Monitoring of early-stage water uptake by hyperspectral imaging and evaluation of nutritional and technological functionality of germinated faba bean (Vicia faba L.) var. minor and var. major as food ingredients

Ulla Holopainen-Mantila¹ | Tuija Sarlin¹ | Outi Mäkinen¹,² | Arja Laitila¹,² | Nesli Sozer¹

¹VTT Technical Research Centre of Finland Ltd., Espoo, Finland
²Valio Ltd., Helsinki, Finland

Abstract
Faba bean (Vicia faba L.) is a potential, sustainable protein alternative. Germination behavior of Vicia faba L. var. minor and Vicia faba L. var. major needs further studies in order to enable larger scale bioprocessing. In this study, early-stage water uptake of two distinct faba bean varieties was assessed by hyperspectral imaging. Nutritional and technological functionality of germinated faba bean ingredients as such and in combination with fermentation were evaluated. Hyperspectral imaging revealed that early-stage water uptake in faba beans occurred evenly from the different sides of the beans. Germination on petri dishes and in pilot-scale showed that smaller faba beans moistened and germinated significantly (p < 0.05) faster and retained water better through germination than larger faba beans. Germinated faba flour of minor-type variety resulted in 72% higher dextran production in Weissella confusa VTT E-143403 fermentation than respective native faba flour. With both types of faba bean varieties, germination as such, and with minor-type variety, germination as combined with fermentation decreased notably the content of raffinose family oligosaccharides. These bioprocessing methods also improved functionality of faba bean ingredients by increasing protein separation efficiency in air classification, protein solubility, foaming capacity, and foam stability. Based on this study, minor- and major-type or small- and large-seeded faba bean varieties set different requirements to the germination process. In addition, germination alone or as combined with fermentation was proved to improve the nutritional and technological quality of faba bean material promoting its use in several food applications including also gluten-free products.

Keywords
air classification, dextran, Faba bean (Vicia faba L.), fermentation, germination, gluten-free bread
1 INTRODUCTION

There is a growing need for finding sustainable protein alternatives for food production. Despite their high protein content, legumes are underutilized as food ingredients. Their seeds serve a well-balanced amino acid composition especially when consumed with cereals typically rich in sulfur-containing amino acids (Shewry, 2000). The reasons for low utilization rate of legume seeds lie in the antinutritional compounds as well as in the poor technological functionality and sensory quality (Vaz Patto et al., 2014).

Faba bean (Vicia faba L.) is a legume species that is cultivated in various climatic conditions from boreal to subtropical zone. Thus, it represents a potential crop to be produced in large quantities for industrial use. Faba bean cultivars represent different phenotypes having, for example, small (Vicia faba L. var. minor) or large (Vicia faba L. var. major) grain size (Crépon et al., 2010). To our knowledge, the bioprocessing behavior of cultivars with larger or smaller seeds has not been compared so far.

Various physical and thermal processing technologies, such as dehulling, cooking, and extrusion, have been tested for improving nutritional quality of legume seeds (Alonso et al., 2000; Ferawati et al., 2019; Khall & Mansour, 1995; Ma et al., 2017; Vidal-Valverde et al., 1998). The most natural alternative for reducing antinutritional compounds in seeds is germination during which the physiological machinery of a seed is activated in order to release nutrients for the growth of the seedling. Germination of legume seeds has been shown to decrease the contents of several antinutritional compounds including phytates (Alonso et al., 2000; Luo et al., 2012; Luo & Xie, 2014), condensed tannins (Alonso et al., 2000; El-Adawy et al., 2003), glycosides vicine, and convicine (Jamalian, 1999; Jamalian & Ghorbani, 2005) as well as enzyme inhibitors (Alonso et al., 1998; Alonso et al., 2000; Urbano et al., 2005). In addition, an increase in the amounts of B-vitamins, antioxidant activity, and the digestibility of legume proteins as an effect of germination has been reported (Alonso et al., 2000; El-Adawy et al., 2003; Hefni et al., 2015; López-Amorós et al., 2006; Urbano et al., 2005). Importantly, raffinose family oligosaccharides (ROFs) causing flatulence and thus reducing the utilization of pulses are shown to be degraded during germination (Nyyssölä et al., 2020; Urbano et al., 2005; Vidal-Valverde et al., 2002, 1998). Besides nutritional value, germination has been reported to influence on techno-functional properties of pulses. The most pronounced changes have been found in the water absorption, pasting, emulsifying, and foaming properties of flours made from germinated pulses (Ferawati et al., 2019; Setia et al., 2019). However, the tendency of the reported changes is somewhat contradictory in particular regarding emulsion activity and foaming capacity. Fermentation as a bioprocessing tool has also been shown to impact the chemical composition of pulses, for example, by reducing the amount of ROFs and altering the phenolic and protein profiles (Di Stefano et al., 2019; Nyyssölä et al., 2020).

Bread is a staple food in our diet, which is mainly produced based on white wheat flour that has inferior nutritional profile but better overall sensory experience compared with wholegrain or bran supplemented bread (Heiniö et al., 2016). The challenges associated with nutritional and sensory profile are pronounced especially in gluten-free (GF) breads. GF breads are mainly based on starch, have low volume, dry, hard, and crumbly texture with bland flavor lacking also micronutrients, dietary fiber, and protein (Melini et al., 2017). Germinated pulses with improved nutritional profile (e.g., less ANFs, more micronutrients, and minerals) such as faba beans are potential protein and dietary fiber sources for bread including GF products (Sozer & Poutanen, 2015). Germination has been applied to soy flour (Shin et al., 2013), yellow field pea (Ma et al., 2018), and chickpea (Ouazib et al., 2016) to improve their applicability in GF breads. Limited hydrolysis of storage proteins during germination alters their technofunctional properties and consequently, their behavior in food matrix. Further bioprocessing of germinated flours with lactic acid strains producing extracellular polysaccharides can boost the formation of dextrans (an in situ hydrocolloid) as germinated seeds contain elevated levels of saccharose which is needed during the bioconversion of dextran (Chen et al., 2019). Dextrans were found to increase the water-binding capacity, reduce bread staling, and result superior structural effects in both wheat and GF breads (Wang et al., 2018).

In this study, a pilot-scale germination process was established both for Vicia faba L. var. minor and Vicia faba L. var. major using automated malting equipment to enable homogenous germination. To our knowledge, this is the first study in which germination behavior of two distinct faba bean varieties was studied systematically by hyperspectral imaging enabling monitoring of detailed spatial distribution of early-stage water uptake. It is hypothesized that this essential hydration phase initiating germination takes place mainly via embryo in faba beans. The aim of the study was to evaluate the nutritional and technological functionality of germinated ingredients as such and in combination with fermentation with lactic acid bacteria strains in situ producing exopolysaccharides and their applicability in GF baking.

2 MATERIALS AND METHODS

2.1 Plant material

Both minor- and major-type cultivars of faba bean (Vicia faba L.) were used in this study. Beans of small-seeded faba bean cv. Kontu were grown in Finland, and large faba bean seeds were of cv. Hangdown grown in Sweden.

2.2 Detection of early-stage moisture distribution by hyperspectral NIR-imaging

Spatial distribution of moisture was measured in faba beans soaked in 1% H2O2 for 1 or 3 h at +20°C in 50-ml test tubes with native beans as a reference. Beans were cut into two halves either longitudinally (n = 10) or via embryonic axis (n = 10). Cutting surfaces were positioned towards line-scanning near infrared (NIR) hyperspectral camera (Specim Ltd., Finland) with a mercury cadmium telluride detector for measuring simultaneously 320 spatial pixels (width 9 mm) and
256 wavelength channels with spectral range of 880–2490 nm. Utilising a 45°/0°/45° measurement geometry, the cutting surface was scanned horizontally by moving it linearly with an ISEL 3D positioning system (Germany) in front of the NIR camera resulting in spatial pixel size approximately 28 μm × 28 μm. The data were collected with an in-house built software using GPU-accelerated computations and chemometric preprocessing algorithms. The appearance of the beans is presented in pseudo RGB images with RGB coordinates calculated as the negative areas between a baseline point and three spectral regions in the absorbance spectrum: baseline region 1060–1160, red 1250–1340, green 1617–1717, and blue 1890–2010 nm. To visualize the spatial distribution of moisture on the measured surface, the ratio between the baseline corrected areas of two absorption bands was calculated: baseline region 1590–1690, water band region 1890–2010, and reference band region 2080–2160 nm. The resulting moisture maps were scaled allowing visual comparison across all measurements and visualized using the “parula” colormap where yellow/blue means high/low intensity value.

### 2.3 Germination of faba beans on plates and in pilot-scale

Germination trials were conducted both on plates and in pilot-scale utilizing automated germination equipment. The experimental setups of plate and pilot-scale germination trials are described in detail in Figure 1. In brief, the beans were first soaked in two-step process with H₂O₂ for cleaning seed surface followed by soaking in H₂O. The germination phase lasted until 72 h from the beginning of soaking. Moisture content and germinated seeds were assayed for monitoring the process. For moisture analysis was dried overnight in +105°C, ground using Fritsch Pulverisette 14 sample mill (Fritsch GmbH, Germany) with a 0.5-mm sieve and dried again for 2 h in +131°C. Beans germinated in pilot-scale were dried up to 60°C for 18.5 h for analyses and further processing.

The germinated samples produced with different soaking procedures are indicated as follows: Germinated 3 h [in 1% H₂O₂] + 0 h or 9 h or 21 h [in H₂O].

#### 2.4 Compositional analysis

The concentrations of ROFs, namely, raffinose, stachyose, and verbascose were analyzed by high-performance anion exchange chromatography (HPAEC) equipment Dionex ICS-3000 with pulse amperometric detection (PAD) (Dionex Corporation, Sunnyvale, CA, USA) according to methods described by Tenkanen et al. (1997) and Tenkanen and Siika-aho (2000). The results were calculated by using corresponding oligosaccharide standards: raffinose (Fluka), stachyose (Sigma), and verbascose (Megazyme).

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**FIGURE 1** Experimental setup for the plate and pilot-scale germination trials of faba beans of cv. Kontu and cv. Hangdown
Starch contents of the samples were analyzed using Total Starch assay kit (Megazyme) according to AACC method 76-13.01.

Protein contents were analyzed based on total nitrogen content (N × 6.25) by Kjeldahl method according to the AOAC method 2001.11 using an autoanalyzer (Foss Tecator Ab, Höganäs, Sweden).

For analyzing the content of free amino nitrogen (FAN), 700 mg of flours from native and germinated faba bean samples were extracted with 6 ml of water for 30 min at +4°C with constant shaking. Extracts were centrifuged (3000 × g, 10 min), and supernatant was filtered with a syringe filter with 0.45-μm cut-off. FAN content of the extract was assayed according to Analytica-EBC (European Brewing Convention, 2007; method 4.10).

2.5 | Dehulling, milling, and air classification of faba bean material

Native and germinated faba beans of cv. Kontu and Hangdown were dehulled by crushing with a Retsch SM300 cutting mill (Retsch, Germany; speed: 700 rpm, 4 × 4 mm sieve) and removing hulls by air classification with Minisplit classifier (British Rema Manufacturing Company Ltd, UK; classifier speed: 1000 rpm, air flow: 220 m³/h). Dehulled faba beans were milled twice by pin disc milling (17,800 rpm) with Hosokawa Alpine 100UPZ-Ib fine impact mill (Hosokawa Alpine AG, Germany).

Air classification of flours from native and germinated faba beans of cv. Kontu was carried out with Minisplit classifier (classifier speed: 10,000 rpm, air flow: 220 m³/h). Mass yield of the air classification and protein separation efficiency (PSE) were calculated as presented in Equations 1 and 2 (Tyler et al., 1981).

\[
\text{Mass yield} \left(\%\right) = \frac{\text{dry weight}_{\text{fraction}}}{\text{dry weight}_{\text{raw material}}} \times 100 \quad (1)
\]

\[
\text{Protein separation efficiency (PSE)} \left(\% \text{dm}\right) = \frac{\text{dry weight}_{\text{fraction}} \times \text{protein}_{\text{fraction}} \left(\% \text{dm}\right)}{\text{dry weight}_{\text{raw material}} \times \text{protein}_{\text{raw material}} \left(\% \text{dm}\right)} \times 100 \quad (2)
\]

All air classification procedures were carried out in duplicate.

2.6 | Fermentation with dextran producing Weissella confusa

Flours of native and germinated dehulled faba beans of cv. Kontu were fermented with Weissella confusa VTT E-143403 (E3403) originally isolated from faba bean and known to produce dextran from sucrose. Prior to fermentation, W. confusa E3403 was refreshed from frozen stock culture in MRS broth (Oxoid, UK) and passaged twice in general edible medium containing sucrose (Saarela et al., 2004). The fermentation inoculum was prepared by collecting cells from an overnight culture by centrifugation (10,000 × g, 10 min) and resuspending in autoclaved tap water. Mixtures of native or germinated faba flour (450 g) and sterile tap water (900 g) were inoculated at an initial viable cell count of 10⁶ colony forming units (cfu)/g and fermented statically at 25°C for 20 h in triplicate. The fermentations were sampled for lactic acid bacteria cell counts and pH at 0 and 20 h and for organic acid determination at 20 h from the beginning of fermentation. The fermented samples were dried with Christ Epsilon 2-25 freeze drier (Martin Christ Gefriertrocknungsanlagen GmbH, Germany) and ground as described in Chapter 2.5 for analyzing contents of dextran, ROFs, and total starch content. Dextran was analyzed from the fermented samples with an enzyme-assisted method according to Katina et al. (2009) with a slight modification of quantifying the glucose formed in enzymatic hydrolysis of dextran with HPAEC-PAD. Methods utilized for analyzing ROFs and total starch are described in the chapter 2.4.

2.7 | Microbiological analyses

Microbial quality of faba bean samples taken before and after both germination and fermentation was analyzed in triplicate. In addition, microbial analyses were carried out from dehulled samples in duplicate in order to study how much microbes were removed by peeling. An aliquot of 1 g of milled bean sample was mixed with 9 ml of Ringer solution (Merck) and spread on the selective agar plates. Coliforms were enumerated on Chromocult agar plates (Merck) in aerobic conditions at 37°C for 24 h, aerobic spore-forming bacteria on Trypticase Soy Agar plates (Beckton Dickinson GmbH) in aerobic conditions at 30°C for 3 days including preheating of samples at 80°C for 10 min in order to inactivate the vegetative cells, lactic acid bacteria on MRS-agar (Oxoid Ltd.) in anaerobic conditions at 30°C for 3–5 days, and yeast and molds on YM agar plates (Difco Laboratories) supplemented with chlortetracycline and chloramphenicol (both at 0.01%) to prevent the bacterial growth in aerobic conditions at 25°C for 3–5 days. The bacteria and fungi results are expressed as cfu/gram of sample.

2.8 | Protein properties and foamyability

Protein solubility was determined at pH range of 3–9, after pH adjustment samples were centrifuged (3000 × g, 10 min) and the amount of soluble protein was determined from the supernatant by DC protein assay kit (Bio-Rad Laboratories Inc, Hercules, USA). Protein solubility was calculated as percentage of protein in supernatant in relation to protein in the suspension (Kjeldahl value).

Changes in protein patterns were studied by SDS-PAGE. Samples were extracted in 0.5-M NaCl, 6-M urea, and 2% SDS, and insoluble material was removed by centrifugation (3000 × g, 5 min). Supernatants were diluted with sample buffer (Bio-Rad), denatured at 100°C for 5 min, and centrifuged (10,000 × g, 1 min) before loading them in Criterion TGX stain-free precast gel (4%-20%, Bio-Rad, USA). Gels were run at 250 V and visualized with Criterion stain-free imaging system (Bio-Rad) including Bio-Rad Precision plus 10- to 250-kDa standard.
Foaming properties were assessed by whipping 100-ml aqueous 5% solution for 1 min at speed 5 and 1 min at speed 6, using a Kenwood KM300 mixer (Kenwood Ltd., UK) equipped with a whisk. The resulting foams were poured into 250-ml graduated cylinders. Initial foam volume and volume of drained liquid were determined visually using scales on the cylinders. Foam capacity and drainage were calculated as presented in Equations 3 and 4.

$$\text{Foam capacity} \% = \frac{\text{volume of the foam immediately after whipping}}{\text{volume of the solution before whipping}} \times 100 \quad (3)$$

$$\text{Drainage} \ [\text{ml}] = \frac{\text{volume of drained liquid (measured after 0.5, 10, 20 and 30 min)}}{\text{volume of the solution before whipping}} \times 100 \quad (4)$$

### 2.9 Baking of GF breads

A GF flour mix was made combining faba flours of cv. Kontu and maize starch (Maizena, Unilever; Table 1). Germinated (3 h + 21 h) faba flour and germinated-fermented faba flour were added in addition levels of 15% and 30% of the flour mix. Control sample contained only native faba flour and maize starch mixture as flour.

The proportion of the rest of the ingredients was fixed for each bread recipe and was as follows: sugar 2.54%, baking powder 1.07%, shortening 2.18%, baker’s yeast 2.18%, salt 0.75%, xanthan gum 0.87%, emulsifier 0.27%, and water 45.68% (based on % total weight). The recipe, mixing, proofing time, and baking conditions were optimized based on Sozer et al. (2019). The dry ingredients were first mixed together and then shortening, yeast and water were added. The temperature of the water was adjusted so that the final dough temperature after mixing was 26 ± 1°C. Kneading was done for 4 min at high speed (level 2) with B30CT planetary mixer (Ningbo Sybo Machinery Co., China). Small tins (9 × 5 × 6 cm) were filled with 160 g of batter and then proofed for 45 min at 28°C and 85% RH. The breads were baked in duplicate in a rack oven (Sveba Dahlen, Sweden) at 180°C for 21 min with 30 s of steaming in the beginning.

### 2.10 Bread characteristics

#### 2.10.1 Bread volume, bake loss, water activity, and moisture content

The specific volume of the bread loaves was measured from six loaves of each bread type by infrared light scanning (Bread Vol Scan, Germany). Bake loss was calculated as presented in Equation 5.

$$\text{Bake loss} \ [%] = \frac{\text{weight of the loaf before baking} - \text{weight of the loaf after baking and cooling}}{\text{weight of the loaf before baking}} \times 100 \quad (5)$$

### TABLE 1 Gluten-free flour mix proportion based on % total weight of the recipe

| Share of flours of total weight (%) | Control | Germ15 | Germ30 | Ferm15 | Ferm30 |
|------------------------------------|---------|--------|--------|--------|--------|
| Maize starch                        | 22.23   | 22.23  | 22.23  | 22.23  | 22.23  |
| **Flours of cv. Kontu**            |         |        |        |        |        |
| Native                             | 22.23   | 15.56  | 8.89   | 15.56  | 8.89   |
| Germinated (3 h + 21 h)            | 0       | 6.67   | 13.34  | 0      | 0      |
| Germinated (3 h + 21 h), fermented | 0       | 0      | 0      | 6.67   | 13.34  |

Note: Addition levels of germinated or germinated-fermented faba flour were 15% and 30% of the flour mix. Steeping procedure used for the germinated samples is shown in parentheses.
Moisture content was determined by drying bread crumb samples in an oven at 105°C for 3 h, and water activity was measured by using Aqua Lab Series 3 water activity meter (Decagon devices, Pullman, USA).

2.10.2 | Texture analysis

Uniaxial compression test was performed for two slices (2.5-cm thickness) of bread from the center of each loaf with a TA-XT Plus texture analyzer (Stable micro systems, UK) equipped with a 25-kg load cell and a 30-mm diameter aluminum cylindrical probe. The pretest, test, and posttest speeds were 2, 0.83, and 10 mm/s, respectively. A strain value of 67% strain with 120-s hold time and 10-g trigger force was applied. Resulting force-deformation curves were used to extract the hardness (N) value reported as average of eight replicate measurements.

2.11 | Statistical analysis

The results were calculated as averages of the replicates. For the air classification results, pooled standard deviations were calculated based on two replicate experiments. Where applicable, the results were examined statistically using independent t-test with confidence interval of 95% or analysis of variance (ANOVA) with Tukey’s honestly

FIGURE 2 Moisture distribution of faba beans of cv. Kontu (a–d) and cv. Hangdown (e–h) after 3 h of soaking in H₂O₂ measured by hyperspectral imaging. Images a, c, e, and g are pseudo RGB images and images b, d, f, and h are parula colormaps of the same images showing spatial moisture distribution with global scaling (blue: lower moisture content; yellow: higher moisture content). (a, b) cv. Kontu, longitudinal cut plane; (c, d) cv. Kontu, cut plane through embryo; (e, f) cv. Hangdown, longitudinal cut plane; (g, h) cv. Hangdown, cut plane through embryo. The width of each subimage is 9 mm (320 pixels). The height of the images varies
significant difference post hoc test with significance level of $p > 0.05$
(IBM SPSS Statistics software for Windows v26.0; IBM Corporation,
New York, USA).

3 | RESULTS

3.1 | Early-stage water uptake of faba beans measured by hyperspectral imaging

The moisture contents of beans of cv. Kontu and Hangdown beans
after 1 h of soaking in H$_2$O$_2$ were 21% and 17% and after 3 h of
soaking in H$_2$O$_2$ were 27% and 28%, respectively. The longitudinal
cross-cut surfaces of cv. Kontu and Hangdown faba beans showed a
high variation in shape and size (Figure 2a,e). The thick hull surrounding
loosely the cotyledons and the embryo was clearly visible in the images
of both varieties. After 1 h of soaking, very few moistened areas were
detected in cv. Kontu beans and in cv. Hangdown hardly any water
was observed in cotyledons (data not shown). In both cultivars, beans
without any moistened area were detected irrespective of the cutting
angle. After 3 h of soaking, all beans of cv. Kontu cut longitudinally had
clear moisture gradients from the peripheral parts towards the center
of the bean (Figure 2b). Water absorption into cv. Kontu beans seemed
to be rather even from different sides of the bean. In the cv. Kontu
beans cut via embryo, moisture gradients did not show as even pattern
as in the longitudinal cross-cut surfaces (Figure 2d). No clear patterns
of water uptake were observed in Hangdown beans (Figures 1f,h). The
results showed that the embryo side of the cotyledons was not more
readily moistened than other parts of the bean.

3.2 | Germination characteristics of faba beans

Germination behavior of faba beans representing cv. Kontu and
Hangdown was first studied in the experiments carried out on petri
dishes. In the preliminary trials, various soaking times were applied for
Kontu beans. It was found that soaking only in water resulted in faster
water uptake compared with a soaking procedure starting with a step
with H₂O₂ (data not shown). Step with H₂O₂ was applied in order to clean the bean outer layers as moist germination conditions typically enhance the microbial growth.

Faster water uptake of smaller faba beans of cv. Kontu was observed already after 3 h of soaking in H₂O₂ (Figure 3a) compared with cv. Hangdown. In that time, moisture content of faba beans of cv. Kontu had increased up to 35% from the initial 12.6%, while the beans of cv. Hangdown showed significantly (p < 0.05) slower increase from 9.2% to 22%. During germination phase starting after soaking, faba beans of both cultivars showed gradual increase in the moisture content. Irrespective of the soaking treatment, the moisture content of faba beans of cv. Kontu was significantly (p < 0.05) higher than that of beans of cv. Hangdown. The length of soaking period did not affect the water uptake of smaller beans of cv. Kontu, while in larger beans of cv. Hangdown the shorter soaking treatment of 3 h + 9 h led to lower moisture content than the longer soaking of 3 h + 21 h. The numbers of germinated beans revealed that smaller cv. Kontu beans germinated significantly (p < 0.05) faster in comparison with cv. Hangdown. After 24 h from the beginning of soaking, approximately 55%–60% of cv. Kontu beans had germinated, while the same figure for Hangdown was only approximately 10% (Figure 3b). After 48 h from the beginning of soaking, beans of cv. Kontu had reached already germination rate above 96%. Germination rates of cv. Hangdown beans were significantly (p < 0.05) lower. In addition, larger cv. Hangdown beans soaked with 3 h + 21 h scheme showed significantly (p < 0.05) higher germination rates than those soaked for a shorter time after 48 and 72 h. Seven days was needed to achieve the number of germinated cv. Hangdown beans >90% with a soaking procedure of 3 h + 9 h (data not shown). On the basis of plate germination assays, bean moisture content of approximately 60% was needed for successful germination indicated by germination rate >90%.

Based on the plate germination trials, a soaking procedure of 3 h + 21 h followed by a 2-day germination step was applied for faba beans of cv. Kontu and Hangdown in the trials carried out in an automated germination equipment. Germination behavior of the faba beans in the automated germination equipment was very similar to that of observed in plate germination assays (Table 2). The moisture contents of 58%–61% achieved during soaking were maintained during 3 days from the beginning of soaking. Similarly, to the plate germination assays, faba beans of cv. Kontu had significantly (p < 0.05) higher germination rate in comparison with faba beans of cv. Hangdown, which did not exceed germination rate of 90% within 3 days from the beginning of soaking.

### 3.3 The effect of germination on faba bean composition

Regarding the amount of ROFs, germination of 3 days including soaking of 3 h in H₂O₂ followed by 21 h in H₂O had a statistically significant, cultivar-dependent effect. In native beans, the levels of ROFs were significantly (p < 0.05) higher in beans of cv. Hangdown than in beans of cv. Kontu. During germination, the contents of stachyose, verbascose, and total ROFs decreased with 99% in Kontu beans, while decrease of total ROFs was 86% in Hangdown (Table 3). In particular, raffinose was degraded to a lower extent in beans of cv. Hangdown when comparing with corresponding changes in those of cv. Kontu. The effect of soaking time in degradation of ROFs was detected significantly (p < 0.05) in the samples of cv. Kontu soaked for 3 h in H₂O₂ only or followed by 9 h in H₂O. The former resulted in decrease of 39% of total ROFs, while the latter reached decrease of 66%. In overall, the hydrolysis of ROFs in beans of cv. Kontu proceed fastest with verbascose followed by stachyose and raffinose being the least hydrolyzed in all the samples.

The impact of germination on proteolysis was clearly indicated by the amount of FAN present in the native and germinated beans of cv. Kontu and Hangdown (Table 4A). Both cultivars showed a significant (p < 0.05) increase of 70% in FAN due to germination. In starch content, the influence of germination was much lower with the decrease of 8%–13% depending on the cultivar (Table 4A).

Microbiological quality of faba bean was not significantly changed during germination (Table 4B). Microbial counts were in general low in native faba beans, and after germination, counts were unchanged regarding aerobic spore formers, lactic acid bacteria and fungi (yeasts and molds). Only the number of coliforms were increased significantly (p < 0.05) during germination. In addition, dehulling was noticed to significantly (p < 0.05) decrease microbial counts of germinated faba beans.

### TABLE 2 Moisture contents and the proportions of germinated faba beans of cv. Hangdown and cv. Kontu in germination trials carried out in pilot-scale

| Sample               | Moisture content(%) | Proportion of germinated faba beans(%) |
|----------------------|---------------------|----------------------------------------|
|                      | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h |
| cv. Hangdown         |      |      |      |      |      |      |
| Germinated (3 h + 21 h) | 61 ± 1.0 b a | 60 ± 1.1 b | 60 ± 2.9 a | 12 ± 5 a | 68 ± 14 a | 84 ± 11 a |
| cv. Kontu            |      |      |      |      |      |      |
| Germinated (3 h + 21 h) | 58 ± 0.2 a | 58 ± 0.5 a | 58 ± 0.9 a | 75 ± 4 b | 93 ± 4 b | 94 ± 1 a |

Note: Time points 24, 48, and 72 h are calculated from the beginning of soaking including 3 h in 1% H₂O₂ and 21 h in H₂O (3 h + 21 h). Values are averages of triplicate germination batches ± standard deviation.

*Different letters within each column indicate statistically significant difference (p < 0.05) between the samples based on independent t-test.
TABLE 3 Contents of individual raffinose family oligosaccharides (ROFs) and their sum in dehulled native, germinated, air-classified, and fermented faba bean samples of cv. Hangdown and cv. Kontu

| Sample                        | cv. Hangdown | cv. Kontu | cv. Hangdown | cv. Kontu |
|-------------------------------|--------------|-----------|--------------|-----------|
|                               | Raffinose    | Stachyose | Verbasco     | Total α-galactosides |
|                               | mg/g dm      | mg/g dm   | mg/g dm      | mg/g dm   |
| Native (3 h + 21 h)           | 2.4 e^b      | 14.6 f    | 27.4 e       | 44.3 g    |
| Germinated (3 h + 21 h)       | 1.0 c        | 1.0 a     | 4.3 b        | 6.3 b     |

Air-classified fine fractions (ff)

| Sample                        | Native, ff    | Germinated (3 h + 0 h), ff | Germinated (3 h + 9 h), ff |
|-------------------------------|--------------|---------------------------|---------------------------|
|                               | 1.7 d        | 1.6 d                     | 1.0 c                     |
|                               | –2           | –8                        | –42                       |
|                               | 9.4 e        | 6.0 d                     | 3.9 bc                    |
|                               | 16           | –26                       | –52                       |
|                               | 21.8 d       | –37 (20)                  | –74                       |
|                               | 9            | 22.4 e                    | 11.2 c                    |
|                               | 32.9 f       | 25 (23)                   | 62 (11)                   |

Samples after fermentation

| Sample                        | Native, fermented                       | Germinated (3 h + 21 h), fermented |
|-------------------------------|----------------------------------------|-----------------------------------|
|                               | 0.7 b                                   | –100                              |
|                               | –58                                     | 0.20 a                            |
|                               |                                         | –98                               |
|                               |                                         | 0.09 a                            |
|                               |                                         | –100                              |
|                               |                                         | 0.29 a                            |
|                               |                                         | –99                               |

Note: Values represent averages (n = 3). Steeping procedure used for the germinated samples is shown in parentheses.

| Sample                        | Raffinose | Stachyose | Verbasco | Total α-galactosides |
|-------------------------------|-----------|-----------|----------|----------------------|
|                               | mg/g dm   | mg/g dm   | mg/g dm  | mg/g dm              |
| Native (3 h + 21 h)           | 2.4 e^b   | 14.6 f    | 27.4 e   | 44.3 g               |
| Germinated (3 h + 21 h)       | 1.0 c     | 1.0 a     | 4.3 b    | 6.3 b                |

3.4 Air classification of nongerminated and germinated faba bean material

Protein enrichment by air classification was carried out with flours of dehulled faba beans of cv. Kontu as native or germinated with two soaking procedures (3 h + 0 h and 3 h + 9 h). Protein contents of these flours were similar being 34%–35% dry matter (dm) (Table 5). In air classification with standardized parameters, the mass yields of the fine fractions from the germinated samples were higher in comparison with flour of native faba beans indicating higher share of smaller particles in the germinated samples. Respectively, the protein contents in the fine fractions of the germinated samples were lower than in the fine fraction of the native sample (Table 5). However, PSE calculated for the fine fractions was notably improved by the germination. Differences were not observed between the germinated samples in air classification due to the different soaking procedures.

ROFs stachyose and verbascose present in the flours of both nongerminated and germinated samples were enriched into the fine fractions in air classification with increase of 9%–34%, while the content of raffinose was not clearly changed (Table 3). The increase in the total content of ROFs was 10%–23%.

3.5 Dextran production in nongerminated and germinated faba bean material

Flours of native and germinated (3 h + 21 h) faba beans representing cv. Kontu were utilized in fermentation trials with W. confusa E-3403. The number of W. confusa increased 2 log units during 20-h fermentation (10^5 viable cells/g flour in final freeze-dried samples) indicating a successful proliferation of this lactic acid bacteria (Table 4B). Neither the counts of lactic acid bacteria nor the decrease in the pH during fermentation were dependent on the faba bean germination process. The pH was 6.3–6.4 before the fermentation and 5.5 after the fermentation for both the native and germinated material. Microbial quality of the fermented flours was good; fermentation reduced significantly (p < 0.05) the amount of coliforms, and no or only small numbers (<200 cfu/g) of aerobic sporeformers, yeasts, and molds were detected in the final samples (Table 4B).

Fermentation increased concentrations of organic acids as the contents of acetic and lactic acid analyzed from the samples after fermentation were higher than in the respective nonfermented samples. The increase of organic acids did not differ between native and germinated samples. The contents of acetic acid were in both samples approximately 13–15 µg/mg dm before fermentation and approximately 35 µg/mg dm after fermentation. For lactic acid the respective contents were approximately 1 µg/mg dm and approximately 5 µg/mg dm.

Fermentation of flour from nongerminated, dehulled beans of cv. Kontu with W. confusa E-3403 increased the amount of FAN significantly (p < 0.05) by 52% up to 2.6 mg/g (Table 4A). In flour of germinated faba beans, the fermentation increased FAN content significantly (p < 0.05) from 3.0 to 4.0 mg/g (1.3-fold change). In addition, fermentation decreased significantly (p < 0.05) the amount of ROFs in native beans of cv. Kontu with reductions of 58%, 67%, and 39% in raffinose, stachyose, and verbascose, respectively (Table 3). As
the germination had already diminished the ROFs to a very low level, no further reduction in ROFs was detected in the fermented samples (Table 3). After fermentation, 1.3% dm dextran was measured from fermented native flour (Table 4A). Germinated flour enhanced dextran production to 2.2% dm, having significantly ($p < 0.05$) higher content by 72% than in native flour after fermentation.

### TABLE 4

| A) Sample                  | FAN (mg/g dm) | Change (%) | Starch (% dm) | Change (%) | Dextran (%) | Change (%) |
|----------------------------|---------------|------------|---------------|------------|-------------|------------|
| **cv. Hangdown**           |               |            |               |            |             |            |
| Native                     | 1.2 $^{a,b}$  | -          | 40.3 ab       | -          | -           | -          |
| Germinated (3 h + 21 h)    | 2.1 bc        | +73        | 36.9 $^a$     | -8         | -           | -          |
| **cv. Kontu**              |               |            |               |            |             |            |
| Native                     | 1.7 $^{ab}$   |            | 43.2 $^b$     |            | -           | -          |
| Native, fermented           | 2.6 $^{cd}$   | +52        | -             | 1.3 $^a$   | -           | -          |
| Germinated (3 h + 21 h)    | 3.0 $^d$      | +74        | 37.6 $^{ab}$  | -13        | -           | -          |
| Germinated (3 h + 21 h), fermented | 4.0 $^{e}$ | +132      | -             | 2.2 $^b$    | +72         |           |

Note: Values are averages ($n = 3$).

*Proportional change in the content compared with the native sample.

*Different letters within each column indicate statistically significant difference ($p < 0.05$) between the samples based on Tukey's HSD post hoc test or independent t-test.

*After inoculation and before fermentation.

### TABLE 5

| Raw material       | Loss in dehulling (%) | Protein content (% dm) | Mass yield (% dm) | Protein content (% dm) | Protein separation efficiency (% dm) |
|--------------------|-----------------------|------------------------|-------------------|------------------------|-------------------------------------|
|                    | Fine                  | Coarse                 | Fine              | Coarse                 | Fine                                | Coarse                             |
| **cv. Kontu**      |                       |                        |                   |                        |                                     |                                    |
| Native             | 24.4                  | 35.3                   | 27.5              | 68.8                   | 63.5                                | 22.7                               | 54.2                               | 48.4                               |
| Germinated (3 h + 0 h) | 25.6                 | 34.2                   | 33.4              | 57.4                   | 56.9                                | 19.7                               | 60.0                               | 35.7                               |
| Germinated (3 h + 9 h) | 28.7                 | 34.4                   | 33.0              | 56.9                   | 57.9                                | 19.5                               | 59.4                               | 34.5                               |

Note: Values represent averages ($n = 2$).

$^a$Pooled standard deviation.
3.6 | Technological functionality of flours obtained from bioprocessed faba beans and their applicability in GF bread baking

Influence of germination on molecular weight of faba proteins could not be seen in SDS-PAGE (supporting information Figure S1). Fermentation led to weakening of the main band (approximately 50 kDa) and the appearance of a strong band just below 50 kDa. This band likely represents monomers of the major storage protein, legumin, and can also contain monomers of vicilin. Fermentation also led to the disappearance of some high molecular weight proteins (MW between 75 and 100 kDa) and low molecular weight proteins.

FIGURE 4  Protein solubility (a) of faba bean samples (cv. Kontu) as function of pH as well as foaming capacity (b) and drainage (c) of 5% faba bean solutions. Samples: native (▲), germinated (3 h + 21 h) (●), fermented (○) and germinated (3 h + 21 h), fermented (x). Different letters within indicate statistically significant difference (p < 0.05) between the samples based on Tukey’s HSD post hoc test.
(MW between 20 and 35 kDa) that were present at lower quantities. It appears that germination-induced protein hydrolysis is very moderate after 2 days. Fermentation clearly caused some hydrolysis of legumin, but it appeared to decrease slightly in molecular weight rather than being completely cleaved to smaller peptides.

Protein solubility of native and bioprocessed faba flour as a function of pH followed an S-shaped curve (Figure 4a). The minimum solubility was between pH 4 and 4.5 in all samples. At its lowest, the protein solubility of native faba flour was <10%. Solubilities of bioprocessed flours were significantly ($p < 0.05$) higher at their lowest, around 15% to 20%. The solubilities started increasing over pH 6, reaching its maximum at pH 7. The maximum solubility of native faba bean protein was approximately 80% but bioprocessing increased solubility to 96%–100%. The effect on solubility was the same with germinated, fermented, and both germinated and fermented samples.

Foam capacity indicates the volume increase obtained when a liquid is whipped into a foam. Drainage (volume of water drained out of the foam) indicates the stability of the foam. Native faba flour had significantly ($p < 0.05$) the lowest foaming capacity (23%), and all bioprocessing methods improved it (Figure 4b). Germination and fermentation increased it to 69% and 117%, respectively. Combination of germination and fermentation increased foaming capacity to 158%. High drainage of liquid from native faba flour means poor foam stability: almost 70% of the liquid had drained out and the foam had collapsed after 5 min (Figure 4c). Germination did not improve foam stability, and the foam had lost 61% of liquid after 5 min. Fermentation as well as combined germination and fermentation decreased and slowed down drainage significantly ($p < 0.05$).

Cross-section digital images of GF breads made with native, germinated, or fermented-germinated faba flour at different addition levels are shown in Figure 5. The volume of control bread made with native faba flour was the highest (1.38 ml/g), followed by 15% addition level of germinated (Germ15), fermented-germinated flours (Ferm15), and Germ30 bread (Table 6). However, a significant ($p < 0.05$) reduction in bread volume was observed in Ferm30 bread in comparison with control bread. Increasing the faba flour content from 15% to 30% and further bioprocessing of germinated faba flour with fermentation interfered with the physical features of the bread, leading to less homogeneous crumb structure and curvy loaf edges. Germ30 and Ferm30 breads had also more compact, doughy crumb structure closer to crust edge (Figure 5). Bake loss, water activity, and moisture content of breads were similar. The hardness of crumb for Ferm30 bread was significantly ($p < 0.05$) the highest initially and after 1 day of storage in room temperature (Table 6). For all bread samples, crumb hardness increased by 20%–33% after 1 day of storage, the highest increase in crumb hardness being observed for Ferm30 bread (Table 6).

**DISCUSSION**

In this study, faba beans were successfully germinated both on plate germination assay and in automated germination device. However, the water uptake, especially regarding shorter soaking procedure (3 h + 12 h) with cv. Hangdown, was not probably efficient enough with respect to enzyme activity indicated by low germination rate. In overall, it was shown that large seeds of var. major type of cv. Hangdown clearly germinated more slowly in comparison with smaller seeds of cv. Kontu representing var. minor. Based on our results, large-seeded faba beans may require, for example, 1 day longer germination compared with small-seeded varieties.

Hyperspectral imaging showed that the moisture was not evenly distributed in the faba beans. Therefore, the total moisture content

![FIGURE 5](image-url) Digital cross-section images of gluten-free breads with addition levels of 15% or 30% of germinated or germinated and fermented faba flour of cv. Kontu

| Table 6 | Characteristics of gluten-free breads with addition levels of 15% or 30% of germinated or germinated and fermented faba flour of cv. Kontu |
|---|---|---|---|---|---|---|
| Bread | HardnessDay 0(N) | HardnessDay 1(N) | Volume(ml/g) | Bake loss(%) | Water activity | Moisture content(%) |
| n = 8 | n = 8 | n = 6 | n = 10 | n = 3 | n = 3 |
| Control | 10.3 a | 11.8 a | 1.37 c | 10.4 bc | 0.970 a | 52.1 ab |
| Germ15 | 9.8 a | 12.2 a | 1.31 bc | 10.5 c | 0.970 a | 52.5 b |
| Germ30 | 10.2 a | 11.8 a | 1.28 ab | 9.6 a | 0.968 a | 52.0 ab |
| Ferm15 | 10.7 a | 12.9 a | 1.28 ab | 10.0 ab | 0.968 a | 51.9 a |
| Ferm30 | 12.3 b | 15.5 b | 1.21 a | 9.9 ab | 0.967 a | 51.6 a |

Note: Values are averages of replicates.

*Different letters within each column indicate statistically significant difference ($p < 0.05$) between the samples based on Tukey’s HSD post hoc test.
did not indicate the moisture level in the inner bean structures. In faba beans, thick hull protects the cotyledons and embryo, which probably inhibited water uptake. When the moisture content of the bean was measured, some of the water may be situated as free in the space between the hull and the cotyledons but was interpreted as the moisture absorbed by the bean. Therefore, the total moisture content does not indicate whether the water is absorbed into bean structures or not. In addition, faba beans were observed to be individual by their shape, which may challenge the simultaneous water uptake. Homogeneity in water uptake between the beans would cause homogeneity in the onset of germination, and further simultaneous germination would ensure uniform quality which is important property in bioprocessing.

A minimum soaking time of 12 h was required to increase the beans’ moisture contents to around 50%, which resulted in sufficient share of germinated faba beans within 3 days. This is in accordance with the studies of Luo et al. (2012) and Luo and Xie (2014) in which soaking of 12 or 24 h followed by a germination step of 3 days was used for faba beans. These germination experiments were carried out at 20°C. Due to high risk of undesired microbial growth, the water uptake cannot be improved by raising the germination temperature. If temperature needs to be decreased, it is probable that germination time needs to be increased, respectively.

Regarding industrial germination of faba beans, small faba beans could be more favorable raw material as they are more homogenous in size and shape and represent faster germination than the faba bean varieties with large seeds. Varieties having medium seed size, for example, between the size of large- and small-seeded varieties would be interesting material for industrial scale germination.

Our findings on the effect of germination on ROFs, known to cause stomach discomfort, correspond with findings of the previous studies. Vidal-Valverde et al. (1998) reported that germination of faba beans for 6 days caused a sharp reduction in α-galactosidase: Only 12% of raffinose was left, while stachyose and verbascose were completely degraded. In addition, a clear reduction in the content of ROFs in faba bean cotyledons has been observed already after 2 days of germination by Goyoaga et al. (2011). A significant decrease in stachyose content of faba beans has also been reported by Khalil and Mansour (1995) after soaking for 12 h followed by germination for 72 h. In addition to ROFs, the results on changes of FAN and starch contents in faba beans due to germination were in line with the earlier reported results. The FAN content of faba bean has been observed to increase after 20 h soaking and germination for 72 h (Luo et al., 2014) and during the 7-day germination period (Kirmizi et al., 2006). The decrease in starch content is in good accordance with the findings of Vidal-Valverde et al. (1998) who reported that the starch content in faba beans decreased with 15% during germination for 6 days.

Air classification of legume flours including faba bean has been well studied earlier (Coda et al., 2015; Elkowicz & Sosulski, 1982; Saldanha do Carmo et al., 2020; Sosulski & McCurdy, 1987; Tyler et al., 1981; Tyler & Panchuk, 1982). However, processing of flour of germinated faba beans by air classification has not been reported earlier. In this study, flour of faba beans germinated in pilot-scale was shown to have higher PSE in comparison with flour of native faba beans. This is likely a consequence of general perishing of the bean structures and proteolysis indicated by increased FAN content occurred during germination.

In comparison with flours of native legume seeds, flours of germinated legume seeds have been reported to have higher oil holding, water holding, water absorption, and gelation capacities, whereas emulsifying and foaming capacities were lower (Benitez et al., 2013). However, germination has also been reported to improve emulsifying properties and foaming capacity probably due to mild proteolysis (Ghavidel & Prakash, 2006). Production of microbial exopolysaccharides in situ is a potential way to further modify the physicochemical properties of legume flours. Bacteria of the genus Weissella typically produce a high molecular weight dextran from sucrose, which can be utilized as a gelling, viscosifying, and emulsifying agent in food applications (Leemhuis et al., 2013). In situ dextran production has been studied in multiple cereal and pseudocereal flours but less in pure legume matrices. Here we observed production of technologically significant amounts of dextran during fermentation in both native and germinated faba flour without sucrose addition, which indicates presence of intrinsic sucrose in the flour. Furthermore, our results suggest that the ROF reduction during germination is due to α-galactosidase activity which hydrolyzes ROFs to galactose and sucrose, producing more substrate for dextran synthesis. The increase in dextran content between fermented and germinated faba flours is a little less than but well in line with the theoretical yield assuming full conversion of first ROFs to galactose and sucrose during germination and then complete conversion of sucrose into dextran and fructose. Hybrid bioprocessing of faba beans by germination and fermentation thus shows synergistic effects in eliminating antinutritional ROFs and turning them into hydrocolloids beneficial for downstream food applications.

The protein solubility of native faba bean was already fairly good above neutral pH (approximately 80%), but the bioprocessing methods applied in this study rendered the protein fully soluble in water probably due to limited hydrolysis. Solubility is an important factor that largely determines the applicability of a plant protein ingredient in food products, as the function of proteins on interfaces is often limited by low solubility and aggregation (Murray, 2020). Protein solubility of the native sample was higher than usually reported for faba bean protein isolates (36%–90% at pH 7.0), as isolates have undergone various processing steps that can alter solubility (Karaca et al., 2011; Nivala et al., 2017; Vogelsang-O’Dwyer et al., 2020). Storage proteins are eventually mobilized during germination, but it may only become visible on SDS-PAGE after a longer germination time (Savelkoul et al., 1992).

Native faba flour had a low foaming capacity, but all bioprocessing methods improved it significantly. Especially the combination of germination and fermentation was efficient—a nearly sevenfold increase in foaming capacity was achieved with this treatment. The resulting foams were not very stable, although fermentation and germination improved their stability to some extent. Bioprocessed faba bean could be used as a foaming agent but would require stabilization from other components. The effect on foaming is likely to be caused by improved solubility, hydrophobic groups revealed by hydrolysis, and the lower pH of the samples. Nisov
et al. (2020) reported that a degree of hydrolysis of 1.5%–1.9% of initially very insoluble rice protein isolate efficiently improved foaming properties, while further hydrolysis decreased them as proteins were cleaved into peptides too small to effectively stabilize the air-water interface. In addition to hydrolysis effect, lower pH of fermented faba flours could improve foaming properties. Chandra-Hioe et al. (2016) reported a decrease in foaming properties of faba bean after fermentation with Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus. In their study, the foaming test was carried out at pH 7, while in our study, foaming was performed at native pH of the samples, namely, 6.3–6.4 in native and germinated faba bean and 5.5 in both fermented samples. It appears that the notable improvement seen in foaming properties of fermented samples may be a pH effect or a combination of limited hydrolysis and lower pH.

GF breads are typically based on starch resulting in dense, crumbly, and hard texture with low loaf volume mainly associated with the lack of gluten. These breads are also inferior in terms of color, flavor, and overall nutritional profile (Melini et al., 2017). Faba flour due to its high protein content (35%) is a potential ingredient in improving structure, texture, and nutritional quality of GF breads. Consistency of GF bread dough is rather similar to a batter where the structure formation mechanism is based on emulsifying and foaming forming capacity of added proteins (Sozer et al., 2019). Therefore, in GF baking porous texture and high specific volume of the final product requires high foaming capacity. The foam must be stable enough to support the texture until a solid starch network has formed during baking and cooling. Liquid drainage in batter can lead to, for example, formation of a gummy layer (Chang et al., 2020). Although protein solubility and foaming capacity increased by germination and combined effect of fermentation with germination, this was not reflected to the GF bread loaf volume and crumb structure. Specific volume of GF bread made of germinated chickpea flour (Ouazib et al., 2016) and germinated rice flour (Cornejo & Rosell, 2015) was found to be lower than the control breads. Besides excessive protein hydrolysis, germination and fermentation can result in excessive amylase activity, starch liquefaction, and dextrinization which might lead to wet rubbery crumb (Cornejo & Rosell, 2015). In this study, although a rather short proofing time was used compared with conventional bread (45 min vs. 90 min), the initial fermentation time applied for the germination faba flour was 20 h. The presence of wet crumb towards the edges of breads made of germinated and fermented faba breads (Ferm15 and Ferm30) might have been overcame by reducing the initial fermentation time for the germinated faba flour.

Hardness of bread samples was in general lower when compared with previous literature, for example, germinated chickpea breads (289N) (Ouazib et al., 2016) or breads made of pseudo cereals (26–43N) (Wolter et al., 2014) which might be associated with the intrinsic differences between the various raw materials or the recipe and baking protocol. As expected, specific volume of breads was much lower than it is for wheat bread (4.2 ml/g) (Salmenkallio-Marttila et al., 2001) but also a bit lower than what was reported for GF chickpea (1.53 ml/g) (Ouazib et al., 2016), pea protein, or soy concentrate supplemented GF breads (2.7–2.2 ml/g) (Ziobro et al., 2016). The total protein content in those breads was higher than it is in this study. The chickpea flour had a protein content of 19% and was used as such without any starch addition, whereas in the latter case although the supplementation level of protein concentrates was around 10% (flour weight), the high level of protein (approximately 72%) in the concentrates increased the final protein content in the breads. Higher protein content in GF bread recipes is critical due to emulsification properties and providing structural support to starch based matrix (Ziobro et al., 2016).

5 CONCLUSIONS

In this study, we successfully established a pilot-scale germination process for faba beans. Based on the results, it seems that minor- and major-type or small- and large-seeded faba bean varieties set different requirements to the germination process, for example, regarding time needed for reaching suitable moisture content for germination process. Although no substantial improvement in terms of structure and texture of GF breads was achieved through germination or fermentation of faba flour, it is essential to note the outstanding performance of germination followed by fermentation on reducing the anti-nutritional factors, such as ROFs, which are a major factor giving stomach discomfort. Therefore, germination alone or as combined with fermentation improves the nutritional and technological quality (protein solubility and foaming) of faba bean material and thus enables its use in several food applications.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHOR CONTRIBUTIONS

Conceptualization: TS, AL, and NS; Methodology: URM, TS, OEM, AL, and NS; Validation: URM and TS; Investigation: URM, TS, and OEM; Writing-Original Draft: URM; Writing-Review & Editing: URM, TS, OEM, AL, and NS; Visualization: URM, OEM, and NS; Supervision: AL and NS; Project Administration: AL; Funding acquisition: AL and NS.

ETHICS STATEMENT

The manuscript does not contain such data that would have needed ethical approval.
DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID
Ulla Holopainen-Mantila https://orcid.org/0000-0003-1109-0569

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