Cereal Chemistry

RESEARCH ARTICLE OPEN ACCESS

Fluorescence Spectroscopy of Flour Fractions and Dough: Analysis of Spectral Differences and Potential to Improve Wheat Quality Prediction

Denise Ziegler[1] | Lukas Buck[2] | Katharina Anne Scherf[2,3,4] | Lutz Popper[5] | Bernd Hitzmann[1]

[1]Department of Process Analytics, Institute of Food Science and Biotechnology, University of Hohenheim, Stuttgart, Germany | [2]Department of Bioactive and Functional Food Chemistry, Institute of Applied Biosciences, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany | [3]Leibniz Institute for Food Systems Biology at the Technical University of Munich, Freising, Germany | [4]TUM School of Life Sciences, Professorship of Food Biopolymer Systems, Technical University of Munich, Freising, Germany | [5]Mühlenchemie GmbH & Co. KG, Ahrensburg, Germany

Correspondence: Denise Ziegler (denise.ziegler@uni-hohenheim.de)

Received: 10 May 2024 | **Revised:** 11 October 2024 | **Accepted:** 5 March 2025

Funding: This work was supported by the Forschungskreis der Ernährungsindustrie (AiF 21711N).

Keywords: chemometrics | dough | flour fractions | fluorescence spectroscopy | rheology | wheat quality

ABSTRACT

Background and Objectives: Spectroscopy of wheat kernels and flour has been used as a rapid tool to assess wheat quality, but predictions still lack in accuracy for most quality parameters except for protein content. To enable an improved prediction of further quality characteristics, new approaches are needed. This study investigates if the preprocessing of flour into flour fractions (by air classification, sieving) or dough and subsequent spectroscopic analysis of these types of samples could be a new way to improve wheat quality predictions. For this purpose, spectral differences are investigated and predictions of farinograph parameters are compared for fluorescence spectra of flour, flour fractions, and dough.

Findings: A wide variety of fluorophores present in cereal products was identified. Their peak intensities significantly differed for flour, flour fractions, and dough. Flour and sieve fractions were superior in predicting water absorption ($R^2_{CV\ flour} = 0.79$; $R^2_{CV\ 32–50\,\mu m} = 0.81$), while gluten and dough samples strongly improved predictions of rheological properties, especially dough development time ($R^2_{CV\ flour} = 0.64$; $R^2_{CV\ dough} = 0.90$; $R^2_{CV\ gluten} = 0.84$).

Conclusion: Preprocessing of flour samples greatly alters their composition (e.g., protein enrichment), which is also reflected by spectral differences. Spectra of different sample types therefore contain different information and have the potential to improve the prediction of wheat quality.

Significance and Novelty: This is the first study that investigates spectral differences of a large number of different flour fractions and dough using fluorescence spectroscopy and subsequently underlines the potential of this novel approach to improve wheat quality prediction in the future.

1 | Introduction

Wheat quality is commonly determined using various analytical and rheological measurements as well as baking tests (Edwards 2007; Klingler 2010). In practice, this is costly and time consuming and requires a large amount of flour, which is problematic for mills and breeders in particular (Pojić and Mastilović 2013). This is why a first estimation of wheat quality is often done using correlations of the quality characteristics of interest to the flour protein content. However, the predictive ability of this method has often proven to be poor (Gabriel et al. 2017; Nagel-Held et al. 2024). Because of this, new approaches involve

using spectroscopy of wheat kernels and flour in combination with chemometric modeling to improve the prediction of wheat quality characteristics (Nagel-Held et al. 2022; Pojić and Mastilović 2013). Spectroscopy is a rapid, non-destructive technique that has become increasingly important in science and industrial applications (Lakowicz 2006). For quality control in the cereal sector, near-infrared (NIR) spectroscopy is an already established method to predict flour protein content (Pojić and Mastilović 2013). Slowly, more research is being done in this area also involving fluorescence and Raman spectroscopy (Ahmad et al. 2016; Nagel-Held et al. 2022; Nawrocka et al. 2016).

However, until now, mostly the flour protein content can be predicted really well using spectroscopy. For the majority of other quality parameters, the predictive quality is not sufficiently high to replace reference measurements (Nagel-Held et al. 2022, 2024). This is why more research is being done to find new ways to improve the prediction of wheat quality characteristics. One novel approach includes the spectroscopic analysis of flour fractions that differ in composition compared to flour. Studies have shown that protein enrichment and the enrichment of polyphenolic compounds in various fractions is possible by separating flour particles according to particle size and/or density using air classification or sieving (S. A. Jensen et al. 1982; Jones et al. 1959; Youssef and Abo-Dief 2022). By enriching and depleting specific compounds in those fractions, the spectral signals of them could be changed and the strong superimposition of spectral signals may be reduced, which might in turn improve the predictions. Furthermore, the pre-processing of flour to dough could be another way to improve wheat quality prediction. During the formation of dough structure, various processes and reactions take place that can be detected using spectroscopy (Ahmad et al. 2016; Albanell et al. 2012). This could make it possible to obtain more information about the rheological behavior of the sample, which might also have a positive effect on the prediction quality. Fractionating the dough into gluten and starch may further increase the information content of the obtained spectra.

Farinograph parameters are one of the most common and important indicators of wheat quality determined in farinograph measurements (Cauvian and Young 2007; Edwards 2007). So far, however, only water absorption can be predicted with acceptable accuracy using NIR spectroscopy of flour (Dowell et al. 2006; Nagel-Held et al. 2024). Farinograph parameters are related to other rheological properties and baking quality (Dowell et al. 2008; Koksel et al. 2009; Van Bockstaele et al. 2008). Consequently, the predictive quality of farinograph parameters using spectra of flour fractions and dough can give an important indication for the potential improvement of the prediction of other wheat quality characteristics using this novel approach.

This study applies fluorescence spectroscopy and farinograph measurements to carry out preliminary investigations using air-classified fractions, sieve fractions as well as gluten, starch, and dough prepared from 50 commercially available wheat flour samples representing mixtures of many different wheat cultivars. The aim of this study is to confirm that fluorescence spectra of flour fractions and dough contain different information compared to flour spectra, that are based on compositional differences, and that this information can provide a valuable new approach to improve the prediction of wheat quality parameters such as baking volume in future research.

To facilitate this, possible fluorophores are identified and the following hypotheses are tested:

1. The modified composition of flour fractions and dough is reflected by spectral differences of fluorescence spectra.
2. The fluorescence spectra of flour fractions and dough contain complementary information about water absorption and rheological properties of samples, enabling an improved prediction of farinograph parameters.

2 | Materials and Methods

2.1 | Flour Samples and Reference Analyses

Fifty commercially available wheat flour samples were provided by Mühlenchemie GmbH & Co. KG (Ahrensburg, Germany). Therefore, no individual cultivars but commercially available mixtures of cultivars were used. Available information about harvest year, country of origin and wheat class are shown in Table 1. The ash content had been adjusted to approximately 0.60% after milling of samples. Protein contents ranged from 9.2% to 16.5% and were determined by near-infrared reflectance spectroscopy of flour according to ICC No. 159. Farinograph measurements of samples were performed according to ICC No. 115. All reference measurements were performed in duplicate. Statistics for farinograph parameters are shown in Table 2.

TABLE 1 | Information on flour samples regarding country of origin, harvest year, and wheat class.

Country of origin	Number of samples	Harvest year	Wheat class[a]
Germany	27	2019, 2020, 2021, 2022	E, A, B, A/B, B/C, unknown
Australia	8	2020, 2021	AH1, AH2, APH, APW, unknown
USA	5	2021	HRS, HRW
Latvia	2	2021	Unknown
Lithuania	2	2020, 2021	Unknown
Mexico	2	2021	Unknown
India	1	2021	Unknown
Poland	1	2021	Unknown
Romania	1	2021	Unknown
Ukraine	1	2021	Unknown

[a]German classification system (A = quality, B = bread making, C = lowest quality, E = elite).
Abbreviations: AH1 = Australian Hard 1, AH2 = Australian Hard 2, APH = Australian Prime Hard, APW = Australian Premium White, HRS = hard red spring, HRW = hard red winter.

TABLE 2 | Descriptive statistics of farinograph parameters ($n = 50$; for stability S $n = 49$).

Parameter	Mean	Standard deviation	Minimum	Maximum	Range
Water absorption WA (%)	58.2	2.7	54.3	65.3	11.0
Dough development time, DDT (min)	2.70	2.05	1.18	8.35	7.17
Stability S (min)	7.31	5.46	1.54	24.77	23.23
Dough softening, DS 10 min (BU)	46.8	27.9	2.5	111.5	109.0

2.2 | Flour Fractionation and Dough Preparation

Flours were air classified into a fine and coarse fraction. The Hosokawa Alpine AG (Augsburg, Germany) air classifier 1213/25 D was used to process 3.5 kg of flour using a classifier wheel speed of 15,000 rpm and a feed rate of 30 g flour/min. After the first run, the obtained coarse fraction was classified for a second time. In the end, the proportion of fine fraction was about 6%–8%. D [4,3] of particle size distributions of fine fractions were below 10 μm and Dv (90) below 15 μm. After air classification, fine and coarse fractions were stored in airtight containers. For three samples, no coarse fraction was available for analysis.

For the sieve fractions, 70 g of flour was air jet sieved using the 200LS-N Hosokawa Alpine AG (Augsburg, Germany) air jet sieve machine at 2500–2600 Pa. The following sieves from Haver&Boecker OHG (Oelde, Germany) were used: 32, 50, 75, and 100 μm. Each fraction was sieved for 10 min. Then, the material that had passed through the sieve was stored in an airtight container while the remaining material on the sieve was transferred to the next larger sieve for further sieving. In this way, the following five sieve fractions were obtained: < 32, 32–50, 50–75, 75–100, and > 100 μm.

Furthermore, dough was prepared from each flour in a 50 g farinograph using 30 g of flour (14% moisture) and 0.60 g of salt at 30°C and 63 rpm. The amount of water and the kneading time were adjusted for every sample to optimum water absorption and dough development time determined according to ICC No. 115. Subsequently, two times 15 g of the dough were washed out using a Glutomatic 2202 (Ing. Stefan Kastenmüller GmbH, Martinsried, Germany) according to ICC No. 155 using 80 μm metal sieves. The remaining dough as well as the glutens after the washing procedure were covered and rested for 10 min each in a temperature-controlled chamber at 25°C. Afterwards, they were frozen at −28°C. This process was repeated three times for each flour sample. Then, the obtained starch slurry was centrifuged at 3046 rcf for 1 min using a VWR Mega Star 600R centrifuge (VWR International GmbH, Darmstadt, Germany). The supernatant was discarded and the precipitate was also frozen at −28°C. The next day, frozen dough, gluten, and starch samples were freeze-dried for 24 h in a Christ Alpha 1-4 (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). Afterwards, they were milled to powders using the Ultra Centrifugal Mill ZM 200 (0.5 mm sieve, 12,000 rpm) (Retsch GmbH, Haan, Germany). After freeze-drying and milling, samples were stored in airtight aluminum containers to prevent permeation of water vapor.

Flours and fractions were all stored in the dark at around 15°C. Twenty-four hours before spectroscopic analysis, they were stored at room temperature (21 ± 1°C).

2.3 | Spectroscopic Analysis

Two-dimensional fluorescence spectra were recorded using the BioView Sensor (Delta Light & Optics, Hørsholm, Denmark). Excitation was scanned between 270 and 550 nm and emission between 310 and 590 nm using 20 nm increments each. For each measurement, the settings were: three repetitions per wavelength combination, high sensitivity for all wavelength combinations, 1350 gain for all wavelength combinations of the excitation wavelengths 270 and 290 nm and 1050 gain for the remaining wavelength combinations. Each sample was placed in a Petri dish (Ø 6 cm) and evenly compacted with a 600 g weight before fluorescence spectra were taken at six different positions.

2.4 | Chemometric Analyses

All analyses were performed using MATLAB (R2021b, The MathWorks Inc.).

For the analysis of spectral differences, no preprocessing was applied as there was little interference in fluorescence spectra and the original information about peak shape and height should be preserved. Mean spectra were plotted and analyzed with principal component analysis (PCA). Furthermore, boxplots of spectral intensities at specific wavelength combinations identified from spectra and PCA analysis were generated. Variability of sample medians was indicated by notches. Notches that do not overlap have significantly different medians at the 5% significance level.

For prediction of farinograph parameters, principal component regression (PCR) and partial least-squares regression (PLSR) were carried out. Different spectral preprocessing routines including detrending, standard normal variate transformation (SNV), multiplicative scatter correction (MSC), extended multiplicative scatter correction (EMSC), min–max normalization as well as 1. norm and 2. norm normalization were tested. After preprocessing, mean spectra were calculated and used for regression analyses. One to 10 components were tested for modeling. If model predictions were negative, values were set to zero, as farinograph parameters cannot become negative. The best models were determined by the minimum Root mean square error (RMSE) of leave-one-out cross-validation. Cross-validation metrics were calculated according to Equations (1–3):

$$R^2_{\mathrm{CV}} = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2}, \quad (1)$$

$$\mathrm{RMSECV} = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n}}, \quad (2)$$

$$\mathrm{NRMSECV}_{\mathrm{range}} = \frac{\mathrm{RMSECV}}{y_{\max} - y_{\min}} \quad (3)$$

with R^2_{CV} being the coefficient of determination of cross-validation; RMSECV the Root mean square error of cross-validation; $\mathrm{NRMSECV}_{\mathrm{range}}$ the range normalized RMSECV; n the total number of samples; y_i the measured value of ith sample; $\hat{y}_i$ the predicted value of ith sample; $\bar{y}$ the mean value of sample set; $y_{\max}$ the maximum value of sample set; and $y_{\min}$ the minimum value of sample set.

3 | Results and Discussion

3.1 | Identification of Fluorophores and Analysis of Spectral Differences

3.1.1 | Mean Spectra and PCA

Visual inspection of mean spectra in Figure 1 and PCA score plots in Figures 2 and 3 reveal differentiation of sample types based on their fluorescence signals related to their composition. While flour, gluten, starch, and dough samples can be differentiated well along PC1, 2, and 3, scores of sieve and air-classified fractions overlap to a certain extent. They show a curve-like trend with the 50–75 µm fraction at the maximum, indicating a possible enrichment and/or depletion of specific compounds according to this trend. This fraction is also most similar to flour, although they can be differentiated to some extent along PC3 as well. The fine fraction is located near the smaller sieve fractions, while the coarse fraction is positioned among the larger sieve fractions.

Based on the mean spectra in Figure 1 and PCA loadings in Figures 2 and 3, a number of different visible and hidden fluorescence peaks can be identified that contribute to this differentiation by PCA. The main peaks that are visible in loadings and spectra are located at EX290/EM350, EX330/EM430&450, EX370/EM450, and EX450/EM510. Furthermore, loading plots reveal important signals present at EX330/EM390 and EX390/EM450.

As wheat samples are a complex matrix, a large number of different fluorophores can be assigned to the observed peaks. Based on literature review, a list of possible fluorophores is compiled in Table 3. These are also visualized in Figure 4.

The peak at EX290/EM350 can be attributed to tryptophan fluorescence (Christensen et al. 2006; Lakowicz 2006), although it has previously been reported that different phenolic acids present in wheat could also contribute to this signal (Sergiel et al. 2014; Xue and Tan 2022). Phenolic acids were also attributed to the peaks at EX330/EM430&450 (S. A. Jensen et al. 1982; Sergiel et al. 2014), together with NAD(P)H fluorescence (Lakowicz 2006). Furthermore, phenolic acids (especially ferulic acid; Garcia et al. 2016; S. A. Jensen et al. 1982; Sergiel et al. 2014) and different oxidation products of lipids, vitamins, and phenolic acids (Garcia et al. 2016; P. Jensen et al. 2004) seem to be responsible for the peak visible at EX370/EM450. Some NAD(P)H may also contribute here, although the absorption

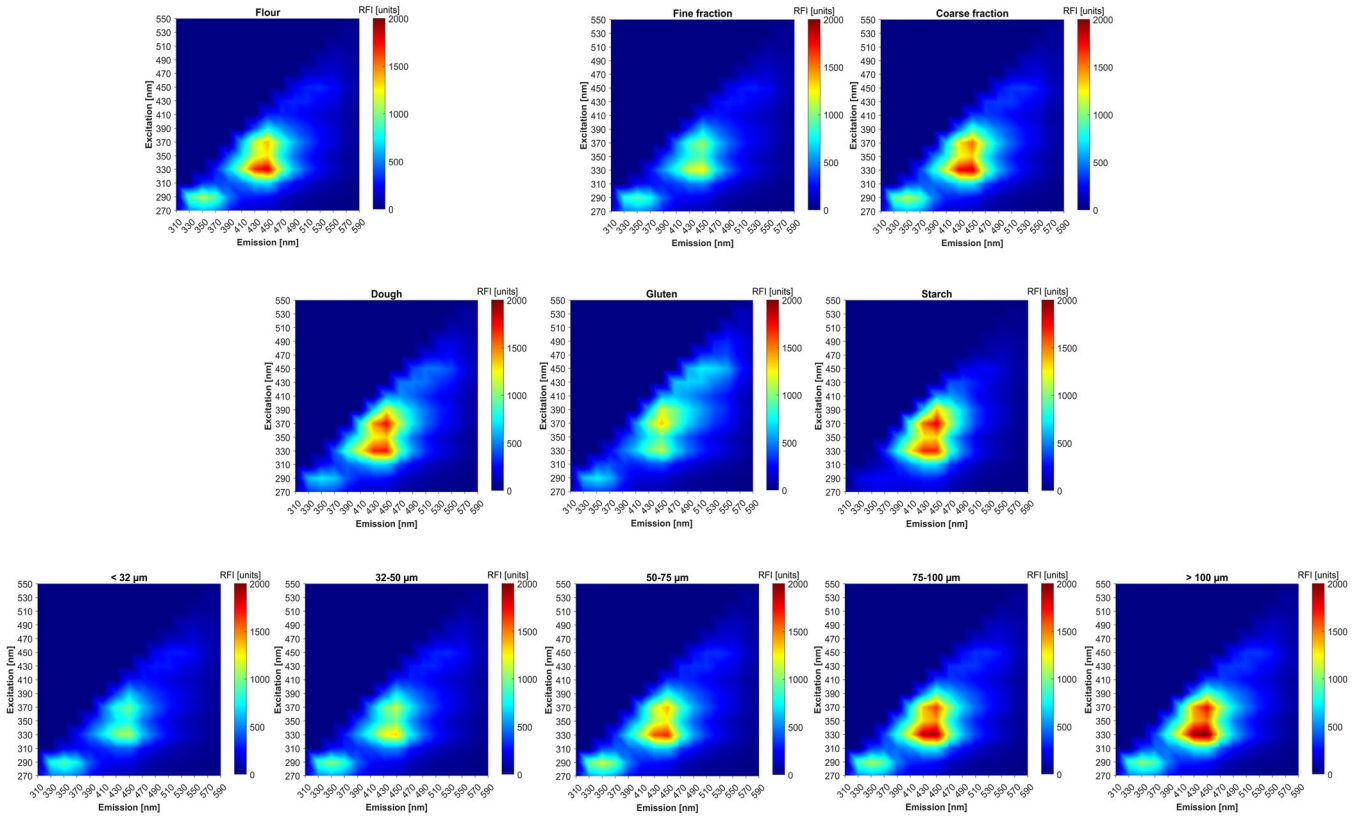

FIGURE 1 | Mean fluorescence spectra of flour, flour fractions, and dough ($n = 50$; for coarse fraction $n = 47$). [Color figure can be viewed at wileyonlinelibrary.com]

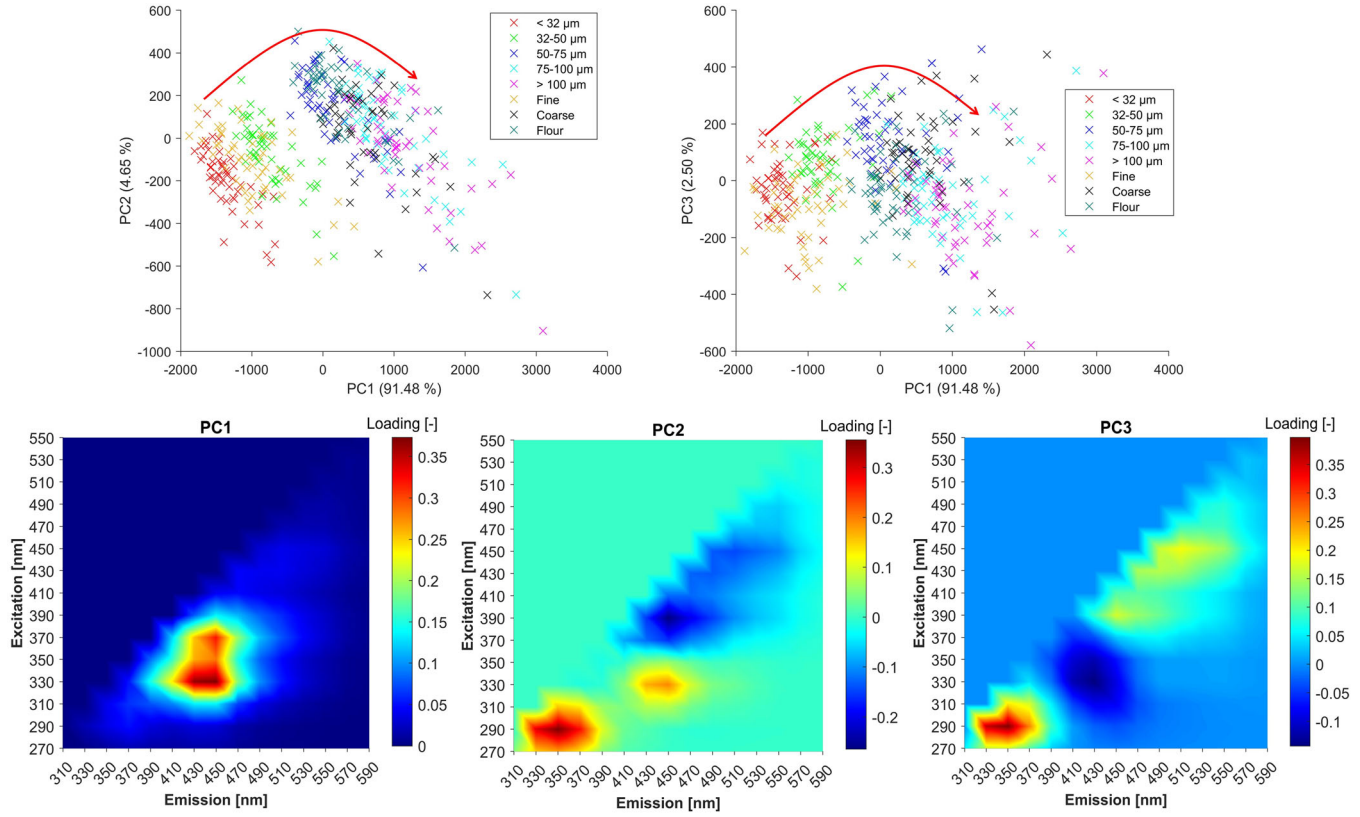

FIGURE 2 | Score plots (top) and loading plots (bottom) of PCA using fluorescence spectra of flour, sieve, and air-classified fractions. [Color figure can be viewed at wileyonlinelibrary.com]

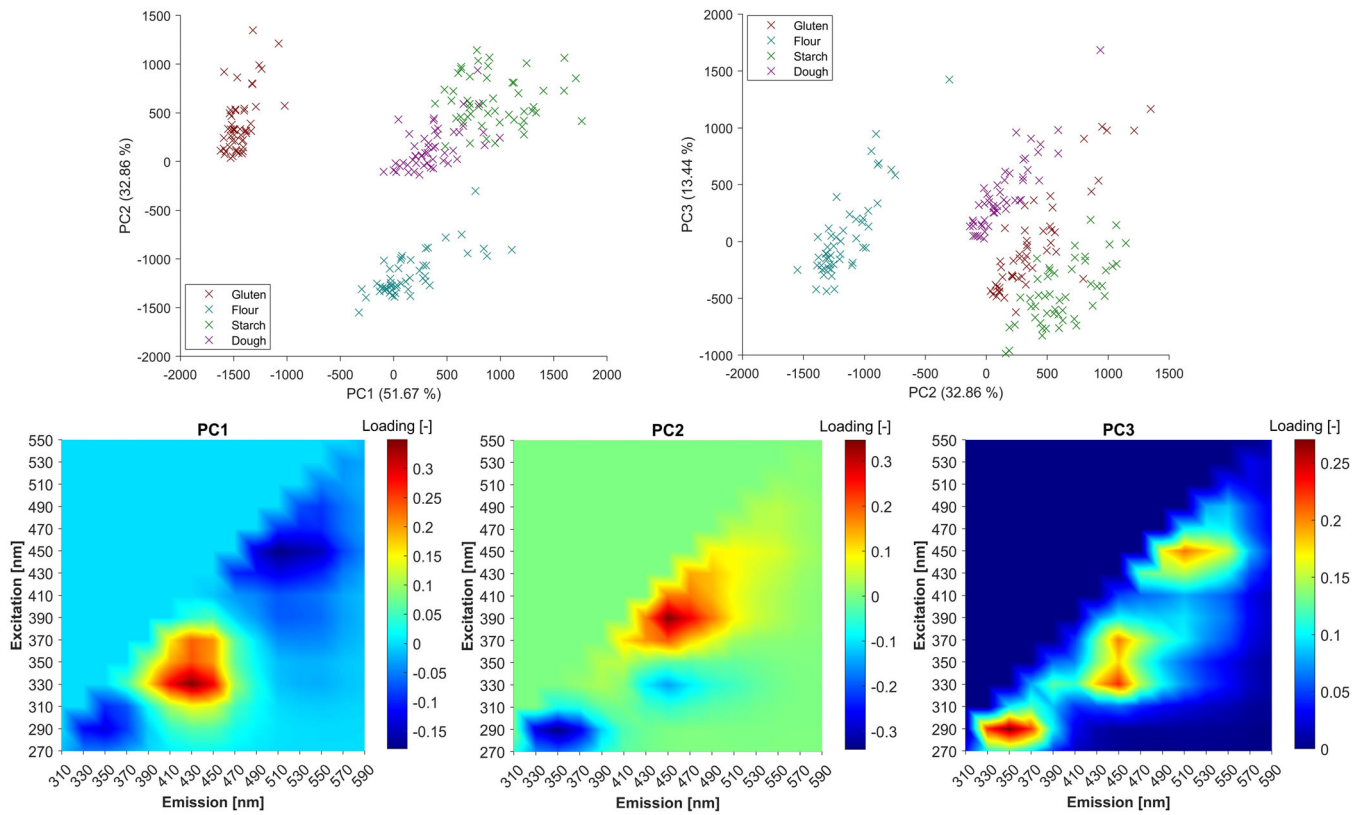

FIGURE 3 | Score plots (top) and loading plots (bottom) of PCA using fluorescence spectra of flour, gluten, starch, and dough. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 | Possible fluorophores identified in wheat and other food products.

Fluorophore	EX	EM	Reference
Protein			
Tryptophan	280	357	Lakowicz (2006), Christensen et al. (2006)
Cofactors/Coenzymes			
NAD(P)H	340	460	Lakowicz (2006)
FAD, FMN	450	525	Lakowicz (2006)
Pigments			
Carotenoids	450	520	S. A. Jensen et al. (1982)
	450	470–750	Soulat et al. (2020)
Oxidation products			
Oil	310–490	350–550	Ammari et al. (2012)
Marine foods	393	463	Aubourg (1999)
Cheese	380	405–480	Karoui et al. (2007)
Oatmeal	379	450	P. Jensen et al. (2004)
Lumichrome	370	450	Miquel Becker et al. (2003)
(Riboflavin photooxidation product)	380	444–479	Karoui et al. (2007)
Dityrosine	300	400	Takasaki et al. (2005)
	325	400–420	B. Li et al. (2023)
Vitamins			
α-Tocopherol (vit. E)	298	326	Christensen et al. (2006), Duggan et al. (1957)
Riboflavin (vit. B_2)	270, 382, 448	518	Christensen et al. (2006), Duggan et al. (1957)
	470	530	S. A. Jensen et al. (1982)
Pyridoxine (vit. B_6)	328	393	Christensen et al. (2006)
	340	400	Duggan et al. (1957)
Phenolic acids			
4-Hydroxybenzoic acid	275–280	315–340	Sergiel et al. (2014)
2,4-Dihydroxybenzoic acid	305–325	435–475	Sergiel et al. (2014)
Ferulic acid	355–365	415–460	S. A. Jensen et al. (1982), Sergiel et al. (2014)
p-Coumaric acid	330	435	Garcia-Sanchez et al. (1988)
Vanillic acid	260–280	320–375	Sergiel et al. (2014)
Gallic acid	305–315	365–405	Sergiel et al. (2014)
Caffeic acid	365–375	445–470	Sergiel et al. (2014)
Syringic acid	260–280	345–385	Sergiel et al. (2014)
Chlorogenic acid	355–365	445–475	Sergiel et al. (2014)

maximum is found at shorter wavelengths (Lakowicz 2006). The peak at EX450/EM510 is due to carotenoids, riboflavin, FAD, and FMN (S. A. Jensen et al. 1982; Lakowicz 2006).

The peak at EX330/EM390 originates from pyridoxine (Christensen et al. 2006; Duggan et al. 1957). Furthermore, it is known that dityrosine crosslinks of gluten proteins are formed during dough formation (Stauffer 2007; Takasaki et al. 2005). Dityrosine fluorescence can be found at EX300–325 with maximum emission at EM400-420 (B. Li et al. 2023; Takasaki et al. 2005), although the emission can extend from 375 to 475 nm (Al-Hilaly et al. 2013). Therefore, it is possible that dityrosine fluorescence also contributes to this peak, especially in gluten and dough samples, although it has never been discussed in direct relation to fluorescence signals of cereal products before.

The peak at EX390/EM450 has been reported multiple times before for fluorescence spectroscopy of cereal products without clear assignment (Garcia et al. 2016; P. Jensen et al. 2004; Zandomeneghi 1999). Garcia et al. (2016) observed an increase based on the addition of ferulic acid, so possibly, ferulic acid and/or reaction products of it might be responsible. P. Jensen et al. (2004) attributed the peak to secondary lipid oxidation products, as they observed an increasing intensity over the storage period in cereal products, which had previously been reported for marine foods (Aubourg 1999) and cheese (Karoui et al. 2007).

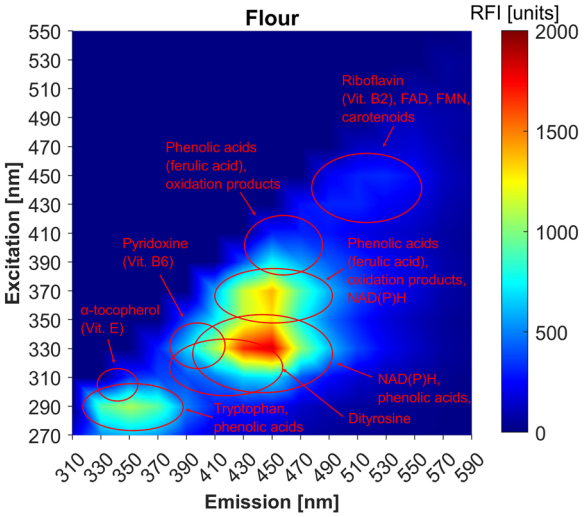

FIGURE 4 | Assignment of possible fluorophores based on references in Table 3. [Color figure can be viewed at wileyonlinelibrary.com]

3.1.2 | Boxplots of Spectral Intensities at Specific Wavelength Combinations

After identification of peaks and possible fluorophores, a more systematic investigation of spectral differences was carried out. For this, boxplots of spectral intensities were generated that are shown in Figure 5. No boxplot is shown for EX330/EM430 as its results were equivalent to EX330/EM450. As the fluorescence intensity is generally nonlinear due to quenching and inner filter effects (Lakowicz 2006), peak intensity differences are discussed in the context of what is known about possible fluorophores and their changes in flour, flour fractions, and dough upon processing.

The protein peak intensities (EX290/EM350) of the sieve fractions show the same trend as the PCA scores in Figure 2. It was confirmed by Osborne fractionation that sieve fractions are protein enriched compared to flour. This enrichment follows the same trend and is largest for the 50–75 μm fraction (results not shown). In contrast, Osborne fractionation also revealed that the protein content of the fine fraction is even higher than that of the 50–75 μm fraction, although its protein peak intensity is significantly lower. Possibly, quenchers have also enriched in the fine fraction, lowering the fluorescence intensity. Additionally, inner filter effects are observed for high concentrations of fluorophores, reducing the measured fluorescence signal (Lakowicz 2006). Furthermore, gluten, starch and dough samples show a significantly lower protein fluorescence than flour. Starch is protein depleted, but gluten and dough are not. While inner filter effects could also influence the gluten protein signal, quenching effects upon hydration and dough formation are another explanation for this observation (Ahmad et al. 2016; Garcia et al. 2016). Tryptophan is easily quenched by external quenchers and nearby groups in the same protein (Lakowicz 2006). As protein structure changes upon water addition and mechanical energy input, quenching effects are increased (Schefer et al. 2021; Stauffer 2007). For example, it was observed that ferulic acid binds covalently and non-covalently near tryptophan residues in the protein and quenches tryptophan fluorescence (Schefer et al. 2021).

For sieve and air-classified fractions, all non-protein peaks show the same trend of an increase in fluorescence intensity from smaller to larger particle sizes. Those peaks are mostly due to vitamins, carotenoids, and phenolic compounds (especially the most abundant ferulic acid; L. Li et al. 2008; Shahidi et al. 2022) that are concentrated in the bran, germ, and aleurone layer of the cereal grain (S. A. Jensen et al. 1982; Shahidi et al. 2022). Bran, germ, aleurone, and endosperm differ not only in their chemical compositions but also in their mechanical properties in milling (S. A. Jensen et al. 1982). It was observed before that those outer layers tend to have larger particle sizes than the endosperm (S. A. Jensen et al. 1982). Therefore, also the content of vitamins, carotenoids, phenolic compounds and associated oxidation products increases with particle size. Possibly, also the increased oxygen input for larger sieve fractions due to subsequent air jet sieving could have accelerated oxidative reactions here.

For gluten, starch, and dough, no clear trend is visible for non-protein peaks. For EX330/EM390, a decrease in fluorescence intensity would be expected for dough, as this peak has been attributed to pyridoxine (Christensen et al. 2006; Duggan et al. 1957) and the pyridoxine content decreases upon kneading due to oxidation (Batifoulier et al. 2005). The observed increase could be due to reduced quenching effects upon dough formation. Possibly, also other fluorophores, like the formation of dityrosine bonds, influence the fluorescence signal in this region (B. Li et al. 2023; Stauffer 2007; Takasaki et al. 2005). Second, for EX330/EM450, gluten shows a significantly lower fluorescence intensity than other samples. This peak is mainly due to phenolic acids (S. A. Jensen et al. 1982; Sergiel et al. 2014) that are mostly washed-out from gluten. Additionally, consumption and production of NAD(P)H by various enzymes in dough affect the fluorescence signal in this region (Joye et al. 2009; Lakowicz 2006). Changes in NAD(P)H concentration may also affect peak intensities for EX370/EM450, but more importantly here, various phenolic acids and oxidation products of vitamins, phenolic acids, and lipids contribute to fluorescence signal intensity (Garcia et al. 2016; P. Jensen et al. 2004; Sergiel et al. 2014). For the peak at EX390/EM450, gluten, starch, and dough all show a significantly higher fluorescence intensity compared to flour. As literature suggests, this may be due to oxidation products of lipids, ferulic acid, and possibly other compounds (Garcia et al. 2016; P. Jensen et al. 2004). Increased lipid oxidation could be present in gluten samples, as the lipid content of gluten is higher than that of starch and dough due to the binding of lipids to gluten proteins upon dough formation (Klingler 2010; Stauffer 2007). Interestingly, the peak at EX450/EM510 shows an increased intensity for gluten and dough. For gluten, this can be explained by carotenoids remaining in the gluten during the washing procedure, as they are not water-soluble (Klingler 2010). It was also observed that especially large, brownish particles became incorporated in the gluten network. The intensity increase for dough suggests that either, during kneading, non-identified compounds that fluoresce here are synthesized or that quenching effects of other compounds are reduced.

3.2 | Prediction of Farinograph Parameters

Fluorescence spectra of flour, flour fractions, and dough were used to predict farinograph parameters. The best models are

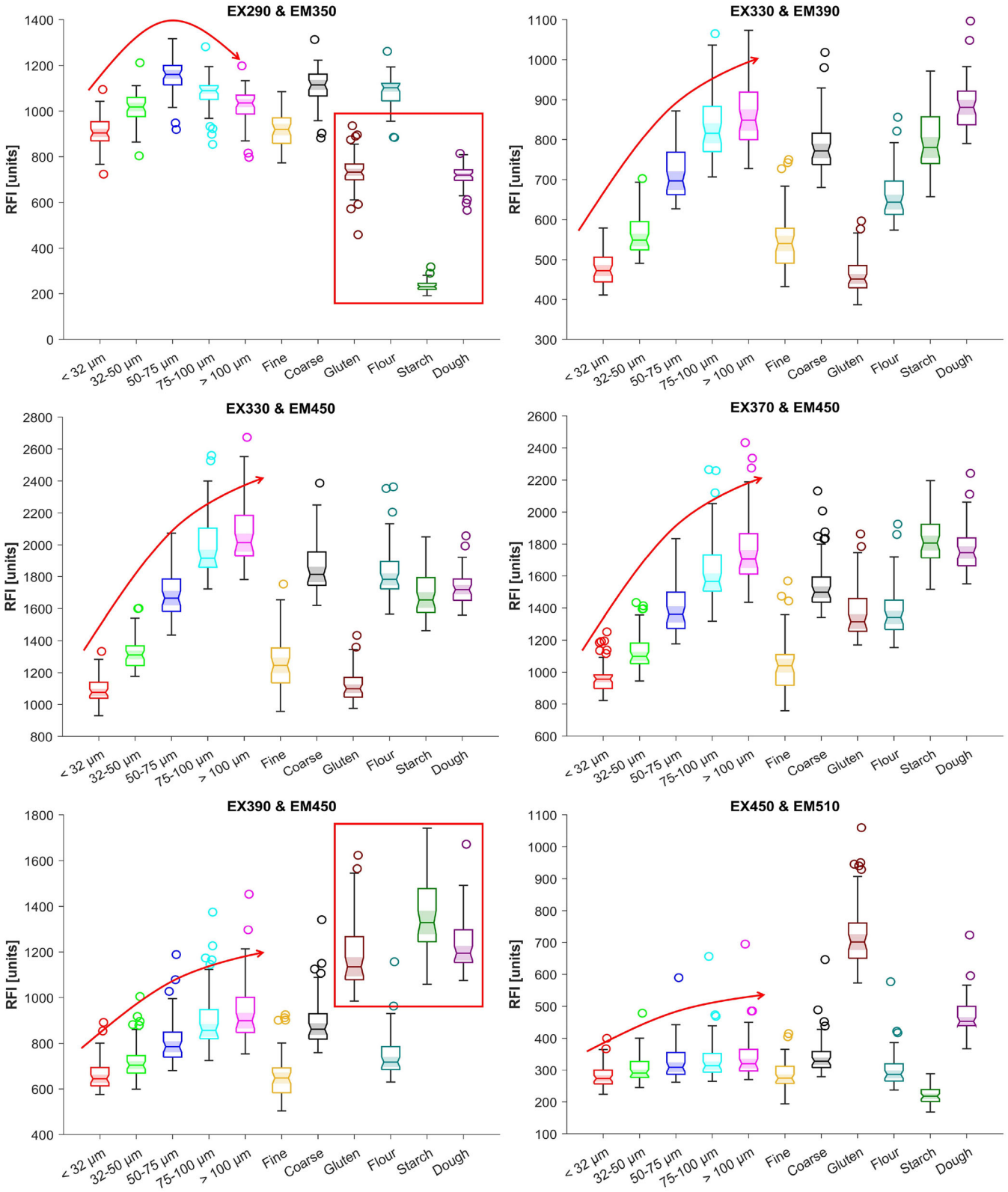

FIGURE 5 | Boxplots of spectral intensities of different sample types at specific wavelength combinations. Notches indicate significant differences of sample medians at the 5% significance level. [Color figure can be viewed at wileyonlinelibrary.com]

listed in Tables 4 and 5 and the best fits for all parameters are compared in Figure 6.

Water absorption can already be predicted well using spectra of flour with $R^2_{CV} = 0.79$. While the prediction using the 32–50 µm fraction shows a similar predictive quality, spectra of gluten, starch, and dough do not improve the prediction. In contrast, dough development time can only be predicted really well from spectra of dough and gluten with an R^2_{CV} of 0.90 and 0.84, respectively. R^2_{CV} of flour is only 0.64 here. A smaller

TABLE 4 | Model specifications of best predictions for water absorption and dough development time using fluorescence spectra of flour, fractions, and dough.

	Water absorption WA ($n = 50$; for coarse fraction $n = 47$)				Dough development time DDT ($n = 50$; for coarse fraction $n = 47$)			
Sample type	**Preprocessing; model; number of components**	**R^2_{CV} (−)**	**RMSECV (%)**	**$NRMSECV_{range}$ (%)**	**Preprocessing; model; number of components**	**R^2_{CV} (−)**	**RMSECV (min)**	**$NRMSECV_{range}$ (%)**
Flour	Detrend (1. order) + min–max normalization; PCR; 8	0.79	1.2	10.91	Detrend (1. order) + min–max normalization; PCR; 8	0.64	1.22	17.02
< 32 μm	Detrend (1. order) + min–max normalization; PLSR; 7	0.67	1.5	13.64	Min–max normalization; PLSR; 8	0.61	1.26	17.58
32–50 μm	Detrend (1. order) + min-max normalization; PCR; 8	0.81	1.2	10.91	Detrend (1. order) + min-max normalization; PCR; 8	0.69	1.14	15.90
50–75 μm	Detrend (1. order); PCR; 7	0.70	1.5	13.64	None; PCR; 9	0.65	1.20	16.74
75–100 μm	MSC; PCR; 8	0.64	1.6	14.55	Detrend (1. order) + min-max normalization; PCR; 8	0.64	1.21	16.88
> 100 μm	EMSC; PCR; 5	0.64	1.6	14.55	EMSC; PCR; 5	0.59	1.30	18.14
Fine	Detrend (1. order) + 2. norm normalization; PLSR; 7	0.36	2.1	19.10	Detrend (1. order) + 2. norm normalization; PLSR; 7	0.36	1.63	22.74
Coarse	MSC; PCR; 5	0.66	1.5	13.64	MSC; PCR; 5	0.50	1.33	18.55
Dough	Detrend (1. order) + min–max normalization; PLSR; 9	0.74	1.4	12.73	Detrend (1. order) + min-max normalization; PLSR; 9	0.90	0.64	8.93
Gluten	None; PLSR; 10	0.52	1.9	17.28	Detrend (1. order); PLSR; 10	0.84	0.83	11.58
Starch	None; PLSR; 7	0.59	1.7	15.46	1. norm normalization; PCR; 10	0.50	1.43	19.95

TABLE 5 | Model specifications of best predictions for stability and dough softening using fluorescence spectra of flour, fractions, and dough.

	Stability S ($n = 49$; for coarse fraction $n = 47$)				Dough softening DS 10 min ($n = 50$; for coarse fraction $n = 47$)			
Sample type	**Preprocessing; model; number of components**	**R^2_{CV} (–)**	**RMSECV (min)**	**$NRMSECV_{range}$ (%)**	**Preprocessing; model; number of components**	**R^2_{CV} (–)**	**RMSECV (BU)**	**$NRMSECV_{range}$ (%)**
Flour	MSC; PLSR; 4	0.50	3.82	16.45	1. norm normalization, PLSR; 4	0.70	15.3	14.04
< 32 μm	Detrend (1. order); PLSR; 8	0.61	3.39	14.60	None; PLSR; 6	0.75	13.7	12.57
32–50 μm	Min–max normalization; PCR; 8	0.57	3.54	15.24	None; PLSR; 5	0.76	13.7	12.57
50–75 μm	EMSC; PCR; 5	0.39	4.21	18.13	Detrend (1. order); PCR; 9	0.69	15.4	14.13
75–100 μm	SNV; PCR; 7	0.43	4.10	17.65	Detrend (1. order) + min–max normalization; PLSR; 5	0.68	15.8	14.50
> 100 μm	Min–max normalization; PLSR; 10	0.39	4.23	18.21	Detrend (1. order) + 1. norm normalization; PLSR; 6	0.68	15.7	14.41
Fine	Detrend (1. order) + 1. norm normalization; PCR; 10	0.36	4.34	18.69	Detrend (1. order) + 1. norm normalization; PCR; 10	0.48	19.9	18.26
Coarse	Min–max normalization; PLSR; 10	0.45	4.09	17.61	SNV; PCR; 5	0.59	17.6	16.15
Dough	Detrend (1. order) + min–max normalization; PCR; 4	0.61	3.36	14.47	Detrend (1. order) + min–max normalization; PCR; 8	0.79	12.7	11.66
Gluten	Min–max normalization; PCR; 4	0.61	3.37	14.51	None; PLSR; 10	0.76	13.5	12.39
Starch	SNV + EMSC; PLSR; 10	0.43	4.08	17.57	Detrend (1. order) + 1. norm normalization; PLSR; 8	0.59	17.8	16.34

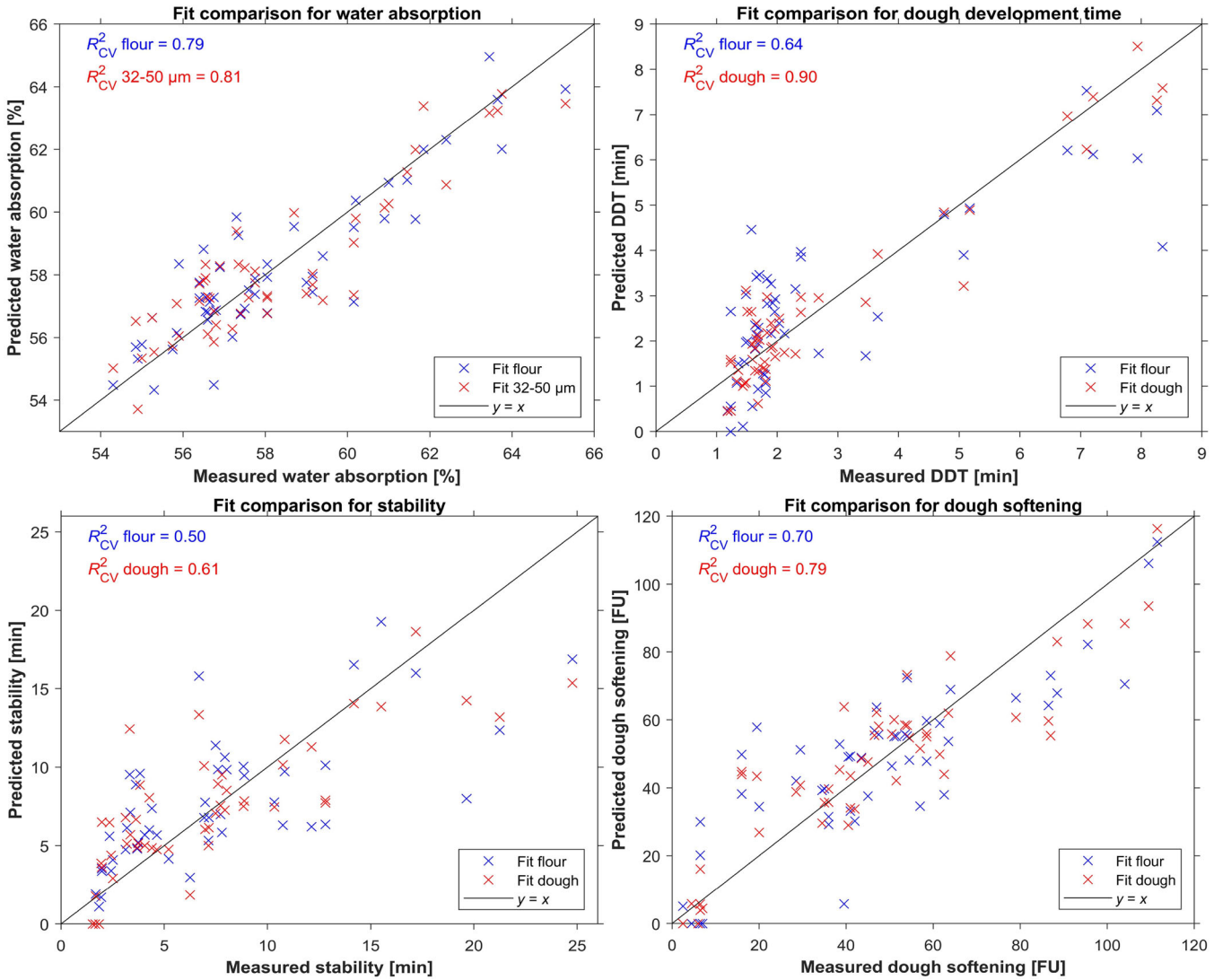

FIGURE 6 | Measured versus predicted values of cross-validation for best models and flour farinograph parameters. For every parameter, the fit for flour spectra is compared with the best fit for fractions or dough spectra. [Color figure can be viewed at wileyonlinelibrary.com]

improvement to $R^2_{CV} = 0.69$ is achieved by the 32–50 µm fraction. For dough softening, the largest improvement in prediction is also achieved using spectra of dough with $R^2_{CV} = 0.79$, while R^2_{CV} is 0.70 for flour spectra. On the other hand, stability cannot be predicted well from any of the used sample types with the applied methods, as the best R^2_{CV} is only 0.61 for gluten, dough, and the < 32 µm fraction compared to an R^2_{CV} of 0.50 for flour. Interestingly, the worst predictions for all four parameters are always observed for the fine fraction with R^2_{CV} ranging from 0.36 to 0.48.

These differences in the predictions indicate that the observed spectral differences strongly influence predictions, both positively and negatively. Because water absorption occurs in the first phase of dough preparation and is based on flour components in their native state (Stauffer 2007), it is not surprising that it can be predicted better using spectra of flour or sieve fractions. Dough development time, stability, and dough softening on the other hand are parameters characterizing the behavior of flour after addition of water under mechanical energy input (Belitz et al. 2009). The improved predictions of those parameters using spectra of dough and gluten compared to flour spectra show that information about the changes during the kneading process can be found in their fluorophores, can consequently be detected by means of fluorescence spectroscopy and from this, especially the dough development time can be predicted really well that was in turn used to prepare the samples. As shown before, besides tryptophan peak intensity, also peak intensities for NAD(P)H, phenolic acids, oxidation products, riboflavin and pyridoxine, possibly dityrosine and other non-identified fluorophores are significantly different for gluten and dough compared to flour and other fractions. Those peaks indirectly measure chemical and enzymatic changes that affect gluten network structure, for example, by changes in coenzyme concentration and generation of oxidation products that can further react with gluten proteins (Joye et al. 2009; Schefer et al. 2021). This was also confirmed by the study of Ahmad et al. (2016). They were able to classify different phases of the kneading process based on fluorescence spectra recorded while kneading. The prediction of farinograph parameters from spectra of gluten and dough is not relevant for practical application, as farinograph measurements have to be performed for the preparation of those sample types. Nevertheless, the strong

ability of gluten and dough samples to improve predictions compared to flour spectra can be of use for the prediction of other rheological parameters (e.g., from extensograph measurements) and more complex quality traits like the baking volume that are strongly influenced by dough rheological properties.

A possible explanation for the worse predictions of the fine fraction compared to all other sample types could be that the proteins enriched in this fraction are mainly free "wedge" proteins. Those proteins are interstitial endosperm proteins that are milled to really small particle sizes and therefore enrich in the fine fraction. In contrast, "adherent" proteins retain larger particle sizes, as those proteins are tightly connected to starch granules and covered by a lipid layer (Coulson and Sim 1965; Jones et al. 1959). This could indicate that the composition of free "wedge" and "adherent" proteins is different and that the free "wedge" proteins are not as important for the dough rheological behavior.

4 | Conclusion

In this study, spectral differences of sieve and air-classified fractions, gluten, starch, and dough compared to flour as well as their potential to improve predictions of wheat quality characteristics were identified.

It was confirmed that fluorophore composition, concentration as well as interactions (quenching, inner filter effect) and therefore also the microenvironment of the fluorophores are significantly different for flour fractions and dough compared to flour. Separation based on particle size and/or density, gluten washing as well as chemical reactions during those processing operations and dough formation are able to significantly alter the fluorescence signal by enriching and depleting a wide variety of fluorophores present in cereal products. In this way, protein- as well as vitamin- and phenolic-enriched fractions are obtained, although it seems that air classification enriches different proteins than air jet sieving. Furthermore, it was shown that dough and gluten spectra contain more information about dough rheological properties, enabling an improved prediction of the farinograph parameters dough development time, stability, and dough softening. Spectra of flour and sieve fractions on the other hand are superior in predicting water absorption as a property related to the native state of flour. In contrast, air-classified fractions did not improve the predictions of any of the farinograph parameters tested. In fact, the fine fraction always yielded the worst predictions.

The results indicate that spectroscopy of flour fractions and dough can be a valuable new approach for improving the prediction of wheat quality characteristics like the baking volume in future studies. For this, besides fluorescence spectroscopy, other spectroscopic techniques as well as advanced data evaluation methods like other types of regression models and feature selection methods can be tested. Also, data fusion approaches could further improve predictions, as different fractions provide complementary information. Besides this, the results also show further potential applications of fluorescence spectroscopy for monitoring and investigating changes during milling and kneading processes in the future. Further studies with a larger number of samples and validation of the results are required for the commercial application of wheat quality predictions based on fluorescence spectroscopy of flour, flour fractions, and dough.

Acknowledgments

This study was funded within an IGF project of the Research Association of the German Food Industry (FEI) that was supported via AiF (AiF 21711N) within the program for promoting the Industrial Collective Research (IGF) of the German Ministry of Economics and Climate (BMWi), based on a resolution of the German Parliament. The authors are grateful for the support of the project partners Mühlenchemie GmbH & Co. KG (Ahrensburg, Germany) for providing flour samples and conducting reference analyses (protein content, farinograph measurements) and GoodMills Deutschland GmbH (Hamburg, Germany) for air classification of flour samples. Open Access funding enabled and organized by Project DEAL. Open Access funding enabled and organized by Projekt DEAL.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be made available on request.

References

Ahmad, M. H., M. Nache, S. Waffenschmidt, and B. Hitzmann. 2016. "Characterization of Farinographic Kneading Process for Different Types of Wheat Flours Using Fluorescence Spectroscopy and Chemometrics." *Food Control* 66: 44–52. https://doi.org/10.1016/j.foodcont.2016.01.029.

Albanell, E., B. Miñarro, and N. Carrasco. 2012. "Detection of Low-Level Gluten Content in Flour and Batter by Near Infrared Reflectance Spectroscopy (NIRS)." *Journal of Cereal Science* 56, no. 2: 490–495. https://doi.org/10.1016/j.jcs.2012.06.011.

Al-Hilaly, Y. K., T. L. Williams, M. Stewart-Parker, et al. 2013. "A Central Role for Dityrosine Crosslinking of Amyloid-β in Alzheimer's Disease." *Acta Neuropathologica Communications* 1, no. 1: 83. https://doi.org/10.1186/2051-5960-1-83.

Ammari, F., C. B. Y. Cordella, N. Boughanmi, and D. N. Rutledge. 2012. "Independent Components Analysis Applied to 3D-Front-Face Fluorescence Spectra of Edible Oils to Study the Antioxidant Effect of *Nigella sativa* L. Extract on the Thermal Stability of Heated Oils." *Chemometrics and Intelligent Laboratory Systems* 113: 32–42. https://doi.org/10.1016/j.chemolab.2011.06.005.

Aubourg, S. P. 1999. "Recent Advances in Assessment of Marine Lipid Oxidation by Using Fluorescence." *Journal of the American Oil Chemists' Society* 76, no. 4: 409–419. https://doi.org/10.1007/s11746-999-0018-2.

Batifoulier, F., M. A. Verny, E. Chanliaud, C. Rémésy, and C. Demigné. 2005. "Effect of Different Breadmaking Methods on Thiamine, Riboflavin and Pyridoxine Contents of Wheat Bread." *Journal of Cereal Science* 42, no. 1: 101–108. https://doi.org/10.1016/j.jcs.2005.03.003.

Belitz, H.-D., W. Grosch, and P. Schieberle. 2009. *Food Chemistry*. Springer.

Van Bockstaele, F., I. De Leyn, M. Eeckhout, and K. Dewettinck. 2008. "Rheological Properties of Wheat Flour Dough and the Relationship With Bread Volume. I. Creep-Recovery Measurements." *Cereal Chemistry* 85, no. 6: 753–761. https://doi.org/10.1094/CCHEM-85-6-0753.

Cauvian, S. P., and L. S. Young. 2007. *Technology of Breadmaking*. Springer.

Christensen, J., L. Nørgaard, R. Bro, and S. B. Engelsen. 2006. "Multivariate Autofluorescence of Intact Food Systems." *Chemical Reviews* 106, no. 6: 1979–1994. https://doi.org/10.1021/cr050019q.

Coulson, C. B., and A. K. Sim. 1965. "Proteins of Wheat Flour." *Nature* 208, no. 5010: 583–584. https://doi.org/10.1038/208583a0.

Dowell, F. E., E. B. Maghirang, R. O. Pierce, et al. 2008. "Relationship of Bread Quality to Kernel, Flour, and Dough Properties." *Cereal Chemistry* 85, no. 1: 82–91. https://doi.org/10.1094/CCHEM-85-1-0082.

Dowell, F. E., E. B. Maghirang, F. Xie, et al. 2006. "Predicting Wheat Quality Characteristics and Functionality Using Near-Infrared Spectroscopy." *Cereal Chemistry* 83, no. 5: 529–536. https://doi.org/10.1094/CC-83-0529.

Duggan, D. E., R. L. Bowman, B. B. Brodie, and S. Udenfriend. 1957. "A Spectrophotofluorometric Study of Compounds of Biological Interest." *Archives of Biochemistry and Biophysics* 68, no. 1: 1–14. https://doi.org/10.1016/0003-9861(57)90320-X.

Edwards, W. P. 2007. *The Science of Bakery Products*. Royal Society of Chemistry.

Gabriel, D., C. Pfitzner, N. U. Haase, et al. 2017. "New Strategies for a Reliable Assessment of Baking Quality of Wheat—Rethinking the Current Indicator Protein Content." *Journal of Cereal Science* 77: 126–134. https://doi.org/10.1016/j.jcs.2017.08.002.

Garcia, R., A. Boussard, L. Rakotozafy, et al. 2016. "3D-Front-Face Fluorescence Spectroscopy and Independent Components Analysis: A New Way to Monitor Bread Dough Development." *Talanta* 147: 307–314. https://doi.org/10.1016/j.talanta.2015.10.002.

Garcia-Sanchez, F., C. Carnero, and A. Heredia. 1988. "Fluorometric Determination of p-Coumaric Acid In Beer." *Journal of Agricultural and Food Chemistry* 36, no. 1: 80–82. https://doi.org/10.1021/jf00079a020.

Jensen, P., J. Christensen, and B. Engelsen. 2004. "Oxidative Changes In Pork Scratchings, Peanuts, Oatmeal and Muesli Viewed by Fluorescence, Near-Infrared and Infrared Spectroscopy." *European Food Research and Technology* 219, no. 3: 294–304. https://doi.org/10.1007/s00217-004-0954-x.

Jensen, S. A., L. Munck, and H. Martens. 1982. "The Botanical Constituents of Wheat and Wheat Milling Fractions. Quantification by Autofluorescence." *Cereal Chemistry* 59, no. 6: 477–484. https://www.cerealsgrains.org/publications/cc/backissues/1982/Documents/chem59_477.pdf.

Jones, C. R., P. Halton, and D. J. Stevens. 1959. "The Separation of Flour Into Fractions of Different Protein Contents by Means of Air Classification." *Journal of Biochemical and Microbiological Technology and Engineering* 1, no. 1: 77–98. https://doi.org/10.1002/jbmte.390010108.

Joye, I. J., B. Lagrain, and J. A. Delcour. 2009. "Endogenous Redox Agents and Enzymes That Affect Protein Network Formation During Breadmaking—A Review." *Journal of Cereal Science* 50, no. 1: 1–10. https://doi.org/10.1016/j.jcs.2009.04.002.

Karoui, R., É. Dufour, and J. De Baerdemaeker. 2007. "Front Face Fluorescence Spectroscopy Coupled With Chemometric Tools for Monitoring the Oxidation of Semi-Hard Cheeses Throughout Ripening." *Food Chemistry* 101, no. 3: 1305–1314. https://doi.org/10.1016/j.foodchem.2006.01.028.

Klingler, R. W. 2010. *Grundlagen der Getreidetechnologie*, 2., vollst. überarb. Aufl. Behr.

Koksel, H., K. Kahraman, T. Sanal, D. S. Ozay, and A. Dubat. 2009. "Potential Utilization of Mixolab for Quality Evaluation of Bread Wheat Genotypes." *Cereal Chemistry* 86, no. 5: 522–526. https://doi.org/10.1094/CCHEM-86-5-0522.

Lakowicz, J. 2006. *Principles of Fluorescent Spectroscopy*. Springer. https://doi.org/10.1007/978-0-387-46312-4.

Li, B., Y. Yang, Y. Ding, et al. 2023. "Dityrosine in Food: A Review of Its Occurrence, Health Effects, Detection Methods, and Mitigation Strategies." *Comprehensive Reviews in Food Science and Food Safety* 22, no. 1: 355–379. https://doi.org/10.1111/1541-4337.13071.

Li, L., P. R. Shewry, and J. L. Ward. 2008. "Phenolic Acids in Wheat Varieties in the HEALTHGRAIN Diversity Screen." *Journal of Agricultural and Food Chemistry* 56, no. 21: 9732–9739. https://doi.org/10.1021/jf801069s.

Miquel Becker, E., J. Christensen, C. S. Frederiksen, and V. K. Haugaard. 2003. "Front-Face Fluorescence Spectroscopy and Chemometrics In Analysis of Yogurt: Rapid Analysis of Riboflavin." *Journal of Dairy Science* 86, no. 8: 2508–2515. https://doi.org/10.3168/jds.S0022-0302(03)73845-4.

Nagel-Held, J., K. El Hassouni, F. Longin, and B. Hitzmann. 2024. "Spectroscopy-Based Prediction of 73 Wheat Quality Parameters and Insights for Practical Applications." *Cereal Chemistry* 101, no. 1: 144–165. https://doi.org/10.1002/cche.10732.

Nagel-Held, J., L. Kaiser, C. F. H. Longin, and B. Hitzmann. 2022. "Prediction of Wheat Quality Parameters Combining Raman, Fluorescence, and Near-Infrared Spectroscopy (NIRS)." *Cereal Chemistry* 99, no. 4: 830–842. https://doi.org/10.1002/cche.10540.

Nawrocka, A., A. Miś, and M. Szymańska-Chargot. 2016. "Characteristics of Relationships Between Structure of Gluten Proteins and Dough Rheology–Influence of Dietary Fibres Studied by FT-Raman Spectroscopy." *Food Biophysics* 11, no. 1: 81–90. https://doi.org/10.1007/s11483-015-9419-y.

Pojić, M. M., and J. S. Mastilović. 2013. "Near Infrared Spectroscopy—Advanced Analytical Tool in Wheat Breeding, Trade, and Processing." *Food and Bioprocess Technology* 6, no. 2: 330–352. https://doi.org/10.1007/s11947-012-0917-3.

Schefer, S., M. Oest, and S. Rohn. 2021. "Interactions Between Phenolic Acids, Proteins, and Carbohydrates-Influence on Dough and Bread Properties." *Foods (Basel, Switzerland)* 10, no. 11: 2798. https://doi.org/10.3390/foods10112798.

Sergiel, I., P. Pohl, M. Biesaga, and A. Mironczyk. 2014. "Suitability of Three-Dimensional Synchronous Fluorescence Spectroscopy for Fingerprint Analysis of Honey Samples With Reference to Their Phenolic Profiles." *Food Chemistry* 145: 319–326. https://doi.org/10.1016/j.foodchem.2013.08.069.

Shahidi, F., R. Danielski, S. O. Rhein, et al. 2022. "Wheat and Rice Beyond Phenolic Acids: Genetics, Identification Database, Antioxidant Properties, and Potential Health Effects." *Plants (Basel, Switzerland)* 11, no. 23: 3283. https://doi.org/10.3390/plants11233283.

Soulat, J., D. Andueza, B. Graulet, et al. 2020. "Comparison of the Potential Abilities of Three Spectroscopy Methods: Near-Infrared, Mid-Infrared, and Molecular Fluorescence, to Predict Carotenoid, Vitamin and Fatty Acid Contents in Cow Milk." *Foods (Basel, Switzerland)* 9, no. 5: 592. https://doi.org/10.3390/foods9050592.

Stauffer, C. E. 2007. "Principles of Dough Formation." In *Technology of Breadmaking*, edited by S. P. Cauvian and L. S. Young, 299–332. Springer.

Takasaki, S., Y. Kato, M. Murata, S. Homma, and S. Kawakishi. 2005. "Effects of Peroxidase and Hydrogen Peroxide on the Dityrosine Formation and the Mixing Characteristics of Wheat-Flour Dough." *Bioscience, Biotechnology, and Biochemistry* 69, no. 9: 1686–1692. https://doi.org/10.1271/bbb.69.1686.

Xue, S. S., and J. Tan. 2022. "Rapid and Non-Destructive Composition Analysis of Cereal Flour Blends by Front-Face Synchronous Fluorescence Spectroscopy." *Journal of Cereal Science* 106: 103494. https://doi.org/10.1016/j.jcs.2022.103494.

Youssef, m, and m Abo-Dief. 2022. "Air Classification: Eco-Friendly Separation Technique in Food Technology—An Article." *Alexandria Journal of Food Science and Technology* 19, no. 1: 10–14. https://doi.org/10.21608/ajfs.2022.255779.

Zandomeneghi, M. 1999. "Fluorescence of Cereal Flours." *Journal of Agricultural and Food Chemistry* 47, no. 3: 878–882. https://doi.org/10.1021/jf981047v.