Fluorescence Spectroscopy and Chemometrics: An Innovative Approach for Characterization of Wheat Flour and Dough Preparation

Dissertation for Obtaining the Doctoral Degree of Natural Sciences (Dr. rer. nat.)

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Coauthors

All the scientific work presented in this thesis is accepted in the peer reviewed journal and Prof. Dr. Bernd Hitzmann served as a main supervisor.

Chapter 2: Stepahine Waffenschmidt carried out some analytical and baking parameters evaluation of bread whereas Marius Nache supported in chemometrics modeling used in this chapter.

Chapter 3: Stephanie Waffenschmidt milled the different cultivars of wheat flour whereas Marius Nache assisted in chemometrics modeling present in this chapter

Chapter 4: Chemometric modeling used in this chapter was performed in collaboration with Marius Nache whereas Prof. Jörg Hinrichs served as a co-supervisor.

List of publications

All scientific work presented in this thesis is already accepted in peer reviewed journal with approval and knowledge of the supervisor Prof. Dr. Bernd Hitzmann.

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Other publications by author

4. Zettel, V; Ahmad, M. H; Hitzemann, A; Nache, M; Paquet-Durand, O; Schöck, T et al. (2016): Optische Prozessanalysatoren für die Lebensmittelindustrie. Optical Process Analyzers in the Food Industry. In Chemie Ingenieur Technik 88, pp. 735-745. DOI: 10.1002/cite.201500097.
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8. Ahmad, M. H; N. Marius; Hitzmann. B. (2016). A rapid and non-invasive method for detection of low-levels of gluten in wheat flours using fluorescence spectroscopy. In Jahrestreffen der ProcessNet-Fachgruppe Lebensmittelverfahrenstechnik. 10-11 März 2016. Universität Erlangen, Germany. (**Poster**)
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Summary

Implementation of process analytical technologies (PAT) in food applications has attained a remarkable motivation due to higher quality and safety standards in this field. PAT applications also include rapid and non-invasive approaches which can be obtained from spectroscopic techniques. Fluorescence spectroscopy together with chemometrics is considered to be an outstanding analytical tool for fast and non-invasive technique for food analysis which can be used in various food applications on industrial scale. It is known for its sensitivity and specificity which can analyze the different foods and its ingredients while chemometrics helps to extract the useful information from the spectral data. The different chemometrics tools used for quantitative and qualitative analysis of spectral data, has increased the importance of this spectroscopic technique in generating the new ideas and hypothesis to develop new analytical methods which lead towards betterment in industrial operations for process and quality monitoring. In this doctoral project, fluorescence spectroscopic together with chemometric has been utilized to develop some new methods for determination of different parameters of wheat which provides the central idea of the thesis.

First manuscript presents the potential of fluorescence spectroscopy to predict the analytical, rheological and baking parameters of different wheat flours by just taking the spectral signature without any sample preparation. Twelve different wheat flours milled from wheat cultivars were used to analyze the analytical, rheological and baking parameters using the conventional methods. These measured parameters were predicted from the spectral data taken for different wheat flours using genetic algorithm coupled with partial least square regression. The model obtained for protein, wet gluten and sedimentation value showing high R^2 = 0.90, 0.92 and 0.81 respectively. Similarly, the rheological parameters like dough development time and water absorption were also predicted with low root mean square error of cross validation (RMSECV) and high R^2 = 0.95 and 0.77 respectively while pasting temperature showed R^2 = 0.78. Furthermore, moisture and volume of bread were predicted with high accuracy showing R^2 = 0.86 and 0.95 respectively in the baking parameters. Other rheological and baking parameters like dough stability, softening, farinograph quality number, baking loss, crumb hardness and springiness were not predicted well due to poor correlation and high error.

In the second paper, characterization of complex farinographic kneading process is performed by using the fluorescence spectroscopy in combination with chemometric tools. The aim of this investigation is to determine the impact of hydration of flour onto the spectral signals, classification of farinographic curve and separation of wheat flours based on their bread making performance. Secondly the middle curve of farinograph was predicted out of the fluorescence

spectra using partial least square regression (PLSR) which can help to predict optimal dough development time. The spectra of the flour showed high intensities in protein, NADH and riboflavin regions which reduce to 36 %, 58 % and 61 % respectively after the hydration process depicting its influence due to structural changes in protein and oxidation of NADH. The farinographic curve was divided into four phases and principal component analysis (PCA) has been used to extract the qualitative information regarding the farinographic curve from the fluorescence spectra to categorize all farinographic phases into hydration, dough development, and stability and softening. Similarly, different pre-processing tools like standard normal variate and generalized least square weighting generate good separation of various wheat flours during the farinographic kneading process into different quality groups (E, A, B and C) on the basis of their bread baking performance from the spectral data using PCA. Additionally, PLSR was applied to predict the middle curve of farinograph out of spectral data showing a descent coefficient of determination R^2 = 0.75 with RMSECV of 14 Brabender units. However, more research can lead towards the development of a sensor for determination of optimal dough development time.

In another study, the nutritional parameters of 26 different types of wheat flour obtained from different vendors from the supermarket were predicted using fluorescence coupled with linear and non-linear chemometric tools. PCA applied on the spectral data for different types of the wheat flours showing a clear separation. On the other hand, PLSR was used to quantify the nutritional parameters of different types of wheat flours showing a good prediction for fat, moisture and carbohydrates using cross-validation, with a R^2 of 0.88, 0.86 and 0.89, respectively whereas the protein, sucrose and salt contents presented a little correlation in PLSR. Therefore, locally weighted regression, a non-linear chemometric tool improves the prediction ability of all of the nutritional parameters by decreasing the error with an increasing R^2. The energetic value, protein, fat, carbohydrate, moisture, sucrose, salt and saturated fatty acid contents showed R^2 of 0.96, 0.93, 0.99, 0.99, 0.98, 0.88, 0.95, and 0.99 respectively, for different wheat flours.

The aforementioned results clearly demonstrate the potential of the fluorescence spectroscopy in determination of analytical, rheological, baking and nutritional parameters of the wheat flours. They present that it can be used to characterize and categorize the farinographic kneading process, which is important in the bread-baking industry. More research in this direction can result in developing a sensor for predicting the quality parameters and processing operations in the cereal based industries rapidly and non-invasively which are important for regulatory and screening of the wheat on quality characteristics for marketing and end product evaluations.

Zusammenfassung

Aufgrund immer höherer Anforderungen an Produktqualität und Sicherheitsstandards ist eine Integration von aktueller Prozessanalysentechnik (PAT) in der Lebensmittelverarbeitung von zunehmender Bedeutung. Methoden der PAT umfassen unter anderem echtzeitfähige und kontaktfreie spektroskopische Verfahren wie beispielsweise die Fluoreszenzspektroskopie. In Kombination mit chemometrischen Auswerteverfahren ermöglicht die Fluoreszenzspektroskopie eine schnelle, hoch sensitive und spezifische Analyse von Rohstoffen, relevanten Prozessgrößen und Lebensmitteln. Chemometrische Verfahren werden angewendet, um sowohl qualitative als auch quantitative Analysen durchzuführen. Zudem finden sie Anwendung für eine effektive und sichere Prozessführung und On-line-Qualitätskontrolle. Im Rahmen der vorliegenden Dissertation wurde die Fluoreszenzspektroskopie mit chemometrischen Auswerteverfahren kombiniert, um eine neue Methode zur Bestimmung von Qualitätsparametern für Weizenmehle und -teige zu entwickeln.

In der ersten Publikation wird das Potential der Fluoreszenzspektroskopie aufgezeigt, teigrheologische Eigenschaften und Backeigenschaften aus Spektren von unterschiedlichen Mehlen direkt vorherzusagen. Es erfolgte keinerlei Proben Auf- und Vorbereitung, die 12 Mehle unterschiedlicher Weizensorten wurden direkt vermessen. Zur Auswertung kam eine Kombination aus genetischem Algorithmus und Partial Least Square Regression zum Einsatz. Mit den berechneten chemometrischen Modellen konnten der Proteingehalt ($R^2 = 0,90$), der Feuchtklebergehalt ($R^2 = 0,92$) und der Sedimentationswert ($R^2 = 0,81$) vorhergesagt werden. Die Vorhersage der rheologischen Eigenschaften wie Teigentwicklungszeit ($R^2 = 0,95$) und Wasseraufnahme ($R^2 = 0,77$) gelang ebenfalls mit niedrigen Kreuzvalidierungsfehlern und hohem Bestimmtheitsmaß. Darüber hinaus konnten Feuchtigkeit ($R^2 = 0,86$) und Volumenausbeute ($R^2 = 0,95$) mit hoher Genauigkeit bestimmt werden. Weitere Parameter wie Teigstabilität, Teigerweichung, Farinograph Qualitätszahl, Backverlust, Krustenhärte und Krumenelastizität konnten aufgrund schlechter Korrelationen jedoch nicht gut bzw. nur mit großen Fehlern vorhergesagt werden.

Die zweite Publikation befasst sich mit der Charakterisierung von Knetprozessen mittels Fluoreszenzmessung und chemometrischer Verfahren. Das Ziel dieser Untersuchung war zum einen den Einfluss der Wasseraufnahme während der Teigbildung auf die Fluoreszenz zu untersuchen und zum anderen die Mehle entsprechend der gemessenen Farinograph Knetkurven zu klassifizieren. Dazu wurde die Mittelwert-Farinographkurve aus den Fluoreszenzspektren mittels PLS Regression vorhergesagt. Die Teigentwicklungszeit konnte mit

diesem Verfahren abgeschätzt werden. Die Spektren der Mehle bzw. Teige zeigten eine hohe Intensität in den Protein-, NADH- und Riboflavin-Bereichen. Diese wurden nach der Hydratation jedoch schnell um 36 %, 58 %, und 61 % reduziert, was auf eine Strukturveränderung der Proteine und eine Oxidation von NADH während der Wasseraufnahme schließen lässt. Mittels der Spektren und einer Hauptkomponentenanalyse wurde zudem eine Klassifikation in die 4 Phasen des Knetens Wasseraufnahme, Teigentwicklung, stabile Phase und Teigerweichung durchgeführt. Anhand der Fluoreszenzspektren konnte bestimmt werden, in welcher Phase sich der Teig gerade befindet. Eine Unterscheidung der Mehle entsprechend ihrer Qualität (E, A, B und C) war nach der Anwendung verschiedener Vorverarbeitungsalgorithmen wie „standard normal variate“ Korrektur und „generalised least squares weighting“ ebenfalls anhand der Spektren möglich. Bei der Berechnung der Farinograph-Mittelwertkurve mittels PLS Regression wurde eine Korrelation von 0,75 und ein Kreunzvalidierungsfehler von 14 Brabender Einheiten erzielt. Die Ergebnisse deuten darauf hin, dass basierend auf der Fluoreszenzmessung ein Sensor entwickelt werden kann, mit dem die Teigentwicklungszeit direkt vorhergesagt werden könnte.

In der dritten Publikation wurde untersucht, ob die Nährwertparameter von 26 verschiedenen Weizenmehlsorten und Typen, welche aus unterschiedlichen Quellen bezogen wurden, aus Fluoreszenzspektren vorhergesagt werden können. Dabei kamen sowohl lineare als auch nichtlineare Regressionsmodelle zum Einsatz. Mittels linearer PLS Regression war die Quantifizierung von Fett-, Feuchte- und Kohlenhydratgehalt mit Bestimmtheitsmaßen von jeweils 0,88, 0,86 und 0,89 möglich. Für Salz-, Protein- und Saccharosegehalt war eine quantitative Bestimmung mittels PLSR allerdings nicht möglich. Mittels „locally weighted regression“ einer nichtlinearen Regressionmethode war die Vorhersage von Brennwert ($R^2 = 0,96$) und den Gehalten von Protein ($R^2 = 0,93$), Fett ($R^2 = 0,99$), Kohlenhydraten ($R^2 = 0,99$), Feuchte ($R^2 = 0,98$), Saccharose ($R^2 = 0,88$), Salz ($R^2 = 0,95$) und gesättigten Fettsäuren ($R^2 = 0,99$) erfolgreich.

Die erzielten Ergebnisse zeigen das große Potential der mit chemometrischen Methoden kombinierten Fluoreszenzspektroskopie für die Analyse der Teig- und Backeigenschaften von Weizenmehlen auf. Weiterhin können die entwickelten Techniken die klassischen analytischen Verfahren ergänzen oder diese sogar ersetzen. Analytische-, rheologische- und Nährwertparameter sowie gängige Kennzahlen für die Backqualität konnten bestimmt werden. Weitere Untersuchungen könnten zu einer Entwicklung eines Sensorsystems führen, mit dem alle wichtigen Qualitätsparameter von Mehl sehr schnell und kontaktfrei bestimmt werden könnten. Somit wäre zum Beispiel eine lückenlose On-line-Qualitätskontrolle möglich.

Chapter 1

General Introduction and Outlines

1.1. General introduction

The role of process analytical technologies (PAT) in food applications has grown due to the advancement in industrialization and increased public interest towards the production of high quality food, changes in eating habits and consumer behavior. Furthermore, food and drug administration (FDA) has established some guidelines for the industry in 2004 which leads towards the popularity of PAT from the last decade (van Den Berg et al., 2013). Basically, PAT deals with a well establish system for ensuring quality from raw material to the end product, describing the process attributes and critical points during the whole operation. PAT involves in developing sensors which serve as rapid, non-invasive, cost effective and reagent free analysis. These sensors are important not only for industrial point of view to attain the high demand and advancement in technology but also for safe environmental concerns (Hitzmann et al., 2015). Hence it is the need of the time to develop some PAT measurements in food applications. To achieve this objective, spectroscopic techniques cannot be ignored which are considered as rapid and non-invasive for food production and process monitoring. In this thesis, the potential of fluorescence spectroscopy is explored which is known for its specificity and sensitivity (Sádecká & Tóthová, 2007). It is being used in different fields including food research and analysis. Previously, it was used to quantify only the fluorescent molecules like the riboflavin in cereal (Zandomeneghi et al., 2003) while estimation of non fluorescent molecules in recent applications has increased its importance. For example, it was applied to determine fatty acid profile in meat (Aït-Kaddour et al., 2016) and to estimate sucrose, glucose and fructose in figs (Jiang et al., 2013). The role of chemometric tools cannot be denied to extract the useful information from the spectral data, in developing the new analytical methods for screening of the food operation which is important for its production, marketing and regulatory purpose (Karoui & Blecker, 2010).

The raw material has a strong impact on the final product quality and understanding of their relationship is necessary to achieve process stability. The major ingredient in the baking industry is wheat which is considered one of three most important cereal crops. It is cultivated on one third area covered by cereal cultivation in the world (Hădărugă et al., 2016). Milling of wheat grains result in the flour which is being used in bread, cookies, cakes, pasta and variety of other cereal based products. The characterization of the wheat flour is really important for the production of high quality wheat based food products. Different analytical parameters like protein, wet gluten, sedimentation value and falling number have strong impact on the quality of

the final product. For example protein concentration in flour is important to predict the volume of the bread (Dowell et al., 2008) whereas gluten in wheat flour is responsible for three dimensional network giving the visco-elastic characteristics to the dough which help to achieve the desired quality characteristics of bread (Goesaert et al., 2005). Similarly, sedimentation value describes the quality of the protein present in wheat flour whereas falling number discusses the alpha amylase activity. Determination of both parameters can help the bakers to predict the end quality of wheat based products.

Different rheological parameters (water absorption, dough development time and pasting temperature etc.) play their role in predicting not only the quality of the final products but also describe the behaviors of the wheat flour during the processing operations in the baking industry for better machine ability of the materials (Ktenioudaki et al., 2010). Furthermore, final quality of the product is assured by using baking and bread parameters like volume and moisture of bread, baking loss, hardness and springiness of bread crumb. Moreover there are some process operations like kneading of the dough which is very complex and undergoes many changes. These changes include the development of gluten network which provide the higher quality characteristics to the bread. Furthermore, determination of accurate water absorption of the flour during the kneading process determines the behavior of further processing operations and the quality of end product (Miralbés, 2004). On the other hand, the prediction of nutritional profile of the wheat flour is considered important which not only provide the highlights to the consumer about the nutrition but also give an insight to the miller for proper labeling of the flour according to the labeling law.

Currently, conventional methods are being used in the cereal industry based on time consuming sample preparations. These methodologies are insufficient due to the increasing demand of better productivity and higher quality standards during the entire process operations from raw material to the final product. Hence, the replacement of conventional, time consuming and chemical based analysis in the cereal industry is necessary with advanced instrumentation like fluorescence spectroscopy which characterizes as rapid and non destructive methodology, forms the basis of the present thesis.

1.2. Thesis outlines

This thesis explores the potential of fluorescence spectroscopy for characterization of wheat flour using different chemometrics tools in combination with various preprocessing methodologies.

Chapter 2 deals with the development of an innovative approach for prediction of analytical, rheological and baking parameters of wheat flours using chemometrics tools based on fluorescence spectral data without sample preparations procedures.

Chapter 3 gives a detail overview for separation of different phases of farinographic curve into hydration dough development, dough stability and softening phases for wheat flour using principal component analysis (PCA) out of fluorescence spectral data taken during the kneading process. Furthermore, the potential of fluorescence spectroscopy to classify different cultivars of the wheat into different quality groups (E, A, B and C) based on bread making performance is also described.

Chapter 4 introduces the potential of fluorescence spectroscopy coupled with linear and non linear chemometric tools for quantification of the nutritional profile of 26 different types of wheat flours

Concluding remarks are presented in Chapter 5.

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Chapter 2

A fluorescence spectroscopic approach to predict analytical, rheological and baking parameters of wheat flours using chemometrics

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A fluorescence spectroscopic approach to predict analytical, rheological and baking parameters of wheat flours using chemometrics

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ABSTRACT

The potential of fluorescence spectroscopy for predicting analytical, rheological and baking parameters of twelve wheat flours were investigated. Partial least square regression models coupled with genetic algorithm were applied on spectral data to optimize the prediction of the aforementioned quality parameters using different pre-processing methodologies. Good linear regression models were obtained for protein, wet gluten and the sedimentation value from the analytical parameters group with a R^2 of 0.90, 0.92 and 0.81 respectively. Similarly prediction was obtained for rheological parameters like the dough development time and water absorption, with a very low *root mean square error of cross validation* (RMSECV) and an optimal R^2 of 0.95 and 0.77 respectively while it settled at 0.78 for pasting temperature. Furthermore, baking parameters like the moisture and volume of bread were predicted with a decent accuracy showing a R^2 of 0.86 and 0.95 respectively. Hence, fluorescence spectroscopy can be used as rapid method in predicting the wheat quality and its baking characteristics by just taking the spectra of flour with no sample preparation.

1. Introduction

Wheat is one of the important cereal crops, which is being used as a main ingredient in various foods like bread, pasta, noodles, cakes and biscuits (Mutlu et al., 2011). The quality of the wheat flour is vital for the quality of the end-product, which affects its commercial value. The key determinants of wheat quality characteristics are evaluated by analytical quantification of a range of parameters like wet gluten content, protein concentration, sedimentation value and falling number. All these parameters have their own distinct role in predicting the quality of wheat flour. The protein content play a vital role in describing flour characteristics while the development of gluten after hydration states the ability of flour and its efficiency of retaining gas during fermentation and ensures the successful completion of the final product (Pareyt et al., 2015). Sedimentation value elaborates quantity of the protein (Carter et al., 1999) whereas falling number is used to estimate the alpha (α) amylase activity (Kruger and Tipples, 1980) in wheat flours and both are important to determine the final quality of cereal based food products.

The rheological parameters of the wheat flours are important as well for the prediction of the baking value. Among different devices for estimation of these parameters, the farinograph has a dominant position from the point of view of the cereal scientist and the bakers (Hrušková et al., 2006). The water absorption can be determined with farinograph which is important to describe the mechanical characteristics of dough and successful completion of wheat based cereal products. Other dough profile characteristics like the dough development time (DDT), its stability and softening also have their respective impacts on the further processing of the wheat flours. Among them, the optimum DDT is important, since it transforms into the dough ability for gas retention and expansion during proofing, influencing as well the baking operation for attaining high volume and better final product characteristics (Dempster et al., 2005). Similarly the gelatinization or pasting temperature of various wheat flours is employed to determine rheological parameters which have a significant impact on the finished product characteristics (Ragaee and Abdel-Aal, 2006). Moreover, baking characteristics and the other bread parameters like the volume, the baking loss and the textural profile analysis

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(TPA) have also been used to describe the evolution of the quality of wheat based products (Scanlon and Zghal, 2001).

Hence, all aforementioned analytical, rheological and baking parameters evaluating methods can be employed on large and small scale applications. However their determination requires a lot of time and efforts to perform as they are laborious and destructive in nature. Due to the advancement in technology and awareness of environmental and safety reasons, there is need to develop fast, non-invasive and chemical free estimation methods. Fluorescence spectroscopy (Faassen and Hitzmann, 2015) offers a great alternative for this purpose. The viscosity of a sample influences the fluorescence quantum yield; therefore, even rheological characteristics can be determined using this measurement technique (Sharafy and Muszkat, 1971). It has been extensively used in dairy industry to monitor the changes in milk due to mild and intensive thermal treatments (Kulmyrzaev et al., 2005; Schamberger and Labuza, 2006) as well as in the classification and monitoring the process of different types of cheese (Karoui et al., 2007a, 2007b), prediction of rheological parameters (Karoui and Dufour, 2006), the stability of yogurt during storage (Christensen et al., 2005) and optimization of ice cream formulations (Granger et al., 2006). It is applied in meat industry to classify the meat based on their quality (Sahar et al., 2009) and to determine the freshness of fish (Dufour et al., 2003). Furthermore, it has been employed to determine the origin of honey (Karoui et al., 2007c) and in some other application to quantify the total plate count in chicken meat (Sahar et al., 2011) to ensure the availability of a high quality and safer food to the consumer. As for its application in cereal research is concerned, the fluorescence spectroscopy is being used in small and large scale cereal-based analytical approaches. A series of approaches have been applied to quantify and visualize wheat gluten, starch and air bubbles in different mixing stages of dough (Kokawa et al., 2012) as well as in the pie pastry analytics (Kokawa et al., 2015). It has been used to perform the classification of different types of cereals (Karoui et al., 2006) to determine its power to describe the fluorescence of various fluorophores present in them (Zandomeneghi, 1999) as well as for the classification of red and white wheat kernels (Ram et al., 2004). Monitoring of the on-line pH plus the degree of acidity during sourdough fermentation (Grote et al., 2014), aging of cakes (Botosoa et al., 2013), separation of farinographic phases and classification of wheat cultivars during the kneading process (Ahmad et al., 2016) represent other applications for this type of spectroscopy employed in the cereal research.

Similarly other spectroscopic techniques like near infrared (NIR) spectroscopy has been applied to describe the rheological characteristics like dough development time and baking parameters of wheat flours (Arazuri et al., 2012; Dempster et al., 2005; Dowell et al., 2006). A multivariate evaluation approach is employed to determine the feasibility of NIR and mid-NIR spectroscopy for analyzing the composition of wheat bran (Hell et al., 2016). Due to the accuracy of the spectroscopic techniques coupled with chemometric models proved to be a suitable approach to predict the quality parameters of wheat flour. This approach can therefore help the bakers and the cereals scientists to estimate the analytical, rheological and the baking parameters of different wheat flours in a fast and non-invasive way by just acquiring a spectral reading.

Hence, this contribution aims to demonstrate the feasibility of the prediction of the analytical parameters (protein, wet gluten, sedimentation and falling number) out of the fluorescence spectra using chemometric evaluation approaches. Similarly, farinographic (water absorption and dough development time), pasting temperature, baking and bread characteristics of different types of wheat flours will also be estimated using the same approach.

2. Materials and methods

2.1. Raw materials

Eleven different cultivars of winter wheat were obtained from KWS SAAT SE Einbeck, Germany. These cultivars belong to different quality groups of wheat, which were classified after their analytical and baking performance. These groups are elite (*E*), quality (*A*), bread (*B*) and other purposes (*C*). *Bussard*, *Milaneco* and *Montana* are the part of *E* while *Malibu*, *Julius* and *Magic* belong to the *A* group. Similarly the *B* group comprises of *Bonanza*, *Ferrum*, *Salix* and *Loft* whereas the *C* group contains only the *Rockefeller* cultivar. Commercially available wheat flour used for baking purpose was also obtained from the local mill and named as *Rettenmeier flour* (Rettenmeier Mühle GmbH, Horb, Germany).

2.2. Milling of wheat cultivars

All the wheat cultivars were milled for flour using the Quadrate junior laboratory mill (Type 279001, Brabender OHG, Duisburg, Germany). After milling the flour was stored at refrigeration temperature to avoid the changes in the flour.

2.3. Determination of the analytical parameters

Different analytical parameters were determined for all types of wheat flour using the standard methods provided in the *International Cereal Chemist* (ICC) and in the *Approved Analytical Methods of Cereal Chemists* (AACC). The moisture was determined using the Infrared moisture analyzer MA 51 (Sartorius AG, Göttingen, Germany). The protein concentration, the falling number, the sedimentation rate and the wet gluten were measured using the ICC methods (ICC, 1994) 105/1, 107/1, 116/1 and 137/1 respectively. The measurement of the farinographic parameters has been performed using the AACC 54-21 (AACC, 1999) while the pasting temperature was determined using the Rapid Visco-Analyzer (RVA, Newport Scientific) according to the ICC 162 method (ICC, 1994). All measurements were performed in duplicate and mean value was used for the prediction models.

2.4. Bread preparation

Wheat flour breads were prepared using 2 % salt, 1 % sugar, 2 % fat, 1 % dry yeast based on the flour weight and water was used according to the farinographic measurement. The water and other ingredients were added in the mixing bowl of the type SP 12 spiral mixer (DIOSNA Dierks & sons GmbH, Osnabrück, Germany) one after the other, then mixed for 1 min at low speed for uniform distribution of the ingredients and then the kneading process was performed according to the respective dough development time taken from the farinographic measurements. The dough was collected from the mixing bowl and placed it in proofing chamber (Stammkälte, Wachtel GmbH, Hilden, Germany) for bulk fermentation at 30 °C with a relative humidity (RH) of 80 % for 20 min. Then it was manually handled to distribute the air cell in the dough and scaled to 500 g into 2 baking tins of 28 × 10 × 9.5 cm. These were again placed into the proofing chamber for 50 min (30 °C, 80 % RH). After the proofing process, baking was carried out for 35 min at 235 °C in a convection oven (Piccolo DMS, Wachtel GmbH & Co. Hilden, Germany). After baking, the breads were cooled at room temperature for 120 min then the measurements were performed.

2.5. Bread parameters

2.5.1. Volume of bread

After cooling the volume of bread was measured using the VolScan Profiler 600 mm (Stable Micro Systems Ltd., Godalming, England). The rotation speed and vertical step was set at 1 rpm and 5 mm respectively. Two breads were scanned one after the other in one replication and the average value of both has been taken for the volume measurement.

2.5.2. Texture profile of bread

Bread slices of 2.5 cm thickness were cut with a rotary slicer, then the TPA of bread was tested using the Texture-Analyser TA.XT2™ (Stable Micro Systems Ltd., Godalming, England) with a cylinder of diameter 2.5 cm using a test speed and control force of 1 mm/s and 5 g respectively. The relaxation time between two compression cycles was 15 s with a strain of 40 %. All measurements were performed in duplicate. Three slices were used to take the textural profile of every bread and the average value of the texture profile of bread was used.

2.5.3. Moisture of bread

The moisture of breads was measured using the 1700-1 method described in ICC methods (ICC, 1994).

2.6. Fluorescence spectra collection of the wheat flour

50 spectra of each of the wheat flours were taken using 2D-fluorescence spectroscopy BioView (Delta Light & Optics, Hørsholm, Denmark) sensor, equipped with an xenon light source and 15 filter wheels. This setup can measure the florescence spectra in the range between $\lambda_{270-550\ nm}$ and $\lambda_{310-590\ nm}$ for excitation and emission respectively using different filter wheels, which rotate for achieving various wavelength combinations with a bandwidth in step of 20 nm. The spectrum obtained comprised of 120 wavelength combinations.

2.7. The chemometric modeling

2.7.1. Data sets and the computational platform

Data sets using 50 spectra of each type of the flours were arranged as predictor matrix of independent variables containing 600 spectra in total for the chemometric modeling of analytical, rheological and baking parameters of the various types of the wheat flours. The measured analytical, rheological and baking parameters were also taken into the data set as the predicted matrix of the dependent variables. All the data were packed into a matrix and further treatments analyzed on the MATLAB 2013b (The Mathwork TM, MA, United States) platform using the PLS and the statistical toolboxes.

2.7.2. Pre-processing of data

Data pre-processing has become very important to extract the meaningful information and used to enhance the predictive capability for determining the analytical parameters out of the spectral data. Spectral scaling and specific transformation methodologies such as the de-trending, the standard normal variate (SNV), the multiplicative scatter correction (MSC) as well as the generalized least square weighting (GLSW) were used throughout the data evaluation process.

Detrending is a pre-processing technique, which removes the trend of data so that deviations from the overall trend will emerge (Barnes et al., 1989).

SNV is a scatter correction method, which will scale every *i*th value of the spectrum to the standard deviation of the entire spectrum. The transformation is performed according to the Equations (1) and (2).

$$X_{SNVi} = (X_i - \overline{X})/\sigma_X \tag{1}$$

$$\sigma_X = \sqrt{\left(\sum_{i=1}^{n}(X_i - \overline{X})^2\right)\Big/(n-1)} \tag{2}$$

Here $X_i, \overline{X}$, σ_X *and* n are the *i*th spectrum, mean, standard deviation and number of wavelength variables respectively.

Similarly, MSC is also used to remove undesired scatter effect in the spectral data. The concept behind the MSC is based on the correction of the measured spectrum according to a reference spectrum like the average spectrum for instance. MSC is a two-step process applied to all the spectrums in the dataset. First the regression coefficients β and α are computed for all the recorded spectra regressed against a reference spectra $x_{ref.}$ Then all the spectrums will be corrected using the slope and intercept parameters found in the previous step according to the Equation (3) (Nache et al., 2015).

$$x_{corr,i} = (x_i - \alpha)/\beta \tag{3}$$

The GLSW (Zorzetti et al., 2011) is an advanced transformation method. It can be applied as a clutter filters that downweigh the information which is common throughout the spectra, leaving untouched the relevant variance. After many trials the weighting factor α was set at 0.01 which provided the most accurate linear regression model.

2.7.3. The wavelength selection using the genetic algorithm

A raw fluorescence spectrum is composed of 120 wavelength combinations. Among them, some wavelength combinations contain no information for any predictive models increasing thus just the complexity of the evaluation task. Therefore the genetic algorithm (GA) was applied to simplify and enhance the predictive capability of regression model used to predict for various parameters of the wheat flours. The concept of the GA is based on Darwin and Mendelian paradigm, which assume that the evolution will promote only the best genes in the population. GA was here applied for selecting the wavelength combinations on different parameters of various types of wheat flours using a gene block size of one wavelength and a population of 128 individuals evolving for 100 generations and using dual-crossover for breeding and a fixed mutation rate of 0.005.

2.7.4. PLSR modeling

After pre-processing of the spectral data, partial least square regression (PLSR) has been used to predict different parameters of various types of flours. In PLSR modeling the spectra belonging to same flour were put in one block. Hence 12 blocks were made for 12 different types of wheat flours spectra. Eleven blocks were used for calibration and the 12th one has been used for the prediction. Cross-validation has been thus performed until all the spectral blocks were once predicted in process of PLSR modeling. The fitness of the model was evaluated using the coefficient of determination (Equation (5)) and the root means square error of the cross-validation (Equation (4))

$$RMSECV = \sqrt{\sum_{i=1}^{n}\frac{(m_i - p_i)^2}{n}} \tag{4}$$

$$R^2 = 1 - \sum_{i=1}^{n} (m_i - p_i)^2 \Big/ \sum_{i=1}^{n} (m_i - \overline{m})^2 \quad (5)$$

Here $m_i, p_i, \overline{m}$ and n are measured, predicted, mean value and total number of spectra in data set respectively.

3. Results and discussion

Table 1 summarizes the various measured analytical, rheological, and baking parameters of different types of wheat flours. The maximum and minimum values of the parameters of wheat flours describe the broad variability in the data. It was observed that protein, wet gluten, sedimentation value and falling number of different wheat flours varied in the range of 9.1–13.4 %, 21.56–35.82 %, 34.45–61.05 mL and 259–410 s respectively. The rheological and baking parameters also showed differences, which exhibit that various types of wheat flours have significantly different characteristics. The variations in analytical, rheological, and baking parameters are due to the different amounts of proteins, wet gluten and α amylase activity, which has a strong impact on rheological and baking characteristic of the wheat flours. For example, water absorption, DDT and other rheological parameters highly depend on percentage of protein and wet gluten, whereas the final bread characteristics like volume, crumb hardness and springiness have strong correlation with the analytical and rheological parameters of wheat flours. So, it was obvious that the various cultivars of wheat have strong variation in their analytical, rheological and baking characteristics, which was used to get the robust calibration model for estimating them with fluorescence spectroscopy.

3.1. Prediction of the analytical parameters of wheat flours

The prediction of the analytical parameters of the various types of wheat flours using fluorescence spectra with GA-PLSR modeling technique is shown in Table 2. The obtained results for the protein content and wet gluten were accurately predicted providing a low RMSECV of 0.38 % and 1.32 % respectively. The obtained R^2 for the protein content reached 0.90 while it settled at 0.92 for wet gluten using 8 latent variables. Similarly the sedimentation value was moderately predicted with 3.62 mL RMSECV and a R^2 of 0.77. On the other hand, the prediction of the falling number showed a R^2 of only 0.48. This might be due the fact that different types of wheat flours showed high peaks in the protein region of the spectra, which can be correlated towards the predictions of protein, wet gluten and sedimentation value, whereas the falling number is connected to the α amylase activity that probably cannot be directly predictable from the spectral data. In Fig. 1, the measured vs. cross-validated predicted scatter plots are also presented for the protein content (1a), the wet gluten (1b) and the sedimentation value (1c). Due to the lack of studies regarding the use of the fluorescence for the evaluation of the aforementioned parameters, a comparison of the obtained results is problematic to accomplish. However, the prediction of the protein content in the present work shows a very good correlation as compared to the previous findings of Karoui and Dufour (2008) for instance which found an R^2 of 0.65 for the prediction of the protein content in cheese with a portable spectrofluorometer. Similarly, Diez et al. (2008) report an R^2 of 0.9 for soluble protein contents in heat-treated infant formula using fluorescence, which is in line with the results of the present contribution. Moreover, the results are also consistent with previous reports which used NIR spectroscopy for determination of analytical parameters of wheat flour for rapid method development (Miralbés, 2003).

Table 2
Prediction of the analytical parameters of wheat flours using the genetic algorithm and PLSR using maximum 8 latent variables.

Analytical parameters	Pre-processing	RMSECV	R^2_{CV}
Protein [%]	GLSW+ detrending	0.38	0.90
Wet gluten [%]	GLSW+ detrending	1.32	0.92
Sedimentation [mL]	GLSW+ detrending	3.62	0.77
Falling number [s]	MSC+ detrending	32.98	0.48

3.2. Rheological parameters prediction

Table 3 presents measured vs. cross-validated predicted rheological parameters of different cultivars of wheat flour.

Among different rheological characteristics, DDT and water absorption are the most important farinographic parameters as it determines the quality of final product. Protein contents have a strong impact on water absorption and dough development time which strengthen our expectation to get good prediction model from fluorescence spectral data as it shows high peaks in the protein region of the spectra. DDT was predicted with a high R^2 of 0.95 and a low RMSECV and percent RMSECV of 18.91 s and 7.1 % respectively using 6 latent variables (Fig. 2a). Similarly, the obtained results for the water absorption prediction models showed a good value R^2 of 0.77 with a low RMSECV of 1.69 % using 4 latent variables (Fig. 2b). Due to the accurate water absorption, a

Table 1
Statistics of analytical, rheological and baking parameters of different types of wheat flours and their breads.

Parameters	Minimum	Maximum	Mean value	Standard deviation
Moisture [%]	11.68	13.4	12.56	0.56
Protein [%]	9.1	13.4	10.88	1.15
Wet gluten [%]	21.56	35.82	27.38	4.69
Sedimentation [mL]	34.45	61.05	42.11	8.18
Falling number [s]	259	410	351.88	42.34
Pasting temperature [°C]	65.75	87.47	83.95	5.64
Water absorption [%]	54.8	66	61.46	3.47
Dough development time [s]	87	352	138.42	78.39
Dough stability [s]	90	744	369.63	202.42
Dough softening [BU]	40	76	62.54	12.45
Farinograph quality number [mm]	28.17	125.5	61.91	33.75
Volume of bread [mL]	2392.62	3589.79	2808.73	364.3
Baking loss [%]	11.17	12.42	11.8	3.8
Moisture of bread [%]	20.72	32.01	25.49	2.93
Crumb hardness [N]	2.21	6.08	3.93	1.09
Crumb springiness [N]	0.99	1.73	1.07	0.21

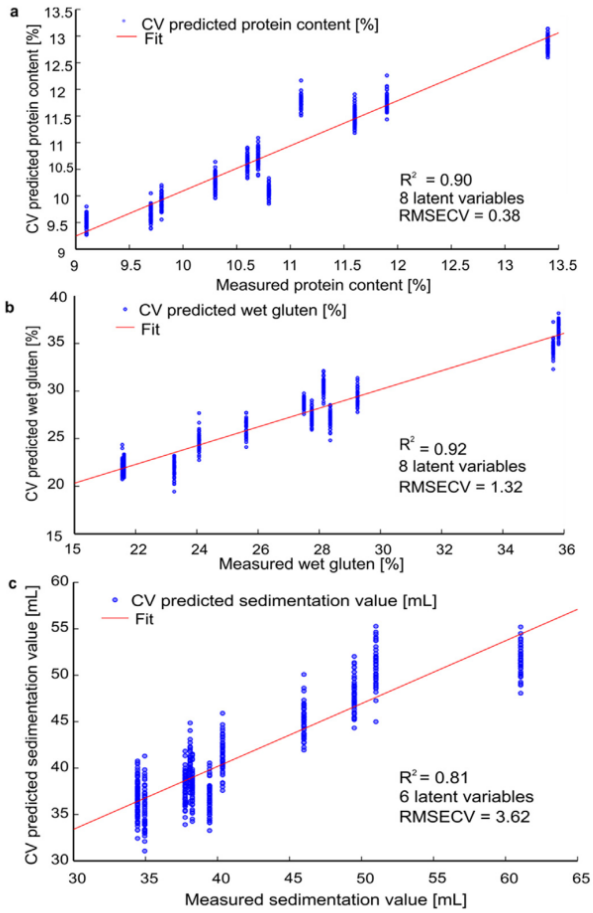

Fig. 1. Scatter plots representation of the protein (a), wet gluten (b) and the sedimentation value (c) prediction models for different cultivars of wheat flours plus Rettenmeier flour.

Table 3
Prediction of the rheological parameters of wheat flours with the genetic algorithm and PLSR after pre-processing with GLSW and detrending using maximum 7 latent variables.

Rheological parameters	RMSECV	R^2_{CV}
Water absorption [%]	1.69	0.77
Dough development time [s]	18.91	0.95
Dough stability [s]	241.25	0.12
Dough softening [BU]	21.38	0.23
Farinograph quality number [mm]	36.4	0.11
Pasting temperature [°C]	2.65	0.78

characteristic farinographic curve obtained which should be in the tolerance range of 480–520 Brabender units (BU). An error in water absorption rejects the farinographic curve due to deviation from the aforementioned tolerance range. Therefore the present prediction model for water absorption shows high accuracy which saves tedious and time consuming procedure for developing farinographic curve. Hence it is obvious that the DDT and water absorption linearly correlated with the spectral data as it was observed from the PLSR model coupled with genetic algorithm approach. Moreover, the other farinographic parameters like the dough stability, dough softening and the farinographic quality

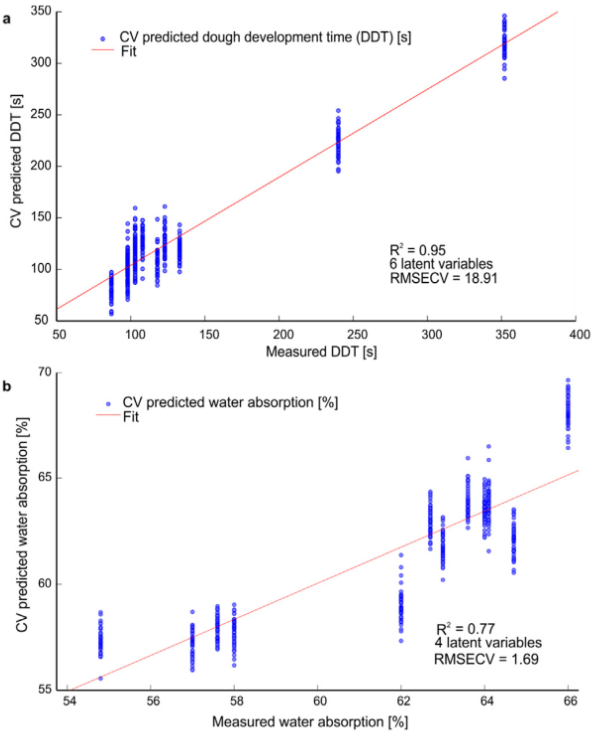

Fig. 2. Representation of measured and cross-validated predicted scatter plots of dough development time (a) and water absorption (b) for different cultivars of wheat flours plus Rettenmeier flour.

number could not be accurately predicted due to the high RMSECV and low R^2 as described in Table 3. Furthermore, the pasting temperature, which is also shown in Table 3 indicates a decent prediction with a RMSECV of 2.65 °C and R^2 settled at 0.78 for different cultivars of wheat flours.

The results showed in the present contribution are comparable with the previous findings of Miralbés (2004) who used NIR spectroscopy on different types of wheat and obtained a prediction error of 0.46 % for water absorption with no prediction of DDT. However, he got good prediction of other farinographic parameters like dough stability, dough softening and farinograph quality number.

Similarly, the DDT, dough stability and softening shows better prediction performance as compared to the findings of Mutlu et al. (2011) who has used neural networks on NIR spectral data of wheat flour to predict these parameters. However, he found higher correlation for water absorption ($R^2 = 0.83$) than the rest of the literature studies. Moreover, the prediction of the pasting temperature is in line with the findings of Bertrand and Scotter (1992) who has applied principal component regression to study starch gelatinization using NIR with good results. Similarly (Cozzolino et al., 2013) found the $R^2 = 0.36$ for pasting temperature in barley using NIR spectroscopy which is much lower than the findings of the present contribution.

3.3. Prediction model for baking and bread parameters

Table 4 presents the results of the baking and bread parameter prediction models of the different wheat flours. Here the cross-validated GA-PLSR models for different types of wheat flours

Table 4
Prediction of the baking parameters of wheat flours with the genetic algorithm and PLSR using maximum 9 latent variables.

Baking parameters	Pre-processing	RMSECV	R^2_{CV}
Volume of bread [mL]	GLSW+ detrending	78	0.95
Baking loss [%]	SNV+ detrending	0.29	0.45
Moisture of bread [%]	GLSW+ detrending	1.08	0.86
Crumb hardness [N]	GLSW+ detrending	0.68	0.57
Crumb Springiness [N]	GLSW+ detrending	0.19	0.16

produced good results for the moisture of bread, whose prediction achieved a RMSECV of 1.08 % and an $R^2 = 0.86$. Similarly the volume of the bread also showed a good RMSECV of 78 mL and a high R^2 of 0.95 as it can be seen in the scatter plots from Fig. 3. Protein content in wheat flour strongly correlated with the volume of baked goods and its fluorescence may be the reason for this linear relation between spectral data and volume of bread. Previous authors have tried to develop the prediction models for the bread volume as a function of the protein, wet gluten sedimentation, falling number and other rheological parameters of wheat grain and flour (Dowell et al., 2008; Renata and Janusz, 2011). But these models still involve the laborious determination of these parameters by conventional methods. The present contribution produces not only better results than the previous findings but also provides a non-invasive methodology for prediction of the loaf volume. Similarly the other spectroscopic methods like NIR in combination with neural networks produced an R^2 of 0.69 for the prediction of the bread volume (Mutlu et al., 2011) which is lesser to the findings of the present contribution.

On the other hand, the baking loss and some textural profile characteristics like crumb hardness and springiness showed a poor correlation and a lower accuracy models and cannot be predicted using this type of approach as presented in Table 4. The results regarding the bread hardness and springiness are not consistent with the previous findings of Allais et al. (2006) who has obtained high R^2 (>0.90) for biscuits and lady finger batter using the fluorescence spectra taken in the region of tryptophan and NADH. The current approach cannot achieve the high R^2 which might be due the fact that crumbs hardness and springiness adherent to the starch and its retrogradation process and is difficult to extract the hidden information from the spectral signature of the wheat flours.

4. Conclusion

Hence, the obtained prediction models for quantification of analytical, rheological and baking parameters of 12 different types of wheat flours using fluorescence spectroscopy coupled with linear regression methods showed the feasibility of this approach. The percent RMSECV for dough development time, water absorption, protein, wet gluten and volume of bread was found less than 10% which indicate the accuracy of this application and opens the way to develop a sensor, which can be used as a rapid and non-invasive method for determining and predicting wheat quality characteristics by just taking their spectral signatures. However, more research is required in this direction to make this approach applicable on industrial scale for screening and regulatory purposes.

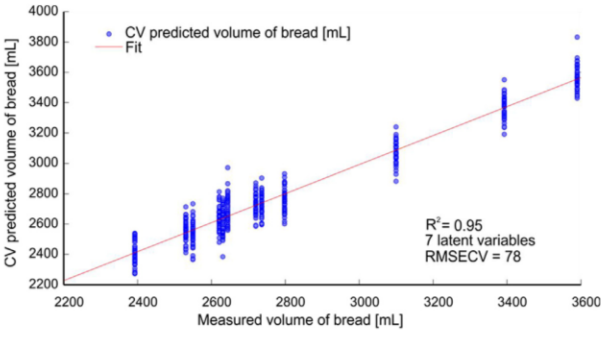

Fig. 3. Representation of measured and cross-validated predicted scatter plots of volume of bread for different cultivars of wheat flours plus Rettenmeier flour.

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Chapter 3

Characterization of farinographic kneading process for different types of wheat flours using fluorescence spectroscopy and chemometrics

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Characterization of farinographic kneading process for different types of wheat flours using fluorescence spectroscopy and chemometrics

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ABSTRACT

Fluorescence spectroscopy provides an ideal tool to explore chemical changes during dough mixing process. This paper aims to make use of this tool to investigate the influence of hydration of flour onto the spectral signals, classification of farinographic curve and separation of wheat flours based on their bread making performance. Secondary the quantitative information regarding the prediction of middle curve out of the fluorescence spectra was attempted using chemometric approaches. The spectral data of *Rettenmeier* flour presents high fluorescence signal in the protein, NADH and riboflavin regions which diminish to 36 %, 58 % and 61 % respectively after the hydration process depicting its influence due to changes in protein structure and oxidation of NADH. The *principal component analysis* (PCA) has been used to extract the qualitative information regarding the farinographic curve from the fluorescence spectra during the hydration phase of the *Rettenmeier flour*. Using this approach all four farinographic phases was clearly separated into hydration, dough development, and stability and softening. Similarly, PCA was used to separate twelve different wheat flours on the basis of their bread baking performance into E, A, B and C groups during the kneading process out of spectral data pre-processed with *standard normal variate* (SNV) and generalized least square weighting (GLSW) methods. Middle curve of farinograph was predicted using the *partial least square regression* (PLSR) modeling approach out of spectral data with a cross-validated error (RMSECV) of 14 *Brabender units* (BU) and a *coefficient of determination* R^2 0.75. The results demonstrate that fluorescence spectroscopy can be used to characterize and categorize the farinographic kneading process, which is important in the bread-baking industry.

1. Introduction

Dough rheological properties are very important describing bread making characteristics and can be measured by using different instruments like the farinograph, the extensograph, mixolab and the amylograph (Xu, Bietz, Felker, Carriere, & Wirtz, 2001). Among them, the farinograph is the most used analytic tool in cereal industry since the last century. It provides important information about the water absorption which is essential for the further processing of the flour and the dough profile characteristics like the dough development time along with its stability and softening (Stojceska & Butler, 2008). It provides a characteristics curve which is generated by the increase in the consistency of the dough during the mixing and can be measured in Brabender units (BU).

During the process of mixing the dough, different types of changes takes place which can be categorized using the fluorescence spectroscopy that is very popular due to its high sensitivity and specificity (Faassen & Hitzmann, 2015; Lenhardt, Bro, Zeković, Dramićanin, & Dramićanin, 2015). It provides the information about the fluorophores like the aromatic amino acids (tryptophan, phenylalanine and tyrosine), vitamins (riboflavin and pyridoxine) and cofactors (NADH, FAD and FMN) which are present in the sample (Andersen & Mortensen, 2008). It is being used in different fields of biological sciences and becoming more frequently applied in the food research (Christensen, Nørgaard, Bro, & Engelsen, 2006). It has become an important tool in combination with multivariate analysis for the prediction and classification of food samples (Karoui & Baerdemaeker, 2007). It is employed in dairy to investigate different types of cheese (Kulmyrzaev et al., 2005) to detect the changes in milk due to heat treatments (Kulmyrzaev, Levieux, & Dufour, 2005) and the storage stability of yogurt (Christensen, Becker, & Frederiksen, 2005). It has been applied for

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determination of fish freshness (Dufour, Frencia, & Kane, 2003; Hassoun & Karoui, 2015), classification and spoilage of meat (Sahar, Boubellouta, & Dufour, 2011; Sahar & Dufour, 2015) and to detect the oxidation of different types of oils (Mabood et al., 2015). Furthermore, it is used to classify the food products according to their origin like in the analysis of honey (Karoui, Dufour, Bosset, & Baerdemaeker, 2007). Similarly, fluorescence spectroscopy has proved its use in different approaches in the cereal research. Cereals can be classified by using synchronous fluorescence spectroscopy (Zeković, Lenhardt, Dramićanin, & Dramićanin, 2012). Front-face fluorescence spectroscopy can be also used to determine and classify the different wheat varieties and its products (Karoui, Cartaud, & Dufour, 2006) while using specific geometry it can determine the different fluorophores in cereals (Zandomeneghi, 1999). Other applications are the determination of the distribution of ferulic acid in different types of cereal grains (Ndolo, Beta, & Fulcher, 2013) and the on-line monitoring of pH and degree of acidity during sourdough fermentation (Grote, Zense, & Hitzmann, 2014). Furthermore, lipid oxidation has been monitored during twenty days storage of cakes using this technique (Botosoa, Chèné, & Karoui, 2013). Hence, fluorescence spectroscopy is being used in a variety of purposes; however this technique has not yet been applied to characterize the farinographic kneading process.

To interpret the fluorescence spectral data and extract the useful information, multivariate data analysis has to be applied (Cozzolino, Cynkar, Shah, & Smith, 2011). *Principal component analysis* (PCA) and *partial least square regression* (PLSR) are well known for their use in variety of purposes. PCA is mainly used for classification and data exploratory processes (Golshan, MacGregor, Bruwer, & Mhaskar, 2010) while the PLSR is a supervised method, which is used to model the correlation of the spectral variables with the measured parameters (Mehmood, Liland, Snipen, & Sæbø, 2012). Several studies have depicted good results using PCA showing its ability in differentiation of barely samples on the basis of year of harvest and location (Cozzolino, Allder, Roumeliotis, & Eglinton, 2012), process control in maize drying (Liu, Chen, Wu, & Zhang, 2006) and many other food applications. However, before any data evaluation is performed, the spectral data set must be pre-processed in order to remove the noise and light scattering effect generated by the data acquiring procedure. Amongst the plethora of the existing pre-processing methods, *standard normal variate* (SNV) is often applied normalization method which well-serve the aforementioned purpose.

In this contribution, the effect of the hydration of the wheat flour will be determined on the fluorescence spectra taken before and after water addition. The farinographic curve will be categorized into different phases like hydration, dough development, stability and softening using PCA applied to the fluorescence spectra. Similarly PCA will be investigated for identifying different cultivars of wheat to differentiate on the basis of quality groups according to German protocol like E (elite), A (quality), B (bread) and C (other purposes). The middle curve of farinogram will be predicted by using PLSR modeling out of the fluorescence spectra for developing the sensor which can give information about dough mixing stages and optimum time of kneading in future researches.

2. Materials and methods

2.1. Raw materials

Eleven winter cultivars of wheat were provided by KWS SAAT SE Einbeck Germany. These belong to different quality groups (*A, B, C* and *E class*). *Bussard*, *Montana* and *Milaneco* belongs to *E* while *Magic*, *Malibu* and *Julius* are the part of the *A* quality group. Similarly, quality group *B* includes *Bonanza*, *Ferrum*, *Salix* and *Loft*. On the other hand, *Rockefeller* is the only cultivar which belongs to *quality group C*. The cultivars of wheat were milled for flour using the *Brabender Quadrumat® Junior* laboratory mill (Type 279001, Brabender OHG, Duisburg, Germany). Additionally, *Rettenmeier flour* was provided by a local mill (Rettenmeier Mühle GmbH, Horb, Germany) which is commercially available for baking purposes.

2.2. Fluorescence spectroscopy

Two dimensional fluorescence spectrometer *BioView®* was used to acquire the fluorescence spectra during the farinographic measurement. The setup consists of two filter wheels mounted with 15 filters. It measures the excitation (270–550 nm) and the emission (310–590 nm) with a 20 nm step bandwidth respectively. The excited light after passing through the first filter enter in the sample through an optical fiber. This excites the fluorophores in the sample and the emission light guided back through a second filter. The light intensity is measured with a photomultiplier and recorded as a raw fluorescence spectrum. A fluorescence spectrum comprised of 120 wavelength combinations.

2.3. Modification of the farinographic bowl for fluorescence measurement

The normal farinographic bowl was modified and equipped with a port for the BioView® (Delta Light & Optics, Hørsholm, Denmark) sensor as shown in Fig. 1a. This was used to acquire the spectra during the entire process of the farinographic measurement as shown in Fig. 1b while Fig. 1c presents the schematic diagram of working setup for the recording of the fluorescence spectra during the mixing with farinograph. All twelve flours were used to make the farinogram in this modified farinographic bowl (FD0234H Farinograph, Brabender® GmbH & Co. KG, Duisburg, Germany) according to method AACC 54-21 (AACC, 1999) while taking the fluorescence spectra. Typically a spectrum was recorded in 53 s, thus 22 spectra were taken during the 20 min process of farinographic measurement. Hence, 220 spectra were taken for 10 replicates of *Rettenmeier flour* in the first phase of farinographic measurement. On the other hand, 264 spectra were acquired for eleven wheat cultivars plus *Rettenmeier flour* in the second phase. Furthermore, *Rettenmeier flour* was also used to take spectra before and after the hydration process to monitor the effect of the water onto the fluorescence signal.

2.4. Classification of the farinographic curves

Farinogram provides a characteristics curve which is developed using the measured increase in consistency in the mixture of water and flour by the application of the force imparted by the Z shaped blades of the farinograph. Fig. 2 shows the farinographic curve for *Rettenmeier* flour which is categorized into four different phases. The hydration phase develops after the addition of water to the *Rettenmeier flour* until it reaches 500 *BU* line. The region around the peak of the curve represents the dough development phase. Dough stability phase starts after the dough development time until the upper curve remains on 500 *BU* line. Last phase is dough softening which began when upper farinographic curve leaves the 500 *BU* line.

2.5. Chemometric data analysis

The spectral data have been imported into a Matlab workstation (The Mathwork™,MA, United States) where all further data evaluation stages were performed. There were two sets of spectral data: one containing the ten measurements of *Rettenmeier* flour (220

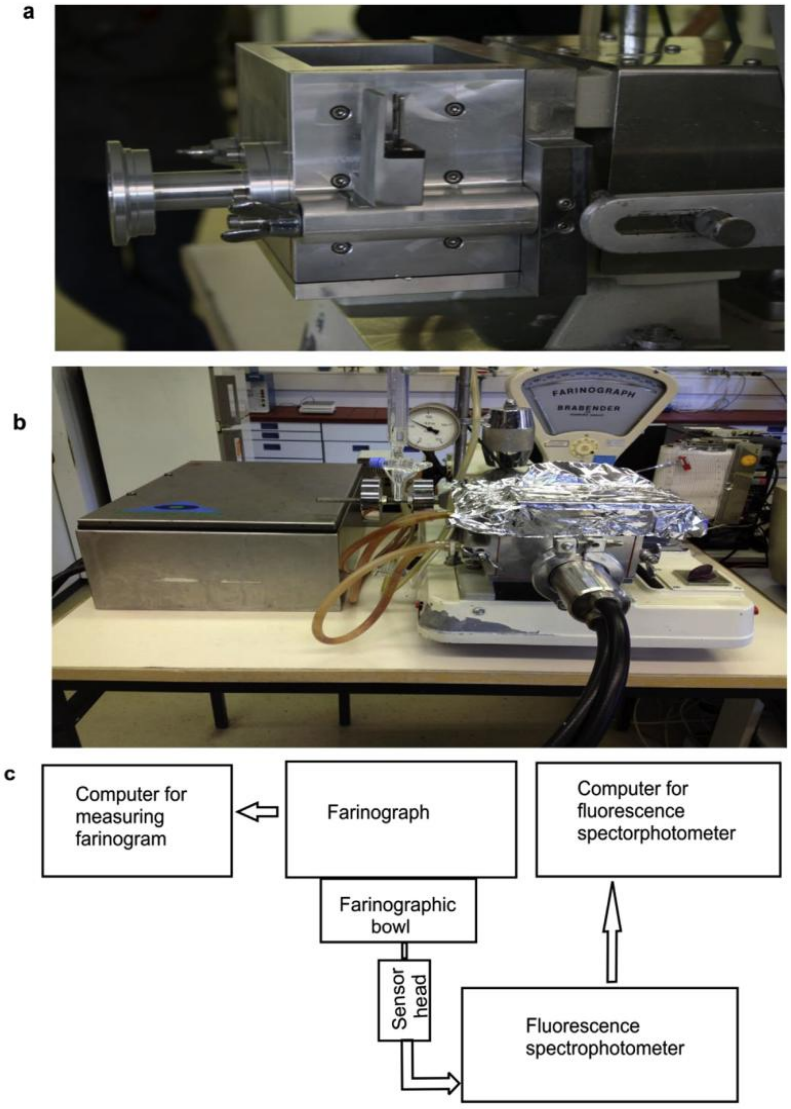

Fig. 1. Chart describing the experimental procedure. Modified farinographic bowl (A), fluorescence sensor connected to farinographic bowl (B) and schematic diagram for taking fluorescence spectra during the kneading process in farinograph (C).

spectra) and the second data set composed of spectra taken from eleven different cultivars plus *Rettenmeier flour* (264 spectra) during the process of farinographic mixing. First spectral data set was used to perform PCA exploratory analysis of the separation into different phases of farinogram. The second data set was subjected to PCA to classify the various types of wheat flours on the basis of bread making performance into different quality groups. Additionally, PLSR modeling was used to predict the middle curve of farinogram.

Before the employment of the PCA and PLSR modeling, the spectral data was pre-processed using *standard normal variate* (SNV) and *generalized least square weighting* (GLSW) transformation.

SNV (Barnes, Dhanoa, & Lister, 1989) transformation is a method of pre-processing which eliminates the variation induced into spectra by the scattering effect due the diversity of particle size. It is applied to take out the mean spectra from the individual variable which is then divided by its standard deviation using the formula shown in Equation (1)

$$X_{SNV_i} = (X_i - \overline{X})/\sigma_X \quad (1)$$

Here X_{SNV_i}, $\overline{X}$, X_i *and* σ_X are the transformed spectral data point, mean, ith intensity value in a spectrum and its standard deviation, respectively.

GLSW is an advanced transformation technique which is used to remove the clutter variance within the class from the spectra (Nache, Scheier, Schmidt, & Hitzmann, 2015). The fluorescence spectra have been filtered here by using GLSW with a down-weighting factor of 0.01 which provides an optimal filtration by retaining the maximum variance in the spectra without any loss of information and achieve a maximum performance in chemometric modeling.

Principal component analysis (PCA) is one of the basic methods

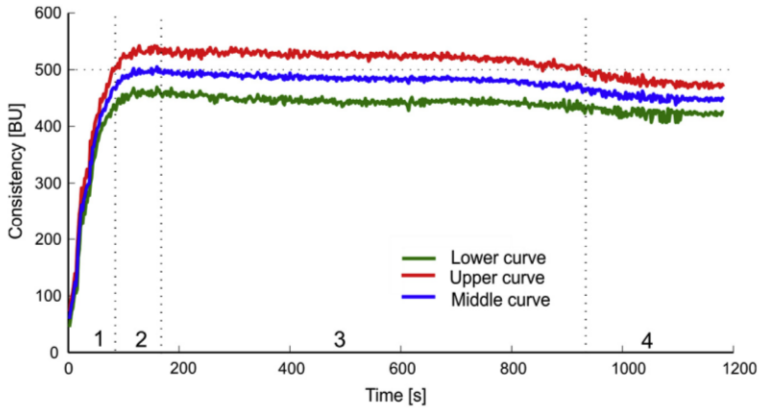

Fig. 2. Representation of the of farinographic phases for Rettenmeier flour: Hydration (1), dough development (2), dough stability (3) and dough softening (4).

of chemometrics used for exploratory analysis of multivariate data. It is applied to reduce the dimensionality of the multivariate data to a number of latent variables, called principal components (PCs) without losing the information (Mehdizadeh, Minaei, Hancock, & Torshizi, 2014). First PC captures the maximum variance which reduces with each successive PCs. PCA was applied on data sets which were pre-processed with SNV and GLSW to separate the farinographic curve and to categorize the different wheat flours into various groups during the kneading process.

PLSR is a supervised method which is applied to get the correlation between the predictor data X and the predicted set of data y (Chung, Heymann, & Grün, 2003). Here X represents the spectral data and y denotes the middle curve of farinogram. PLSR model was applied on the second set of spectral data containing eleven different cultivars plus *Rettenmeier flour* using cross-validation. In this validation method, the spectra belonging to the one flour put in one block (22 spectra). Eleven blocks were used for the calibration while validation was performed using the twelfth block. This process was repeated until all the blocks used for validation purpose one by one.

The performance of the model was estimated by computing the *root mean square error of cross validation* (RMSECV) and the *coefficient of determination* (R^2). These are calculated using the Equations (2) and (3),

$$RMSECV = \sqrt{\sum_{i=1}^{n} (m_i - p_i)^2 / n} \quad (2)$$

$$R^2 = 1 - \sum_{i=1}^{n} (m_i - p_i)^2 \Big/ \sum_{i=1}^{n} (m_i - \overline{m})^2 \quad (3)$$

Here m_i and p_i represents the measured and the predicted value of the ith sample, while $\overline{m}$ and n represent the mean and number of sample respectively. Here number of sample are ($n = 12$) due to the twelve different flours investigated.

3. Results and discussion

Table 1 summarizes the mean, standard deviation and range values of the middle curve of farinogram for first (middle curve of farinogram for ten replicates of Rettenmeier flour) and second (middle curve of farinogram for eleven cultivars of wheat plus Rettenmeier flour) data set. The variation in the data is important for accurate and robust modeling.

3.1. Hydration effect on the fluorescence spectra

The contour plot of the fluorescence spectra for the *Rettenmeier* flour is presented in Fig. 3. The plots show peaks in different regions of the spectra. Before the addition of water, high fluorescence intensities were observed in the NADH-specific area of the spectra defined by $\lambda_{excitation}$ 330–390 nm and $\lambda_{emission}$ 430–490 nm. After the addition of water, there is pronounced decrease in the fluorescence intensity in this region. The highest intensity value dropped from 3640 units to 1520 units after the hydration process, which is the reduction of 58 %. This may be due to the fact that the NADH which is naturally present in the flour oxidizes after the addition of water that lowers the fluorescence intensity in this region (Parmentier, Vandamme, Beauprez, & Arnaut, 2012). Ferulic acid fluorescence can also be observed in the same region as described in the literature (Pussayanawin, Wetzel, & Fulcher, 1988). However, there was an increase in the fluorescence intensity after the hydration process (Garcia et al., 2016) which is contrary to the present findings.

Another peak was visible in the protein area of the spectra which was present in the $\lambda_{excitation}$ 270–310 nm and $\lambda_{emission}$ 310–390 nm before the addition of water. These protein peaks are due to the presence of tryptophan which is highly fluorescent fluorophore present in adequate amount in wheat flour. The quenching of tryptophan fluorescence due to the interaction with tyrosine residues was observed after the hydration of wheat flour (Sironi, Guerrieri, & Cerletti, 2001) which can be allocated towards the structural changes of proteins. The highest peak due to the tryptophan fluorescence was 2230 units which diminished to 1430 units after the hydration of wheat flour with variation of 36% that is similar to the previous findings. Furthermore a distinct peak was observable in the spectral area defined by $\lambda_{excitation}$ 430–470 nm and $\lambda_{emission}$ 510–570 nm which has assigned to different fluorophores like carotenoid pigments (Gillbro & Cogdell, 1989) or glycoflavones in wheat germ (Barnes & Tester, 1987). Furthermore, one author allocated the attributed fluorescence of this region due the riboflavin as he enriched the flour with it which resulted in its determination (Zandomeneghi et al., 2003). Due to the hydration, 61 % reduction of fluorescence signals was observed in this region of spectra. Moreover the pyridoxine is present in wheat flour however its typical peak was masked due to the high intensity in NADH and ferulic acid region of fluorescence spectra in the contour plots shown in Fig. 3. Altogether considering these effects, it sustains the idea that the hydration of the flour directly affects its fluorescence intensity.

Table 1
Representation of mean, standard deviation and ranges values of the middle curve of farinogram developed for different cultivars of wheat plus Rettenmeier flour.

Data set	Mean value of middle curve of farinogram [BU]	Standard deviation [BU]	Range [BU]
[a]First data set	456	27	354–503
[b]Second data set	475	24	397–518

[a] Middle curve of farinogram of 10 replicates of *Rettenmeier flour*.
[b] Middle curve of farinogram of 11 wheat cultivars plus *Rettenmeier flour*.

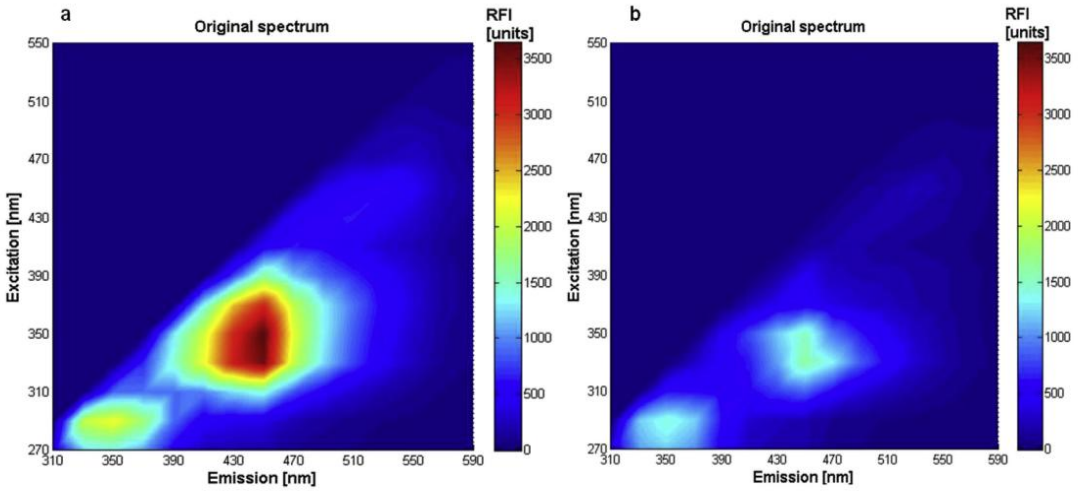

Fig. 3. Contour plot of Rettenmeier flour before (a) and after (b) the hydration process.

3.2. The effect of the pre-processing on the spectral signal

The raw fluorescence spectra recorded during the kneading of wheat flour with farinograph is presented in Fig. 4a. Each spectral line indicates one run of farinographic measurement. Here the distinct peaks can be observed in the various regions of the fluorescence spectra. The highest peak was observed in the NADH and ferulic acid regions of the spectra. NADH and ferulic acid show peaks in excitation 350 nm with emission 450 nm and 440 nm respectively (Schulman, 1985) and exhibit strong relationship with fluorescence in wheat flours as described by previous researches (Garcia et al., 2016; Zandomeneghi, 1999). The riboflavin region of the spectra showed the small peaks and can easily detectable by using these line spectra. Similarly, the peaks obtained due to the fluorescence of pyridoxine were found in the region of excitation 333 nm and emission 375 nm (Nakai & Horimoto, 2006). Furthermore, the peaks in the protein region of the spectra can be allocated due to fluorescence of aromatic amino acids like tryptophan, tyrosine and phenylalanine present in wheat flour (Garcia et al., 2016). Fig. 4b represents spectra which have been normalized and filtered with SNV and GLSW pre-processing techniques.

3.3. Separation of farinographic curve with PCA

PCA was carried out on the first data set that contains the fluorescence spectra of ten replicates of *Rettenmeier flour* during the farinographic kneading process. Fig. 5 presents the distinct separation of the spectral variables into the all four different phases specific to a farinographic curve achieved by *single value decomposition* employed in the PCA computation. These farinographic phases (hydration, dough development, dough stability and softening) have specific characteristics associated with the structural and development changes of gluten network. For example, *hydration* phase hydrates the flour particles and smoothens its way towards the next phase of the farinographic curve, the *dough development*. *Dough development* phase is responsible for the development of the three-dimensional gluten networks which confer visco-elastic properties to the dough. These properties are crucial since they provide the information for the further processing of the dough in the production lines. These structural changes in the protein network can be assumed clear differentiation between *the hydration* and the *dough development* phase. The next phase is the *dough stability*, a span-zone between where the upper curve touches and leaves the 500 BU line. It determines how much the flour is stable during the application of the torque. Some spectra of the *dough stability phase* appeared in *the dough development* phase. This might be due to the fact that *dough development* is considered to be a part of the *dough stability phase*. But for the separation *dough development* and *stability* phases, it was a little bit modified. The last phase is the *dough softening* in which the gluten completely breaks down and the dough become very viscous and adhesive. A few spectra of the *dough stability* and *softening* phases show similarities which might be due to the complexity of the farinographic kneading process as depicted in Fig. 5 as a function of principal component 1 and 2.

In order to investigate the nature of the distinct separation of the farinographic phases attained with the PCA, the eigenvectors associated with the first two principal components were analyzed as shown in Fig. 6. The first principal component which describes the main direction of the variance accounted by the highest peaks observed in the ferulic acid, in the riboflavin region as well as in the protein region of the spectra which was an expected feature due to the application of the GLSW spectral pre-processing. Similarly, the second principal component similarly captures variance describing the same peaks as the main one, however with some extra peaks in the riboflavin area of the spectra. The multitude of the peak

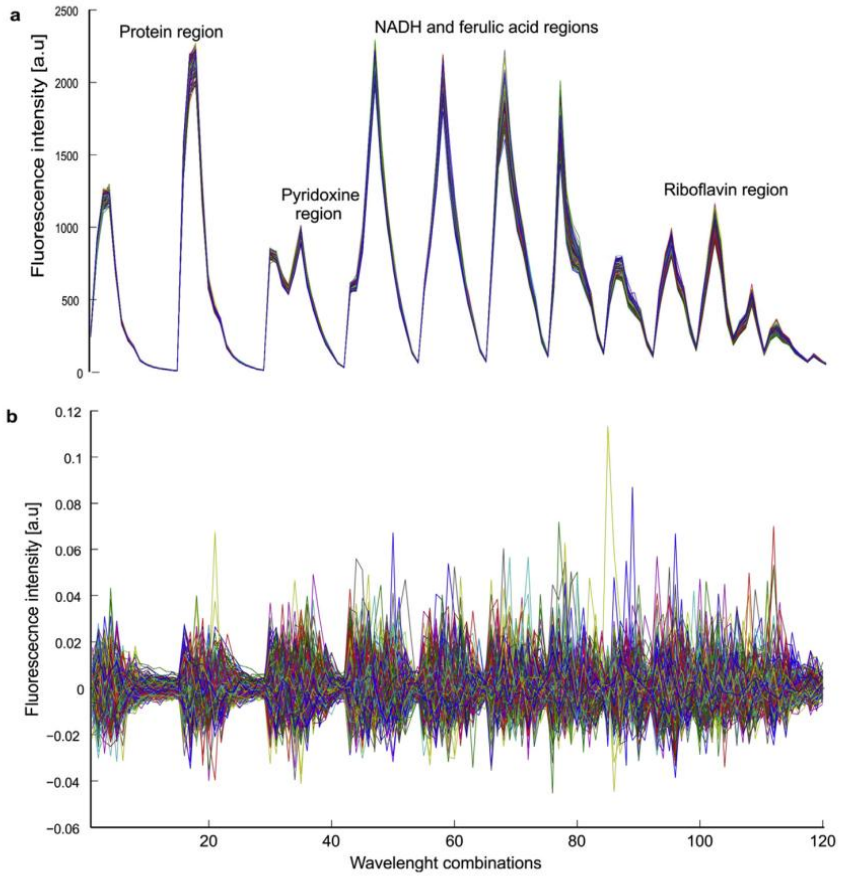

Fig. 4. Pre-processing effect onto fluorescence spectra of Rettenmeier flour: (A) Raw spectra and (B) SNV + GLSW transformed spectra.

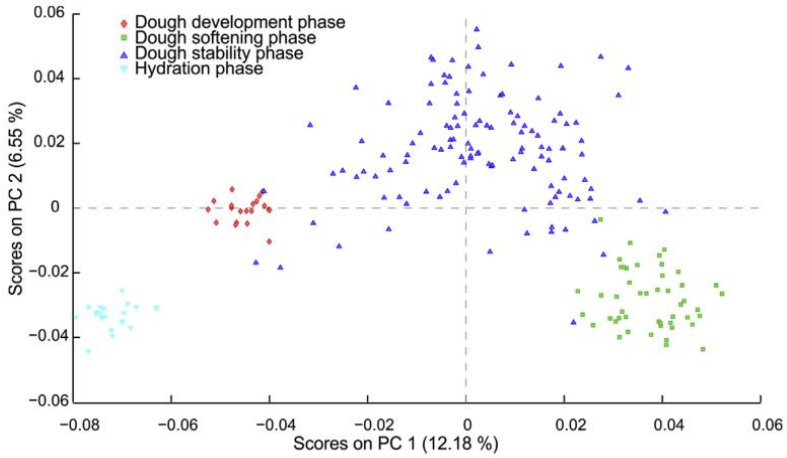

Fig. 5. Principal component analysis (PCA) scatter plot showing the separation of the different phases specific to the farinographic curve during kneading of 10 fluorescence measurements of Rettenmeier flour.

features represented with the help of PCA, suggested that the kneading is a very complex process and the interaction between different fluorophores can be associated and explained by the separation of the farinographic curve into the different phases. Fig. 6 also presents the residuals computed in the PCA process that contain the rest of the variance which in fact has the highest representation of ~82 %. It does not present any relevant information as it mainly contain variance present in the same class, not useful for

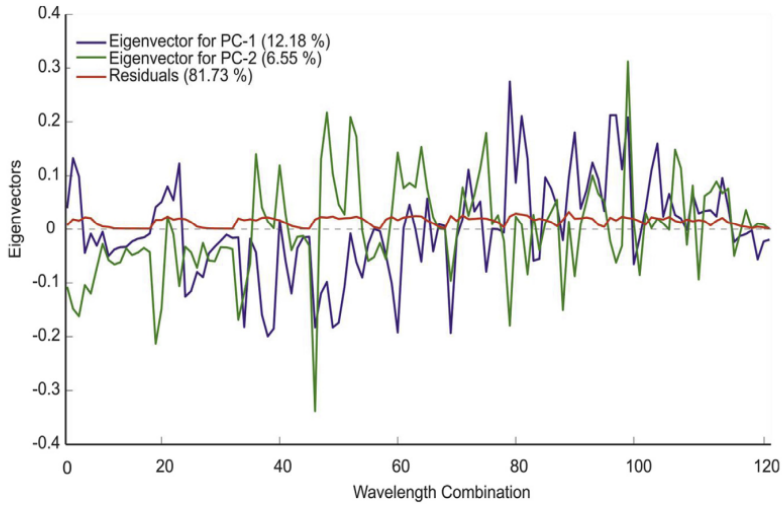

Fig. 6. Representation of the eigenvectors associated with the first two principal components for separation of farinographic phases of Rettenmeier flour out of fluorescence spectra.

the PCA modeling approach which was filtered out of the model by the GLSW pre-processing technique. The pre-processing increases however the variation between the classes which leads to a significant separation of the phases and to a very simple PCA model with only two sufficing factors. The present results revealed the similar trend as was observed in the previous application of this chemometric approach for the classification of soil using different pre-processing methodologies (Rozenstein, Paz-Kagan, Salbach, & Karnieli, 2015).

3.4. PCA for classification of the wheat flours into different groups during kneading

PCA was applied on second data set obtained by taking the fluorescence spectra of eleven different cultivars plus Rettenmeier flour during the farinographic kneading process. Different wheat cultivars used in this contribution were divided into various groups according to their bread-producing performance and analytical parameters. The wheat belonging to the quality group *E (elite)* is considered excellent while quality group *A* is good for bread making performance. The third group which is relatively less suitable for bread making belongs to group *B* and the group *C* is considered to be used for cookies, animal feed and other purposes as it is unsuitable for bread. PCA scatter plots from Fig. 7 shows a complete separation for all the wheat quality groups during the kneading process. First principal component accounts for 18.7 % variation in the data presenting a clear separation of quality group *B* and *C* from the quality group *A* and *E. Rettenmeier flour* was found to be in between these groups showing similarity with the *quality group B*. On the other hand, second principal component explains 8.49 % variation with a clear differentiation of quality group *C* from all other classes of wheat flours during the kneading process. The low explained variance (~27 %) by the first two principal components is due to the application of GLSW as described in the previous section. The eigenvectors associated with first two principal components (results not represented) showed peaks in different regions (proteins, ferulic acid and riboflavin) of the spectra. It can be the reason for differentiation of the wheat cultivars during the kneading process into different quality groups with the help of PCA as they have the different analytical and baking characteristics. The results of the present contribution are in line with the previous contribution of Cozzolino, Roumeliotis, and Eglinton (2014) who has classified barley based on year of harvesting and locality with the help of partial least square discriminant analysis using Rapid Visco Analyser (RVA) data. Similarly, Karoui et al., (2006) has used non-destructive sampling procedure to differentiate various cereal products with the help of fluorescence spectroscopy which lacks in the present contribution but it still provides a novel idea for differentiation of wheat cultivars.

3.5. Prediction of the farinogram middle curve

Middle curve of farinogram usually describes the hydration, dough development time, dough stability and softening phases. As it provides a complete overview about the farinograph, that is why, it was predicted by using PLSR modeling. The PLSR model managed to accurately predict the middle curve of the farinogram using 7 principal components. Fig. 8 represents the model fit results with a cross-validated error of 14 BU and a coefficient of determination R^2 of 0.75 which suggests that the middle curve can be decently predicted from the fluorescence spectra showing the relative error less than 10 %. Therefore, in the fluorescence signals the information regarding the farinographic curve is included. However it is not as easy to extract as more information might be hidden.

The prediction of middle curve of farinograph reported here the feasibility study using PLSR modeling out of fluorescence spectral data. It requires considerable more number of samples and complex chemometrics techniques like non-linear modeling and variable selection methodologies for improvement of the results of the present contribution to replace the farinograph from cereal industry.

4. Conclusion

Farinograph is the most popular method in the baking industry for quality monitoring and therefore for classification of the cereal products. This work presents the potential of the fluorescence spectroscopy for the characterization of the farinographic kneading processes by separating the farinographic curve into four different phases which are characterized due to the developmental and structural changes taken place during the mixing. These phases are hydration, dough development, dough stability and dough

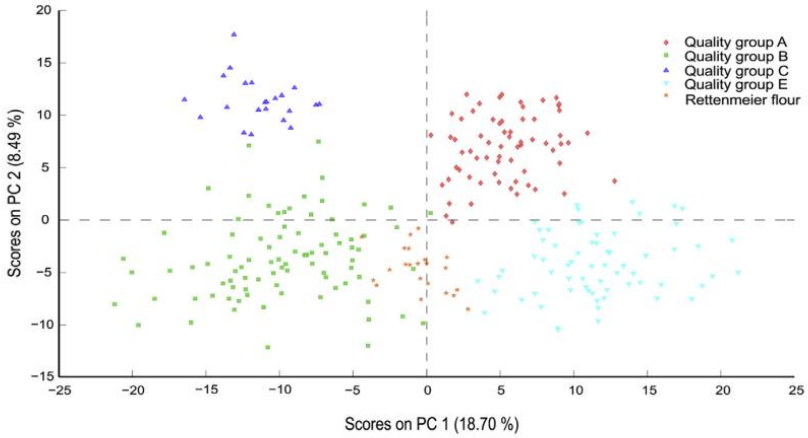

Fig. 7. Principal component analysis (PCA) scatter plot showing the separations of four different classes of wheat plus Rettenmeier flour during the kneading process.

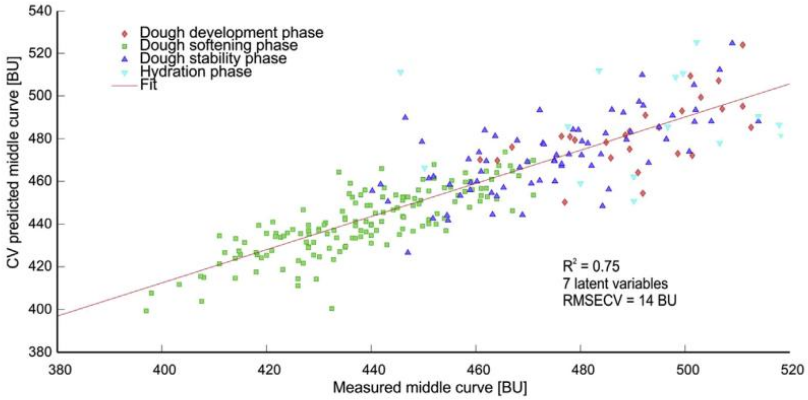

Fig. 8. PLSR scatter plot showing the measured vs. cross-validated predicted farinographic middle curve of the fluorescence spectra for 11 flours of different wheat cultivars plus Rettenmeier flour.

softening. An impact of hydration on different types of fluorophors has been explored which can be acknowledged as a decrease of intensity in the protein, NADH, ferulic acid, pyridoxine and riboflavin regions of spectra. Different quality groups of wheat according to their bread making performance can be categorized into four groups using PCA applied to the florescence spectra. Similarly the middle curve of farinogram can be decently predicted using PLSR modeling on the fluorescence spectroscopic data with an R^2 of 0.75. The characterization of the farinographic kneading process with fluorescence spectroscopy provides a new approach to estimate the changes in the dough using a cheap, fast and non-invasive alternative method. This approach will lead the way into developing soft-sensor applications for identification of the wheat cultivars and the optimum time for mixing of dough.

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Chapter 4

Estimation of the nutritional parameters of various types of wheat flours using fluorescence spectroscopy and chemometrics

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Original article

Estimation of the nutritional parameters of various types of wheat flours using fluorescence spectroscopy and chemometrics

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Summary The purpose of this study is to evaluate the potential of fluorescence spectroscopy to predict the nutritional parameters of twenty-six commercially available wheat flours from different vendors. *Principal component analysis* (PCA) was used to clearly identify the correlations among different types of flours. A *partial least square regression* (PLSR) model gives a good prediction for moisture, fat and carbohydrates using cross-validation, with a R^2 of 0.86, 0.88 and 0.89 respectively. However, the protein, sucrose and salt contents showed little correlation in PLSR. *Locally weighted regression* (LWR) provides a significant improvement in the prediction of all of the nutritional parameters. The error decreases with an increasing R^2 to 0.96, 0.93, 0.99, 0.98, 0.99, 0.88, 0.95 and 0.99 for the energetic value, protein, fat, moisture, carbohydrate, sucrose, salt and saturated fatty acid contents respectively, for different wheat flours. Hence, fluorescence, which is a non-invasive and rapid method, can be used to evaluate the nutritional parameters of different types of wheat flours.

Keywords Chemometrics, fluorescence spectroscopy, nutritional aspects, wheat flour.

Introduction

Cereal-based foods are a complex mixtures of different nutrients that play an important role in human nutrition and have a strong influence on life quality and physical fitness (Kulmyrzaev *et al*., 2007; Topping, 2007). Among the cereal-based foods, wheat is one of the most cultivated cereal crops (Goesaert *et al*., 2005) and is produced and consumed in every part of the world for different products, such as bread, cookies, cakes and pasta. Wheat flour contains macro- and micronutrients, which have a vital role in human nutrition (Topping, 2007). Therefore, the estimation of the nutritional composition of wheat flour represents an important step in the production of a flour-based end-product and is a prerequisite for its marketing. Due to increased public awareness of nutrition, there is a high demand for quick and non-invasive methods for the estimation of nutritional parameters. To date, chemical methods are widely used for these types of analyses, which are laborious, expensive and produce many non-desired waste pollutants. Fluorescence spectroscopy offers a good alternative and is recognised as rapid and efficient tool for monitoring the structural changes in fluorescent molecules (fluorophores) present in the particular food or ingredient as a result of analytical treatment, storage and contamination (Karoui & Blecker, 2011). Chemometric tools (descriptive and predictive methods) are a useful fingerprinting method when mixed fluorophores are scanned in the fluorescence spectrum, as has been performed in cereals (Zeković *et al*., 2012), culinary oils (Sikorska *et al*., 2005) and meat (Sahar *et al*., 2016). Similarly, chemometrics has been applied for online process monitoring of sourdough fermentation to predict the pH and degree of acidity at different temperatures and dough yields (Grote *et al*., 2014).

Theoretically, fluorescence spectroscopy cannot be used to analyse non-fluorescent molecules; however, it has been employed to estimate them indirectly due to the correlation with fluorescent molecules. Fluorescence spectroscopy is applied to estimate glucose during online monitoring of bio-processes (Ohadi *et al*., 2015) and to predict the vitamin C content due to the changes in heat-treated infant formulas (Diez *et al*., 2008). Quantification of glucose, fructose and sucrose in figs (Jiang *et al*., 2013), the prediction of acrylamide in biscuits during the baking process (Sereys *et al*., 2013) and the estimation of fat and fatty acid in meat (Aït-Kaddour *et al*., 2016) have increased the importance of this type of spectroscopy for the determination

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of non-fluorescent molecules. On the other hand, the prediction of riboflavin in yogurt (Becker *et al.*, 2003) and wheat flours (Zandomeneghi *et al.*, 2003) proves the applicability of fluorescence spectroscopy in determining the unique nutritional profile.

Similarly, other spectroscopic techniques are used in directly or indirectly estimating the nutritional parameters for different types of foods. NIR has been employed to determine the energetic value and macronutrients in commercially available homogenised meals (Kim *et al.*, 2007), whereas FTIR is applied for the direct evaluation of the energetic value, carbohydrates, protein and indirect prediction of calcium content of different yogurt samples (Moros *et al.*, 2006). Furthermore, Raman spectroscopy is used in determining fat, carbohydrate and other nutritional parameters in milk powders and infant formulas (Moros *et al.*, 2007). Hence, these types of applications are important in determining the nutritional parameters not only in the food industry, but can also be employed in the hotel management chain, home use and in hospitals for diabetics and other types of patients (Moustakas & Pitris, 2009).

Fluorescence spectroscopy has been used in determining unique nutritional parameters but has never been applied to predict the complete nutritional profile like other spectroscopic techniques. Therefore, in the present study, the potential of fluorescence spectroscopy was analysed to determine the nutritional parameters of wheat flours with the help of linear and non-linear chemometrics. Here, principal component analysis (PCA) is used to differentiate among different types of wheat flours. Nutritional parameters, such as the energetic value, protein, fat, carbohydrates and other nutrients, of different types of wheat flours are predicted using partial least square regression (PLSR) and locally weighted regression (LWR) based on the fluorescence spectra.

Materials and methods

Raw materials

Twenty-six samples of various types (405, 550, 1050 and whole wheat flour) of commercially available wheat flour from different vendors were purchased from the local supermarket. A total of 9, 8, 4 and 5 samples of type 405, 550, 1050 and whole wheat (WWF) flour respectively, were used. This classification of the commercially available wheat flours into different types is based on the different mineral profiles corresponding to the respective extraction rate during milling using the German standard DIN 10355 (Scholenberger *et al.*, 2002). The nutritional parameters of the different types of wheat flours were recorded from the packing label as given in Table 1. The moisture content of the samples of different types of flours was also determined using an infrared moisture analyser (MA 51; Sartorius AG, Göttingen, Germany). Analysis of variance (ANOVA) and hierarchical analysis were applied to interpret the recorded nutritional parameters of the various types of wheat flours.

Data collection by fluorescence spectroscopy

The fluorescence spectra were acquired using the 2D-fluorescence spectrophotometer BioView® (Delta Light and Optics, Hørsholm, Denmark). BioView® uses two filter-wheels mounted with fifteen filters, which are used for the excitation and emission spectra. These filters select the 20 nm step-widths of excitation and emission light in the range of 270–550 and 310–590 nm respectively. A xenon flash lamp is used for excitation and is located in front of the filter-wheel. After passing through the light guide, the excited light goes into the optical well then into the sample. The fluorophores are irradiated with the respective wavelength, and the fluorescent light is sent back through the second optical fibre towards the emission filter. The filter-wheel shifted to the next filter of excitation of wavelength and fluorescence spectrum was taken through the complete cycle of emission. The data are transferred to the computer through an infrared single fibre modem. The computer is equipped with software that controls the BioView sensor and also performs the analysis of the data (Lindemann *et al.*, 1998; Rossi *et al.*, 2012). Three spectra for each wheat flour were taken and then averaged. The data set comprises 104 spectra for all twenty six types of wheat flours, containing four spectra from each sample, which make a block. Hence, twenty-six blocks were created containing four spectra in one block of the same flour sample.

Spectral data analysis

Principal component analysis (PCA), *partial least square regression* (PLSR) and *locally weighted regression* (LWR) chemometric methods were used to evaluate the nutritional parameters from the spectral data sets. The chemometric evaluation was performed on a MATLAB 2013b (The Mathwork™, Natick, MA, USA) platform using PLS toolbox (7.5; Eigenvector Research, Inc., Manson, WA, USA) as well as the *Unscrambler software* (*Version 10.3; CAMO Software AS, Oslo, Norway*).

Pre-processing of spectral data

Pre-processing of the spectral data is a mandatory procedure prior to any type of spectral quantitative or qualitative evaluation to make the extracted information more accessible and to improve the goodness of fit in the chemometric modelling approaches (Nache *et al.*, 2015).

Table 1 Commercial wheat flours from different vendors and their nutritional parameters

Sample no.	Types of flour	Energy value (kJ/100 g)	Moisture (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Sucrose (%)	Salt (%)	Saturated fatty acids (%)
1	405[†]	1457	12.74	10.0	1.0	72.0	0.70	0.010	0.10
2	405	1446	13.43	10.0	1.0	71.0	0.40	0.010	0.20
3	405	1446	12.44	12.0	1.0	69.0	0.40	0.010	0.10
4	405	1480	13.36	11.0	1.0	72.0	0.70	0.010	0.20
5	405	1433	12.34	11.0	1.1	69.0	0.50	0.010	0.10
6	405	1483	13.16	12.8	0.7	71.0	0.50	0.005	0.20
7	405	1470	13.64	10.6	1.0	71.8	0.40	0.005	0.20
8	405	1467	11.94	9.8	1.0	70.9	0.43	0.002	0.15
9	405	1459	13.52	10.0	1.0	72.3	0.70	0.003	0.10
10	550[‡]	1444	11.70	9.8	1.1	70.8	0.43	0.002	0.17
11	550	1474	12.58	11.0	1.1	72.0	1.10	0.010	0.20
12	550	1409	13.33	10.0	1.1	71.0	0.40	0.002	0.20
13	550	1474	14.00	10.6	1.1	72.0	1.10	0.005	0.20
14	550	1480	13.08	11.0	1.1	72.0	1.10	0.010	0.20
15	550	1447	12.05	9.8	1.1	71.0	0.50	0.010	0.20
16	550	1468	11.18	9.8	1.1	70.8	0.43	0.002	0.17
17	550	1468	12.24	9.8	1.1	70.8	0.43	0.002	0.17
18	1050[§]	1459	11.67	11.6	1.8	67.7	0.74	0.002	0.26
19	1050	1454	12.28	12.1	1.7	67.2	0.70	0.005	0.30
20	1050	1383	12.20	12.0	1.8	67.0	0.50	0.002	0.30
21	1050	1459	11.17	11.6	1.8	67.7	0.74	0.002	0.26
22	WWF[¶]	1381	11.61	11.0	1.8	60.0	0.60	0.020	0.30
23	WWF	1373	11.60	11.4	2.4	59.5	0.72	0.003	0.34
24	WWF	1373	11.58	11.4	2.4	59.6	0.70	0.008	0.30
25	WWF	1374	10.85	11.4	2.4	59.5	0.72	0.003	0.34
26	WWF	1376	11.23	11.0	2.4	60.0	0.70	0.010	0.30

[†]Flour type 405 contains less than 0.5% mineral contents.
[‡]Flour type 550 contains 0.5–0.63% mineral contents.
[§]Flour type 1050 contains 0.91–1.2% mineral contents.
[¶]Whole wheat flour contains 1.2–1.8% mineral contents.

Before applying any chemometric evaluation, the spectral data were pre-processed using solo and combinations procedures of *standard normal variate* (SNV) and 2nd derivative to remove the scattering effect induced by the spectra acquiring procedures and changes due to the diversity of particles during the measurements.

SNV transformation (Barnes *et al.*, 1989) is a scattering correction technique in which the mean and standard deviation of each spectrum are used. The mean spectrum is subtracted from each data point and then divided by its standard deviation. Eqns (1) and (2) were used to calculate the SNV transformation

$$X_{\mathrm{SNV}i} = (X_i - \overline{X})/\sigma_X \quad (1)$$

$$\sigma_X = \sqrt{\left(\sum_{i=1}^{n} (X_i - \overline{X})^2\right)/(n-1)} \quad (2)$$

here, $X_{\mathrm{SNV}i}$ is the scattering corrected spectral data point of X_i, which is the ith variable of the spectra. $\overline{X}$ and n are the mean and the total number of variables respectively. Then, SNV transformed data were subjected to the second derivative with fifteen window points using the Savitzky–Golay method.

Chemometric models

Principal component analysis (Clément *et al.*, 2010) is one of the basic methods of exploratory data analysis and reduces a large set of spectral data into a small number of principal components without loss of major information. The first latent variable has the highest variance, and each successive latent variable describes a relatively lesser amount of variance. In our approach, PCA was applied to separate the different types of flour on the set of spectral data, which is pre-processed by SNV.

Partial least square regression is a quantitative chemometric tool that decomposes the spectral data and reference values simultaneously to maximise the covariance between them by compressing the data into

a smaller number of factors, which leads to a linear relationship (El Masry *et al.*, 2012). In the present contribution, PLSR was used on pre-processed spectral data to determine the nutritional parameters of the wheat flours. The spectra belonging to the same flour sample were put into one block. Cross-validation was performed using PLSR by the leave one block out method. Calibration was performed using twenty five blocks every time, and the last block was used for testing the model. This process was repeated until all the blocks were used to predict the nutritional value of wheat flours. In another approach, an SNV transformed data set was divided into 70% and 30% for calibration and prediction respectively, to estimate the nutritional parameters of the wheat flours.

A non-linear regression method was used to predict the nutritional parameters for different types of wheat flours to improve the linear regression modelling results. Locally weighted regression (LWR) (Naes *et al.*, 1990) was applied to estimate the non-linear correlations between some of the dependent variables and the spectral data. LWR is based on the application of the local models around the point of interest on different subsets of the data to predict the y values (Nache *et al.*, 2015). The LWR algorithm was applied using twenty-six local points for weighing to predict every sample using two latent variables in the models. The LWR models were validated using the leave one out cross-validation technique.

Goodness of the fit in linear and non-linear regression

The goodness of a model fit was evaluated using the *root mean square error of cross validation* (RMSECV) and the coefficient of determination (R^2). The RMSECV and R^2 were calculated using eqns (3) and (4) (Zhu *et al.*, 2015).

$$\mathrm{RMSECV} = \sqrt{\sum_{i=1}^{n}(m_i - p_i)^2/n} \qquad (3)$$

$$R^2 = 1 - \sum_{i=1}^{n}(m_i - p_i)^2 \Big/ \sum_{i=1}^{n}(m_i - \overline{m})^2 \qquad (4)$$

here, m_i, p_i, $\bar{m}$ and n are the measured, predicted, mean value and number of observations respectively. The R^2 values ranging from 0.5–0.65, 0.66–0.81, 0.82–0.91 and above 0.91 describe poor, moderate, good and excellent prediction respectively (Kulmyrzaev *et al.*, 2007).

Results and discussion

Table 1 describes the measured nutritional parameters of all the evaluated categories of wheat flour, which shows the variation in the data set. For example, the energetic value of whole wheat flour is very low compared to the other types of flours due to the presence of high dietary fibre, which has no role in the energy value (Topping, 2007). Similarly, other nutritional parameters also show the variation, which can be elaborated by application of analysis of variance (ANOVA). The results of the ANOVA (not shown) show that flours 550 and 405 are not significantly different from each other, although they were from different vendors. However, flour 1050 varies significantly in carbohydrate, protein and fat contents from the aforementioned flour types. Furthermore, whole wheat flour (WWF) is completely distinct from the other types of the flour used in this research. These variations in the data set are important for developing a robust and accurate calibration model.

Characteristics of the fluorescence spectra of flours

In Fig. 1, the contour plots of different types of wheat flours are presented. Each spectrum of the wheat flours shows three distinct peaks that can be attributed to the different types of the fluorophores. The highest peak was observed at $\lambda_{excitation}$ 310–370 nm and $\lambda_{emission}$ 410–470 nm, which can be attributed to ferulic acid (Garcia *et al.*, 2016) and NADH, which are naturally present in the wheat flour. Another peak was observed at $\lambda_{excitation}$ 270–310 nm and $\lambda_{emission}$ 310–370 nm, which belongs to the protein area of the spectra due to the fluorescence of tryptophan, phenylalanine and tyrosine (Grote *et al.*, 2014). This peak has the lowest intensity for whole wheat flour (Fig. 1d) due to the cascade effect as a result of the ferulic acid content (Garcia *et al.*, 2016). The third peak, which is attributed to the riboflavin content of the wheat flour, falls in the range of $\lambda_{excitation}$ 410–450 nm and $\lambda_{emission}$ 510–550 nm (Zandomeneghi *et al.*, 2003). The whole wheat flour has relatively higher intensity in this region of the spectrum compared to the other types of the flour due to the presence of more riboflavin. Pyridoxine and vitamin E also show peaks in their respective regions, but they are masked due to the high intensity in the ferulic acid region of the spectrum. All these differences in the different regions of the fluorescence spectra can be further explored to describe the correlation among various types of flours using principal component analysis (PCA).

Exploratory analysis of the spectral data using principal component analysis

The exploratory evaluation of the spectral data of the different types of wheat flour was performed with the help of the PCA data analysis technique, which is used to reduce the high dimensionality of the spectral data to a set of compressed vectors (principal components, PC) that are easier to evaluate. The presented scatter plots indicate clear separation among the scores belong-

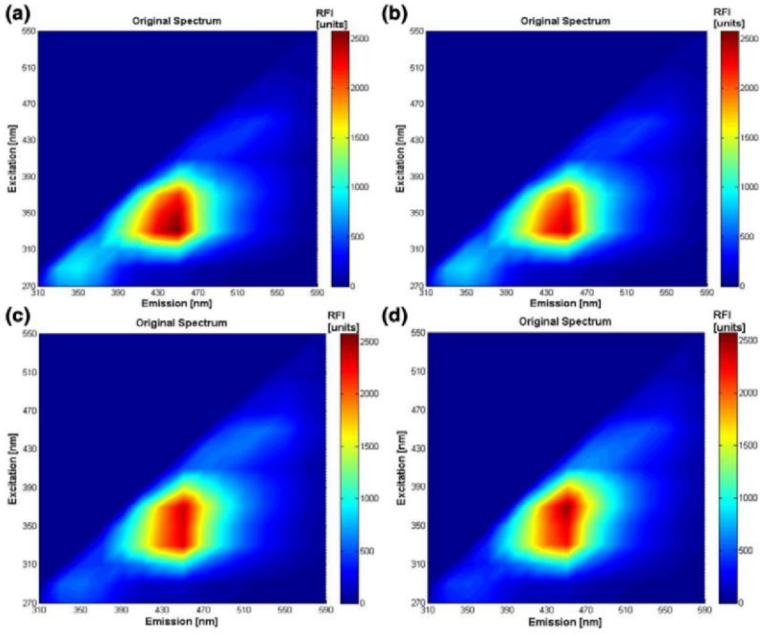

Figure 1 Representation of the contour plots for different wheat flours, type 405 (a), type 550 (b), type 1050 (c) and whole wheat flour (d).

ing to the various types of wheat flours, as shown in Fig. 2. After the SNV transformation, most of the variance present in the data set was captured in the first two principal components, which account for up to 96% of the variance in the data. The scores for flour types 405 and 550 are highly correlated and are located in the same area due to their comparable nutritional values for carbohydrates, fat and the protein. These flour types are in contrast with the remaining flours, type 1050 and the whole wheat flour. The whole wheat flour has different protein, vitamins and cofactors (NADH, FMN and FAD) compared to the other types of flours, which separate themselves in a non-correlated score space region in the plot. Similar results were obtained using the hierarchical analysis applied to the nutritional parameters of the wheat flours (dendrogram not shown), which confirms the results of the PCA.

Nutritional parameters prediction using partial least square regression

Table 2 presents the PLSR, which includes the evaluation results of the cross-validated chemometric models used to estimate the prediction performances of the nutritional parameters of the wheat flours. Here, the fat, carbohydrates and moisture contents provided a good prediction from the fluorescence spectral data, expressing an increased coefficient of determination (R^2) and a decreased prediction error. The prediction of the protein, sucrose and the salt contents produced linear regression models with a lower performance compared with the aforementioned prediction models because the R^2 levels did not reach an acceptable level. However, the energetic value was predicted with the cross-validated model error settled at 22.73 kJ/100 g with an R^2 of 0.65, indicating poor prediction. Similarly, the saturated fatty acid contents of the different types of wheat flours also have a moderate correlation with an R^2 of 0.76.

Figures 3a, b and c show the scatter plots for the measured and predicted parameters. In the case of fat determination, the RMSECV reached 0.18%, whereas the R^2 settled at 0.88 and the carbohydrates prediction gave a 1.49% RMSECV with an R^2 of 0.89. Furthermore, the RMSECV was 0.32% and the R^2 reached 0.86 for the moisture determination. The per cent error was less than 10% in the prediction case for all the aforementioned parameters.

The results of the PLSR modelling by dividing the data set into 70% and 30% for calibration and prediction respectively are given in Table 3. The fat, carbohydrate, moisture and saturated fatty acid can be accurately predicted. The root mean square error of prediction (RMSEP) was 0.13%, 1.04% and 0.33% for fat, carbohydrate and moisture respectively with R^2 in the range of good prediction. The energetic value can be moderately predicted with a RMSEP 18.47 kJ/100 g. The other parameters, including the protein, sucrose and salt contents, did not have good prediction ability, providing a high RMSECV.

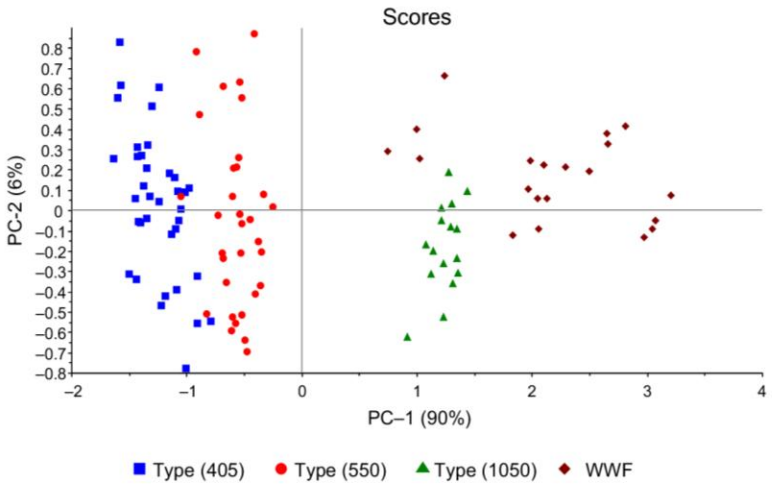

Figure 2 Principal component analysis (PCA) score plot showing the separation of different types of wheat flours using PCA with 2 PCs.

Table 2 Statistics of cross-validated PLSR modelling for prediction of the nutritional parameter of wheat flours

Parameter	RMSECV†	R^2_{CV}‡
Energy value (kJ/100 g)	22.730	0.65
Moisture (%)	0.320	0.86
Protein (%)	0.740	0.24
Fat (%)	0.180	0.88
Carbohydrate (%)	1.490	0.89
Sucrose (%)	0.180	0.26
Salt (%)	0.004	0.17
Saturated fatty acids (%)	0.036	0.76

†Root mean square error of cross-validation.
‡Coefficient of determination for cross-validation.

Non-linear regression approach for the nutritional parameters prediction

LWR was employed to estimate the nutritional parameters of wheat flours with the target of improving the prediction for some of the nutritional attributes, which are not reliably predicted by PLSR.

The results of the LWR technique are shown in Table 4. LWR significantly improves the predictive ability of the nutritional parameters of wheat flours compared with the results of the linear regression approach presented in Table 2 for the fluorescence spectral data. The results regarding all nutritional parameters (except sucrose) showed excellent R^2 with reduced RMSECV (Table 4). With reference to Tables 2 and 4, the R^2 shifts from 0.24 to 0.93 for the protein content, with a reduction of the RMSECV from 0.74% to 0.23% using LWR. Similarly, LWR improved the results of the energetic value and saturated fatty acids by minimising the RMSECV from 22.73 kJ/100 g to 7.61 kJ/100 g and 0.036% to 0.007% respectively showing excellent R^2 compared with the results of the PLSR modelling. Furthermore, the sucrose contents of the wheat flour showed significant enhancement in the predictive model using this type of approach, leading to a good $R^2 = 0.88$, which is slightly lower compared to the other nutritional parameters. The improvement in the results indicates that there is a non-linear relationship between the spectroscopic data and the nutritional parameters of the wheat flours.

Discussion of the results

In this contribution, fluorescence spectroscopy shows strong potential to predict the nutritional parameters of wheat flour. The chemometrics tools, including PCA, PLSR and LWR were applied to extract the useful information from the fluorescence spectra to obtain robust and accurate predictive models. PCA describes the separation of the various types of flours due to the fluorescence of different fluorophores, such as protein, ferulic acid, NADH and vitamins, including riboflavin (Zandomeneghi *et al.*, 2003), because they have different compositions. PLSR modelling has shown a good linear relationship with some of the nutritional parameters of the wheat flours, such as fat, carbohydrate and moisture (Figs 3a, b and c). These parameters are non-fluorescent, and the exact reason for their prediction is based on assumptions. Because flour is a complex mixture of different fluorescent and non-fluorescent molecules whose interaction and correlation can be a reason for the good prediction of the aforementioned parameters. For example, vitamin E is fat soluble, is present in wheat flour (Nielsen & Hansen, 2008) and shows fluorescence in the region of $\lambda_{excitation}/\lambda_{emission}$ = 295/340 nm (Schulman, 1985). The

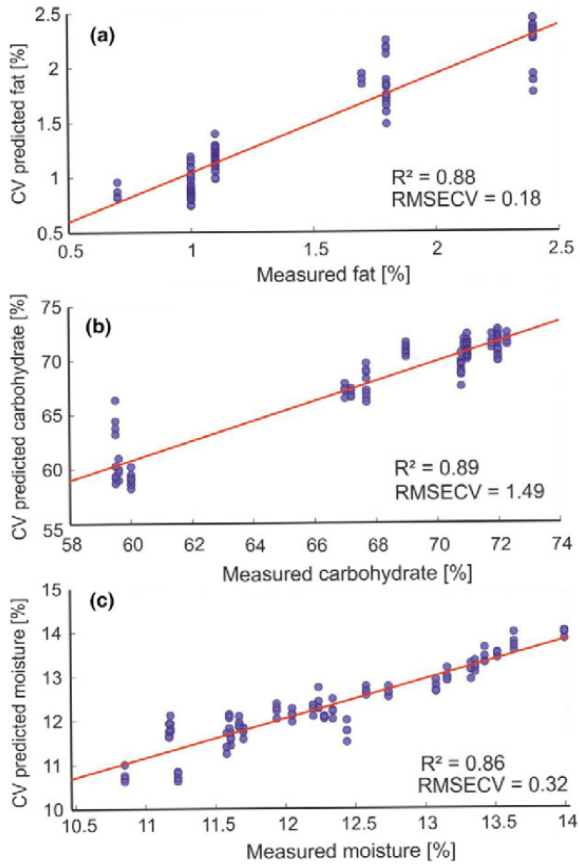

Figure 3 Representation of measured vs. CV predicted plots for fats (a), carbohydrates (b) and moisture (c) using PLSR model for different types of wheat flours (RMSECV in %).

Table 3 Statistics of calibration and validation PLSR modelling for nutritional parameters of wheat flours

Parameter	RMSEC†	RMSEP‡	R^2§
Energy value (kJ/100 g)	15.860	18.470	0.77
Moisture (%)	0.330	0.330	0.86
Protein (%)	0.630	0.740	0.27
Fat (%)	0.130	0.130	0.93
Carbohydrate (%)	1.040	1.140	0.93
Sucrose (%)	0.140	0.170	0.47
Salt (%)	0.003	0.003	0.35
Saturated fatty acids (%)	0.029	0.030	0.81

†Root mean square error of calibration.
‡Root mean square error of prediction.
§Coefficient of determination.

fluorescence of this vitamin may be the reason for the prediction of fat in the different types of wheat flours. The prediction of total lipids (R^2 = 0.68) and the saturated fatty acid composition (R^2 = 0.66) in meat muscles using this technique strengthens the present contribution (Aït-Kaddour *et al.*, 2016). However, taking the full spectra of different types of flour instead of the specific wavelength combinations might be the reason for the better prediction performance of the fat content (R^2 = 0.88) and saturated fatty acids (R^2 = 0.76) using the PLSR modelling in the present work compared to the aforementioned study regarding meat. Furthermore, no sample preparation operations (freeze-drying and powder formation) provide significant advantage over the previous contributions. Similarly, the hydration of the wheat flour has a strong impact on the fluorescence intensity in different regions of the spectra (Garcia *et al.*, 2016), which may be correlated with the estimation of moisture, whereas indirect determination of the glucose during the bioprocess (Ohadi *et al.*, 2015) and in figs (Jiang *et al.*, 2013) using this type of spectroscopy can be related to carbohydrate prediction. The other parameters, such as the protein, sucrose and salt contents, do not have a linear relationship (Tables 2 and 3). Therefore, LWR (a non-linear approach) has strong potential to improve the performance of the model described by (Nache *et al.*, 2015) for the prediction of pH and lactate content of the meat. LWR led to better predictive models (Table 4), which indicates that this approach is adequate for the prediction of nutritional parameters. The results of the present contribution showed the better estimation of the nutritional attributes compared to the previous findings of Moros *et al.* (2006), who has used FTIR for the prediction of the nutritional profile in yogurt samples (unable to predict fat) and Ferreira *et al.* (2013) for the proximate composition of soybeans with NIR spectroscopy (R^2 = 0.63–0.81). However, the results generated by Raman spectroscopy for the nutritional parameters in infant formula are superior to the present contribution due to lower prediction errors (Moros *et al.*, 2007). Moreover, this contribution suggests the

Table 4 *Locally weighted regression* (LWR) for calibration of nutritional parameters of wheat flours using cross-validation

Parameter	RMSECV†	R^2_{CV}‡
Energy value (kJ/100 g)	7.6100	0.96
Moisture (%)	0.0940	0.98
Protein (%)	0.2270	0.93
Fat (%)	0.0350	0.99
Carbohydrate (%)	0.4030	0.99
Sucrose (%)	0.0760	0.88
Salt (%)	0.0009	0.95
Saturated fatty acids (%)	0.0070	0.99

†Root mean square error of cross-validation.
‡Coefficient of determination for cross-validation.

potential of fluorescence spectroscopy for the determination of the complete nutritional profile, which is important for the labelling of food products. The obtained model using LWR with excellent predictive performance for the nutritional parameters could be applicable in the baking and the cereal-based food industries to predict the quality attributes of the end-product.

Conclusion

This contribution discusses the prediction of the nutritional parameters of different types of wheat flour based on fluorescence spectroscopy. Linear regression approaches, such as PLSR, can predict the fat, carbohydrate and moisture contents of all wheat flour types and provide a moderate prediction for the energetic value and saturated fatty acids content. However, the protein, sucrose and salt contents show poor correlation using this modelling technique. Therefore, a nonlinear evaluation technique was applied. LWR showed excellent correlation for proteins, salt and sucrose and improved the prediction of the fat, moisture, and carbohydrate levels and saturated fatty acid content; as well as the prediction of the energetic value of the different types of wheat flours. In short, fluorescence spectroscopy can be used for the prediction of the nutritional parameters of wheat flour. This technique may be applicable to the other types of foods, but more research is needed.

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Chapter 5

Concluding remarks

Concluding remarks

This thesis illustrates the usefulness of fluorescence spectroscopy for characterization of wheat flours and its dough preparations in combination with chemometric tools. This was demonstrated by retrieving hidden information in fluorescence spectra of the wheat flour using chemometrics to predict the critical parameters (protein, dough development time and water absorption etc.) which are important for wheat based industry. This approach is helpful to develop a rapid and non-invasive sensor based on fluorescence to characterize wheat flour by just taking the spectral signature which in turn predicts the final product quality.

Kneading of the dough is a complex process operation which undergoes a number of changes particularly the development of three dimensional gluten networks. Different instruments are being used in kneading of dough like farinograph. It estimates the water absorption and dough profile like dough development time, dough stability and softening. These characteristics are important for prediction of the quality of intermediate and end product during processing. Classification of farinographic curve using PCA into hydration, dough development, stability and softening phases out of fluorescence spectra has open the new horizons for development of sensors which can be helpful for determination of optimum dough development time. Furthermore, quantification of farinographic curve from fluorescence using PLSR modeling can predict the quality characteristics of the dough. Similarly, classification of different wheat cultivars into different groups like E, A, B and C on bread making performance using PCA during the kneading process gives a new idea to categorize the wheat according to its respective use. It is important for the industrial point of view for screening and regulatory purposes.

Food labeling is an important step for marketing due to the high awareness of the consumers towards nutrition. Conventional methods are being used which are laborious and based on chemicals. Prediction of complete nutritional parameters of wheat flour using PLSR and LWR with the help of fluorescence spectroscopy provides a new vision for food labeling by just taking the spectral reading rapidly and non-invasively. More research in this direction leads this approach in other food applications by just taking the spectral signature of the sample.

In short, fluorescence spectroscopy seems to be outstanding analytical tools for characterization of the wheat flour and its dough preparations based on the finding of present investigations. As the above mention approaches were conducted on laboratory scale using a small number of samples. Therefore, it is recommended to increase the number of sample to make them feasible

to adopt by the industry to combat with the growing concerns in technology for attaining high standards of quality and safety. Furthermore, application of the abovementioned techniques can shift the batch process operations to continuous which show a huge advantage what the conventional analytical methodologies can not offer.

UNIVERSITÄT HOHENHEIM

FACULTY OF NATURAL SCIENCES

Annex 2 to the University of Hohenheim doctoral degree regulations for Dr. rer. nat.

Affidavit according to Sec. 7(7) of the University of Hohenheim doctoral degree regulations for Dr. rer. nat.

1. For the dissertation submitted on the topic

   Fluorescence Spectroscopy and Chemometrics: An Innovative Approach for Characterization of Wheat Flour and Dough Preparation

   I hereby declare that I independently completed the work.

2. I only used the sources and aids documented and only made use of permissible assistance by third parties. In particular, I properly documented any contents which I used - either by directly quoting or paraphrasing - from other works.

3. I did not accept any assistance from a commercial doctoral agency or consulting firm.

4. I am aware of the meaning of this affidavit and the criminal penalties of an incorrect or incomplete affidavit.

I hereby confirm the correctness of the above declaration: I hereby affirm in lieu of oath that I have, to the best of my knowledge, declared nothing but the truth and have not omitted any information.

15-04-2016 Hohenheim

Place and Date

Signature