneficial effects on the gas retention capacity of gluten-free dough. Moreover quinoa white flour improved bread quality regarding crust color, loaf volume and sensory attributes. Considering the nutritive value and economic aspects also milling byproducts such as quinoa bran feature a high potential for the application in gluten-free products.

Since gluten-free flours are mostly lacking protein when based on corn (6.58% db) or rice (6.99% db), the targeted application of small quinoa bran amounts can improve the nutritive value without impairing gas holding properties and bread volume (Föste et al., 2014). In addition, the protein extracts can be of relevance as functional food ingredients, when featuring stabilizing or foaming properties as observed for isolated amaranth proteins (Fidantsi and Doxastakis, 2001). Further analyses should cover the impact of quinoa proteins as ingredients in gluten-free bread or dietary supplements in order to enrich protein content and improve food quality.

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## 2.3 Fibres of milling and fruit processing by-products in gluten-free bread making: A review of hydration properties, dough formation and quality-improving strategies

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Review

# Fibres of milling and fruit processing by-products in gluten-free bread making: A review of hydration properties, dough formation and quality-improving strategies

Maike Föste, Christoph Verheyen, Mario Jekle*, Thomas Becker

*Technical University of Munich, Institute of Brewing and Beverage Technology, Research Group Cereal Technology and Process Engineering, Weihenstephaner Steig 20, 85354 Freising, Germany*

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ABSTRACT

Gluten-free (GF) breads often lack proteins, minerals and fibres and have an imbalanced energy value, as they are primarily based on flour or starch. To nutritionally fortify GF bread, dietary fibres from milling and fruit processing by-products can be utilized. However, fibre addition changes sensorial, nutritional and also technological properties, such as dough or batter hydration. This review evaluates and compares different methods for quantifying the hydration properties of GF fibres and the resulting batters. Revelations are that the hydration properties of fibres vary greatly, depending on the utilized measuring technique, thus impeding the calculation of the appropriate water amount for GF batter processing. In addition, bran and fibres increase the loss factor tan δ and delay thermal transformation, compromising the specific loaf volume. Finally, operational strategies, such as enzymatic or extrusion treatments are discussed regarding their efficiency to increase water absorption in order to further improve GF bread quality.

### 1. Introduction

During the past decade, gluten-free (GF) products have attracted considerable attention and gained popularity. According to a new report by Grand View Research (2019), global GF product market size could be increased up to 32.39 billion US dollars by 2025 (https://www.grandviewresearch.com/press-release/global-gluten-free-products-market/Accessed 22 May 2019). A market research study by Mintel pointed out that consumers without coeliac disease purchase the biggest share of GF foods (Topper, 2016). Main reasons are that 27% of tested consumers are gluten-intolerant or sensitive while another 25% ate GF foods for weight loss. Although there is no scientific evidence, 65% of recruited American adults believed that GF foods are healthier than their wheat-containing counterparts (Theethira & Dennis, 2015). Coeliac disease is a chronic enteropathy of the upper small intestine in genetically susceptible individuals. By the ingestion of the prolamin fraction, which is contained in wheat gluten or in rye, barley or possibly oats, an autoimmune-mediated reaction can be triggered from the intestine. This leads to villous atrophy and consequently malabsorption of minerals. To date, the only effective treatment for patients suffering from coeliac disease or a related clinical condition is the complete and life-long elimination of gluten from their diet.

Traditional wheat-based bakery products contain gluten, which can be divided into a glutenin and a gliadin fraction. Gluten has unique network-forming properties, being essential for the final structure in bakery products (Wilderjans, Pareyt, Goesaert, Brijs, & Delcour, 2008). In relation to their protein content, wheat can be classified into weak flour (7–10% protein) and strong flour (10–14% protein). The former is characterized by lower water absorption, which is utilized for cake production. The stronger flour shows higher water absorption, giving the ideal framework for trapping air bubbles in stiffer doughs to produce bread (Jazaeri et al., 2015). Because of these unique characteristics, gluten from wheat flour is applied in a variety of different foods (e.g. bread, bakery and dairy products or emulsions). This makes consumption of processed foods difficult for coeliac patients, as a precise and uniform labelling of these products is indispensable.

In contrast to wheat bread, GF counterparts are primarily based on refined flours and starches. Therefore, their carbohydrate content is high and they often contain additional fat and sugar (Jnawali, Kumar, & Tanwar, 2016; Wild, Robins, Burley, & Howdle, 2010). The lack of proteins and dietary fibres (Miranda, Lasa, Bustamante, Churruca, & Simon, 2014), the imbalanced energy value and the elevated glycemic response (Segura & Rosell, 2011) have been discussed. Hence, fortification with alternative flours from pseudocereals (quinoa, amaranth,

* Corresponding author.
*E-mail addresses:* maike.foeste@tum.de (M. Föste), christoph.verheyen@tum.de (C. Verheyen), mjekle@tum.de (M. Jekle), tb@tum.de (T. Becker).

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**Table 1**
Comparison of different milling and fruit processing by-products as fibre sources for GF bread formulations.

| Source of by-products | SDF (%db) | IDF (%db) | TDF (%db) | Main fibre components | References |
|---|---|---|---|---|---|
| Milling of cereals | | | | | |
| Corn (whole flour) | 1.1 | 6.4 | 7.5 | Cellulose, lignin, hermicellulose | Dreher (1987) |
| Rice bran (defatted) | 2.7 | 30.2 | 32.9 | Cellulose, lignin, hermicellulose | Daou and Zhang (2014) |
| Oat bran | 8.9 | 11.5 | 20.4 | Cellulose, lignin, β-glucan, arabinoxylan | Aprodu and Banu (2015) |
| Milling of non-cereals | | | | | |
| Quinoa (whole flour) | 2.2 | 7.8 | 10.0 | Pectic substances, xyloglycan | Lamothe et al. (2015) |
| Amaranth (whole flour) | 2.5 | 9.0 | 11.5 | Pectic substances, xyloglycan | Lamothe et al. (2015) |
| Psyllium fibre | 71 | 16.6 | 87.6 | Xylose, arabinose, uronic acid | Aprodu and Banu (2015) |
| Pea fibre | 2.1 | 72.9 | 75.0 | Hemicellulose, cellulose, pectin | Aprodu and Banu, (2015) |
| Fruit processing | | | | | |
| Sugar beet fibre | 20.2 | 52.1 | 72.3 | Hemicellulose, pectin, cellulose | Thibault, Renard, and Guillon (1994) |
| Orange bagase | 20.2 | 38.9 | 59.1 | Hemicellulose, pectin, cellulose | Romero-Lopez, Osorio-Diaz, Bello-Perez, Tovar, and Bernadino-Nicanor (2011) |
| Orange pulp | 13.3 | 54.2 | 67.5 | Pectin, cellulose, hemicellulose | Mañas, Bravo, and Saura-Calixto (1994) |
| Lemon pulp | 31.8 | 41.9 | 73.7 | Pectin, cellulose, hemicellulose | Mañas et al. (1994) |

Abbreviations: SDF: soluble dietary fibre; IDF: insoluble dietary fibre; TDF: total dietary fibre; db: dry base.

buckwheat), pulses (soy, pea, chickpea), GF cereals (rice, sunflour, millet), oil seeds (sesame), nuts (Alvarez-Jubete, Arendt, & Gallagher, 2009; Capriles & Arêas, 2014; Pellegrini & Agostoni, 2015) or bran and/or dietary fibre has been recommended (Thompson, 2000; Tsatsaragkou, Protonotariou, & Mandala, 2016).

According to Dubois (1978), the addition of fibres impairs bread quality in terms of texture, loaf volume and appearance. This is attributed to the dilution of gluten in wheat dough, lowering gas retention and decreasing loaf volume. In addition, Pomeranz, Shogren, Finney, and Bechtel (1977) reported modifications of mixing time and water absorption in high fibre-enriched wheat dough. Several authors recommended adding water, whereby the amount can be determined by an established method (Farinograph or Mixograph). Unlike in wheat dough, the structure formation in GF dough is primarily based on the interaction of flour/starch and water-absorbing hydrocolloids. Higher amounts of water are required to form the GF dough, which more resembles a cake batter. Determination of the optimum water addition is complicated, because of the absence of the network-forming proteins.

Water is a necessary component for solubilizing ingredients, as well as for hydrating proteins and carbohydrates (Maache-Rezzoug, Bouvier, Allaf, & Patras, 1998). It may also affect the interaction of molecules during GF batter formation. To date, fundamental knowledge on hydration kinetics, the interrelation between recipe components and the adjustment of water content in GF batter is scanty. Recently, the properties of cereal brans (Chinma, Ramakrishnan, Ilowefah, Hanis-Syazwani, & Muhammad, 2014) and the structural role of adding dietary fibre to GF bread were reviewed (Tsatsaragkou et al., 2016). The application of alternative protein and fibre-enriched flours or fibre fractions from pseudocereals or legumes seems to be promising to nutritionally fortify GF bread (Chauhan, Eskin, & Tschachuk, 1992; Föste, Elgeti, Brunner, Jekle, & Becker, 2015). However, the role of bran and its mode of action in structure-function relationships during GF batter and bread formation should be further evaluated.

The main objective of this review is to discuss the impact of different fibres from the milling and fruit processing industries on dough functional properties during processing. Therefore, special emphasis is placed on the availability of disposable water in GF batter. First, the challenges and the different output variables that arise when determining the hydration properties of bran-enriched GF batter are critically evaluated and compared. Second, the effect of the hydration properties on the formation of GF batter and bread is outlined. Third, strategies for modifying the hydration properties of GF batter by e.g. soaking of native polymers, microbial and enzymatic polymer modification or thermal treatments to improve bread quality are discussed.

## 2. Milling and fruit processing by-products as fibres for GF bread formulations

Dietary fibres are defined as “edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. They include polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibres promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation” as reported by AACC Board of directors in March 2000. Sustainable sources for dietary fibres are the by-products of milling and fruit processing industries, since the cell wall materials from the respective grains (cereals/non-cereals) or fruits are particularly rich in these substances. Extraction methods can further isolate specific fibre components, as reviewed by Elleuch et al. (2011). One of the most common classifications of dietary fibres is according to their solubility in a water-soluble/ well fermentable fibre fraction (SDF) and a water-insoluble/ less fermentable fibre fraction (IDF) (Anita & Abraham, 1997). While fruits mainly consist of the more soluble pectins, gums and mucilage, cereals are made up of the more insoluble lignin, cellulose and hemicellulose (Davidson & Mc Donald, 1998). Depending on their botanical origin and polysaccharide structure, more regular backbone and side chains result in more insoluble dietary fibre and vice versa. Fibres from different by-product origins will be targeted throughout this review and are presented in Table 1.

Cereal brans from, e.g. corn or oat, consist of cell clusters or particles. They can be obtained as milling fractions containing the outer seed tissues. These include the pericarp, testa, aleurone layer, germ and parts of the starchy endosperm (Lebesi & Tzia, 2012). The plant variety, kernel size, shape, conditioning and resting time prior to milling or the milling type affect the specific bran composition and its physical and technological properties (Zittermann, 2003). Due to differences in the kernel structure, brans from cereals or pseudocereals may vary in their composition. High levels of pectic substances and xyloglucans were measured in quinoa and amaranth (Lamothe, Srichuwong, Reuhs, & Hamaker, 2015).

During milling or solvent processing, also heavy metals (e.g. lead, cadmium, mercury and arsenic), micronutrients (copper, zinc, cobalt, iron, manganese, selenium or molybdenum), anti-nutritives (phytic acid, tannins, lectins, trypsin inhibitors) or phytochemicals (phytoestrogens, tocotrienols, carotinoids) may be accumulated in cereal bran or press cake of the food industries (Slepecka, Kalwa, Wyrostek, & Pankiewicz, 2017). Especially, chronic exposure to cadmium can lead to wide-ranging health problems, such as disorder of the immune or

cardiovascular system (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Permissible thresholds of heavy metal ions in foodstuffs have been set by Commission Regulation No 1881/2006, which is a framework for EU legislation. The allowed maximum level of metals in flour is specified as follows: 73.5 mg/kg for zinc, 10.3 mg/kg for copper and 0.20 mg/kg for lead and cadmium. A study on wheat flour and bran in Serbia showed, that lead, arsenic and mercury were within permissible limits prescribed by the regulations (Ludajic, Pezo, Filipovic, Filipovic, & Kosanic, 2016). Recently, a study from Poland pointed out that cereal bran exceeded the allowed cadmium threshold. The researchers also showed increased values for other heavy metals (Slepecka et al., 2017). However, the quantities of xenobiotics in plants and their accumulation vary, depending on the atmospheric pollution, water and soil quality.

Moreover, an in vitro study showed the potential of wheat bran for binding heavy metals and other toxicants, such as heterocyclic amines (Ou, Gao, & Li, 1999). One possible explanation for this is the presence of so called metallothionein, which is a metal-binding protein, involved in several regulation processes, including zinc and copper homeostasis and buffering against toxic metals (Shabb, Muhonen, & Mehus, 2017). In summary, from the nutritional point of view, bran and dietary fibres can be regarded as elementary components to spice up a GF diet. As coeliac patients oftentimes have a lower absorption of minerals, more attention must be paid to the bioavailability of calcium, zinc, and iron. Because of the complex formation between fibres, minerals and phytic acid, bioavailability of these essential minerals may be decreased, which is decisive for patients suffering from coeliac disease.

## 3. Water-binding and hydration properties of different fibres

Depending on by-products' origin, the cell structure, the polysaccharide content and the main fibre components differ. The specific hydration behaviour results from the number of hydrophilic groups, the side chain reactivity and the binding forces. According to Auffret, Ralet, Guillon, Barry, and Thibault (1994), entrapment of water by capillary binding forces is the most important way for dietary fibres to bind water. Further mechanisms for direct water binding are a) polar effects, b) weak hydrogen bonding, c) strong hydrogen bonding, d) ionic interactions or e) hydrophobic (surface) effects, as reviewed by Chaplin (2003). This highlights the complexity of the hydration behaviour, when using different by-products. Moreover, hydration properties depend on and are defined by the methods that are used to measure them. The following paragraphs will compare and evaluate the different parameters with the corresponding techniques.

Swelling capacity is defined as the volume occupied by the fibre in excess water. This can be measured by the settled bed volume technique in a measuring cylinder. In many cases, however, the volume increase during the swelling of particles can be difficult to observe visually. Water absorption describes the kinetics of water migration, utilizing osmotic pressure or dialysis techniques, e.g. in the Baumann apparatus. For both methods, the amount of absorbed water can be measured over time.

Water-binding capacity is synonymous with water-holding capacity. This characteristic is measured by analyzing the amount of water retained by the fibre after low-speed centrifugation with forces of 2000 xg for 10 min (method 56-30.01, AACC International, 1999b). Low amounts of water are used to saturate the sample. In contrast, excess water (25 g per 5 g sample) and longer centrifugation duration (1000 xg for 15 min) are employed to determine the water-retention capacity according to AACC method 56-11.02 (AACC International, 1999a). In general, less bound/retained water was measured when using the centrifugation methods in comparison to the determination of water absorption by means of the Baumann apparatus or swelling capacity (Baumann, 1967; Chen, Rubenthaler, Leung, & Baranowski, 1988). Especially, centrifugation promotes the release of water, because of the utilized external forces. All methods do not directly reveal whether water is bound through capillary or molecular forces.

As a result, there can be huge variations in the hydration properties, depending on the fibres and the applied approach. Table 2 summarizes the hydration properties from different GF milling and fruit processing by-products. The water binding capacity ranged from 2.1 g/ g for oat bran (Chen et al., 1988) to 48.3 g/ g (db) for psyllium fibre (Cappa, Lucisano, & Mariotti, 2013). The difference might be caused by the high amount of mucilage in psyllium which can absorb water and form a gel (Mudgil, 2017). Using the same method for the apparently same fibre component also yields considerable variations. This can be attributed to the plant origin (genotypes) or differences in the implementation of these methods. Aprodu and Banu (2015) determined the water-binding capacity by suspending 0.4 g oat bran in 25 ml of distilled water, centrifuging the samples for 1 h at 14,000 x g and drying them for 15 h at 103 °C. Instead, Chen et al., (1988) utilized 1 g oat bran in 50 ml, centrifuging at 10,000 x g for 15 min and drying the samples at 70 °C for 24 h. Lowest value for water-retention capacity was measured for

**Table 2**
Hydration properties of selected fibres depending on the source of by-product and analysis method.

| Source of by-products | Method | Swelling capacity (mL water/g sample db) | Water-binding capacity (g water/g sample db) | Water-absorption (mL water/g sample db) | References |
|---|---|---|---|---|---|
| Milling of cereals | | | | | |
| Oat bran | Centrifugation | | 2.1 | | Chen et al. (1988) |
| Oat bran | Centrifugation | | 5.8 | | Aprodu and Banu (2015) |
| Oat bran | Hydration volume | | | 4.2 | Aprodu and Banu (2015) |
| Oat fibre | [a]Centrifugation | | 8.0 | | Sabanis et al. (2009) |
| Corn fibre | [a]Centrifugation | | 8.0 | | Sabanis et al. (2009) |
| Milling of non-cereals | | | | | |
| Quinoa bran | [b]Centrifugation | | 1.5 | | Föste et al. (2014) |
| Psyllium fibre | Centrifugation | | 40.6 | | Aprodu and Banu (2015) |
| Psyllium fibre | Hydration volume | | | 41.2 | Aprodu and Banu (2015) |
| Psyllium fibre | Centrifugation | | 48.3 | | Cappa et al. (2013) |
| Sugar beet fibre | Centrifugation | | 8,3 | | Cappa et al. (2013) |
| Pea fibre | Centrifugation | | 3.1 | | Aprodu and Banu (2015) |
| Pea fibre | Hydration volume | | | 3.2 | Aprodu and Banu (2015) |
| Pea fibre | Hydration volume Centrifugation | 4.6 | 3.7 | | Guillon, Renard, Hospers, Thibault, and Barry (1985) |
| Fruit processing | | | | | |
| Apple fibre | Centrifugation | 7.0 | 6.2 | | Guillon et al. (1985) |
| Apple fibre | Centrifugation | | 9.4 | | Chen et al. (1988) |
| Apple fibre | Centrifugation | 6.6 | 3.8 | 3.7 | Robertson et al. (2000) |

db, dry base; a) AACC 56-30; b) AACC 56-11.

quinoa bran with 1.5 g water per g sample (Föste et al., 2014). Since Robertson et al. (2000) suggested that this technique measures the water retained by the insoluble fibres, some of the fibre components in quinoa might not be detectable through this approach since they are soluble. In conclusion, hydration properties can be utilized as indices for water uptake. However, even slight variations of the methods can result in huge differences of swelling capacity, water-absorption, water-binding capacity or water-retention capacity of single fibres. In contrast, GF batter consists of numerous hydrophilic recipe components, which further complicates the determination of an optimum water amount.

## 4. Fibres and their overall functionality in food systems

Dietary fibres from different origin vary in their soluble/ insoluble fibre ratio, leading to changes in hydration properties (see Sections 2 and 3). Moreover, the difference in hydration properties is a decisive value that affects rheological characteristics. Because of its high water absorption and swelling, soluble dietary fibre primarily forms viscous solutions. Instead, insoluble dietary fibre also absorbs and retains water, however, without viscosity increasing effects (Sadeq et al., 2011). Depending on their solubility, dietary fibres vary in their physical, hydration, rheological, functional and nutritional properties, summarized as overall functionality (see Fig. 1).

In general, fibres with a high chain length or molecular weight show increased viscosity. Instead, relatively short-chain and highly branched polymers show low viscosity (Dhingra, Michael, Rajput, & Patil, 2012). Because of their solubility, the majority of pectins, gums and alginates can be utilized as thickening or gelling agents, as well as emulsion- or foam-stabilizers. In addition, pectin does not provide any caloric value and therefore can be utilized to produce low-caloric foods.

In contrast, the more insoluble fibres are utilized for stabilizing and texturizing purposes. They can improve product density, minimize shrinkage, retard staling, control moisture and increase food stability. Thus, fibres from grape pomace have been utilized to protect meat products from lipid oxidation and to prolong their shelf-life (Sayago-Ayerdi, Brenes, Viveros, & Goni, 2009). How bran and dietary fibres from the milling and fruit processing industries affect GF bread production is discussed in Section 5.

## 5. Fibres affect structure-function relationship throughout batter processing

### 5.1. General

The formation of batter, the stabilization of gas bubbles, the thermal transformation and finally also the structure stabilization are key stages for GF bread production. These steps depend on the hydration level, which is strongly influenced by fibre addition. The following section provides an in-depth look at the impact of fibre on batter structure and baking performance.

### 5.2. Batter rheology

The complexity of recipe component interactions, enzymatic reactions and mechanical forces during mixing and hydration impedes the possibility of predicting how single fibres affect the dough rheology (Létang, Piau, & Verdier, 1999). Some studies have analyzed fundamental rheological properties in GF batter with fibres from milling or fruit processing by-products. Oscillatory tests (e.g. strain and frequency sweeps or creep-recovery), provide key values, such as the complex shear modulus G*, the storage modulus G′, the loss modulus G″ or the loss factor tan δ (Gujral & Rosell, 2004; Lazaridou & Biliaderis, 2009; Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007; Marco & Rosell, 2008; Moreira, Chenlo, & Torres, 2011). In most of the analyzed GF formulations, the storage modulus G′, representing the elastic part, was greater than the loss modulus G″, representing the viscous part of the batter (Föste et al., 2014; Hager et al., 2011; Lazaridou et al., 2007; Martínez, Díaz, & Gómez, 2014). The loss factor, tan δ, is defined as the ratio between viscous and elastic parts of the dough. If the loss factor, tan δ, is below one, this represents a solid, elastic-like behaviour. In GF dough, Föste et al. (2014) showed that increasing amounts of quinoa

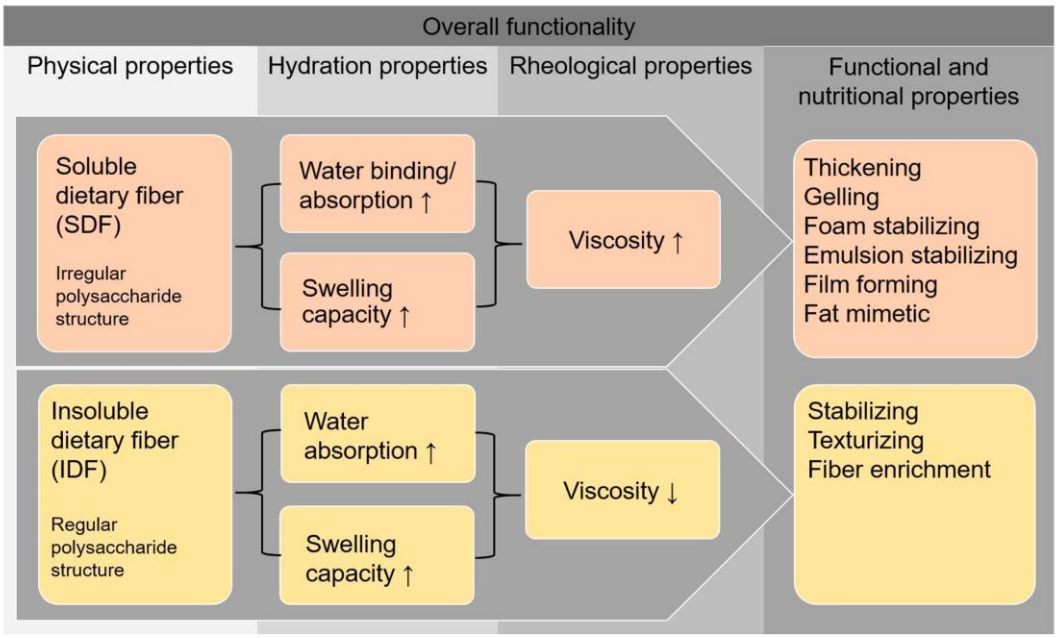


**Fig. 1.** Overview on the physical properties of fibres and their impact on the hydration, rheological, functional and sensorial properties, summarized as overall functionality.

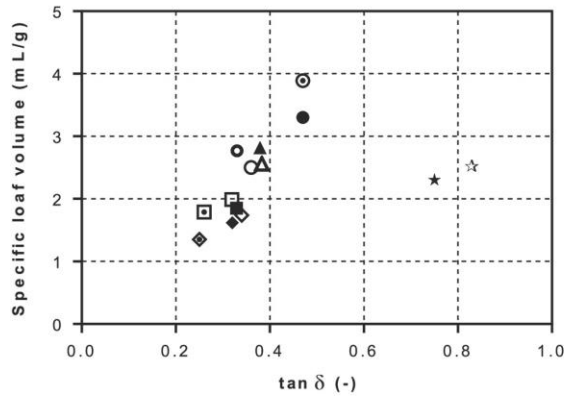


**Fig. 2.** Impact of the loss factor tan δ on specific loaf volume of GF bread. References : ★ Control; ☆ pectin: 2 g/100 g flour; water: 150 g/100 g flour; Lazaridou et al. (2007), ● Control; fibers of ◉ pea, ○ potato and ⊙ fine oat: 10 g/100 g flour; water: 80 g/ 100 g flour; Martínez et al. (2014), ♦ Control; quinoa bran:◇ 10 g and ◈ 80 g/100 g flour; water: 80 g/100 g flour; Föste et al. (2014), ■ Control; □ inulin: 9 g/100 g flour; water: 83 g/ 100 g flour; ⊡ oat β-glucan: 5.6 g/ 100 g flour; water 132 g /100 g flour; control water: 90 g/ 100 g flour; Hager et al. (2011), ▲ Control; Δ oat β-glucan: 2.6 g/100 g flour; water: 78 g/100 g flour; Ronda et al. (2015).

bran decreased loss fractor, tan δ, and simultaneously increased G*.

Orange pomace led to higher dough consistency (torque in Mixolab) and firmness, representing a stronger batter formulation (O'Shea, Doran, Auty, Arendt, & Gallagher, 2013). Martínez et al. (2014) measured higher loss factor, tan δ, after potato or pea fibre addition, both containing higher amounts of insoluble dietary fibres. According to Weipert (1990) dough stiffness and rigidity are represented by lower values for the loss factor, tan δ, and can be attributed to increased water binding and reduced water availability for starch hydration. As opposed to these results, the reduction in psyllium fibre content or the increase in water content showed no clear relation with G′ and loss factor, tan δ (Mancebo, San Miguel, Martínez, & Gómez, 2015). Therefore, these authors recommended utilizing the creep-recovery test as a good predictor of bread specific volume. Depending on the type of fibre and its solubility, dough firmness and the formation of a gel-like structure may vary. Thus, dough hydration and gas-holding properties may change, complicating the determination of batter rheology. These findings highlight, that rheological characterization with a constitutive equation (Burger model, Maxwell model) is insufficient to characterize fibre-enriched GF batters. In fact, a rheological modulation would be necessary to predict the impact of bran on hydration properties in those batters. In addition, the impact of particle size, molecular interaction and deformation-dependent behaviour should be considered as well. To the best of the authors knowledge, no appropriate rheological key value has been identified, which enables the adjustment of the water content in fibre-enriched GF batter.

### 5.3. Aeration and gas retention

In this section, the effect of fibres on the formation and stabilization of gas bubbles in GF batter are discussed. Substrate availability and the presence of saccharide-forming enzymes play a major role in gas formation. The addition of fibres can strongly influence the availability of substrates for the gas production of yeast (Elgeti et al., 2014; Föste et al., 2014; Hager et al., 2012).

Whereas in wheat dough the gluten network contributes to gas retention, in GF batter this function is mimicked by hydrocolloids and an optimal dough consistency. Inferior gas-holding properties can result from excessive dough firmness (indicated by low amounts of disposable water). Second, it can result from too little dough firmness (indicated by an excess amount of disposable water), impairing the stability of the gas bubbles. In general, the water absorption of network-forming wheat flours is measured in a Farinograph. Thereby, the amount of water to yield a dough consistency of 500 Farinographic units (FU) is determined (Teng, Liu, Bai, & Liang, 2015). In contrast, the respective target values for GF dough consistency were fundamentally different when psyllium fibre (200 FU), pectin (500 FU) or oat β-glucan (650 FU) were used (Lazaridou et al., 2007; Londono, Gilissen, Visser, Smulders, & Hamer, 2015; Mariotti, Lucisano, Ambrogina Pagani, & Ng, 2009). The ability of batter to retain gas is limited when the matrix becomes too rigid, as reported by Cappa et al. (2013). These authors investigated a mixture of psyllium and sugar beet fibre (ratio: 2.5% to 0.5%) in a formulation based on corn/ rice starch and rice flour. They reported that a preset dough viscosity of 500 FU or 200 FU, respectively, reduced the dough height by 68% and the retention coefficient from 99.3% to 95.0% (Cappa et al., 2013). Furthermore, Martínez et al. (2014) pointed out that oat, pea, potato or bamboo, which all contain major proportions of insoluble dietary fibre, increased dough consistency and decreased dough rise. Insoluble dietary fibres, especially of a coarse particle size, created rupture points in the dough matrix, favouring gaseous release caused by an impaired gas retention capacity. Hydrocolloids, such as hydroxypropyl-methylcellulose (HPMC), are often added to stabilize GF batter. The incorporation of 5 g HPMC per 100 g flour into a rice cassava dough significantly increased loss factor, tan δ, from 0.2 to 0.8 (Crockett, le, & Vodovotz, 2011).

As mentioned in Section 5.2, loss factor tan δ is one of the key values measured by fundamental rheology in GF batter. The impact of fibres from milling and fruit processing by-products on loss factor tan δ and specific loaf volume in GF bread is visualized in Fig. 2. In consideration of the varying formulations, loss factor tan δ ranged from 0.2 to 0.8. Whereas fibres from pea, potato, 80% quinoa bran or oat β-glucan decreased loss factor tan δ and final loaf volume (Föste et al., 2014; Hager et al., 2011; Martínez et al., 2014; Ronda, Perez-Quirce, Lazaridou, & Biliaderis, 2015)., particularly oat fibre, inulin or 10% quinoa bran improved the loaves although loss factor tan δ was nearly unaffected. The highest value for loss factor tan δ was achieved by adding 2 g of pectin to a rice flour-based formulation, containing 150 g of water per 100 g. While pectins, containing higher proportions of soluble dietary fibre (see Table 1), have been reported to decrease batter viscosity (Ziobro, Korus, Juszczak, & Witczak, 2013), insoluble dietary fibres rather increase dough viscosity, hampering gas holding and consequently reduce final loaf volume. Thus, a clear correlation cannot be shown between loss factor tan δ and specific loaf volume. However, these results indicate that, besides batter rheology, also the interplay of soluble and insoluble fibre components may contribute to increased specific loaf volume.

### 5.4. Thermal transformation

In general, gelatinization of starch in wheat dough and GF batter depends on the amount of disposable water. The relationship between the extent of starch gelatinization and the amount of water has been described by Salmenkallio-Marttila, Roininen, and Autio (2008). In wheat dough, a late start of starch gelatinization, in combination with the protein-related strain hardening, is associated with higher bread volume. This is attributed to a delayed transition from the liquid dough phase to the solid wheat crumb structure, allowing the volume to increase for a longer time period (Kusunose, Fujii, & Matsumoto, 1999; Stauffer, 1990; Wilderjans et al., 2008). As fibre addition changes water availability, the structure-function relationships during thermal transformation might be modified as well. However, this is difficult to assess, since, mostly, not only the fibre content, but also the water addition was varied. Aprodu and Banu (2015) added 77 g water to 100 g flour in fibre-enriched GF batter, whereas Ronda et al. (2015) added up to twice as much (141 g / 100 g).

Sabanis, Lebesi, and Tzia (2009) measured an increase in gelatinization temperature and a decrease in gelatinization enthalpy after replacing oat, corn, barley or wheat bran in a corn-starch rice-flour mixture. It would be interesting to know, whether these enthalpy changes were attributed to the bran addition, to the decreased starch content or to the water restraint from starch granules. Also O'Shea et al. (2013) observed a decreased gelatinization rate and reduced granule swelling ($p < 0.01$) when adding 5.5 g of orange pomace to 100 g of a rice-flour potato-starch and methylcellulose mixture. Possibly, protein aggregates were formed during gelatinization, that reduced the swelling capacity of starch granules.

To counteract the drawbacks of fibre addition on GF batter gelatinization, also Aprodu and Banu (2015) proposed adjusting the water content. An adequate amount of water depends on the specific formulation and the interaction of its components. In conclusion, the addition of fibre has a significant influence on the complex gelatinization behaviour of the GF batter. Moreover, it affects the stabilization of gas bubbles and the final loaf volume. To further elucidate the underlying correlations and mechanisms, standardized recipes and a uniform approach are required.

### 5.5. Structure stabilization

During baking, the temperature profile and the interaction of recipe components promote starch gelatinization and protein coagulation. In this manner, the vapour pressure rises, causing rapid gas expansion in the batter. Water evaporates, so that the final crumb structure is formed. This process of moisture migration from crumb to crust, is known as the firming phenomenon (He & Hoseney, 1990; Ruan et al., 1996). GF bread firming is related to changes in water distribution, water loss, starch retrogradation and the formation of amylose-lipid complexes. Due to the loss of intermolecular attractions between ingredients, GF starches tend to emit the water instead of absorbing/binding it. This promotes an increase in crumb hardness and crumbliness (Ronda & Roos, 2011). In particular, flours and starches with high amylose content retrograde faster during crumb setting. In contrast, amylopectin counteracts this effect and softens the bread crumb (Schober, 2009). This highlights the importance of using appropriate fibres with good hydration properties that do not impair GF batter.

The structure characteristics (crumb firmness, crumb moisture) of GF bread, considering the applied fibre amount and the water addition are presented in Table 3. The crumb became softer after adding psyllium fibre or rice bran (Risolubles) although the water content remained constant (Aprodu & Banu, 2015; Phimolsiripol, Mukprasirt, & Schoenlechner, 2012). Similarly, inulin addition decreased the crumb hardness, independent of the adjusted water amount (Ziobro et al., 2013). All these fibres have in common that they contain higher proportions of soluble dietary fibre.

In contrast, the incorporation of fibres with major proportions of insoluble dietary fibres, e.g. from pea, oat or quinoa bran, increased crumb hardness when the water dosage remained fixed (see Table 3). However, when increasing the water content (from 90 g to 110 g per 100 g flour), the crumb was softened also in the case of oat fibre or quinoa bran (Aprodu & Banu, 2015; Föste, Jekle, & Becker, 2017). These results emphasize the importance of adjusting the water amount in GF formulations, particularly when insoluble fibres are added.

In addition, several studies suggested aiming at a lower dough consistency through water addition, to keep the bread softer during storage (Bechtel & Meisner, 1954; Cappa et al., 2013; Gallagher, Gormley, & Arendt, 2003; Mariotti et al., 2009). The impact on GF bread firming is mostly explained by the ratio of soluble to insoluble fibres. The former dissolve in the aqueous dough phase, enveloping starch granules and impairing their ability to absorb water. Consequently, less starch gelatinizes during baking (Martínez et al., 2014; Phimolsiripol et al., 2012). In addition, hydrogen bonding between fibres and starch may delay starch retrogradation (Sabanis et al., 2009).

Baking loss describes the amount of water, which evaporates during baking. In GF formulations this value varied from 13% to 20% (Capriles & Areas, 2013; Hager et al., 2011; Korus, Grzelak, Achremowicz, & Sabat, 2006; Ronda et al., 2015; Föste et al., 2017). Because of their high water-binding capacity, fibres might reduce water loss during storage. However, no correlation between solubility of fibres and the baking loss has been identified. In fact, other components from the by-products may influence the structure-function relationship in GF bread. Especially fibers rich in fats can form complexes with amylose. This might be the reason for limited starch swelling, amylose leaching and reduced starch retrogradation, softening the crumb. The formation of amylose-lipid complexes and the impact on crumb softening has been

**Table 3**
Impact of fibres on crumb structure characteristics in GF bread.

| Source of by-products | Fibre amount (g/100 g flour) | Water addition (g/100 g flour) | Firmness (g) hardness (N) | | Crumb moisture | References |
|---|---|---|---|---|---|---|
| | | | $t_0$ | $t_x$ | $t_0$ | |
| Oat bran | 12.5 | 76.5 | ↑ | | | Aprodu and Banu (2015) |
| Oat fibre | 3, 6, 9 | 90, 100, 110 | ↓ | ↓ | ↑ | Sabanis et al. (2009) |
| Defatted rice bran | 10 | 100 | ↑[b] | ↑[b] | | Phimolsiripol et al. (2012) |
| [a]Risolubles | 10 | 100 | ↓[b] | ↓[b] | | Phimolsiripol et al. (2012) |
| [a]Rifibre | 10 | 100 | ↑[b] | ↓[b] | | Phimolsiripol et al. (2012) |
| [a]Ribran 100 | 10 | 100 | ↓[b] | ↓[b] | | Phimolsiripol et al. (2012) |
| Oat β-glucan | 3.9 | 111, 121, 131, 141 | ↑ | ↑ | | Ronda et al. (2015) |
| Oat β-glucan | 5.6 | 132 | ↓ | ↓ | ↑ | Hager et al. (2011) |
| Inulin | 9 | 83 | ↔ | ↑ | ↓ | Hager et al. (2011) |
| Inulin | 3, 5, 8 | 120 | ↓ | ↓ | | Korus et al. (2006) |
| Inulin | 4, 8, 12 | 122, 120, 119 | ↓ | ↓ | | Ziobro et al. (2013) |
| Inulin | 4, 8, 10, 12 | 85 | ↓ | ↓ | ↓ | Capriles and Areas (2013) |
| Psyllium fibre | 5 | 76.5 | ↓ | | | Aprodu and Banu (2015) |
| Pea fibre | 20 | 76.5 | ↑ | | | Aprodu and Banu (2015) |
| Quinoa bran | 10, 20, 40, 40 | 80 | ↑ | | | Föste et al. (2014) |
| Quinoa bran | 10 | 80, 90, 100, 110 | ↓ | | | Föste et al. (2017) |

Crumb structure characteristics were determined as follows: Crumb hardness (g) or firmness (N) by means of Texture Profile Analysis and crumb moisture in (g/100 g) by means of gravimetric determination both in dependence of time with $t_0$: after processing and $t_x$: after at least 3 days of storage.
↑, increase in value with addition of fibre source; ↓, decrease in value with addition of fibre source; ↔ no significant difference reported.
[a] Risolubles, Rifibre, Ribran: commercial rice bran sources from NutraCea™ Scottdale, USA.
[b] Staling kinetic parameter firmness (N) during 9-day storage of GF bread fitted to the Avrami equation, values considered were $T_0$ crumb firmness of fresh bread and $T_\infty$ (final crumb firmness in $F_{max}$ (N) compared to GF control.

observed for buckwheat flour addition (Alvarez-Jubete, Arendt, & Gallagher, 2010; Wronkowska, Haros, & Soral-Śmietana, 2013). Determination of proton mobility has already been introduced as a promising tool to clarify phase transition of water during processing in dough formulations (Bosmans et al., 2012). To date, little information exists on the impact of fibres on proton mobility in GF bread. Hitherto, rice and oat flour-based model systems have been studied, showing comparable proton distribution. This approach could help to better understand the firming mechanisms in GF bread (Hager, Bosmans, & Delcour, 2014). Utilization of nuclear magnetic resonance spectroscopy could support the monitoring of fibers' water binding throughout processing of GF bread, to enable a targeted application in the future.

## 6. Strategies to modify hydration properties

### 6.1. General

The following section addresses strategies to modify hydration properties in GF batter. The soaking of native polymers, microbial and enzymatic polymer modification, as well as thermal and mechanical treatments will be compared.

### 6.2. Soaking of native polymers

Soaking is a common practice to soften the grain and accelerate the cooking process of beans (Zamindar, Baghekhandan, Nasirpour, & Sheikhzeinoddin, 2013) or rice (Horigane, Takahashi, Maruyama, Ohtsubo, & Yoshida, 2006). The seed coat and its microstructure determine the speed and quantity of water uptake and the degree of softening during soaking (Taiwo, Akanbi, & Ajibola, 1998). Water penetrates from the outer seed coat through the cotyledons towards the centre of the grain. In bran-containing grains, water movement is slowed by the pericarp tissues and the seed coat, while milled grains enable rapid water infiltration into the cracks or chalky areas (Horigane et al., 2006). Similarly, slow hydration is attributed to hemicellulose and pentosans, which are located in the seed coat of cereals or legumes. These substances prevent the water penetration, while the middle lamella without seed coat may absorb the moisture more quickly (Vasudeva & Vishwanathan, 2010).

As already mentioned in the introduction, the application of wheat bran weakens the dough structure and consequently decreases loaf volume (Lai, Hoseney, & Davis, 1989a, 1989b). One strategy to partly reverse the detrimental effect of fibres or bran is water addition, for instance by means of soaking. According to Lai et al. (1989a), loaf volume was significantly increased by adding 22% of pre-soaked, fine ground wheat bran to traditional wheat dough. Chen et al. (1988) also reported that pre-soaking of 4% apple fibre partly alleviated the negative effect of the untreated fibre on wheat bread loaf volume. Hamada, Aoki, and Suzuki (2012) analyzed the impact of soaking (2 to 48 h) in a flour mixture containing brown rice and vital gluten in the proportion of 80% to 20%. Instead of soaking the pure bran, brown rice grains were soaked and milled afterwards. The authors observed an increased specific loaf volume of rice bran-containing breads after increasing the soaking time up to 48 h. During soaking, also time-dependent changes in enzyme activity may occur that have to be taken into consideration. For instance, Hamada et al., (2012) reported that soaking increased α-amylase activity (5 to 12 times higher), but this surprisingly did not correlate with the specific loaf volume. Instead, a strong interrelation between specific loaf volume and the damaged starch content of brown rice flour ($r = 0.987$) was revealed. The authors concluded that rice bran had little effect on the rising properties of a brown rice/gluten formulation (Hamada et al., 2012). Since GF batters are a neglected issue in this context, further investigations would be valuable to clarify whether soaking of fibers has a positive effect on GF bread quality.

### 6.3. Microbial polymer modification

A second strategy to improve the hydration properties of GF batter is the pre-fermentation of fibres. This microbial approach combines fibre or bran with water and a defined amount of starter strains (lactic acid bacteria or yeasts). In general, for the manufacture of pre-fermented dough, higher initial water amounts are utilized, ranging from 175 to 200 g water/100 g buckwheat flour to 350 g water/100 g wheat bran (Katina, Salmenkallio-Marttila, Partanen, Forssell, & Autio, 2006; Messia et al., 2016; Moroni, Arendt, Morrissey, & Dal Bello, 2010). Depending on substrate availability and the type of lactic acid bacteria (homofermentative/heterofermentative), carbohydrates are metabolized, forming lactic acid or a combination of lactic and acetic acid, as well as ethanol and carbon dioxide. Both acids are hydrophilic components and therefore increase dough hydration. In addition, pre-fermentation enables the activation of endogenous enzymes due to the decreased pH value (see Section 6.4).

Pre-fermentation with *Aspergillus oryzae* for 12 h improved the swelling of rice flour particles in a GF batter formulation and increased the final loaf volume (2.2-fold) compared to the control (zero hours of pre-fermentation) (Hamada, Suzuki, Aoki, & Suzuki, 2013). According to the authors, pre-fermentation significantly increased protease activity, leading to an elevated batter viscosity and slower sedimentation of flour particles. Possibly, aggregations between starch granules and deformed proteins may have caused this change in batter rheology. Furthermore, their RVA measurements revealed an influence on starch gelatinization, as indicated by a decrease in peak and final viscosity (Hamada et al., 2013).

Farahmand, Razavi, Yarmand, and Morovatpour (2015) observed increased loaf volume, softer crumb and extended shelf-life after the addition of pre-fermented rice bran. Also, exopolysaccharides (EPS) can be generated by means of specific lactic acid bacteria. These EPS may increase water absorption (comparable to hydrocolloids), improving GF dough formation and structure stabilization (Arendt, Moroni, & Zannini, 2011; Rühmkorf, Jungkunz, Wagner, & Vogel, 2012). Because of their ability to retain water in the crumb, pre-fermentation of bran might be an appropriate strategy to slow down firming in GF bread.

### 6.4. Enzymatic polymer modification

In some cases, endogenous or exogenous enzymes have been applied to support structure formation in GF batter. Especially, functional groups of amino acid side chains in protein (and fibre)-rich raw materials can be targeted by enzymatically introduced protein modifications. This approach comprises: (1) the addition of organic molecules, including cofactors, oligosaccharides, nucleotides, lipids, and small moieties such as methyl, acetyl, and phosphoryl groups; (2) intramolecular transformations, such as disulfide bond formation and proteolytic processing; and (3) intermolecular cross-linking by covalent bond formation between individual protein molecules (Heck, Faccio, Richter, & Thony-Meyer, 2013). These modifications also affect the hydration properties and consequently the structure of GF batter. The proteins of GF raw materials oftentimes show inferior network-forming capacities and are unable to retain gas during fermentation and baking. The following enzymes may be of interest for modifying the hydration properties: glucose oxidase (GO), oxidoreductases, tyrosinase and laccase or transglutaminase (TG). By treating rice flour with glucose oxidase, the sulfhydryl groups have been decreased by almost 41% and, instead, stabilizing disulfide bridges have been formed (Gujral & Rosell, 2004). As these covalent bonds normally remain unchanged during bread making (Aït Kaddour, Barron, Robert, & Cuq, 2008), this enzymatic polymer modification can have a positive effect on the final bread volume.

Furthermore, proteins can be cross-linked, for instance, by transglutaminase (TG), which mediates protein network formation and increases elasticity. This can improve the water and gas retention, leading

to softer crumb and elongated shelf life (Rosell, 2009). To mediate such a reaction, availability of suitable amines is required, so that water can act as a nucleophile, initiating the deamidation of protein-bound glutamine residues (Kieliszek & Misiewicz, 2014; Lorand & Graham, 2003). Scarnato et al. (2016) reported that the microbial enzyme (mTG), isolated from a *Streptoverticillium* sp. *strain*, was successfully applied in GF flours, from corn, rice, amaranth or lentil, to promote protein network formation. Moreover, these authors for the first time combined mTG and sourdough treatment. They highlighted the formation and accumulation of volatile compounds, which could be of interest for leavening, aroma and preservation of GF bread.

Not only proteins can be targeted by enzymatic treatments. Xylanase can be applied to modify the ratio between soluble and insoluble fibres in favour of desirable soluble fibre, thus improving the hydration properties as proposed by Laurikainen, Härkönen, Autio, and Poutanen (1998). Also, pentosan-containing rye bran, which comprises high contents of arabinoxylan (AX), can be utilized as substrate for enzymatic reactions. As rye bran is a gluten-containing by-product, its suitability for GF bakery application was previously evaluated by Mansberger et al. (2014). These authors studied pentosan extraction from rye bran regarding its protein and residual gluten content. They produced pentosan isolates with a purity of over 65% (m/m), yielding 2.5 g out of 100 g of bran, whereas secalines were below 20 ppm in all isolates. Buksa, Nowotna, and Ziobro (2016) analyzed the impact of cross-linked and hydrolyzed AX in model rye bread. These authors examined the structure formation of rye bread in a model recipe using water-extractable AX with varying molar weights and structural units. The applied mixes resulted in a product closely resembling typical rye bread, even if AX was modified via cross-linking or hydrolysis. Therefore, pentosan isolates may be recommended as a promising structuring agent to improve the hydration properties of GF bread.

### 6.5. Thermal and mechanical treatments

In contrast to the beneficial functions of the enzymes described in Section 6.3, endogenous enzymes from fibre or bran can also have undesirable effects. Thermal treatments are one strategy to inactivate these heat-sensitive components that are inter alia located in the seed coat. For example, heat can be applied to inactivate peroxidase, lipoxygenase or lipase activity, depressing the formation of free fatty acids and consequently extending shelf life due to reduced autoxidation. Indeed, microwave heating (245 MHz, 3 min) prolonged the shelf life of rice bran by up to four weeks (Tao, Rao, & Liuzzo, 1993).

Moreover, especially thermal and mechanical treatments of fibres from the milling and fruit processing industries can influence the water absorption and change the overall functionality . There are different dry (roasting, oven drying, microwave heating) and wet treatments (boiling, autoclaving and extrusion), which can improve hydration properties, independently of the applied source of by-product (see Table 4).

For hydrothermal treatments such as extrusion cooking, it is crucial to know whether the integrity of starch granules is preserved by using heat treatment below gelatinization temperature, or if the molecular order of starch granules is irreversibly destroyed by using temperatures above gelatinization (Gómez & Martínez, 2016). Gelatinization renders the starch molecules more prone to swelling in contact with water (Martínez et al., 2014; Mason, 2009). Surprisingly, the total dietary fibre content in GF recipes with teff flour, apple fibre, beetroot (Stojceska, Ainsworth, Plunkett, & İbanoğlu, 2010) or oat bran (Zhang, Liang, Pei, Gao, & Zhang, 2009) increased after extrusion cooking. Moreover, extrusion reduced the molecular weight of pectin and hemicellulose molecules in sugar beet pulp, thus promoting water solubility (Ralet, Thibault, & Della Valle, 1991). Possibly, high molecular weight fibres are less affected by heating than are fibres of lower molecular weight (Greve et al., 1994). The apparent fibre increase can possibly be ascribed to the formation of complexes between polysaccharides and proteins or phenolic compounds, which are measured as fibre (Takeyama, Yokokawa, & Tanimura, 1996). As a consequence of starch gelatinization during extrusion and its retrogradation after cooling, the formation of resistant starch is favoured. In particular, the amylose/amylopectin ratio and the technological conditions during processing are important factors influencing the solubility and formation of resistant starch (Escarpa, González, Mañas, García-Diz, & Saura-Calixto, 1996; Stojceska et al., 2010; Vasanthan, Gaosong, Yeung, & Li, 2002). It has been observed that insoluble dietary fibres of barley were transformed into soluble dietary fibre by transglucosidation. Thereby 1,4 carbon-oxygen bonds are cleaved and new anhydroglucose linkages are formed (Vasanthan et al., 2002). In summary, the choice of by-products' origin and the processing conditions enable targeted modification of soluble or insoluble dietary fibres. This approach is an efficient way to modify the hydration properties of GF batter, e.g. by increasing the solubility and consequently modifying the water absorption and functional batter characteristics. This facilitates the specific choice of innovative strategies for technological applications in GF bread making for the future.

## 7. Conclusion

The fortification of GF bread with sustainable by-products, such as fibres from the milling and fruit processing industries, addresses the increasing demand for nutritious GF foods. However, the addition of fibres can change the aroma profile, weaken the dough structure and modify volume, texture and shelf-life of GF bread. This review summarizes the varying composition of fibres from different milling and fruit processing industries and compares their influence on batter rheology, gas retention and structure formation as a function of the hydration behaviour.

An initial comparison of different hydration properties revealed the problematic dependence on the adopted measuring technique. The wide range of available definitions and methods essentially complicates the comparability of values from different literature sources. A standardized and uniform approach that comprises the absorption of water

**Table 4**
Studies employing heat treatment to modify hydration characteristics of fibres.

| Source of by-products | Method | Results | References |
|---|---|---|---|
| Oat bran | Heat steam<br>Extrusion | ↑ Ratio of soluble dietary fibres | Zhang et al. (2009) |
| Sugar beet pulp | Extrusion | ↑ Soluble dietary fibres, ↑ Water solubility | Ralet et al. (1991) |
| Apple fibre, beetroot, carrot, cranberry, teff flour | Extrusion | ↑ Total dietary fibre content | Stojceska et al. (2010) |
| Rice grits | Extrusion | ↑ Water absorption | Singh, Gamlath, and Wakeling (2007) |
| Amaranth flour | Extrusion | ↑ Water absorption, water solubility | Menegassi, Pilosof, and Arêas (2011) |
| Corn flour | Extrusion | ↑ Water absorption, ↓ Baking loss | Ozola, Straumite, Galoburda, and Klava (2012) |
| Rice bran | Microwave heating | ↑ Inactivation of lipase<br>↑ Stability during storage | Tao et al. (1993) |

↑, increase in value with addition of fibre source; ↓, decrease in value with addition of fibre source, ↔, no significant difference reported.

over time, as well as the ability of the fibers to retain water when external forces are applied, should be defined for the future. Missing correlations between rheological values and GF bread quality impede the optimization of the water addition via traditional methods, such as the Farinograph. Moreover, results have revealed that the loss factor tan δ, ranging from 0.2 to 0.8, cannot serve as uniform key value to adjust GF loaf volume. Several alternative attempts have been made to adapt the water content in GF formulations to form a batter that is neither too liquid nor to viscous, as both options impair gas retention capacity. In most of the analyzed fibre-enriched GF batters, further water addition was not considered. Instead, an increased dough firmness, a lower gelatinization rate and a reduced starch granule swelling were observed. It can be concluded that, especially for fibre-enriched formulations, there are still gaps and needs that must be elucidated to optimize water addition.

Different strategies to compensate the drawbacks of fibres on GF batter and bread quality were compared. Pre-fermentation and extrusion appear particularly efficient in modifying the hydration properties of fibers and GF batter. Pre-fermentation turned out to be an efficient strategy to change batter rheology, to increase enzymatic activity and to improve starch gelatinization. Lastly, extrusion treatment changed the ratio from insoluble to soluble dietary fibres and consequently modified water absorption. The effects of hydration properties on structure formation have to be further clarified in order to enable a targeted fortification of GF bread with nutritious fibres in the future.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## 2.4 Impact of quinoa bran on gluten-free dough and bread characteristics

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ORIGINAL PAPER

# Impact of quinoa bran on gluten-free dough and bread characteristics

**Maike Föste · Sebastian D. Nordlohne · Dana Elgeti · Martin H. Linden · Volker Heinz · Mario Jekle · Thomas Becker**

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**Abstract** Besides an appealing texture and taste, gluten-free products should feature a well-balanced nutrient profile, since celiac disease or chronic inflammations are likely to induce malnutrition for involved patients. Due to their composition, pseudocereals represent a promising ingredient to improve nutrient profile of gluten-free bread. The objective of this study was to investigate the impact of quinoa bran on gluten-free bread quality, focusing on volume, pore size and sensory acceptance. The impact of quinoa bran was studied in a gluten-free bread formulation. Five different quinoa bran and two whole grain flour concentrations were evaluated and compared to a control formulation based on rice and corn flour. The rheological properties of quinoa bran as well as the effect on dough development up to a replacement level of 80 % were investigated. Baking tests were carried out, and loaf volume, crumb firmness and sensory characteristics were determined. Quinoa fractions significantly increased carbon dioxide formation ($p < 0.05$) due to a higher substrate availability. Gas retention was reduced by increasing bran levels ($p < 0.05$). Oscillation measurements indicated a firming impact of quinoa bran which might have caused a more permeable dough structure, promoting the release of carbon dioxide. With regard to the specific loaf volume significant differences were found across the quinoa milling fractions and the applied levels ($p < 0.05$). Overall this study demonstrated that 10 % bran improved the bread volume by 7.4 % and enhanced the appearance without compromising the taste.

**Keywords** Milling fraction · Protein enrichment · Substrate availability · Carbon dioxide · Celiac disease

M. Föste · D. Elgeti · M. Jekle (✉) · T. Becker
Institute of Brewing and Beverage Technology, Research Group Cereal Process Engineering, Technische Universität München, 85354 Freising, Germany
e-mail: mjekle@wzw.tum.de

S. D. Nordlohne · M. H. Linden · V. Heinz
Research Platform Biotechnology, German Institute of Food Technologies, 49610 Quakenbrück, Germany

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### Introduction

Alternative plant-based protein sources are gaining importance not only for people on a vegetarian diet but also due to the high energy requirements for animal protein production. The protein content in quinoa seeds varies from 14 to 20 % (g/100 g dry basis), being predominantly rich in essential amino acids such as methionine and lysine [1]. In particular, the high protein efficiency ratio of quinoa, which is up to 93 % of that of casein or even up to 105 % when cooked [2], promotes the growing interest, compared to rice or corn. Because of the lack of gluten, this pseudocereal is perfectly suited for celiac patients who are obliged to maintain a lifelong gluten-free diet.

In the last decades, several reports were published on the chemical composition of quinoa [3–5]. As mentioned by Krupa-Kozak, gluten-free products are often characterized by their low protein, mineral and dietary fiber content [6]. Efforts have been made to enrich gluten-free products in micro- and macroelements and proteins [6]. Quinoa and amaranth have been utilized for the manufacture of products such as pastas, bread, cakes and baby foods [7–9] and the incorporation of quinoa flour for the manufacture of enriched gluten-free bakery products has been discussed by Taylor and Parker [10]. Due to the high calcium, iron, zinc or magnesium content, quinoa is of high nutritional value for different target populations. In particular, adults and children benefit from calcium for bones and from

iron for blood functions [3, 11]. From the botanical point of view, proteins, lipids and minerals are mainly localized in the outer grain parts as mentioned by Prego [12]. The separation of cell tissues such as the pericarp, seed coat and embryo from the perisperm through milling has been analyzed by Chauhan [13], resulting in nutritionally different flour and bran fractions. Previous studies focused mostly on white- or whole grain flour from, e.g., buckwheat, pointing out positive effects on specific bread volume and proportional enrichment in proteins and microelements [6, 14, 15]. Furthermore, Gambus et al. [16] focused on protein and fiber enrichment in a gluten-free corn starch formulation. The replacement by 10 % amaranth increased protein content by 32 and mineral content by 152 %, whereas the sensory quality of the bread remains unaffected. Whereas the application of proteins from different sources such as soybean, milk and egg or whole grain flours has been investigated [17, 18], studies on the incorporation of bran in gluten-free bread are rare. Quinoa bran consists of the outer cell tissues which include the pericarp and/or seed coat and the embryo, representing about 40 % of manually dehulled quinoa seed [13]. However, containing mainly worthwhile components, the addition of cereal bran cause severe technological challenges when applied in such amounts that health benefits can be expected. As reported by Seibel for wheat bread, the addition of wheat bran decreases bread volume, resulting in a tense and non-elastic crumb and flavor changes depending on the kind of fiber [19]. Although the application of cereal bran in some gluten-free foods has been reported for rice [20], the addition of quinoa bran has not been investigated yet.

The objective of this study was to investigate the impact of quinoa bran on gluten-free dough and bread characteristics. Therefore, the focus was to incorporate a maximum level of quinoa bran in the gluten-free dough matrix, whereby bread characteristics should be improved. Targeted figures for the evaluation of gluten-free bread quality were a high bread volume, a soft crumb, a medium pore size and sensory acceptance. Moreover, viscoelastic dough and gas holding properties were analyzed in order to determine reasons for the changing quality characteristics in gluten-free bread.

## Materials and methods

### Raw materials and ingredients

Whole grain rice flour from brown rice of the plant *Oryza sativa L.*, corn flour of *Zéa mays L.* and corn starch were purchased from Davert (Senden, Germany). Organic Royal Quinoa seeds (*Chenopodium quinoa*, free of saponins due to a removal of the pericarp) from Bolivia were purchased from Ziegler & Co. GmbH (Wunsiedel, Germany). Quinoa seeds were ground to whole grain flour in an ultra-centrifugal mill type Retsch ZM 200 (Haan, Germany) with a mesh of 500 µm. Prior to the fractionation in a Brabender Quadrumat Junior mill (Duisburg, Germany), seeds were conditioned in an airtight box to 15 % moisture content and kept at room temperature for 20 h at 20 °C. Separation into bran and quinoa white flour was performed by sieving in a rotating sifter (mesh of 200 µm).

Further ingredients for dough and bread formulation were food grade hydroxypropyl methylcellulose (HPMC) by K4M, The Dow Chemical Company (Midland, USA), NaCl purchased by esco (Hannover, Germany), baking margarine by CSM Deutschland GmbH (Bingen am Rhein, Germany) and dry yeast of the species *Saccharomyces cerevisiae* (Casteggio Liveti, Italy).

### Analysis of ingredients and water retention capacity

The analytical composition of quinoa whole grain flour and quinoa bran was determined according to AACC methods as follows: protein (AACC 46-16, N × 5.54), ash (AACC 08-12) and moisture content (AACC 44-01). In addition, water retention capacity (WRC) was determined according to AACC method 56-11 [21].

### Analysis of protease and α-glucosidase activity

The activity of endogenous proteolytic activity was assayed using a method for staining amino acids with a ninhydrin reagent [22]. Sample preparation was performed as follows: Milling fractions were mixed with an equal amount of distilled water in a sterile beaker. Chloramphenicol and cycloheximide purchased from Carl Roth GmbH & Co. KG, (Karlsruhe, Germany) were both added in a concentration of 0.02 % before kneading for 10 min. The dough was incubated in a 30 °C tempered water bath for 26 h. The development of yeast, lactobacilli strains and pH-value was monitored in the beginning and at the end of incubation. For a determination of the kinetic of proteolysis, dough samples were taken every 2 h, centrifuged (25,876 rcf, 4 °C for 10 min) and the supernatants were diluted 1:100 with distilled water. Furthermore, 300 µL of sample material or blank with distilled water were added to 600 µL cadmium-ninhydrin reagent (0.8 g ninhydrin, 80 mL ethanol, 10 mL acetic acid), all provided by Carl Roth GmbH & Co. KG (Karlsruhe, Germany), and 1 mL of the following solution: 1 g/mL distilled water (cadmium chloride-hemipentahydrate solution) purchased by Sigma-Aldrich Chemie GmbH (Seelze, Germany) and incubated at 84 °C for 5 min. According to Krauss after cooling on ice, the absorption rate was measured at 507 nm [22] and a calibration curve was constructed with glycine by neoLab Migge

**Table 1** Gluten-free bread formulations

| Recipe | Control | Proportion of quinoa milling fraction (%) | | | | |
|---|---|---|---|---|---|---|
| | | 10 | 20 | 30 | 40 | 80 |
| Rice flour | 50 | 45 | 40 | 35 | 30 | 10 |
| Corn flour | 25 | 22.5 | 20 | 17.5 | 15 | 5 |
| Corn starch | 25 | 25 | 25 | 25 | 25 | 25 |
| Milling fraction | – | 7.5 | 15 | 22.5 | 30 | 60 |
| Margarine[a] | 3 | 3 | 3 | 3 | 3 | 3 |
| Dry yeast[a] | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| NaCl[a] | 2 | 2 | 2 | 2 | 2 | 2 |
| HPMC[a] | 2 | 2 | 2 | 2 | 2 | 2 |
| Distilled water[a] | 80 | 80 | 80 | 80 | 80 | 80 |

Milling fraction: either quinoa bran or quinoa whole grain flour

*HPMC* Hydroxypropyl methylcellose

[a] Parts per 100 parts flour

Laborbedarf-Vertriebs GmbH (Heidelberg, Germany). One unit is defined as the amount of enzyme that releases 1 µmol of free amino acids from the substrate per hour and gram dough at the defined pH and temperature.

The endogenous α-glucosidase activity was determined using 1.0 g of the sample, which was extracted for 20 min at 40 °C with 10 mL 200 mM NaAc provided by Merck (Darmstadt, Germany) at pH 4.5. After centrifugation at 20 °C and 30,790 rcf for 10 min, the supernatant was analyzed with the assay of amyloglucosidase using p-nitrophenyl-β-D-maltoside plus thermostable β-glucosidase (Megazyme, Ireland). One unit is defined as the amount of enzyme that releases 1 µmol of p-nitrophenol from the substrate per minute at the defined pH and temperature.

### Preparation of gluten-free dough and bread

The composition of eight different gluten-free dough and bread formulations are summarized in Table 1. According to the AACC approved method 44-01, the moisture content of all flours was adjusted to 14 % and added water was tempered to produce dough of 28 °C [21]. All ingredients were mixed at 100 rpm for 2 min and kneaded at 200 rpm for 2 min in a SP 12 A-3 spiral kneader (Diosna Dierks & Söhne GmbH, Osnabrück, Germany). After weighing 250 g into baking tins (resulting in 4 tins per recipe), samples were proofed at 30 °C and 80 % relative humidity for 30 min and baked at 220 °C for 35 min with initial 0.5 L steam in a deck oven (Matador MD 120, Werner & Pfleiderer, Dinkelsbühl, Germany). Gluten-free breads were stored on a wooden rack at room temperature for 2.5 h before volume and crumb firmness measurements were taken and 24 h before sensorial evaluation was conducted.

### Dough fundamental rheology

Rheological measurements were taken with a controlled stress rheometer (ARG2, TA Instruments, West Sussex, UK). Gluten-free dough was prepared as explained above but without the addition of yeast. The rheological properties of the samples were analyzed using a parallel plate geometry, which consisted of a 40 mm diameter corrugated sample and plate. The tests were performed with a gap of 3,000 µm between the plates. After loading, excess sample was trimmed and a thin layer of paraffin oil was applied to the sample edges. Prior to the analysis at 30 °C, samples had a 10 min conditioning time. Deformation sweeps were performed in the range of 0.001–100 % strain on all samples in order to determine the linear viscoelastic region, which was determined with 0.005 %. Therefore, frequency sweeps were performed in the range of 0.1–10 Hz with 0.005 % strain on all samples. The fundamental rheological properties of the dough samples were evaluated through the complex shear modulus $G^*$(Pa) and the loss factor tan δ (–). All determinations were carried out in triplicate, and the average values and standard deviations were adopted.

### Dough development characteristics

The dough development and gaseous release during fermentation were determined using a Rheofermentometer (Chopin, Villeneuve-La-Garenne, France). The preparation of dough samples was done in the same manner as for the baking trials, and analysis was carried out immediately after mixing. Measurements were taken with 315 ± 0.5 g dough at 30 °C for 3 h, without the use of a cylindrical weight. Registered were as follows: Hm = maximum dough height; H′m = maximum height of gaseous production; Tx = time of dough porosity, which indicates the point in time when gas starts to escape the dough and conforms to permeability of the gluten-free dough matrix [23]. All determinations were made in duplicate, and the average values and standard deviations were adopted.

### Bread characteristics

With regard to the determination of bread volume loaves of one batch were measured with a laser-based volumeter

**Table 2** Analytical composition and water retention capacity

| Flours and milling fractions | Moisture content (%) | Protein content (% db) | Mineral content (% db) | Water retention capacity (%) |
|---|---|---|---|---|
| Rice flour | 12.54 ± 0.09b | 6.99 ± 0.09b | 1.37 ± 0.01c | 109.75 ± 1.12a |
| Corn flour | 12.02 ± 0.06a | 6.58 ± 0.08b | 0.45 ± 0.02b | 114.97 ± 1.77b |
| Corn starch | 12.41 ± 0.05b | 0.44 ± 0.04a | 0.10 ± 0.01a | n.d. |
| Quinoa whole grain flour | 12.40 ± 0.06b | 11.75 ± 0.11c | 2.39 ± 0.02d | 109.13 ± 1.09a |
| Quinoa bran | 13.83 ± 0.18c | 18.08 ± 1.77d | 5.15 ± 0.02e | 151.94 ± 2.25c |

A number of replicates are means with standard deviation ($n = 3$). Different letters indicate significant differences between means in the same column (ANOVA, $p < 0.05$)

*db* dry basis; *n.d.*: not detectable

(BVM-L370, Perten Instruments, Hägersten, Sweden). For calculation of specific bread volume (mL/g), loaves were divided by their weight. Crumb firmness was determined with regard to AACC method 74-09 [21]. Therefore, bread was sliced using a Tendenza T16 Genio bread slicer (Graef GmbH & Co.KG, Amsberg, Germany) to obtain uniform thickness of 1.25 cm and placed one above the other. Texture profile analysis (TPA) was determined with a TVT-300 XP texture analyzer (Perten Instruments, Hägersten, Sweden) equipped with a 20 mm aluminum cylindrical plug. Bread slices with a height of 2.5 cm were compressed by 40 % in two subsequent cycles with 15 s intermediate rest in the center. Four replicates from two different sets of baking were analyzed and averaged. All baking trials were performed twice on two different days.

Sensorial evaluation of gluten-free bread was performed by a panel of non-celiac panelists ($n > 10$). According to the DIN standard method 10961, panelists were trained with regard to color, flavor, taste and texture [24]. Each type of gluten-free bread was evaluated two times on different days ($n > 20$). Therefore, a scale from zero to ten was applied. Sensory attributes were rated by the panelists with a score from zero to ten (highest intensity). Sensory evaluation was carried out as follows: one slice of bread, identified by code numbers, was served to each panelist under normal (daylight) illumination. Evaluated attributes are defined as follows: Crust color: dark (10), pore size: fine distribution (10), firmness: firm (10), juiciness: juicy (10), odor intensity: high (10), bitterness: high (10), off-flavor: high (10) and overall acceptability: high acceptance (10).

Images of the bread slices were captured 2.5 h after baking using a scanner (Canon Scan N67OU) and supporting software (Canon Scan Toolbox version 4.1).

### Statistical analysis

Statistical analysis was performed with Prism 5 software (version 5.03, GraphPad Software, Inc.) on all data using one-way analysis of variance (ANOVA). The Tukey–Kramer test was utilized to describe statistical differences between means at ($p < 0.05$) significance level.

## Results and discussion

### Protein and mineral enrichment

Depending on the choice of raw materials, gluten-free bread often has a low nutritive value. With the focus on an improvement in protein and mineral content, in this study, all utilized flours and milling fractions were analyzed and results are represented in Table 2.

The protein and mineral content in rice and corn flour were significantly lower than in quinoa whole grain flour. Through fractionation, both values were significantly increased in quinoa bran ($p < 0.05$). Due to the seed structure of quinoa, in particular, the seed coat and the embryo, being rich in minerals and protein, were concentrated in the bran as also indicated by Chauhan [13]. The replacement of rice and corn flour by 10 % quinoa bran increased the protein content of gluten-free bread by 17 %. This phenomenon has been already described in the literature by Krupa-Kozak who reported a proportional enrichment in proteins and microelements when buckwheat flour for bread preparation was utilized [6]. Due to the higher protein content in quinoa bran, it seems reasonable to assume that not only end product quality, but also gluten-free dough characteristics will be influenced. In contrast to rice or corn flour, the major seed protein fraction of quinoa, amaranth or oat is represented by water-soluble globulins, which do not possess the requisites to confer dough elasticity [25, 26]. Regarding the water retention capacity, quinoa bran revealed a significantly higher value ($p < 0.05$) than rice or corn flour (Table 2). Therefore, it can be assumed that due to varying viscoelastic dough properties also gas holding properties and gluten-free bread quality will be affected.

### Enzymatic activity

Proteolytic activity in quinoa bran was nearly doubled in comparison to whole grain flour through fractionation as shown in Table 3. In contrast to rice and corn flour, quinoa bran revealed a significantly higher proteolytic activity

**Table 3** Endogenous enzyme activities

| Endogenous enzyme activity | Milling products | | | |
|---|---|---|---|---|
| | Corn flour | Rice flour | Quinoa whole grain flour | Quinoa bran |
| Proteolytic activity (U/g flour) | 0.10 ± 0.12b | 0.06 ± 0.10a | 0.83 ± 0.09c | 1.76 ± 0.24d |
| a-Glucosidase activity (U/g flour) | 0.50 ± 0.06b | 0.01 ± 0.01a | 9.60 ± 1.04d | 5.86 ± 1.29c |

A number of replicates are means with standard deviation ($n = 2$). Different letters indicate significant differences between means in the same row (ANOVA, $p < 0.05$)

($p < 0.05$). The degradation of proteins by enzymes could influence the water absorption of gluten-free dough, improve digestibility and influence the crust color or flavor of the end product. As reported by Martínez-Anaya proteases produce peptides and amino acids, which participate in metabolic and thermal reactions. Therefore, they can be a source of bitter peptides influencing the bread flavor [27]. The liberation of free amino acids by, e.g., exoproteases can support the browning of the crust as they undergo Maillard-type reactions with reducing sugar to form pigments [28].

Carbon dioxide formation by yeast can be elevated by providing more substrates in the form of mono- and disaccharides. Since the amylolytic activity was significantly higher in quinoa bran than in rice or corn flour ($p < 0.05$), this is an indication for a higher availability of these substrates. Elgeti et al. demonstrated that the higher substrate availability indeed leads to higher bread volume. Glucosidase and sucrose were separately added to a gluten-free control recipe, resulting in a similar volume increase compared to the use of quinoa white flour [15].

## Viscoelastic properties

The rheology of gluten-free dough is a particularly important physical property affecting the product quality. The effect of quinoa bran on viscoelastic dough characteristics is visualized in Fig. 1. The complex shear modulus ($G^*$) was measured as it is linked to the dough firmness. Depending on the bran concentration, a positive correlation with dough firmness was found ($R^2 = 0.96$, $p < 0.001$). This means that by increasing the amount of quinoa bran up to 80 % dough firmness was elevated.

The loss factor tan δ represents the relation between the storage modulus G′ (elastic part) and the loss modulus G″(viscous part). A low value indicates more elastic dough than observed for amaranth by Houben et al. [29]. Regarding the amount of quinoa bran, gluten-free dough became more elastic (Fig. 1), which is also in accordance with earlier studies on the addition of up to 30 % husked buckwheat flour or plant protein [30, 31], respectively. The elasticity of dough goes hand in hand with its firmness [32]. It can be softened by a higher amount of unbound water available in the dough matrix. Due to the significantly higher water

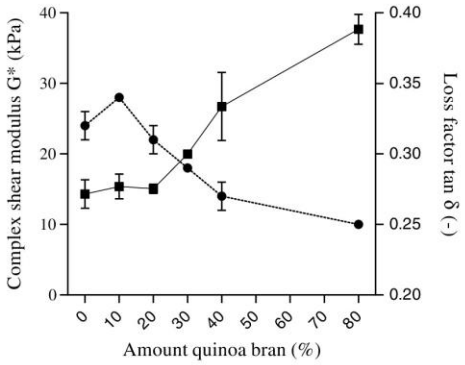


**Fig. 1** Rheological dough properties as a function of replaced rice and corn flour by quinoa bran. Presented are means with standard deviation ($n = 3$). Symbols are as follows: (*square*) $G^*$ and (*circle*) tan δ

**Table 4** Rheological dough properties

| Milling fractions | Complex shear modulus $G^*$ (kPa) | Loss factor tan δ (–) |
|---|---|---|
| Quinoa whole grain flour | | |
| 10 % | 8.97 ± 1.44a | 0.38 ± 0.01d |
| 80 % | 13.94 ± 1.07b | 0.31 ± 0.01b |
| Quinoa bran | | |
| 10 % | 15.39 ± 1.76b | 0.34 ± 0.00c |
| 80 % | 38.28 ± 2.12c | 0.25 ± 0.00a |

A number of replicates are means with standard deviation ($n = 3$). Different letters indicate significant differences between means in the same column (ANOVA, $p < 0.05$)

retention capacity (WRC) of quinoa bran in comparison to quinoa whole grain, rice flour and corn flour (Table 2), this could lead to firmer dough. A positive correlation ($R^2 = 0.82$) between water absorption and the proportion of amaranth or quinoa flour was observed by Tömösközi et al. [33]. In addition, Zhang and Moore as well as Sudha et al. [34, 35] demonstrated that the addition of wheat or other commercial cereal bran fractions to dough resulted in a higher water absorption rate. This was attributed to the higher amount of hydroxyl groups present in bran [36].

Due to greater hydrogen bonding, more water is accumulated, which favors a firmer dough matrix. This could be a possible explanation for the softer gluten-free dough when quinoa whole grain flour was utilized (Table 4). In contrast to quinoa bran, the water retention capacity of whole grain flour was lower, possibly resulting in less water binding and therefore in significantly lower values of $G^*$ ($p < 0.05$).

### Dough development

The influence of quinoa bran on carbon dioxide formation and gas holding properties was determined by the rheofermentometer as shown in Fig. 2.

With quinoa whole grain flour or quinoa bran, considerably more gas was produced as visualized by the maximum height of gaseous production (H′m). The total volume of carbon dioxide was nearly three times as much when quinoa whole grain flour or quinoa bran was utilized, compared to the control (data not shown). This was attributed to the higher substrate availability. However, the maximum height of gaseous production was negatively influenced by the bran level (H′m: 10 % QB = 82.10 mm; 80 % QB = 69.70 mm). Furthermore, higher bran levels lead to an earlier point in time at which gas retention capacity was exceeded. In comparison to 40 % quinoa whole grain flour, an equal amount of bran revealed that the starch-based gluten-free dough matrix could better retain the carbon dioxide. In addition, rheological properties can be taken into consideration in order to explain the gas hold-up. Especially the increased firmness through quinoa bran might have impaired the foam stability of the dough. Moreover, it is possible that due to increased carbon dioxide formation, gas pressure in the dough matrix accumulated and contributed to a structural break. This might be the reason why the maximum dough height was negatively influenced by quinoa bran despite the higher gas production. Compared to the control, the maximum dough height significantly decreased with higher bran levels. Only in the case of 10 % quinoa bran, the effect of higher substrate availability seems to have overruled the structure weakening.

Wang et al. [37] related the reduced height in wheat dough to an increase of the permeability to carbon dioxide. For wheat dough, the addition of particulate components, especially bran and epicarp fibers, resulted in a physical disruption of the gluten protein matrix [38, 39]. As a possible explanation, it was proposed that fibers act as points of weakness or stress concentrations within the expanding dough cells. Furthermore, gas holding properties depend on the viscoelastic dough characteristics. With regard to the data of viscoelastic dough properties, it seems reasonable that firmer dough weakens the dough structure, resulting in higher release of carbon dioxide.

### Gluten-free bread characteristics

One of the major quality deficits in gluten-free bread is their poor structure due to bad gas holding properties which particularly affect the bread volume and the crumb density negatively [29]. The impact of quinoa bran on the specific bread volume and the crumb firmness is presented in Table 5. Quinoa whole grain flour (80 %) significantly increased the bread volume by up to 6.8 % in comparison to the control ($p < 0.05$). With regard to the incorporation of the same amount of quinoa bran, loaf volume was significantly decreased. The gas holding properties were negatively influenced by quinoa bran and possibly as a result, air bubbles entrapped through mixing and fermentation were not stabilized throughout baking due to the firm and porous dough matrix. Accordingly, the resulting bread volume was impaired. This effect might have counteracted the substrate availability when more than 10 % of bran was utilized.

Another explanation for the reduced volume might be the high amount of dietary fiber in quinoa bran. Dietary fiber can increase the water absorption of flour [37] and indeed it was shown that quinoa bran had a particularly high water retention capacity (Table 2). Due to the competition of dietary fiber and starch for water, the starch swelling and gelatinization could be limited [40]. The sudden increase in viscosity due to starch gelatinization might be required to reduce the final gas volume fraction in the crumb. Surprisingly, 10 % quinoa bran affected the volume to the same extent as in the case of 80 % whole grain flour. Due to the high amylolytic activity in quinoa milling fractions, the production of carbon dioxide as it was indicated in Fig. 2 was nearly increased to the same extent in both quinoa milling products. It was shown that small amounts of quinoa bran do not necessarily have a negative impact on gas holding properties in gluten-free dough but rather promote volume increase when higher amounts of substrate are available.

Because of the low specific volume, the bread was denser and had a tightly packed crumb structure. This resulted in a higher crumb firmness which negatively correlated with the specific bread volume ($R^2 = -0.76$, $p < 0.05$). Similar results have been previously reported by Gallagher et al. [17] who studied the effect of wheat fiber in gluten-free bread. The explanation probably lies in the thickening of the walls surrounding the air bubbles in the crumb [41].

### Sensory evaluation

Sensory attributes particularly influence consumer's acceptance. The impact of quinoa bran on sensory evaluation and overall acceptability in gluten-free bread is presented in

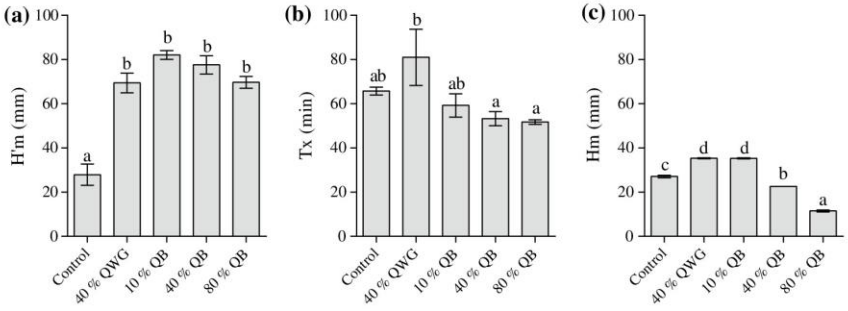


**Fig. 2** Influence of quinoa bran on dough development. **a** Maximum height of gaseous production = H′m, **b** time of dough porosity = Tx; **c** maximum dough height = Hm. A number of replicates are means with standard deviation ($n = 2$). Different letters indicate significant differences between means (ANOVA, $p < 0.05$). *QWG* Quinoa whole grain flour, *QB* Quinoa bran

**Table 5** Impact of quinoa bran on specific bread volume and crumb firmness

| | Specific bread volume (mL/g) | Firmness (N) |
|---|---|---|
| Control | 1.62 ± 0.04c | 16.72 ± 2.01b |
| Quinoa whole grain flour | | |
| 40 % | 1.87 ± 0.07f | 10.63 ± 2.08a |
| 80 % | 1.73 ± 0.10ed | 10.70 ± 1.58a |
| Quinoa bran | | |
| 10 % | 1.74 ± 0.04e | 12.95 ± 1.77ba |
| 20 % | 1.68 ± 0.03d | 14.78 ± 1.84b |
| 30 % | 1.43 ± 0.06b | 20.49 ± 2.48c |
| 40 % | 1.38 ± 0.02a | 27.01 ± 2.35d |
| 80 % | 1.35 ± 0.04a | 32.04 ± 2.58e |

A number of replicates for determination of specific bread volume are means with standard deviation ($n = 12$). A number of replicates for texture profile analysis are means with standard deviation (n = 8). Different letters indicate significant differences between means in the same column (ANOVA, $p < 0.05$)

Table 6. Because of the increasingly bitter taste, quinoa bran has only been replaced by up to 40 %.

The biggest pores were obtained when 10 % of quinoa bran has been incorporated. This means that the dense crumb of the control recipe turned to a medium pore size, as initially set as an aim in this study. Depending on bread type, a finer distribution of pores is common in wheat bread, whereas whole grain bread typically features a coarser pore distribution. In contrast, the crumb of gluten-free bread with 40 % bran was marked as dense and compact due to its small pores. The images in Fig. 3 visualize the porosities of the respective bread slices. Moreover, the gluten-free bread crumb was estimated to be softer when 10 % quinoa bran was applied, while elevating bran levels increased the firmness.

In addition, crust color of the different recipes was assessed. Panelists evaluated the quinoa containing breads with a darker crust compared to the yellowish control

**Table 6** Sensory characteristics of gluten-free bread

| Sensory attributes | Control | Amount of quinoa bran (%) | | | |
|---|---|---|---|---|---|
| | | 10 | 20 | 30 | 40 |
| Pore size | 6.93 ± 1.76cde | 4.40 ± 1.17a | 5.75 ± 1.52bd | 6.95 ± 1.47de | 7.35 ± 1.81e |
| Firmness | 6.51 ± 1.67c | 4.25 ± 0.85a | 5.35 ± 1.18ab | 6.55 ± 0.94bc | 6.90 ± 1.41c |
| Crust color | 1.83 ± 1.14a | 3.47 ± 0.84b | 4.26 ± 0.87bc | 5.16 ± 1.30cd | 5.68 ± 0.89d |
| Odor intensity | 3.40 ± 1.62a | 5.05 ± 1.36b | 5.30 ± 1.56bc | 5.65 ± 1.46bc | 6.50 ± 1.19c |
| Juiciness | 3.99 ± 1.52a | 5.55 ± 1.00b | 5.55 ± 1.15b | 6.30 ± 0.98b | 6.65 ± 1.18b |
| Bitterness | 0.75 ± 0.83a | 0.95 ± 1.05ab | 1.70 ± 0.80b | 3.05 ± 1.05c | 4.10 ± 0.91d |
| Off-flavor | 1.40 ± 1.19a | 2.35 ± 1.04b | 3.00 ± 1.41b | 4.60 ± 1.27c | 5.90 ± 1.29d |
| Overall acceptability | 3.43 ± 1.48a | 6.45 ± 1.00c | 5.75 ± 0.85bc | 5.20 ± 1.05b | 5.05 ± 1.05b |

Evaluated attributes are defined as follows: crust color: dark (10), pore size: fine distribution (10), firmness: firm (10), juiciness: juicy (10), odor intensity: high (10), bitterness: high (10), off-flavor: high (10) and overall acceptability: high acceptance (10). A number of replicates are means with standard deviation ($n \geq 20$). Different letters indicate significant differences between means in the same column (ANOVA, $p < 0.05$)

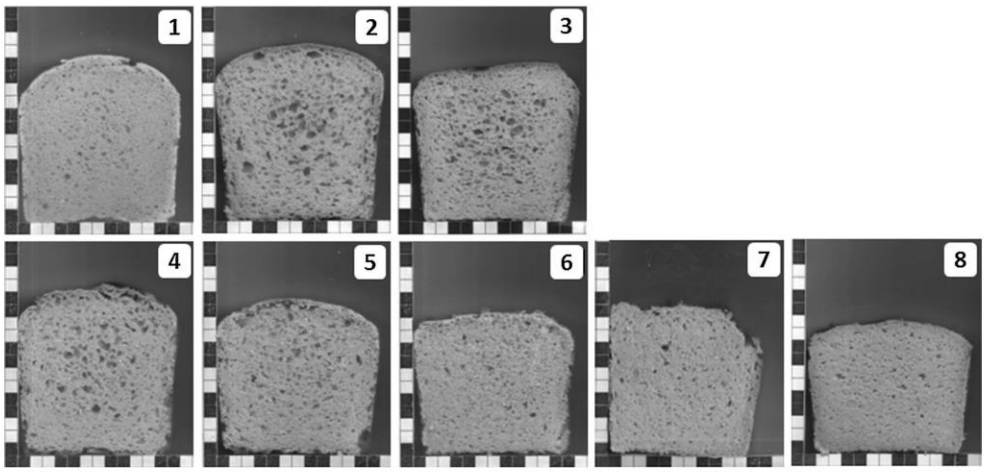


**Fig. 3** Images of scanned bread slices. **1** Control, **2** 40 % quinoa whole grain flour, **3** 80 % quinoa whole grain flour, **4** 10 % quinoa bran, **5** 20 % quinoa bran, **6** 30 % quinoa bran, **7** 40 % quinoa bran, **8** 80 % quinoa bran. Images were taken 2.5 h after baking. One square of the scale is 0.5 cm

bread. This darkening effect was desirable, as gluten-free bread, when based on rice or corn flour exhibits a pale, yellowish color [17]. Moreover, they rated the gluten-free breads with an intensified olfactory impression. These two effects could be ascribed to the higher proteolytic and amylolytic activity, promoting the production of free amino acids or simple saccharides as determined by Elgeti et al. [15] for the latter. During baking, these substrates can be transformed into aroma compounds through biochemical processes and thermal reactions. Therefore, the production of Maillard products is an explanation for the darkened crust and the intensified odor. Additionally, Sabanis et al. [42] mentioned that dietary fiber affected mouthfeel and flavor release. They observed intensified flavor when adding different bran types to wheat bread.

Panelists found significant differences in juiciness between the starch-based control and the bran formulation. Depending on the amount of quinoa bran, gluten-free breads tended to be evaluated as juicier. An explanation for this could be the confirmed higher water binding capacity of quinoa bran due to its enriched protein and conceivably dietary fiber content. Moreover, an excess bran amount (40 %) significantly intensified the bitterness and also promoted an off-flavor ($p < 0.05$), which probably affected overall acceptability negatively. Farfan et al. [43] ascribed the so called earthy taste of quinoa to its polyamine content, which are nitrogen storage compounds. Despite a higher bitterness and the promoted off-flavor in quinoa breads compared to the control, the improved acceptance can be explained by crust color and juiciness, which might have overcome the negative quality characteristics of the gluten-free control recipe.

## Conclusion

In this study, the quality of gluten-free bread (nutrient profile, volume, crumb firmness and sensory acceptance) was improved by low levels of quinoa bran. It was observed that quinoa bran featured a different analytic composition, enzyme activities and functionalities in comparison to whole grain flour as a consequence of fractionation. The replacement of rice and corn flour by quinoa bran firmed the gluten-free dough matrix significantly when more than 40 % bran was applied as shown by the high $G^*$ values. In addition, a decrease in gas holding properties as shown by the lower values for Hm was determined. Specific volume of gluten-free bread was greatly affected by increasing the amount of bran up to 80 %, whereas 10 % quinoa bran significantly improved loaf volume. The pore size of the crumb was coarser resulting in reduced crumb firmness and moreover improved sensory acceptance. Overall, it was found from this study that lower amounts of quinoa bran have a positive effect on the quality of gluten-free bread. Since typically consumers of gluten-free products are justifiably concerned with the value of their nutrition, for them the outcome will be of special interest.

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**Conflict of interest** None.

**Compliance with Ethics Requirements** This article does not contain any studies with human or animal subjects.

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## 2.5 Structure stabilization in starch-quinoa bran doughs: The role of water and gelatinization

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Research Paper

# Structure stabilization in starch-quinoa bran doughs: The role of water availability and gelatinization

Maike Föste, Mario Jekle*, Thomas Becker

*Technical University of Munich, Institute of Brewing and Beverage Technology, Research Group Cereal Process Engineering, 85354 Freising, Germany*

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ABSTRACT

Bran is a promising ingredient for nutritional fortification in starch-based dough systems. However its incorporation is a technological challenge favoring a shift in dough functionality. The objective of this study was to elucidate the impact of bran on baking performance independent of dough firmness and start of gelatinization. Therefore, corn starch was replaced by quinoa bran (10% to 50%) and water addition (80–110 g/100 g flour) was standardized on a fixed complex shear modulus ($G^*$) and start of gelatinization ($T_{Onset}$) based on a corn starch reference dough. A destabilizing effect by bran particles was counteracted in corn starch dough by adjusting the water content up to 110 g/100 g flour. Moreover, a negative correlation between $T_{Onset}$ and loaf volume was determined ($r = -0.9042$), thus an early $T_{Onset}$ should be aspired in order to prevent gas release and to stabilize corn starch- quinoa bran dough.



### 1. Introduction

The incorporation of milling by-products in bread enables product improvement not only from a nutritional point of view but also in regard to economic aspects. Due to celiac disease and even more because of current dietetic trends, the demand for gluten-free bakery products steadily increases. Its production is challenging, because of the high starch content and the lack of gluten which is, among other things, responsible for gas retention and thus also specific loaf volume in gluten-containing bread. In the case of gluten-free bread, final loaf volume depends on 1) incorporation of gas through the choice of mixing settings or yeast and 2) the gas holding characteristics of the starch-based dough systems (Elgeti, Jekle, & Becker, 2015). These factors are influenced by viscoelastic dough properties during proofing and pasting characteristics during baking; both highly dependent on disposable water. Consequently, hydration of recipe components such as starch can be seen as one of the driving forces of structure formation in gluten-free bread. In wheat dough, a late start of starch gelatinization is associated with higher bread volume; since a delayed transition from the liquid dough phase to the solid wheat crumb structure was said to allow volume increase for a longer period of time (Wilderjans, Pareyt, Goesaert, Brijs, & Delcour, 2008). Increased gelatinization temperature was observed in rice starch dough as moisture concentration decreased Chungcharoen & Lund (1987). Furthermore, in sorghum batter delayed gelatinization led to decreased loaf volume Onyango, Mutungi, Unbehend, & Lindhauer, 2011. In spite of that, influences on dough functionalities for starch-based dough systems enriched with bran have been insufficiently elucidated. Complex carbohydrates such as bran and some hydrocolloids are known to be hydrophilic biopolymers, absorbing huge amounts of water and influencing the rheological behavior as shown for HPMC (Demirkesen, Mert, Sumnu, & Sahin, 2010). Recently, 22% of quinoa was identified as soluble dietary fiber, in contrast to about 15% in wheat or maize (Lamothe, Srichuwong, Reuhs, & Hamaker, 2015). Research data showed that soluble dietary fiber is mainly composed of arabinose-rich pectic polysaccharides (35–55%), which can be associated with increased water uptake. Föste et al. (2014) observed that starch replacement by quinoa bran increased dough firmness, attributed to the high water absorption of bran. This resulted in a destabilized gluten-free dough system as well as reduced gas retention, and finally decreased loaf volume. However, a lack of knowledge regarding the mechanistic interrelation of water binding and structure formation is still present. To counteract detrimental effects of bran addition through water competition between bran and other recipe components, specific water adjustment has been recommended (Seyer & Gélinas, 2009). Anyhow, knowledge about optimum water compensation for targeted processing of gluten-free dough still does not exist. Therefore, elucidation regarding the interrelation

* Corresponding author. Tel.:+ +49 8161 71 3669; fax: +49 8161 3883
*E-mail addresses:* mjekle@tum.de, info@jekle.de (M. Jekle).

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of adjusted dough firmness, start of gelatinization and the amount of disposable water in starch-based dough systems could enhance fundamental knowledge and further on will lead to improve the quality of final baked goods. The aim of this research was to: *(i)* evaluate the viscoelastic dough and gelatinization characteristics in relation to the amount of disposable water and its relation to the final loaf volume in dough systems with limited water content; *(ii)* elucidate the mechanistic relation between an increasing bran amount and its structure weakening effects when complex shear modulus ($G^*$) and start of gelatinization ($T_{Onset}$) were adjusted to the reference dough by percentage water addition up to 110 g/100 g flour; *(iii)* investigate the hypothesis that gas holding properties are also improved in starch-bran-based dough systems by lower dough firmness and/or delayed gelatinization as in wheat dough. In addition, viscoelastic dough and gelatinization characteristics were correlated to the values for baking performance (e.g. specific volume, crumb hardness). Thus, mechanisms favoring destabilization of corn starch-quinoa bran dough systems should be disclosed, to improve baking performance of gluten-free bread.

## 2. Materials and methods

### 2.1. Materials

Corn starch was delivered by Davert (Senden, Germany) and organic royal quinoa grains (*Chenopodium quinoa*, free of saponins due to pericarp removal) from Bolivia were purchased from Ziegler & Co. GmbH (Wunsiedel, Switzerland). Quinoa was fractionated according to Föste, Elgeti, Brunner, Jekle, & Becker (2015). The resulting bran fraction contained 27.8% proteins, 14.6% lipids, 5.4% ash on dry base, and 12.9% moisture as determined by the following AACC approved methods: 46–10, 30–25, 08–01, and 44–15, respectively (AACC, 2002) and using a conversion factor of N × 5.45 (Fujihara, Sasaki, Aoyagi, & Sugahara, 2008).

### 2.2. Methods

#### 2.2.1. Starch dough preparation

For dough preparation a recipe based on corn starch and a constant water content of 80% was applied as reference. In order to analyze the impact of bran on gluten-free dough systems, increasing amounts of corn starch were replaced by quinoa bran (10% to 50%). To 100 g of flour mixture, further ingredients were added: distilled water (80 g), margarine (3 g), hydroxypropylmethylcellulose (HPMC) (2 g), NaCl (2 g) and dry yeast (1.5 g). According to the AACC approved method 44-01 (AACC, 2002) the water content of all flours was determined in order to adapt the amount of tempered water (30 °C) based on 14% flour moisture. For dough preparation all ingredients were kneaded at 100 rpm for 2 min and 200 rpm for 2 min in a lab scale z-blade mixer, before analysis of viscoelastic and gelatinization characteristics were performed. Emerging from preliminary trials, the water content for the adjustment of dough firmness ($G^*$) and start of gelatinization ($T_{Onset}$) varied in between 90–110 g/100 g flour; higher water addition resulted in inadequate dough formation.

Water activity of dough samples was evaluated by using the Aqualab analyzer (Decagon Devices Inc., Washington, USA). Samples were placed in plastic dishes and analyzed at 30 °C. Analyses were performed in quadruplicate.

#### 2.2.2. Determination and standardization of viscoelastic starch dough properties

Rheological measurements were conducted with a controlled stress rheometer (ARG2, TA Instruments, West Sussex, UK). All measurements were performed at 30 °C, using 40 mm parallel plates. Dough samples were prepared as described in Section 2.2.1, without the addition of yeast. Subsequently, a sample was placed between the plates, the gap was adjusted to 3 mm, the edges were trimmed with a knife and a thin layer of paraffin oil was applied to the sample edges. Prior to analysis, the dough samples had a 10 min conditioning time. In order to determine the linear viscoelastic region, deformation sweeps for all dough samples were performed in the range of 0.001–100% strain, resulting in 0.01%. Dynamic oscillatory tests for frequency sweeps (0.1–10 Hz at 0.01% strain) were conducted. The fundamental rheological properties of the dough samples were evaluated through the complex shear modulus $G^*$ (Pa), being indicative for dough firmness. All determinations were carried out in triplicate and the average values and standard deviations were calculated.

Experimental data of $G'$ as a function of frequency were further described by the power law equation (Georgopoulus et al., 2004), $G'(w) = K' \times \omega^{n'}$ where $G'$ represents the storage modulus (Pa) and n' is the dimensionless power law exponent (the corresponding slope), $\omega$ is the angular frequency ($s^{-1}$) and $K'$ is the point of intersection with the y-axis (Pa $s^n$). The constants were obtained from the linear regression analysis after a logarithmic transformation of the raw data.

#### 2.2.3. Determination and standardization of thermal starch dough properties

Thermal properties of corn starch dough and quinoa bran dough samples with 10–50% bran were monitored with differential scanning calorimetry (DSC). Measurements were carried out with the DSC 6 (Perkin Elmer Inc., Wellesley, USA) and data were analyzed with the Pyris Manager Thermal Analysis Software. Equipment was calibrated with Indium (MP 196 °C) and *n*-Octadecane (MP 8.5 °C). Approximately 20 mg dough samples were weighed in aluminum DSC pans of 40 μL (Perkin Elmer) and sealed. An empty pan was used as an inert reference and differential heat flow between sample and reference was measured at atmospheric pressure. Pans were heated from 30 °C to 220 °C at a constant rate of 10 °C/min. The thermal properties were evaluated by start of gelatinization $T_{Onset}$ (°C) and gelatinization enthalpy $\Delta H$ (J/g). Three different dough samples were analyzed and results were expressed as means with standard deviation (n = 3).

#### 2.2.4. Thermal transformation from starch dough to bread and its quality assessment

The production of each gluten-free recipe was performed in a KitchenAid (5KSM150, KitchenAid, St. Joseph, USA). For dough preparation all ingredients were kneaded at 100 rpm for 2 min and at 200 rpm for 2 min before weighing 250 ± 0.05 g into baking tins (resulting in 4 tins per recipe). Dough samples were tempered in a proofing cabinet (30 °C; 80% relative humidity) for 30 min. Subsequently, baking tins were placed in a deck oven (Matador MD 120, Werner & Pfleiderer, Dinkelsbühl, Germany) and baked at 220 °C for 35 min with initial 3.6 l/$m^3$ steam. Two independent batches resulting in a total of 8 bread loaves were processed and each was analyzed 2.5 h after baking. The specific bread volume (mL/g) was calculated by dividing the volume, which was determined in a laser-based volumeter (BVM-L370, Perten Instruments, Hägersten, Sweden), with the weight of each loaf. Bake loss was calculated according to the following equation: ([weight of loaf before baking – weight of loaf after baking]/[weight of loaf before baking] × 100). Crumb hardness was determined in agreement with AACC method 74-09 (AACC, 2002). Loaves were sliced using a Tendenza T16 Genio bread slicer (Graef GmbH & Co.KG, Amsberg, Germany) to obtain a uniform slice thickness of 1.25 cm. Two slices were placed, one above the other, underneath a 20 mm aluminum cylindrical plug before crumb hardness was determined with a TVT-300 XP texture analyzer (Perten Instruments, Hagersten, Sweden). Bread slices with a total height of 2.5 cm were compressed by 40% (or com-

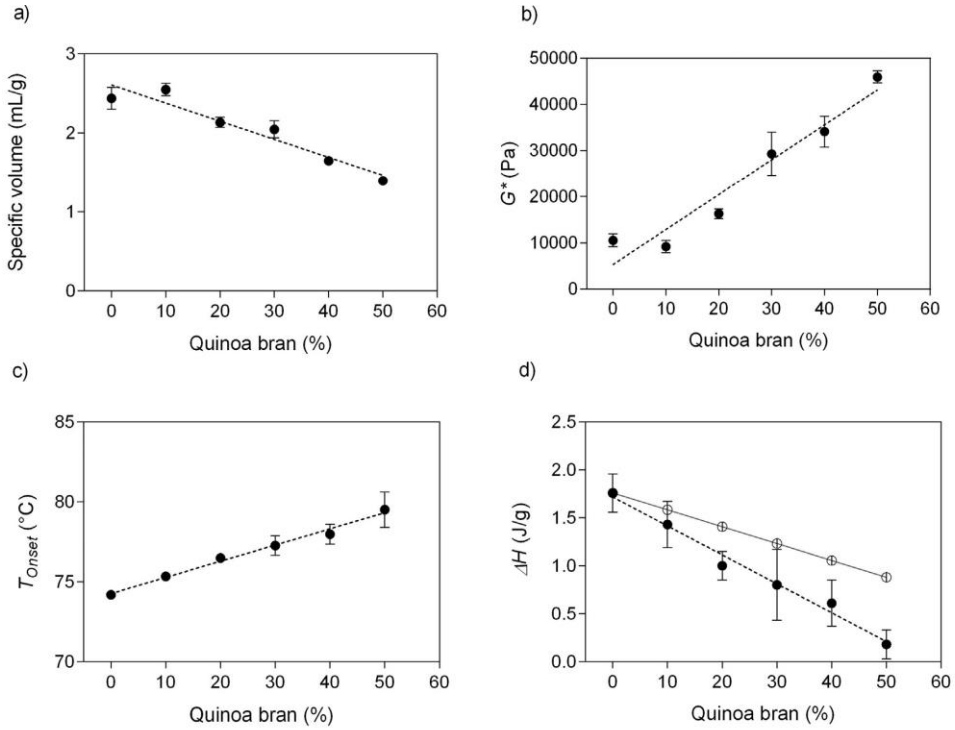


**Fig. 1.** Impact of quinoa bran on bread volume, rheology and gelatinization characteristics in corn starch dough. Presented is the impact of 10–50% bran on a) Specific bread volume; b) Complex shear modulus $G^*$, c) $T_{Onset}$ and d) $\Delta H$. The dough was based on corn starch with 80 g water per 100 g flour. Dotted lines: linear regression with $R^2 = 0.9189$; $p < 0.0025$ for (a) with $n = 8$; $R^2 = 0.9350$; $p < 0.0016$ for (b) with $n = 3$, $R^2 = 0.9889$; $p < 0.0001$ for (c) with $n = 6$; $R^2 = 0.9840$; $p < 0.0001$ for (d) with $n = 6$. (◯): Calculated reduction of $\Delta H$ due to decreased starch content of the gluten-free recipes.

pressed to 60% of their original height) in two subsequent cycles with 15 s intermediate rest in the center. Six replicates from two different sets of baking were analyzed and averaged. Gluten-free bread with holes and/or a doughy crumb were excluded for correlation analysis.

#### 2.2.5. Statistical analysis

Statistical analysis was performed with the aid of Prism 5 (Version 5.03, GraphPad Software, Inc.). To detect significant differences between samples, variances were analyzed (ANOVA) with separation of means by the Tukey-Kramer test ($p < 0.05$). Correlation analysis served to quantify the degree of relation between two variables. For correlation of data the Spearman coefficient was applied.

## 3. Results and discussion

### 3.1. Impact of quinoa bran on dough functionality and bread properties in relation to disposable water

The impact of quinoa bran on gas stability during baking, dough firmness and gelatinization characteristics are presented in Fig. 1

Percentage increase of corn starch substitution by quinoa bran at constant water addition of 80 g/100 g flour significantly decreased specific loaf volume and increased dough firmness, pointing at a linear correlation with $R^2 = 0.9189$; $p < 0.0025$ for specific bread volume and $R^2 = 0.9350$; 0.0016 for $G^*$ (see Fig. 1a, b). In addition, determination of $a_w$-values (see Section 2.2.1) revealed a decrease in disposable water from $0.983 \pm 0.001$ to $0.976 \pm 0.004$ (data not shown), when corn starch was replaced by 50% quinoa bran, respectively.

As non-starch hydrocolloids are known to modify starch gelatinization by limiting the hydration of amorphous regions in starch granules (Tester & Sommerville, 2003), gelatinization properties of starch-based dough systems were analyzed. Starch replacement by 50% quinoa bran delayed $T_{Onset}$ by 7.2 °C rel., starting its gelatinization at 79.52 °C. Consequently, these results supported the assumption that quinoa bran lowered the amount of disposable water, resulting in higher dough firmness and delayed start of gelatinization (see Fig. 1c). In a gluten-free formulation, containing rice flour and corn starch, substitution of 1% to 3% of different fiber sources delayed start of gelatinization (79.89 °C) in contrast to the reference (75.83 °C) Sabanis, Lebesi, & Tzia (2009).

By increasing the amount of quinoa bran up to 50%, reduced gelatinization enthalpy by 89.77% rel. was observed. This reduction in enthalpy was attributed to the following two effects: 1) lower starch content in bran dough and 2) a negative impact of bran on gelatinization. The latter was sustained by calculating the theoretically required energy for gelatinization depending on the amount of starch (Fig. 1d). As visualized by the calculated values for $\Delta H$ with regard to the lower starch content, 50% quinoa bran dough would require 0.880 J/g starch for gelatinization; however, the analyzed $\Delta H$ value was 0.180 J/g starch. For a differentiated consideration whether specific loaf volume depends on dough firmness or start of gelatinization the following approaches aimed to adjust dough functionality.

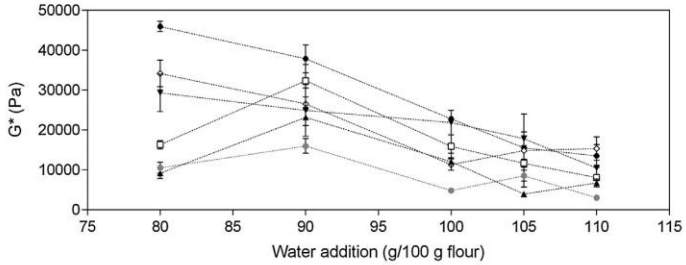


**Fig. 2.** Impact of water addition on complex shear modulus with varying quinoa bran content. Reference: corn starch (●); 10% QB (▲); 20% QB (□); 30% QB (▼); 40% QB (◊); 50% QB (●). Means with standard deviation for $G^*$ (n = 3).

### 3.2. Adjustment of dough firmness by water addition

Gas bubble stabilization during fermentation and consequently final loaf volume after baking depends on viscoelastic dough characteristics. To clarify the impact of bran amount on dough functionality, independent of dough firmness, $G^*$ was standardized by variation of water addition from 80 to 110 g/100 g flour (Fig. 2).

In dough containing 10% to 30% quinoa bran, water addition had to be increased up to 100, 105 and 110 g/100 g flour, respectively, to adjust $G^*$ to the reference (10.500 Pa). Higher bran amounts (>30%) would have required water addition above 110 g/100 g flour to adjust $G^*$; however a water addition of such an extent resulted in insufficient dough formation. Therefore, for highly substituted starch-based dough systems, values for $G^*$ couldn't be achieved. In this study, elevated water contents resulted in softer doughs by a decrease in both viscous and elastic moduli, which has also been determined for gluten-free dough systems by Ronda et al. (2013) and Mancebo et al. (2015). Contrary to the expectations, $G^*$ slightly increased after water addition of 90 g/100 g flour for the reference and 10% to 20% quinoa bran dough. A possible explanation could be the high water absorption of HPMC and quinoa bran. It is conceivable that these hydrophilic biopolymers underwent increased swelling, thus grew in particle size and consequently increased $G^*$. In addition, the varying particle size distribution of quinoa bran might have resulted in higher standard deviation.

However, an increase in water addition from 90 to 110 g/100 g flour in 30% quinoa bran dough decreased $G^*$. Furthermore, it was clearly visible that this decrease occurred faster within water addition from 80 to 100 g/100 g flour than from 100 to 110 g/100 g flour. The following two aspects should be taken into consideration for explaining this effect. Firstly, less water was absorbed by quinoa bran possibly due to saturation and secondly, less starch was available for swelling, so that excess water reduced dough firmness to a higher extent. Hence, this phenomenon could be attributed to the dilution of constituents within elevated water addition, as it was also stated for gluten-containing dough (Autio, Flander, Kinnunen, & Heinonen, 2001). The power law provides information about the frequency-dependent structural strength by the variables $n'$ and $K'$. For clarity, selected values of the power law indice $n'$ depending on water addition are presented in Fig. 3.

Since higher quinoa bran amounts increased the elastic modulus (see. Fig. 1b), the power law index for $G'$ was calculated. Increased bran amounts with regard to water addition of 80 g/100 g flour decreased $n'$, offering values in the range of 0.40–0.18 (see Fig. 3). However $R^2$ varied between 0.49 and 0.91. Corn starch-based dough systems showed higher values for $G'$ than for $G''$:, which was in agreement with the observations in rice dough (Ronda, Pérez-Quirce, Angioloni, & Collar, 2013). According to the definition of Ferry (1980), values below two can be attributed to a higher elastic part of the dough. Elevated $n'$ values were determined when water content was 110 g/100 g flour, indicating a weaker formation of a gel like structure, being ascribed to the increased dilution of dough components and the structure weakening through quinoa bran particles.

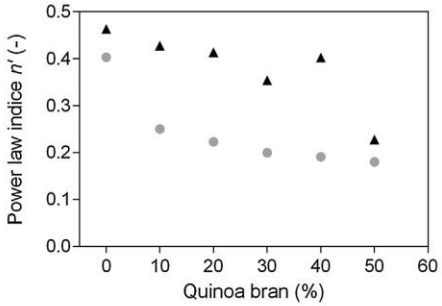


**Fig. 3.** Impact of bran addition and water content on power law indice $n'$ of storage modulus $G'$ analyzed by power law equation. Water content: 80 g/100 g flour (●); Water content: 110 g/100 g flour (▲). Presented are means for $n'$ of $G'$ (n = 3).

### 3.3. Adjustment of dough gelatinization properties by water addition

Start of gelatinization for quinoa bran doughs and selected values for enthalpy $\Delta H$ with regard to starch content are presented in Fig. 4.

In comparison to the dough firmness, for the adjustment of $T_{Onset}$ to the reference (74 °C), 110 g water per 100 g flour was already required for 20% quinoa bran dough (see Fig. 4a). The adjustment of 30% quinoa bran dough to the reference would have required an even higher water amount above the maximum limit. Independently of the bran amount, a decrease in $T_{Onset}$ was achieved with water addition from 80 to 110 g/100 g flour. The strongest impact on $T_{Onset}$ was observed in 50% quinoa bran dough with a reduction by 4.71% rel. in contrast to the appropriate reference (1.43% rel.). The reduced values for start of gelatinization within water addition can be explained by the following to facts. Firstly, water addition resulted in a lower starch/water ratio, accelerating starch gelatinization. Secondly, bran is disposed to absorb large amounts of water. However, when placed under stress such as heating water release can occur rather quick, due to its low affinity, promoting the amount of disposable water in the dough. In addition, quinoa bran is limited in its water uptake, which could have promoted a higher amount of disposable water as a consequence of saturation.

Reference dough revealed the highest enthalpy value for gelatinization $\Delta H$, (3.31 J/g starch). By elevating the water content (110 g/100 g flour) $\Delta H$ values were further increased by 129% rel. (see Fig. 4b). Starch gelatinization was promoted, as a consequence of elevated water content and lower starch content, resulting in a lower starch/water ratio. Quinoa bran dough (50%) revealed a

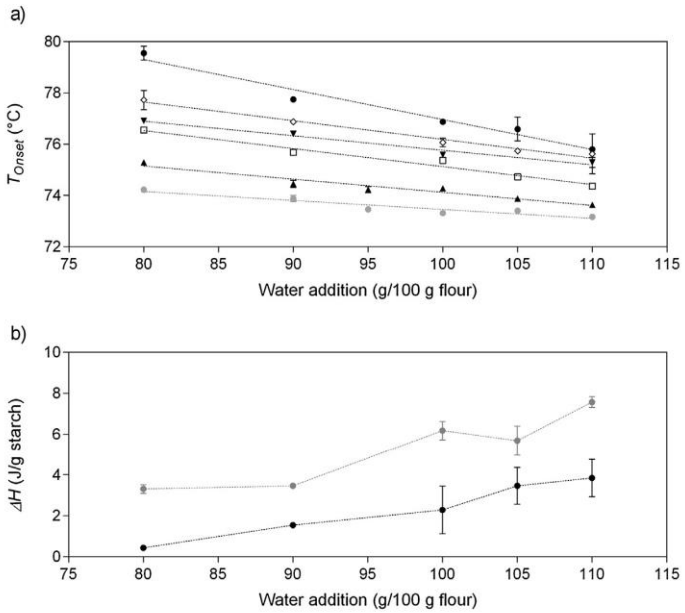


**Fig. 4.** Impact of water content on dough gelatinization properties depending on the amount of quinoa bran. A) Impact of water content on $T_{Onset}$ (°C) and b) Impact of water content on $\Delta H$ (J/g starch). Reference: corn starch (● ); 10% QB (▲); 20% QB (□); 30% QB (▼); 40% QB (◊); 50% QB (●) Abbreviation: Quinoa bran: QB. Presented are means with standard deviation (n = 3).

decrease in $\Delta H$ by 49% rel., although water incorporation was increased. It can be assumed that a decrease in $\Delta H$ with a lower starch/water ratio is attributed to limited swelling of the starch granules due to higher water binding ability of bran. To investigate the impact of reduced gelatinization and delayed start of gelatinization as well as elevated dough firmness on end product quality, baking trials were performed.

### 3.4. Baking performance of starch-quinoa bran bread

The specific bread volume, baking loss and crumb texture are important key factors regarding consumer's acceptance. Specific loaf volume of the reference significantly decreased by 28% rel. within water addition from 80 to 110 g/100 g flour (see Table 1), supporting the formation of holes and/or a doughy crumb (see Table 2).

Small amounts of quinoa bran (10%) significantly increased loaf volume and decreased crumb hardness ($p < 0.05$) with 80 g water addition per 100 g flour. In particular quinoa bran is fortified in its protein-, saccharide content and enzymatic activity (protease; a-glucosidase), which offered higher substrate availability and promoted gas formation during fermentation as recently shown by Elgeti et al. (2015) and Föste et al. (2014). Furthermore, it can be assumed that quinoa milling fractions might contain surface active components, improving gas bubble stabilization and promoting an even pore distribution when less than 20% bran was incorporated (see Table 2).

However, higher bran amounts (>20%) at constant water addition decreased specific loaf volume and elevated crumb hardness. This structure weakening effect was counteracted by the addition of water (90–110 g/100 g flour). In this context, a weakening of dough functionality in relation to gas holding properties and the final loaf volume is addressed. Thus, specific loaf volume was increased in the range of 13% to 36% rel., in comparison to the appropriate reference (Table 1). Finally, the formation of progressively coarser pores was promoted through water addition, in contrast to the dense and compact pore structure obtained at 80 g water addition per 100 g flour (see Table 2).

The simple adaption of dough firmness or start of gelatinization by a variation of water amount was not a suitable approach to standardize the baking performance of starch-quinoa bran dough. As shown in the previous chapters, depending on the amount of bran, different water concentrations were required to adjust $G^*$ or $T_{Onset}$. However, according to ANOVA results, loaf volume was significantly higher in 10% quinoa bran bread (2.78 mL/g) in comparison to the reference (2.44 mL/g), considering the required amount of water (100 g/100 g flour) to adjust $G^*$ (see Table 1).

In summary, starch-quinoa bran dough systems required additional water for structure stabilization to counteract the structure weakening effect by bran. However, suitable parameters for adapting water content on bran addition have to be developed.

### 3.5. Correlation of dough rheology and gelatinization characteristics with baking performance

Analysis of multivariate data supplied useful information about the significantly correlated dough characteristics with their baking performance to elucidate mechanisms favoring structure weakening of corn starch-quinoa bran bread. Using Spearman correlation analysis, a range of correlation coefficients (r) from 0.2560 to −0.9143 was obtained for the evaluation of interrelation between dough firmness, gelatinization characteristics and baking performance (Table 3).

In particular the amount of quinoa bran was negatively correlated with specific loaf volume ($r = -0.9123$; $p < 0.05$), which can be traced to the structure weakening effect. The results stated that varying starch/bran and water proportions influenced the amount of disposable water in gluten-free dough, which might be the reason for a shift in water uptake of single recipe components.

**Table 1**
Baking performance of starch-quinoa bran bread depending on water addition.

| Baking performance | Quinoa bran (%) | Water addition (g/100 g flour) | | | | |
|---|---|---|---|---|---|---|
| | | 80 | 90 | 100 | 105 | 110 |
| Specific volume (mL/g) | 0 | 2.44 ± 0.14 d; z | 2.25 ± 0.07 c; y | 2.05 ± 0.09 b; x | 1.75 ± 0.09 a; w | 1.75 ± 0.08 a; v |
| | 10 | 2.55 ± 0.08 a; z | 2.66 ± 0.11 ab; z | **2.78 ± 0.12 b; z** | 2.84 ± 0.19 b; z | 2.87 ± 0.12 b; z |
| | 20 | 2.13 ± 0.06 a; y | 2.29 ± 0.09 a; y | 2.51 ± 0.17 b; y | **2.54 ± 0.15 b; y** | 2.52 ± 0.05 b; y |
| | 30 | 2.05 ± 0.11 a; xy | 2.22 ± 0.24 ab; y | 2.40 ± 0.06 ab; y | 2.24 ± 0.09 b; x | **2.35 ± 0.15 b; x** |
| | 40 | 1.65 ± 0.03 a; w | 2.02 ± 0.12 b; x | 2.04 ± 0.08 b; x | 2.13 ± 0.11 b; x | 2.09 ± 0.06 b; w |
| | 50 | 1.39 ± 0.01 a; v | 1.61 ± 0.08 b; w | 1.79 ± 0.06 c; w | 1.81 ± 0.06 cd; w | 1.89 ± 0.07 d; v |
| Baking loss (%) | 0 | 16.60 ± 2.53 b; z | 15.60 ± 1.97 ab; z | 14.30 ± 0.88 a; xy | 13.85 ± 0.95 a; xy | 13.35 ± 0.74 a; xy; |
| | 10 | 14.25 ± 0.60 a; y | 14.65 ± 0.74 a; z | **15.95 ± 0.66 ab; z** | 18.55 ± 3.31 b; z | 18.55 ± 2.73 b; z |
| | 20 | 13.15 ± 0.62 a, xy | 13.95 ± 1.27 ab; y | 14.55 ± 0.83 ab; xy | **15.49 ± 0.50 b; y** | 15.30 ± 0.70 b; y |
| | 30 | 14.05 ± 1.61 a; y | 15.35 ± 2.61 a; z | 15.30 ± 0.93 a; yz | 14.55 ± 0.56 a; xy | **15.50 ± 0.97 a; xy** |
| | 40 | 11.40 ± 0.93 a; x | 13.35 ± 0.83 b; y | 13.40 ± 0.71 b; wx | 14.15 ± 0.98 b; xy | 14.55 ± 1.45 b; xy |
| | 50 | 11.30 ± 0.73 a; x | 12.10 ± 0.97 ab; y | 12.90 ± 0.85 b; w | 12.90 ± 0.90 b; x | 13.40 ± 0.91 b; x |
| Crumb hardness (N) | 0 | 9.19 ± 3.23 a; x | 8.41 ± 0.89 ab; y | 12.10 ± 1.58 ab; z | 11.82 ± 3,76 b; z | 14.70 ± 1.06 b; z |
| | 10 | 4.36 ± 0.71 b; w | 3.95 ± 0.55 ab; x | **4.23 ± 0.26 b; wx** | 3.58 ± 0.34 a; x | 4.35 ± 0.61 b; x |
| | 20 | 5.08 ± 0.77 c; w | 3.72 ± 0.39 b; x | 2.98 ± 0.60 ab; w | **2.89 ± 0.77 a; x** | 3.17 ± 0.75 ab; x |
| | 30 | 8.23 ± 2.10 c; x | 4.06 ± 0.58 b; x | 4.11 ± 1.10 b; wx | 2.32 + 0.84 a; x | **3.89 ± 0.64 b; x** |
| | 40 | 12.22 ± 1.68 d; y | 7.27 ± 0.99 c; y | 5.20 ± 1.20 ab; x | 4.94 ± 1.17 a; x | 6.66 ± 1.45 bc; y |
| | 50 | 26.96 ± 1.92 e; z | 14.72 ± 2.15 d; z | 10.18 ± 1.12 c; y | 9.18 ± 2.01 bc; y | 6.66 ± 1.34 a; y |

[a–e] Mean values ± SD (n = 8) labelled with a different letter in the same row are significantly different at a 5% level according to Tukey's test ($p < 0.05$).
[v–z] Mean values ± SD (n = 8) labelled with a different letter in the same column are significantly different at a 5% level according to Tukey's test ($p < 0.05$).
Bold: Baking performance of gluten-free dough matrices after the adjustment to the firmness of the reference dough.

**Table 2**
Overview of crumb and pore structure of starch-quinoa bran bread depending on water addition.

| Bran (%) | Water content (g/100 g flour) 80 | 90 | 100 | 105 | 110 |
|---|---|---|---|---|---|
| 0 |  |  |  |  |  |
| 10 |  |  |  |  |  |
| 20 |  |  |  |  |  |
| 30 |  |  |  |  |  |
| 40 |  |  |  |  |  |
| 50 |  |  |  |  |  |

[a] Standardized dough firmness comparable to reference dough (water content 80 g/100 g flour).
[b] Excluded for correlation due to holes, doughy crumb.

Additional analysis by nuclear magnetic resonance spectroscopy might be helpful for further elucidation, giving information about the mobility of water.

The varying water content would have influenced the amount of disposable water in starch-based dough systems. However, no correlation was found between the calculated water content of each

**Table 3**
Correlation coefficients between viscoelastic and gelatinization characteristics of starch-quinoa bran recipes and bread quality.

| | | | Dough gelatinization and viscoelastic characteristics | | | | |
|---|---|---|---|---|---|---|---|
| | | | $G^*$ (Pa) | $T_{Onset}$(°C) | ΔH(J/g starch) | Bran (%) | [a]Calc. water content |
| Bread characteristics | Specific volume | *r* | **−0.6524** | **−0.9042** | **0.7159** | **−0.9123** | 0.2560 |
| | (mL/g) | *p* | 0.0004 | 0.0001 | 0.0001 | 0.0001 | 0.2167 |
| | Baking loss | *r* | **−0.6445** | **−0.9143** | **0.7677** | **−0.7666** | 0.4013 |
| | (%) | *p* | 0.0005 | 0.0001 | 0.0001 | 0.0001 | 0.0468 |
| | Crumb hardness | *r* | 0.4115 | **0.6336** | **−0.7185** | **0.6201** | −0.3930 |
| | (N) | *p* | 0.0410 | 0.0007 | 0.0001 | 0.0009 | 0.0520 |

Dough firmness and gelatinization characteristics of each starch-quinoa bran dough were determined by means of oscillatory measurements and differential scanning calorimetry, respectively, independent from baking trials. Correlation analysis was performed with Spearman correlation coefficients. Values for r above 0.5 with $p < 0.05$ are given in bold.

[a] Calculated water content: Water, which should be added to each of the recipes, depending on the starch/bran ratio and the adjustment to the corresponding moisture content up to 14%.

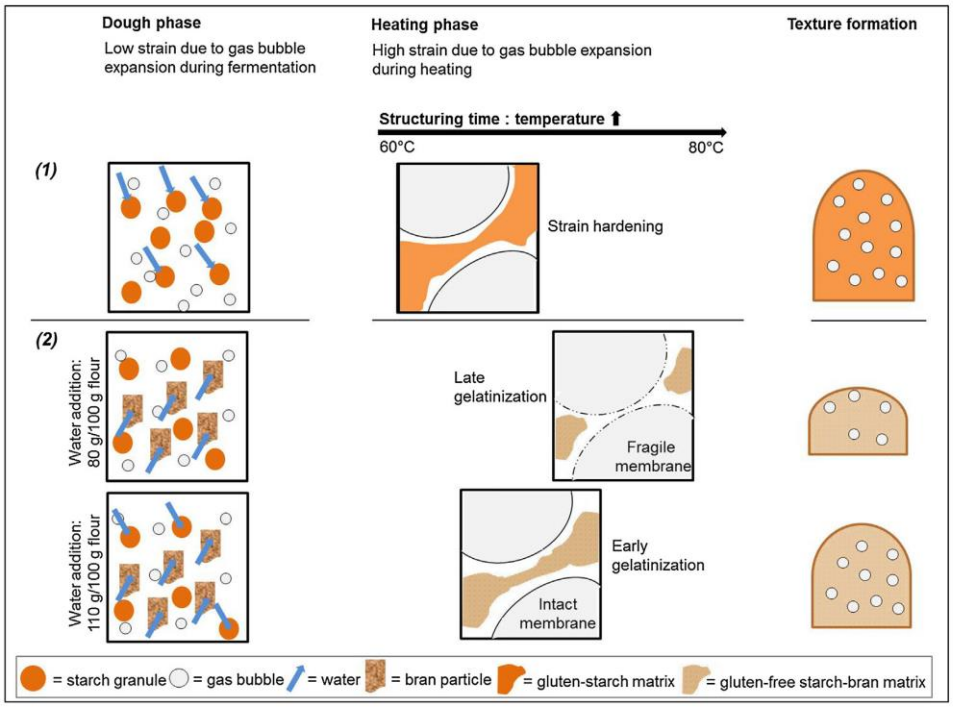


**Fig. 5.** Schematic overview on texture formation of dough matrices during baking with regard to gas expansion. 1) Wheat dough 2) Gluten-free starch-based dough (50% corn starch and 50% quinoa bran).

starch-quinoa bran dough and specific loaf volume or baking loss. This indicated, that baking performance is independent of the calculated water content and is even more influenced by the amount of quinoa bran.

In view of the initial hypothesis, specific loaf volume was negatively correlated with $G^*$ ($r = -0.6524$; $p < 0.05$), meaning that firmer dough favored volume decrease, confirming that the lower $G^*$ before baking, the higher specific volume of final loaves. Moreover, specific loaf volume was negatively correlated with the start of gelatinization ($r = -0.9042$; $p < 0.05$). In contrast to wheat dough, where gas bubbles are stabilized during the baking phase due to strain hardening of the protein-starch matrix (Vliet, Janssen, Bloksma, & Walstra, 1992), stabilization of gas bubbles and structure formation of starch-based dough systems was favored by other dough components and the early start of gelatinization (Fig. 5). Thereby, increased water content decreased $T_{Onset}$, meaning that transformation from paste-like dough consistency led to a solid crumb structure. During heat induced gas expansion, the membranes of the growing gas bubbles remained intact, which allowed them to travel upward. Whereas a more fragile membrane favored volume decrease, the escape of gas bubbles from the starch-based dough was delayed because of their intact membrane, improving final loaf volume.

## 4. Conclusion

Incorporation of quinoa bran without water adjustment, increased dough firmness and decreased start of gelatinization. This was attributed to a lower amount of disposable water in corn starch dough, owing to the increased water absorption of quinoa bran (see Section 3.1). The higher dough firmness led to more fragile gas bubble membranes, which for example promoted coalescence and the release of carbon dioxide, finally reducing specific loaf volume. The approach to adjust $G^*$ or $T_{Onset}$ by increasing water addition from

80 to 110 g/100 g flour was unconvertible and did not offer the opportunity to standardize bread quality characteristics. However, a dominant impact on the reduction of $T_{Onset}$ (4.71% rel.) in 50% quinoa bran dough was observed when water content varied from 80 to 110 g/100 g flour in comparison to the equivalent corn starch-based reference (1.43% rel.). Maximum water addition restricted water uptake by quinoa bran due to saturation, supporting the dilution of corn starch dough and consequently decreased $G^*$ and $T_{Onset}$. Thus, bread loaf volume was improved by enhanced water addition, possibly because of the more intact membrane of gas bubbles as a consequence of the modified dough properties ($G^*$ and $T_{Onset}$). In particular the interrelation of these two variables as well as gas formation and retention capacity has to be taken into account. The initially mentioned hypothesis for wheat dough is not transferable to corn starch-quinoa bran dough. A negative correlation between $T_{Onset}$ and specific loaf volume ($r = -0.9042$; $p < 0.05$) was observed. In corn starch-quinoa bran dough, a later start of gelatinization would mean a higher release of carbon dioxide during baking, as indicated by coarser pore size distribution (see Table 2). Considering the applied amount of quinoa bran, improved loaf volume and pore size were achieved when up to 20% bran was incorporated. To counteract the structure weakening effect by quinoa bran particles and to stabilize corn starch-based dough systems, an earlier $T_{Onset}$ should be aspired, which can be achieved by the addition of water. Thereby, coalescence and gas bubble escape can be minimized, aiming to keep the gas bubbles in the weak starch-based dough system.

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# 3 Discussion, conclusion and outlook

In the course of sustainable concepts, the valorization of bran gained more and more attention in recent years. Although the mild fractionation of quinoa is only a niche area, the composition of this Andean seed shows great potential for its application in GF food. As quinoa is rich in protein, minerals and dietary fiber it guarantees better nutritive value than most commonly used GF flours from rice or corn, which is of major concern for nutrient deficient GF bread. Especially the fat and starch content in GF bread is often very high. This inadequate composition is of particular interest for the number of people suffering from CD, patients with other types of GF sensitivity and of course for those, who consume GF foods of their own choice. A link between people suffering from CD and the initiation of a GF diet promoting overweight, obesity or new-onset insulin resistance were observed in several studies (Reilly et al., 2011; Rewers, 2005; Tucker et al., 2012). It is assumed that the worldwide annual net sales of the GF market could be increased up to 43.7 billion US dollars by 2027 (GrandViewResearch, 2020, February 01.). Against this background, a nutritional quality improvement of GF bread should be in the focus, to reduce potential associated diseases and to minimize additional arising costs for the health care system.

Research has focused on different strategies to improve GF bread quality in recent years. From the processing side, alternative strategies such as mechanical aeration (Elgeti, Peng, Jekle, & Becker, 2017; Elgeti, Yu, Stüttgen, Jekle, & Becker, 2017) or the application of particle-stabilized foams (Yano, 2019) were discussed. From the raw material side, the addition of protein-rich flours, bran and fiber products, concentrates or isolates from pseudocereals or legumes and their effects on GF dough and bread were analysed (Arslan, Rakha, Xiaobo, & Mahmood, 2019; Elsohaimy et al., 2015; Föste, Verheyen, Jekle, & Becker, 2020; Miñarro et al., 2012). During the last decades, the application of bran in classical gluten-containing bakery products such as wheat bread has been studied and oftentimes showed technological challenges. Especially dough rheology, gas retention and gelatinization were impaired by utilization of bran. In contrast, only few studies have been conducted on bran-enriched GF dough. Literature survey of the second study in this thesis showed, that specific loaf volume, crumb texture and sensory attributes of fiber-enriched GF bread were impaired by fibers from the milling and fruit processing industries (Föste et al., 2020). Research on mechanical relations between material-based properties in dough systems and final product quality are of decisive importance. Hereby, the link between the hydration level of the formulation and dough functional properties could be dominant, however, were considered to a low extent in GF dough. To elucidate relationships between viscosity, gelatinization or gas retention in GF dough, also the hydration properties of the raw material, competition of recipe components for disposable water and possible mechanisms that stabilize/ destabilize the

dough matrix should be considered. The present thesis delivers a fundamental contribution to understand the potential of quinoa bran to stabilize and destabilize GF dough with regard to functional properties (viscosity and gelatinization) considering the hydration level. Revelations gained in the course of this thesis are summarized in the following.

- ✓ Protein content of quinoa bran was successfully increased by the adjustment of tempering settings prior to milling (as a gentle dry fractionation process) and revealed a concentrate by means of aqueous extraction with 68% protein on db.
- ✓ The critical review clearly pointed out the challenges in determination of water absorption and hydration properties of by-products from the fruit and milling processing industries and the transfer of these properties to GF dough systems. Depending on the by-product in use and the initial hydration level of the GF formulation, dough viscosity, starch gelatinization and gas bubble stabilization were affected. Moreover, several strategies (thermal treatment, microbial polymer modification) to modify hydration properties of bran-enriched GF dough were reviewed.
- ✓ The amount of 10% quinoa bran was identified to be the lowest concentration before a structure loss is triggered by quinoa bran. This amount simultaneously improved texture properties and sensory attributes of GF bread. The incorporation of > 20% bran to GF dough impaired the hydration properties, which resulted in higher dough firmness and a dough height decrease during inflation.
- ✓ The adjustment of dough firmness $G^*$ and start of gelatinization $T_{Onset}$, respectively, by a property-adapted hydration level clearly showed that these dough functional properties could not be easily standardized. In GF dough with > 20% bran, excess water (> 110 g/ 100 g flour) would have been needed to standardize $G^*$ or $T_{Onset}$. However these amounts are not used in practice as they are far above the level required for GF dough formation.
- ✓ The structure of GF dough was stabilized during processing by water addition in an application-oriented range (90-110 g/100g flour) and clearly improved final product quality.

**Hypothesis 1: Dry fractionation of quinoa as a very gentle approach enables protein enrichment in the bran fraction and subsequent aqueous extraction of proteins from quinoa bran enables to achieve a concentrate with a protein content ≥ 65% db**

Previous studies on quinoa comminution showed large differences in the composition of milling fractions depending on the method used. The small kernel size of quinoa seeds and its oval shape require specific grinding adjustments. The use of a conventional roller mill to separate seed tissues into protein, fiber and starch-rich fractions has been investigated by Chauhan et al. (1992) and Ando et al. (2002). While the adjustment of moisture content or resting time prior milling is a standard method for wheat (Edwards, Osborne, & Henry, 2008), this is nevertheless a neglected issue when fractionating niche raw materials. Therefore, in the first study of this thesis, separation of quinoa was adapted and optimized to achieve functional seed tissues, given as white flour and bran (Föste, Elgeti, et al., 2015). The adjustment of moisture content and resting time revealed an increase in the protein content. In comparison to whole grain flour with a protein content of 11.8% db, quinoa seeds without tempering yielded a flour to bran ratio of (70 to 30%) with a protein content of 23.9% db in the bran fraction. By elevating the moisture content of quinoa seeds to 15%, these settings yielded a flour to bran ratio of (65 to 35%) with a protein content of 27.9% db in the bran fraction (Föste, Elgeti, et al., 2015). Thus, the first hypothesis of this thesis is confirmed that the adjustment of tempering settings before a gentle comminution procedure such as grinding is a useful tool for enriching the protein content in bran. Later investigations, which focused on wet-fractionation by introducing a wet milling stage in a roller mill, achieved more than 90% protein recovery in the germ (Mufari, Miranda-Villa, & Calandri, 2018). Investigations by Ballester-Sánchez, Gil, Fernández-Espinar, and Haros (2019) pointed out that steeping time and temperature are essential for starch recovery and quality. These findings once more underline the importance for seed tempering to better separate seed tissues.

According to the Osborne fractionation high occurrence of albumins and globulins were observed in quinoa (see chapter 1.2). These two protein fractions were primarily located in the outer seed tissues as pointed out by D'Amico et al. (2019), who used an abrasive disc mill to separate quinoa seeds from the outer to the inner part. To further extract proteins from flour, whole grain flour or bran, knowledge on the presence of protein fractions (albumins, globulins, prolamins, glutelins) is required. With regard to the solubility of these protein fractions, the extraction medium (water, salt solution, organic solvents) has to be selected. In recent years, the valorization of milling by-products (bran), dried distillery grains or fruit processing by-products (press cakes) gained incredible attention in research, focusing on biorefinery and sustainability concepts (Contreras et al., 2019; Roth, Jekle, & Becker, 2019). These

approaches focus on the extraction of valuable macronutrients (protein, dietary fibers and oil) from milling or fruit processing by-products. Within this approach, generated extraction co-products can be further re-used for food and non-food applications. The feasibility of protein isolation from quinoa bran was investigated in the first study of this thesis and revealed a protein solubility of 67%, when using quinoa bran milled to 250 µm and optimized extraction settings (pH 10; 1 h) (Föste, Elgeti, et al., 2015). In consideration of 100 g quinoa bran (27.9% protein db), thus 18.69 g protein could be solubilized within the first step. Simultaneously, this means that 33% of the protein remained insoluble in the residue. A possible explanation could be the presence of phytochemicals as introduced in section 1.2. For instance, phytates show high affinity to interact with proteins, which promote the formation of insoluble protein-phytate complexes, lowering its solubility. In addition, bioavailability of multivalent minerals for e.g. zinc, ferrum, magnesium or calcium may be decreased after chelating with phytic acid (Cheryan, Anderson, & Grynspan, 1983; Cheryan & Rackis, 1980; Fox & Tao, 2012). Consequently, further approaches to optimize protein extraction from quinoa bran might consider the dephytinization. This can be performed by reducing the pH value or particle size and hydrothermal treatments stated by Özkaya et al. (2017) and Majzoobi, Pashangeh, Farahnaky, Eskandari, and Jamalian (2014).

Also the interaction with polyphenols (phenolic acids, flavonoids, tannins), which can be assumed due to the change hence to a darker colour in the supernatant of solubilized proteins, might be the cause of reduced protein solubility (see Fig. 7).

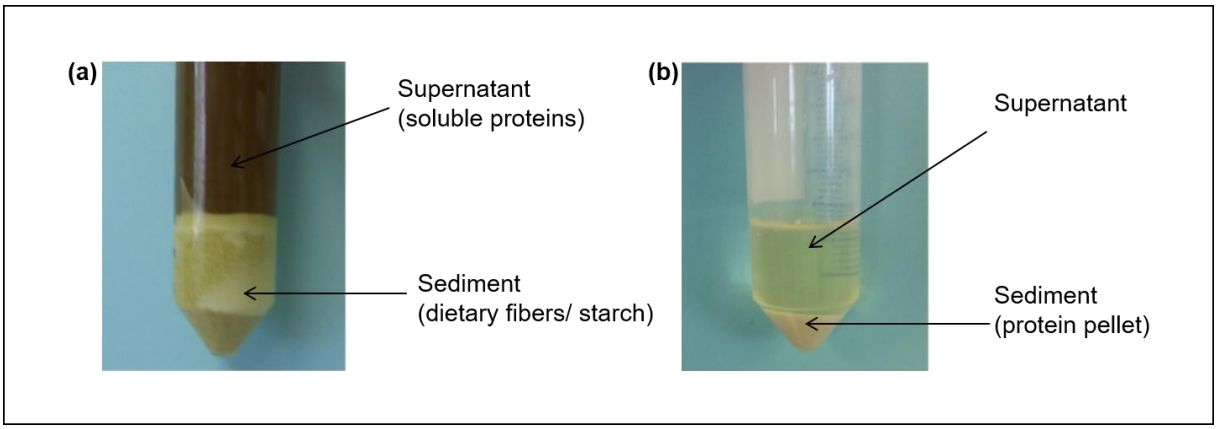



**Figure 7: Appearance of solubilized and precipitated quinoa proteins.** (a) Extraction phase and (b) Precipitation phase.

After solubilization, proteins were precipitated (pH 4, 1 h, 20 °C) and revealed a protein solubility of about 60% at pH 4 (Föste, Elgeti, et al., 2015). Simultaneously, the decrease in pH-value changed the dark colour of the supernatant hence to a clear supernatant. By means of this purification approach a total protein yield of 12.27% was achieved and the protein content of the freeze-dried concentrate was 68% db. With regard to the protein content of

quinoa bran, this means that the isolation process yielded 42.91% of the initial proteins. By further optimization of precipitation settings for e.g. ultrafiltration or thermal precipitation, a higher protein yield and protein content of the concentrate could be achieved. These revelations as well confirm the first hypothesis of this thesis as by means of aqueous extraction a concentrate of ≥ 65% was obtained. From an economic point of view, the actual price development of quinoa seeds (2 Euro/ kg (2011) to 9 Euro/ kg (2018)) and additional costs for protein extraction might be the reasons for high prices of concentrates/ isolates. In comparison to present prices of isolates from soy or pea (3 Euro/ kg) and wheat gluten (1.20 Euro/ kg) available on the market (Mulder et al., 2016), concentrates or isolates from quinoa bran would not be competitive. Consequently, the use of bran is more efficient than the concentrate, so that ongoing investigations focused on the impact of quinoa bran on GF dough formation in consideration of the hydration level.

**Hypothesis 2: A critical review on milling and fruit processing by-products reveals the differences in hydration properties, the impact of fibers and bran on dough formation and shows strategies for modifying bran-enriched GF bread quality**.

The second hypothesis was confirmed in the second study of this thesis, where the challenges in determining hydration properties of raw materials, its water absorption and as well its transfer to GF dough systems have been reviewed (Föste et al., 2020). The authors highlighted that GF formulations greatly vary in their water content (70-110 g water per 100 g flour). Water as a polar substance interacts with other polar ingredients such as starches, hydrocolloids, proteins or amino acids. In a dough formulation, it serves as a solvent medium for polysaccharides and enzymes, improves the homogenizing of recipe components and supports the swelling and gelatinization of starch (Schiraldi & Fessas, 2012). The water amount, its type of binding, interactions between recipe components and the temperature profile during processing play a major role in dough structure formation. While, the composition and water addition are known input data for the processing of GF dough, its functional properties and GF bread quality are the measurable output data (see Fig. 8). However, material-based interaction for e.g. between starch and hydrocolloids, starch and proteins, proteins and hydrocolloids or hydrocolloids and fibers are unknown. Depending on the initial hydration level, water absorption of recipe components will greatly vary. The knowledge on which of these interactions is more prominent is still missing. Therefore, the impact of material-based interactions on structure formation in GF dough are entitled with “unknown mechanisms” in this thesis.

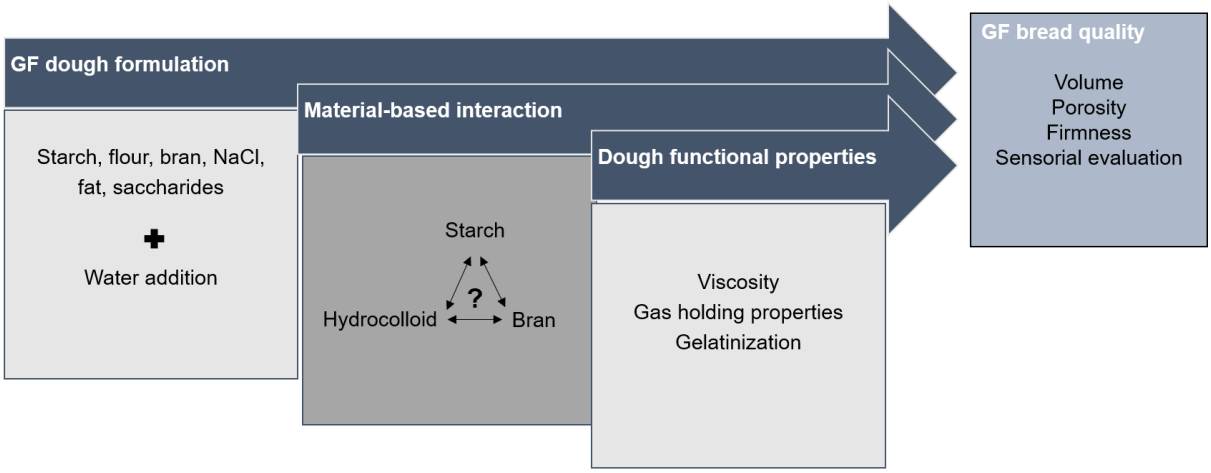



**Figure 8: Input and output data in gluten-free dough processing considering material-based interactions entitled as "unknown mechanisms".**

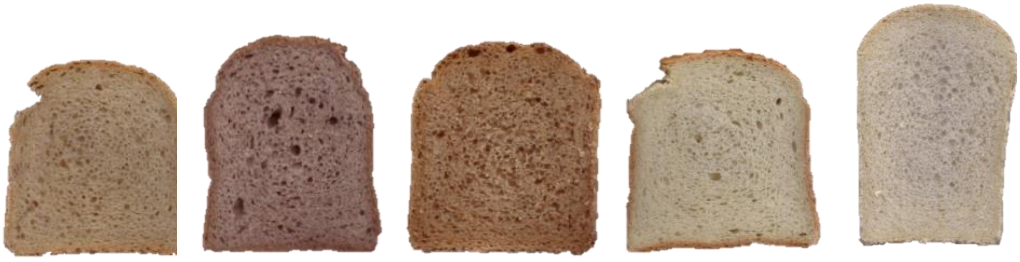
It is state of the art that a limitation in the starch-to-water ratio will increase gelatinization temperature (Schirmer, Jekle, & Becker, 2015). Limitations in dough hydration level can be induced by a lower initial water content of the formulation or by the choice of recipe components with higher water absorption. Depending on the applied starch, flour, hydrocolloids or bran, the type of water binding (free/bound) may vary as well. If the water is disposable (free available), it acts as a dispersing agent and can be easily removed by drying. In contrast, water can be tightly held by fibers and proteins (cell wall tissues/ protoplasm) through intermolecular hydrogen bonds around hydrophilic molecules and thus cannot be easily removed. In addition, also interactions between recipe components may occur, for e.g. competition for water between starch and protein, thus leading to reduced swelling of starch granules (Schirmer, 2014). Finally, during baking the initial water content in GF dough is reduced to 45-50 g/ 100 g sample.

A suitable measuring technique to determine water absorption in GF dough, comparable to a Farinograph for gluten-containing dough, is only recently available (Sahin, Wiertz, & Arendt, 2020). Different GF flour-hydrocolloid combinations were investigated regarding to their water absorption in an adjusted Farinograph. The values achieved from water absorption measurements were compared to those revealed by measuring the water holding capacity (WHC) of single recipe components (Sahin et al., 2020). However, as these analyses did not consider the adjustment of water absorption in GF flours enriched with bran, a matching measuring technique to estimate water absorption in these kind of GF dough systems is still missing. So far, quantification of the required water amount to form GF dough is mostly arbitrarily. Inter alia, determination of hydration properties could give a first impression of the required water dosage (Abugoch et al., 2008). To characterize the hydration properties of by-products from the fruit and milling processing industries, several methods (e.g. swelling

capacity (SC), water binding capacity (WBC) and water absorption were discussed by Föste et al. (2017). However, the authors pointed out that these key values could only give an indication of the required water amount in GF dough as the composition of bran and/or dietary fibers and the applied approach to determine the hydration properties showed huge differences and therefore cannot be easily transferred to the dough matrix.

In general, bran is used only in a neglected number of GF formulations. Föste, Muthmann, Jekle, and Becker (2015) analyzed commercial GF baking mixtures, which mostly contained starch or flour components of corn or rice, hydrocolloids and greatly varied in their hydration level (see Tab. 4). It is noteworthy that the list of ingredients was oftentimes very long and the prices varied in between 1.90 to 2.49 Euro per 500 g. While bran from rice or pea were used more often in GF bread, the application of quinoa bran as a potential additive to GF bakery products was not analyzed yet. Depending on the formulation and the final product, huge variation in the hydration level for baking mixtures (80 to 100%) or in GF bread (70 to 110%) were observed (Föste, Muthmann, et al., 2015; Föste et al., 2020).

**Table 4: Composition and quality characteristics of gluten-free baking mixtures in comparison to quinoa bran bread.**

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| **List of ingre-dients** | Corn starch, potato starch, corn flour, chestnut flour, GF sourdough, guar gum | Corn starch, linseed flour (12%), buckwheat flour (8%), pea bran, rice bran, apple fiber, salt, saccharides, guar gum | Corn starch, sweet lupine powder, guar gum, dextrose, skimmed milk powder, salt, caramel, glucona-delta-lactone | Corn starch, rice flour, lupine protein, dextrose, apple fiber, HPMC, salt | Corn starch, quinoa bran, HPMC, fat, salt, |
| **Hydration level (%)** | 80 | 100 | 90 | 100 | 80 |
| **Price (€/ 500g)** | 2.25 | 2.33 | 2.49 | 1.90 | |
| **Crumb** |  | | | | |

Modified from Föste, Muthmann, et al. (2015). HPMC: Hydroxy-propyl-methyl-cellulose.

This of course raises the question if it is preferable to achieve a more solid-like dough or a liquid-like batter? While, for practical purposes in the food industry it is more common to use firmer dough, those dough systems considered in this thesis varied from a liquid-like batter to

a more solid-like dough consistency. In general, GF formulations contain hydrocolloids, which support the formation of a weak gel network and contribute to increased viscosity and improved gas retention (Rosell, Rojas, & Benedito de Barber, 2001). Concurrent, this could raise the question if only hydrocolloids have such potential to improve GF bread quality or if there might be alternative fibers/bran that show somehow similar batter or dough-improving characteristics. Furthermore, due to the variety of recipe components, it must be assumed that interrelation between starch-hydrocolloid, starch-protein or protein-protein occur. These multitude interactions will limit the water content in GF dough as a consequence of the competition for water among all these recipe components.

Besides changes in dough functional properties and final bread quality, also sensorial characteristics will change enormously by incorporation of huge bran amounts. Sensorial impression, however, is a decisive factor for consumers' purchase decision. Depending on the botanical origin, bran impacts color, odor, taste or mouthfeel. While rice bran impaired wheat bread quality (Hu, Huang, Cao, & Ma, 2009), a limited number of publications pointed out a positive impact of fibers and/or bran on GF bread quality. Phimolsiripol et al. (2012) observed improvements in sensory color, smell, taste, texture and overall impression of GF bread enriched with 10% rice bran and a hydration level of 100%. Also in GF bread containing 10% quinoa bran with a hydration level of 80%, a softer crumb, a darker crust color, higher juiciness and overall acceptability were identified (Föste et al., 2014). Nevertheless, a slight off-flavor was noticeable, which significantly enlarged by increasing the bran amount up to 40% (Föste et al., 2014). Pursuant to supplier specification, the herein used quinoa seeds were free of saponins, and therefore the increasing off-flavor has to be traced back to other phytochemicals or endogenous enzymes. Therefore, further investigations should consider the presence of polyphenols and/or lipase and its effect on sensory quality in quinoa enriched GF bread. The relationship between a bitter somehow grassy taste of other dicotyledonous plants, such as pea or broad beans, and the presence of lipases, lipoxygenase or peroxidase was reviewed by Roland, Pouvreau, Curran, van de Velde, and de Kok (2017). Especially, free fatty acids, which are generated by lipid hydrolysis, are prone to oxidation and lead to the formation of a large number of volatile compounds (Pico, Hansen, & Petersen, 2017; Roland et al., 2017).

Several strategies were stated to overcome this sensorial deterioration. Thermal inactivation of endogenous enzymes by blanching, steam heating or dry heating the bran is only one approach to control the formation of enzyme-generated off-flavor compounds. Moreover, this method simultaneously modifies dough hydration behavior, reviewed by Föste et al. (2020). In particular, extrusion turned out to be a potential technology to improve dietary fiber contents in ready-to-eat snacks and to improve hydration properties of the formulation (Stojceska, Ainsworth, Plunkett, & İbanoğlu, 2009). The sensorial evaluation of those kind of products is

surely a worthwhile focus for future research. The application of sourdough fermentation is a well-known strategy to improve sensory characteristics, of fiber-enriched GF bread. Unlike extrusion, the suitability of fermenting flours and bran has been subject of lots of research studies (Coda, Rizzello, & Gobbetti, 2010; Moore, Bello, & Arendt, 2008; Moroni, Bello, Zannini, & Arendt, 2011; Salmenkallio-Marttila, Katina, & Autio, 2001; Zannini, Pontonio, Waters, & Arendt, 2012), however regarding to quinoa bran can be counted on one hand. To fill this gap, quinoa bran was fermented with *Lactobacillus plantrarum* ($10^7$ colony forming units (cfu)/g dough) for 24 hours at 30 °C with a dough yield of 200. Sourdough application showed great potential to reduce the off-flavor and to increase overall acceptability of final products (see Fig. 9).

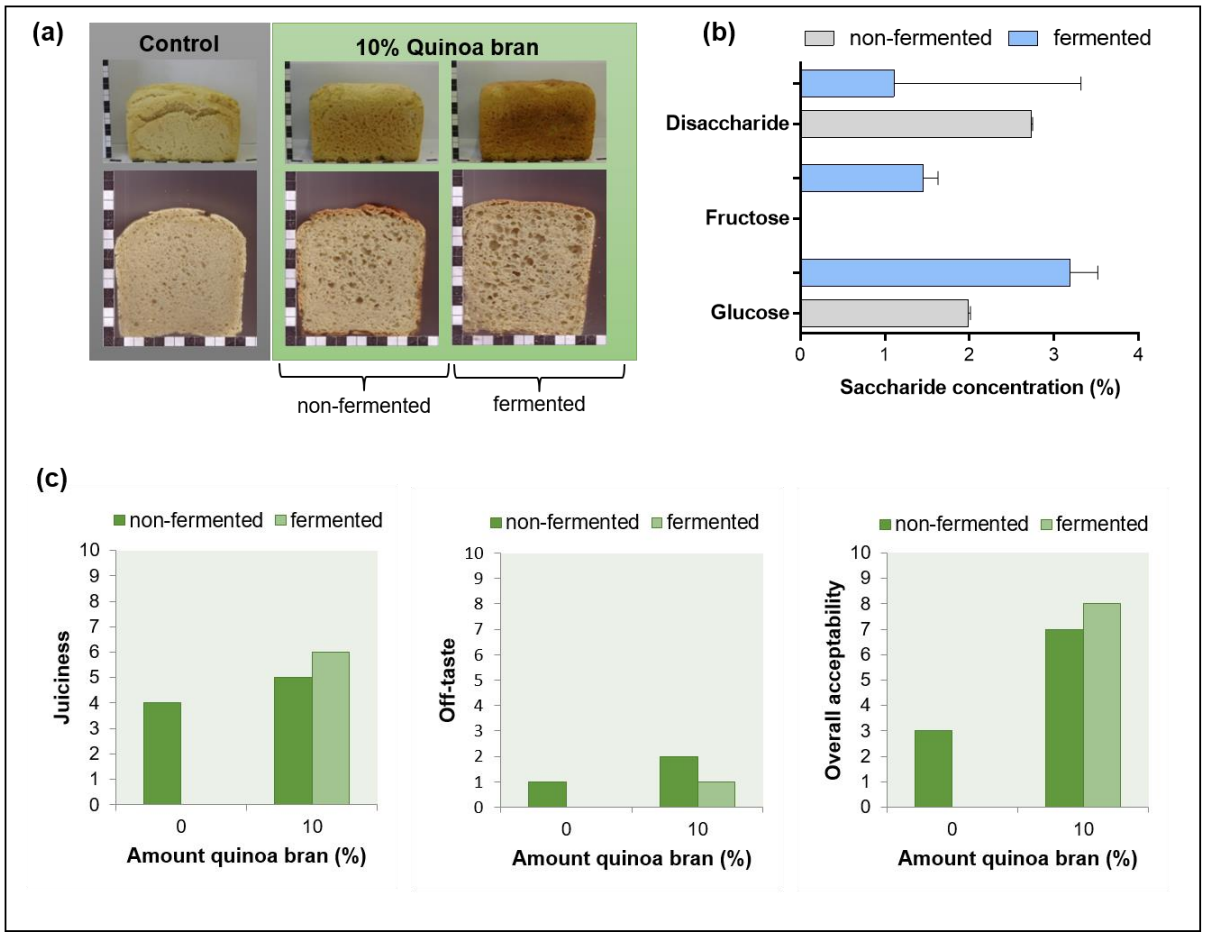



**Figure 9: Differences between non-fermented and fermented gluten-free quinoa bran bread.** (a) Appearance of GF bread and crumb slices. (b) Concentration in saccharides. Data presented are means with SD of (n ≥ 2). (c) Sensorial evaluation of non-fermented and fermented gluten-free bread. Data presented are means with SD of (n = 15). Modified from Föste, Elgeti, Jekle, and Becker (2013).

Investigations by Föste et al. (2013) showed that fermentation of quinoa bran resulted in a darker crust due to maillard reaction and a more aerated crumb structure, visualized in Figure 9a. Further investigations on saccharide concentration revealed a decrease in disaccharides and an increase in fructose and glucose with regard to fermented quinoa bran (see Fig. 9b). In addition, a sensorial evaluation with a trained panel (n = 15) was conducted

to assess the quality attributes of non-fermented and fermented GF bread. Panelists rated GF bread containing 10% fermented quinoa bran with a higher juiciness, a lower off-flavor and finally with a higher overall acceptability (see Fig. 9c). In addition, this kind of microbial polymer modification can also be used as a strategy to improve hydration properties and therefore as well confirm the second hypothesis of this thesis (Föste et al., 2020). In particular protease activity might be promoted by fermentation, leading to elevated batter viscosity and slower flour particle sedimentation as observed in GF rice flour (S. Hamada, Suzuki, Aoki, & Suzuki, 2013).

**Hypothesis 3: Incorporation of excessive bran in GF dough favors a weaker dough structure because of changes in dough functionality or destabilization by bran particles**.

The third hypothesis was confirmed by the third study of this thesis (Föste et al., 2014). The effect of quinoa bran on GF dough viscosity, gas holding properties and gelatinization were analyzed and evaluated with regard to the hydration level. Therefore, in their third study, Föste et al. (2014) used a GF formulation consisting of a corn and rice flour mixture containing HPMC with a hydration level of 80% as reference. Oscillatory measurements in a fundamental rheometer showed that complex shear modulus $G^*$ significantly increased after replacing the GF flour mixture with up to 40% quinoa bran and constant water addition of 80 g per 100g flour (Föste et al., 2014). The authors identified 10% quinoa bran to be the lowest amount before an increase in $G^*$ was observed. Independent of GF dough viscosity, 10% quinoa bran significantly improved GF bread quality, indicated by a higher specific loaf volume and a uniform distribution of crumb pores compared to the reference. Besides HPMC, which is known as a surface-active hydrocolloid absorbing huge amounts of water, it is conceivable that quinoa bran might also contain surface-active proteins that contribute to improved gas bubble stabilization (see section 1.2) and also observed in quinoa white flour by Elgeti, Peng, et al. (2017).

Another aspect of interest is the endogenous enzyme activity, which is depending on the water amount and the present temperature during proofing. Investigations on GF dough containing quinoa white flour revealed significant α-glucosidase activity, providing adequate substrate for yeast fermentation (Elgeti et al., 2014). Also Föste et al. (2014) observed either amylolytic enzyme activity, but also high proteolytic activity in quinoa bran. The modification of the protein network through proteolytic activity caused by flour or other protein-rich raw materials was observed by Armero and Collar (1997) and Corsetti et al. (1998). For instance, rice batter treated with protease was more prone to swelling than the control formulation, which did not rise and showed impaired gas retention (S. Hamada et al., 2013).

The incorporation of more than 20% bran significantly increased *G** and simultaneously promoted a structure loss indicated by the decrease in dough height during proofing (Föste et al., 2014). This revelation can be attributed to the following aspects: 1) Increasing water absorption through the presence of quinoa bran and 2) Piercing of gas bubbles by quinoa bran. The former aspect might result in a lower amount of disposable water for hydrocolloid gel network formation or starch gelatinization. In particular, hydrophilic macronutrients such as polysaccharides or protein fractions (see section 1.2) may have favored water absorption. Investigations on water retention capacity (WRC) revealed considerably higher values for quinoa bran (151.9%) in comparison to rice flour (109.75%) or corn flour (114.97%) (Föste et al., 2014). The higher WRC of quinoa bran can be traced back to the composition of the seeds (see section 1.2). Thus, quinoa consists of numerous hydrophilic macronutrients and phytochemicals, most of them located in the outer seed tissues. A special emphasis has to be taken on albumin and globulin fractions, whose contents are higher in quinoa compared to corn or rice flour. In addition, a principal component analysis performed by Tomić et al. (2015) showed that the albumin fraction of 15 to 30 kDa especially affected wheat dough functional properties being related to water absorption. What is more, in cashew nut protein, huge amounts of glutelin were found, which led to higher water binding capacity (WBC) (15.85 g/g) in comparison to the cashew nut albumin fraction, which achieved a WBC of 1.22 g/ g (Liu et al., 2018). These researches pointed out that glutelins of cashew nut had a flaky microstructure that enhanced the interaction with water. This could contribute to a tight water binding and may reduce availability of water in the dough, thus promoting an increase in dough firmness (*G**). Unlike cashew nut protein, for milled quinoa seeds a glutelin content of 31.6% db is reported (Ando et al., 2002). A detailed analysis of the glutelin structure from quinoa could give additional indications, whether the protein structure is advantageous for tight water binding. Furthermore, in comparison to cereals, no significant amount of xylans were detected in quinoa (Lamothe et al., 2015). As pointed out by Lamothe et al. (2015) seed tissues of quinoa rather resemble those of legumes, fruits and vegetables. Especially the high occurrence of water-absorbing pectic substances (see Tab. 2) may be an explanation for the increased hydration properties of quinoa bran compared to rice or corn flour.

The second point refers to the rigid and inflexible structure of bran particles. By increasing the bran amount in GF dough, especially its rigid structure may promote piercing the gas bubbles, leading to a reduction in dough height and loaf volume. This so-called structure weakening effect in GF dough has been addressed by Schober (2009). To exclude polymer interactions, which may result from the combination of different flours and starches, GF formulations should be simplified. Investigations on a model formulation based on rice flour or corn starch revealed higher tan $\delta$ values in bran-enriched corn starch dough than for appropriate mixtures based on rice flour (see Fig. 10).

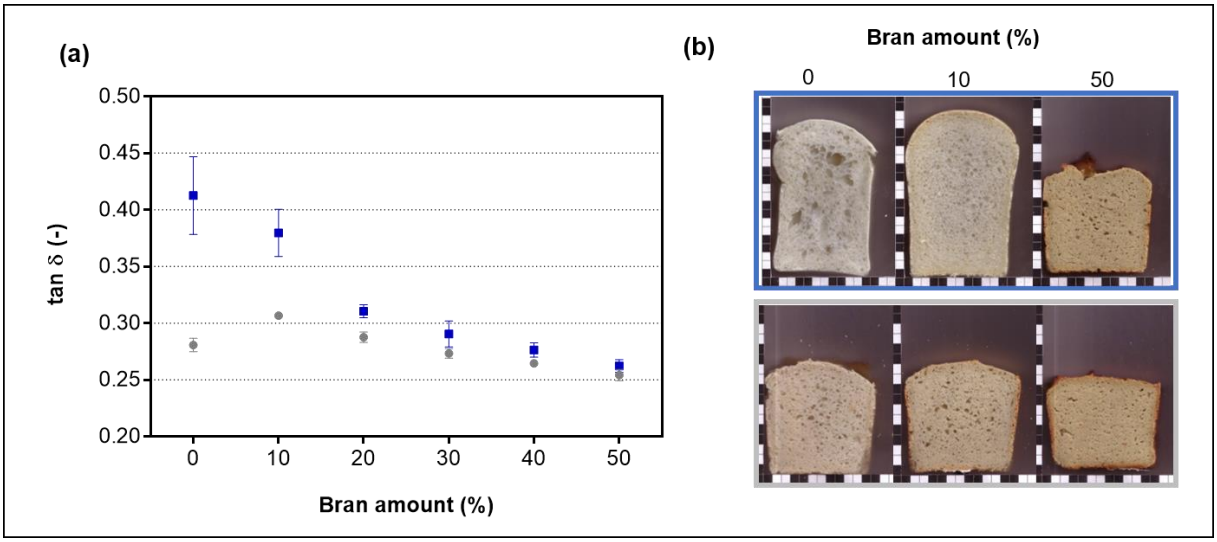



**Figure 10: Impact of bran on dough viscosity and bread quality.** (a) Tan δ of GF dough depending on the bran amount with a hydration level of 80%. (b) Pictures of crumb slices from the reference formulation and with 10% and 50% quinoa bran. Presented are values for corn-starch dough (■) and rice flour dough (■). Values presented are means with SD of (n = 3). Crumb slices for corn starch bread are adapted data from Föste et al. (2017).

While in rice flour dough, interactions between starch-hydrocolloid, starch-protein, protein-hydrocolloid and protein-protein from flour and bran may be present, the latter can be excluded in the corn starch formulation. Notwithstanding, the difference in dough rheology depending on the botanical origin (rice, corn) indicate that GF dough viscosity plays a major role in stabilizing gas bubbles (Elgeti, Peng, et al., 2017). From the processing side, the knowledge on the optimum range of dough viscosity would be beneficial for targeted adjustment to keep gas bubbles as long as possible in the dough and to modify structure formation during baking. The idea to establish a defined key value for dough viscosity to improve GF bread quality has been considered only by few researchers. With the focus on kneading conditions, Aprodu et al. (2016) suggested to strive dough consistency of 0.33-0.50 Nm to obtain beneficial volume and texture properties in GF bread. The findings by Föste et al. (2020) highlighted that only one key value such as tan $\delta$ is insufficient to predict GF bread quality. However, if tan $\delta$ varied between 0.35 to 0.45 improved final bread quality was observed in comparison to values below or above this range (see section 2.3). To conclude, the determination of single key values is not expedient and maybe a rheological modulation could be helpful to predict the impact of bran on hydration properties in GF dough and batter (Föste et al., 2020).

According to the findings of an increased WRC for quinoa bran, an increase in dough viscosity after replacing corn starch by 10% quinoa bran would have been expected. Therefore, the impact of quinoa bran on water activity was investigated in a starch-based model recipe (see Fig. 11).

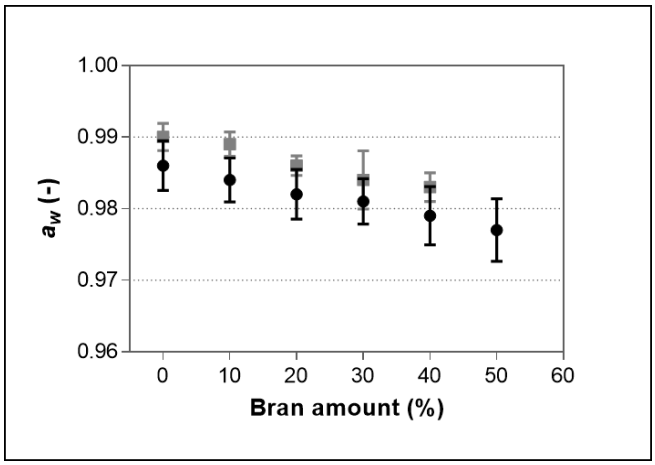



**Figure 11: Water activity of GF corn starch dough.** Visualized are values for water activity for the reference formulation and after partial replacement with quinoa bran depending on the hydration level 80% (■) and 100% (■). Presented are means with SD of (n ≥ 3). Adapted from Föste et al. (2017).

In tendency, $a_w$ decreased in those formulations with a higher bran amount (up to 50%) in comparison to the pure starch formulation. Moreover, $a_w$ was higher in GF dough with elevated water content of 100 g water/100 g starch. In summary, the elevation of quinoa bran led to increased $G^*$, although $a_w$-values did not significantly differ. From these results it can be concluded that not only dough rheology and $a_w$, but also further dough functional properties determine final crumb structure. Detailed investigations by means of nuclear magnetic resonance spectroscopy would have been appropriate, as this tool supports visualization of water mobility in GF dough. Therefore, future research should focus on visualization of proton movement between specific recipe components, for e.g. between starch and proteins. As highlighted by Föste et al. (2020) the required water addition in fiber and/or bran-enriched GF formulations is very often overlooked. Nevertheless, functional properties (dough firmness; gelatinization) of GF dough are greatly affected by the hydration level of the formulation. To evaluate the impact of bran on GF formulations, regardless of dough viscosity and gelatinization characteristics, $G^*$ and $T_{Onset}$ were adjusted.

**Hypothesis 4: Adjustment of the dough firmness *G** and/or the start of gelatinization $T_{Onset}$ enables standardizing of the dough functional properties and bread quality characteristics.**

The fourth hypothesis was declined in the fourth study of this thesis (Föste et al., 2017), as the results clearly showed that dough functional properties could not be easily standardized by adjustment of the hydration level. In GF dough containing 30% quinoa bran, 110 g water per 100 g flour was required to adjust dough firmness to the reference formulation, as indicated by *G** values. By increasing the bran amount (> 30%) water addition far above the property-adapted hydration level would have been required to adjust *G*.* As well to adjust gelatinization characteristics in GF dough containing 20% quinoa bran, excess water (> 110 g/ 100 g flour) would have been needed to standardize $T_{Onset}$. However, these amounts are not used in practice as they are far above the level required for GF dough formation.

**Hypothesis 5: An optimized hydration level counteracts a structure-weakening effect by quinoa bran and stabilizes the GF dough.**

The fifth hypothesis was confirmed in the fourth study of this thesis (Föste et al., 2017). Apart from dough viscosity, also gelatinization of starch is affected by the presence of water. To enlighten the effect of bran on water availability, gelatinization profile of varying starch-bran to water ratios was analyzed. Therefore, Föste et al. (2017) investigated the impact of bran on $T_{Onset}$ and the enthalpy ΔH during gelatinization, based on a simplified model formulation, containing corn starch, 2% HPMC and 80 g water per 100 g flour. While gelatinization of this reference formulation started at 74 °C, replacing corn starch with 50% quinoa bran significantly delayed the $T_{Onset}$ to 79°C (Föste et al., 2017). Depending on the type of starch and its composition, different transition temperatures and enthalpies during gelatinization may be achieved (Biliaderis, Maurice, & Vose, 1980). Considering pure corn starch, $T_{Onset}$ varied between 62 to 66 °C and ΔH between 8 to 14 J/g (Schirmer, Höchstötter, Jekle, Arendt, & Becker, 2013; Singh, Singh, Kaur, Singh Sodhi, & Singh Gill, 2003), being lower than the herein observed $T_{Onset}$ values for the corn starch-based reference formulation. Further, the replacement of corn starch with 50% quinoa bran significantly lowered ΔH (Föste et al., 2017). In addition to the measured values for ΔH, also the enthalpy, which is theoretically required for gelatinization considering the lower starch amount in the respective formulation, was calculated to show the impact of bran on gelatinization. While estimation of ΔH in GF dough containing 50 % quinoa bran revealed 0.180 J/g for gelatinization, calculation of the enthalpy gave 0.880 J/g (Föste et al., 2017). This gap between the measured and the calculated

gelatinization enthalpies underlined the influence of quinoa bran on ΔH as a consequence of a lower amount of disposable water (Föste et al., 2017).

Both GF formulations used in this thesis contained 2% HPMC based on 100 parts of flour or starch, respectively, whereby the flour/starch basis was replaced with different amounts of quinoa bran (Föste et al., 2017; Föste et al., 2014). Therefore, further interaction between starch and hydrocolloids or starch and hydrophilic bran components (protein, polyphenols, fibers) must be considered. In addition to the overview of texture formation of dough matrices during baking presented by Föste et al. (2017), Figure 12 illustrates the dominant structuring mechanisms of gluten-containing and GF dough after bran addition. For this contemplation, different scales are used as transformation of β-sheet and α-helix structure (nanoscale) were considered to be the most important transformation steps in gluten-containing dough. With regard to GF dough, macroscopic scale was used to point out the changes in water uptake by hydrophilic bran components, starch and hydrocolloid gel network considering as well stabilization and/or destabilization of gas bubbles.

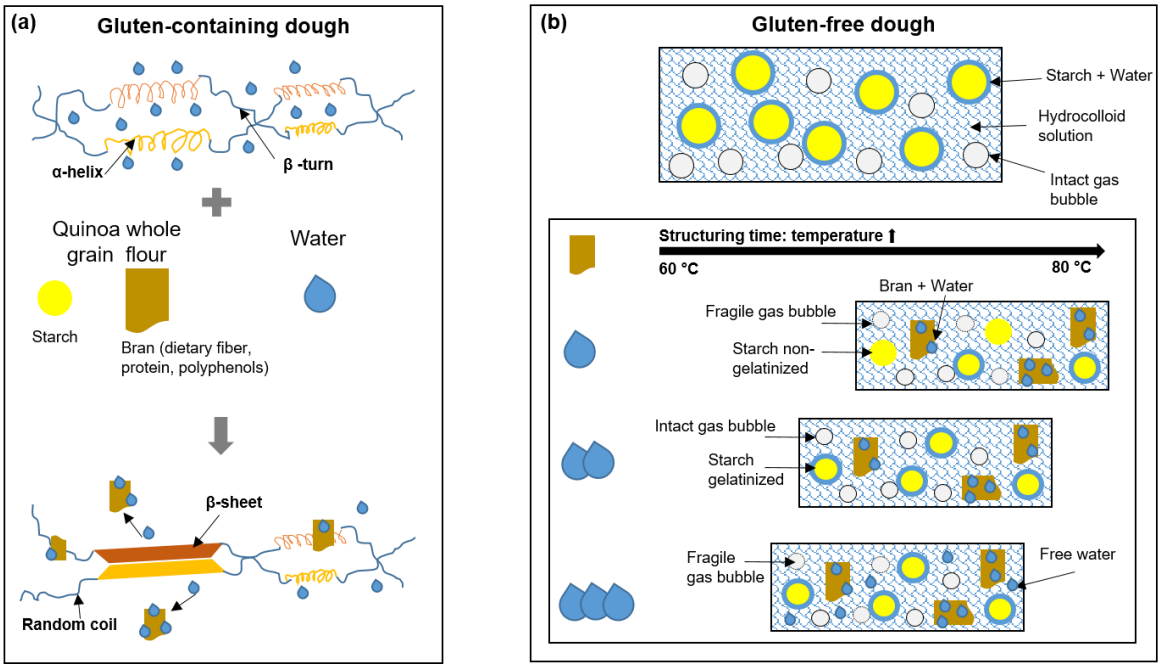



**Figure 12: Dominant structure formation mechanisms in gluten-containing and gluten-free dough after bran addition.** (a) Part of a protein in nanoscopic scale for gluten-containing dough. Modified from Xu et al. (2019) (b) Part of the hydrocolloid gel network in macroscopic scale for GF dough. The box includes the three scenarios for water content in GF dough, representing the shift in thermal structuring, synonymous with an earlier start of gelatinization. Modified from Föste et al. (2017).

Bran addition in gluten-containing dough was reported to change gluten conformation from the more elastic α-helix hence to the more inelastic β-sheet aggregates (Bock et al., 2013; Xu et al., 2019) (see Fig.12a). These studies revealed a decrease in viscoelasticity and a reduction in final loaf volume. As introduced in section 1.4, structure formation in GF bread is a combination between starch being embedded in a hydrocolloid solution, forming a gel-like

structure to increase viscosity and retaining gas bubbles. By replacing starch with quinoa bran, it must be assumed that either HPMC or proteins/ dietary fibers from quinoa bran may reduce the water content. To visualize the changes in GF dough, considering limitation in water content, the following three scenarios were depicted (see Fig. 12b).

If the **hydration level was kept constant** (see Fig. 12 b, box/ first line), the replacement of corn starch with 50% quinoa bran clearly pointed out that starch gelatinization was delayed, indicated by the increased $T_{Onset}$ (Föste et al., 2017). In addition, because of the higher dough firmness, gas bubbles might be more fragile, ending up in a low loaf volume, which can simultaneously be ascribed to lower disposable water in the dough. The findings by Maaruf, Che Man, Asbi, Junainah, and Kennedy (2001) support this assumption. These authors observed sago starch suspensions with varying water volume fraction from 0.80 to 0.37 and found an increase in $T_{Onset}$ (63.6-74.7 °C) and a decrease in ΔH (5.0-1.6 J/g) with lower water volume. In addition, investigations on a GF formulation, containing corn-cassava starch in the ratio 75:25 and as well 1.5% HPMC revealed that $T_{Onset}$ varied from 70.4 to 74.5 °C depending on the water content (80 or 110%) (Kobylañski, Pérez, & Pilosof, 2004). Even though, these values are lower than the herein observed $T_{Onset}$ after replacing corn starch with quinoa bran, this once more demonstrates that material-based interactions between starch and hydrocolloid, respectively starch and proteins or dietary fibers from bran may limit hydration level in GF dough. The following example should illustrate the changes in dough hydration level by increasing the bran amount. Presuming a hydration level of 80% in the GF model formulation, the use of corn starch will lead to interactions primarily based on hydrogen bonds between carbohydrates (starch/ HPMC) and water (Syamaladevi et al., 2016). By the presence of quinoa bran, the proportion of hydrophilic macronutrients and phytochemicals in relation to the constant hydration level of 80% will increase. Furthermore, the replacement of corn starch with 50% quinoa bran will lead to the formation of hydrogen bonds between either carbohydrate (starch/HPMC) and water, protein and water and as well phytochemicals and water. While for regular corn starch a WRC of ~59 % (w/w) was determined (Schirmer et al., 2013), quinoa bran revealed a WRC of ~152% (Föste et al., 2014). In conclusion, it can be assumed that the presence of hydrophilic macronutrients and phytochemicals contained in quinoa bran will lower the disposable water amount in the GF formulation. As a result, dough firmness increased, $T_{Onset}$ was delayed and GF bread quality was deteriorated.

In view of an **increased hydration level** resulting from excess water addition (see Fig. 12 b; box/ third line), bran and HPMC will absorb most of the water and starch granules will be gelatinized (see Fig. 12b, third line). The studies on adjustment of dough functional properties ($G^*$ and $T_{Onset}$) in this thesis revealed that water amounts higher than 110 g per 100 g flour did not result in the structure formation in those kind of GF batters (Föste et al.,

2017). It was assumed that excess free water promoted coalescence of the more fragile gas bubbles so that gas retention was impaired.

Considering **optimum hydration level** (see Fig. 12 b; box/ second line), water will be evenly distributed between HPMC and quinoa bran. It is assumed that temperature increase will lead to uncontrolled starch gelatinization and intact gas bubbles due to an early start of gelatinization. By adjusting the water content in the range from 90 to 110 g per 100 g flour structure formation in GF bread was improved (Föste et al., 2017; Sabanis et al., 2009; Ziobro et al., 2013). Unlike a fixed hydration level of 80%, elevated hydration level of 110% decreased $T_{Onset}$ and increased ΔH. The results of the fourth study clearly showed that ΔH positively correlated with specific loaf volume (r= 0.7159; $p < 0.0001$) and $T_{Onset}$ negatively correlated with the loaf volume (r=-0.9042; $p < 0.0001$) (Föste et al., 2017). Finally, the improvement of bran-enriched GF bread quality regarding specific loaf volume and crumb texture, requires water addition in an application-oriented range to counteract the structure-weakening effect promoted by quinoa bran. Consequently, the awareness that $T_{Onset}$ appears to play a major role in stabilizing GF dough was an important outcome of this thesis.

To conclude, this study provided fundamental knowledge on the application of quinoa bran to improve GF bread quality considering the challenges of dough hydration and dough functional properties. Under processing aspects, 10% quinoa bran in combination with a hydration level of 80% was sufficient to stabilize GF dough. However, by increasing the bran amount, GF dough was destabilized, indicated by higher dough viscosity and lowered gas retention capacity, impairing final bread quality. In the case of quinoa bran, containing huge amounts of protein, dietary fiber and phytochemicals, the findings clearly showed increased affinity to absorb water and simultaneously reduce hydration level of GF dough. To avoid destabilizing of GF dough, this study impressively demonstrated that an earlier start of gelatinization was an efficient key value to improve final GF bread quality. By adjusting the hydration level in GF dough up to 100%, final loaf volume and crumb texture were improved. Moreover, by elevating the hydration level, corn starch could be replaced with 20% bran. The use of higher amounts of quinoa bran is synonymous with further enrichment in protein and fiber. However, because of the increased hydration level no negative effect on GF dough matrix was observed. To further enlighten the ”unknown mechanisms” between material-based interactions for e.g. starch and hydrocolloids or hydrocolloids and bran and the disposable water amounts in GF bread, future studies should consider further simplification of the GF formulation. A possible approach could be to exclude HPMC in the GF formulation, avoiding additional competition for disposable water between hydrocolloids and bran or starch, respectively. Another methodical approach could be to fractionate the bran into its individual macronutrients (protein fractions, fat, dietary fibers). Thereafter, it would be possible to investigate the particular impact of for

e.g. only protein or dietary fibers on hydration properties and gelatinization of GF dough. Consequently, a clearer assignment between material-based interactions would be conceivable. Besides technological aspects, from the nutritional point of view, 10% replacement by quinoa bran would mean elevation in protein, minerals and dietary fibers by many times over. This once more underlines the worthwhile application as recipe component in GF formulations. Simultaneously, the valorization of milling by-products such as bran mirrors the spirit of the time in sustainability and likewise fulfils the trend of the market in developing alternative food products in the future expanding “free-from” sector (vegan, gluten-free).

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# 5 Appendix

The numbering of the following non-reviewed publications is continued from the referenced publications (page II).

## 5.1 Non-reviewed paper

7. Föste, M., Muthmann, I., Jekle, M. & Becker, T., Glutenfreie Backmischung – Bewertung von Inhaltsstoffen, Review. Innovations, (2015), 68-79.
8. Föste, M., Muthmann, I., Jekle, M., & Becker, T., Gluten-free: innovation or confusion by mixture components. Review. Innovations. (2014), 82-91.
9. Föste, M., Elgeti, D., Jekle, M., Becker, T.: Manufacture of gluten-free breads - a question of the substrate? Baking+biscuit 6 (2013), 46-49.
10. Föste, M., Elgeti, D., Jekle, M., Becker, T.: Herstellung glutenfreier Brote – alles eine Frage des Substrats? Brot+Backwaren 5 (2013), 66-70.

## 5.2 Oral presentations

11. Föste, M., Jekle, M., Becker, T.: Application of Pseudocereals' different Milling Fractions and their Rheological Influence in Gluten Free Dough. 10th European Young Cereal Scientists and Technologists Workshop, Helsinki, Finland, 2011-05-25.
12. Föste, M., Jekle, M., Becker, T.: Potential der Fraktionierung von Quinoa und Buchweizen. 1. WIG Frühjahrstagung, Freising, Germany, 2012-03-29.
13. Föste, Jekle, M., Becker, T.: Amarant – Potenzial in Backwaren. Diskussionsforum „Verträglich, sicher, gesundheitsfördernd-die Lebensmittel von morgen“, Salenstein, Schweiz. 2012-06-06.
14. Föste, M., Elgeti, D., Jekle, M., Becker, T.: Anwendung von fermentierten Mahlfraktionen zur Herstellung glutenfreier Quinoabrote. 2. WIG Frühjahrstagung, Freising, Germany, 2013-03-21/22.
15. Föste, M., Elgeti, D., Jekle, M., Becker, T.: Evaluation of structure weakening effects by quinoa bran in gluten-free bread. AACC Annual Meeting, Albuquerque, New Mexico, USA, 2013-10-02.
16. Föste, M., Muthmann, I., Jekle, M. & Becker, T. Marktscreening glutenfreier Brote – die Backmischung macht’s. 3. WIG Frühjahrstagung, Freising, Germany, 2014-04-02.
17. Föste, M., Jekle, M., & Becker, T. Fermentierte Mahlfraktionen – gezielte Verbesserung der Qualität von glutenfreien Broten. GDL-Forum Sauerteig V, Münster, Germany, 2014-05-20.
18. Elgeti, D., Föste, M., Jekle, M., Becker, T.: Einsatz von Pseudocerealien zur Aroma- und Inhaltsstoffaufwertung - Seminar: Einfluss der Vermahlungstechnologie auf Backwaren, Bakery Innovation Center, Uzwil, Switzerland, 2014-07-18.

19. Föste, M., Vogt, S., Holtz, K., Gratzl, R. Jekle, M. & Becker, T. Hochdruck zur Hydratisierung von Rohstoffen – eine Methode der Zukunft? 4. WIG Frühjahrstagung, Freising, Germany, 2015-04-22.
20. Föste, M., Elgeti, D., Jekle, M., & Becker, T. Processing strategies for gluten-free bread improvement. Training Workshop – creating value in wheat and gluten-free based bakery production chain. Cork, Ireland, 2015-05-15.

## 5.3 Poster presentations

21. Föste, M., Elgeti, D., Jekle, M., Becker, T.: Impact of fermented quinoa fractions on dough and baking performance in gluten-free bread, 3rd International Symposium on Gluten-Free Cereal Products and Beverages, Wien, Austria, 2013-06-12./14.
22. Föste, M., Elgeti, D., Jekle, M. & Becker, T. Fraktionierung von Pseudocerealien, 3.WIG Frühjahrstagung, Freising, Germany, 2014-04-22.
23. Föste, M., Brunner, A. K., Elgeti, D., Jekle, M., & Becker, T. Protein extraction from gluten-free milling fractions for food applications. 13$^{th}$ European Young Cereal Scientists and Technologists Workshop, Freising, Germany, 2014-05-14.