Hyperspectral imaging system to online monitoring the soy flour content in a functional pasta

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Abstract

Pasta enriched with soy flour can be considered as a functional food, due to its content in nutraceutical compounds such as isoflavones, carotenoids, and other antioxidants. The quantification of the amount of a functional ingredient is an important step in food authenticity. The availability of non-destructive techniques for quantitative and qualitative analyses of food is therefore desirable. This research aimed to investigate the feasibility of hyperspectral imaging in reflectance mode for the evaluation of the soy flour content, also to investigate the possibility of implementing a feed-back control system to precisely dose the soy flour during the industrial production of pasta. Samples of pasta in shape of spaghetti were produced with durum wheat semolina and soy flour at increasing percentages (0, to 50%, steps of 5%). A feature selection algorithm was used to predict the amount of soy flour. The most influent wavelengths were selected, and a six-term Gauss function was trained, validated, and tested. The identified transfer function was able to predict the percentage of soy flour with high accuracy, with an R^2_{adj} value of 0.98 and a Root Mean Square Error of 1.31. The developed system could represent a feasible tool to control the process in a continuous mode.

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Introduction

Pasta is a very popular food obtained by extrusion, lamination, or shaping on a belt, generally followed by drying, of a mixture of semolina and/or wheat flour and water. The amount of world pasta production is reported to be around 17 million tons and the main producer is the European Union (32,8%), followed by the other European countries (17.9%), Central and South America (17.7%), Africa (13.8%), and North America (12.9%) (IPO, 2021). Always in 2021, the top 5 consumers are Italy (23.5 kg), Tunisia (17 kg), Venezuela (15 kg), Greece (12.2 kg), and Perù (9.9 kg). Despite the data referred to production and consumption are considerable and although pasta has been included within the intangible cultural heritage list (Giannetti *et al.*, 2021), this traditional product is in the maturity stage of its life cycle. This state of things implies that the price can be secondary to quality in consumer choice. In addition, despite having a long-shelf-life and being a good and cheap source of carbohydrates, pasta lacks proteins and functional bioactive compounds, factor that can slow down its consumption by people interested in a healthier lifestyle (Bianchi *et al.*, 2021). In order to increase purchase and expand consumer targets, new pasta products have been developed including functional pasta, which can be consumed as part of the normal diet but that contains bioactive components able to enhance health or reduce risk of disease (Di Monaco *et al.*, 2004). Functional foods are gaining an increasing interest with an annual average growth rate of about 8.5%. and a global market of around 300 billion dollars (Bogue *et al.*, 2017). Although, functional dairy products have the biggest market share, followed by beverages and cereals (Stein and Rodríguez-Cerezo, 2008), according to the World Health Organization and the Food and Drug Administration, the fortification of pasta with valuable ingredients assumes great nutritional importance since pasta is a useful carrier for compounds acting as nutrition enhancers or improving some physiological functions. It can be functionalized by fortification with vitamins, minerals, vegetable fibers, probiotics, or prebiotics or by partial replacement of wheat flour with other ingredients (Fares *et al.*, 2015; Omeire *et al.*, 2014; Pasqualone *et al.*, 2016; Romano *et al.*, 2021; Wang *et al.*, 2021). The demonstration of its suitability is given by the fact that pasta has a well-documented, long-standing research history of partial or total substitution of the durum wheat semolina with ingredients from vegetable and animal sources, including insects (Duda *et al.*, 2019; Nilusha *et al.*, 2019). Although pasta produced from durum wheat flour has excellent rheological properties of the dough and high cooking and sensorial quality, the addition of flours from various high-protein sources allows the improvement of its nutritional properties (Fuad and Prabhasankar, 2010). In particular, the replacement with soy (*Glicine max* L.) flour is attractive because it is abundant and inexpensive if compared to animal proteins. Furthermore, soybean seeds contain: over 40% protein with essential amino acids, whose amounts often closely match those required for humans or animals, 30-35% carbohydrates, 20% fat with an interesting content of linoleic acid; many bioactive compounds, including vitamin B6, folate vitamin E, isoflavones, lecithin, saponins, oligosaccharides, and phytosterols (Dulger and Hallac, 2020; Tripathi and Mangaraj, 2011). Thanks to this variety of compounds, soy is able to exert beneficial effects by reducing risks of coronary heart disease and some cancers and lowering cholesterol and glycemic index (Messina, 2003; Wietrzyk *et al.*, 2005). Furthermore, soy proteins have remarkable technological effects, having emulsifying, gelling, water, and oil-holding capacity (Nishinari *et al.*, 2014; Wietrzyk *et al.*, 2005). Concerning the impact of soy flour addition on pasta, according to Baiano *et al.* (2011), it can increase the optimal cooking time and decrease the release of organic matter whereas the sensory response is similar for semolina and semolina-soy spaghetti. A different opinion was expressed by Kamble *et al.* (2019), who found that increasing the addition of soy flour reduces pasta's optimum cooking time, increases cooking loss, and alters all sensory characteristics. The amount of a functional ingredient in a food is of fundamental importance since it contributes both to the nutritional and economic value of the products. In addition to destructive analytical techniques, techniques that allow the evaluation of the sample in a rapid and non-destructive way are taking place. Hyperspectral imaging (HSI) integrates conventional imaging and spectroscopy to obtain spatial and spectral information of an object (Gowen *et al.*, 2011). An HSI system can be used for quantitative and qualitative analyses by recording the spectral characteristics of samples and for classifying objects based on their spectral properties through the building of mathematical model based on algorithms (Liu *et al.*, 2017). Multispectral and hyperspectral imaging have been successfully used for the identification and quantitation of durum wheat grain samples in relation to pasta authenticity. These techniques were suitable to rapidly distinguish between durum wheat and common wheat cultivars and to assign percentage adulteration levels (Wilkes *et al.*, 2016).

This research aimed to investigate the feasibility of the application of hyperspectral imaging for the estimation of the amount of soy flour in the semolina-soy flour mixtures used to produce functional pasta. This is to find an easy and precise way to control the dosing of functional ingredients during the pasta production process. In particular, the hyperspectral imaging system was used to distinguish between durum wheat flour (semolina) and soy flour based on their spectral signatures, using pasta samples in which increasing amounts of semolina were replaced by soy flour. A mathematical model was developed to assess the correlation between the reflectance of pasta samples and the percentage of soy flour used for their production in the way to develop a mathematical model to be used as a transfer function to control the correct dose of soy flour in feed-back continuous mode.

Materials and Methods

Flour samples and pasta production

Commercial durum wheat semolina and green soy flour were found on the local market. Pasta was produced in a 2-kg pilot plant (NAMAD, Rome, Italy) consisting of a mixer and an extruder. The flours were mixed with tap water (for 10 min) to obtain a dough having a water content of about 45%. The extrusion conditions were the following: temperature 50±5°C; kneading time 15 min; pressure 60-125 atm as a function of the specific formulation; vacuum degree 700 mmHg. A Teflon die-plate suitable for spaghetti production was used. Spaghetti had a diameter of 1.70±0.03 mm. Eleven types of spaghetti were produced: a control, made of 100% (named 0% soy flour, SF) durum wheat semolina and spaghetti in which 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50% of durum wheat semolina was replaced with SF. Ten batches were produced for each type of spaghetti.

Hyperspectral image system and acquisition mode

The hyperspectral imaging system used in reflectance mode - linear scanning type – consisted of a progressive scan camera (AVT F100 B, Allied Vision Technologies, DE) equipped with a 16-bit Charge-Coupled Device (CCD) chip (Kodak KSI 1020, DE), spectrograph (ImSpector V10, Specim Ltd, NL). The acquisition field was in the range of 400-1000 nm with spectral resolution of 5 nm to obtain the reflectance values corresponding to 121 wavelengths. The excitation source consisted of two halogen lamps whose light was conveyed on the acquisition plane by two linear diffusers (placed behind and forward the line of view of the spectrograph). Before hyperspectral image acquisition, an electronic correction was made by setting the 0 and 100% values of reflectance. The 0% reflectance was set by placing a black cap on the objective and recording the camera response. In this way, it was possible to exclude the electronic noise of the CCD detector, which is thermally sensitive. The 100% reflectance intensity was set by recording the reflectance of a standard with reference (Teflon white board, 99% reflectance). A factory software (DV s. r. l., IT) converted the data acquired by the hyperspectral system in a hypercube made of the 121 component images quantized with 4096 grey levels. Before data analysis, the pixel intensity of each component image was corrected using the related white and dark reference component images. The corrected image (R) was estimated using the following equation (Elmasry *et al.*, 2007):

$$R_{c\lambda} = \frac{R_{\lambda} - D_{\lambda}}{W_{\lambda} - D_{\lambda}} \qquad (1)$$

where R_{λ} is the recorded hyperspectral image, D_{λ} is the dark component image (0% reflectance) acquired by turning off the lighting source with the lens of the camera completely closed by a black cap, and W_l is the white reference component image (acquiring the white board reference). Equation (1) was iteratively applied for all 121 component images to obtain the corrected component images ($R_{c\lambda}$). These corrected images were repacked into a hyperspectral image. The choice to use a hyperspectral system was dictated by the opportunity to investigate a large area of the product under investigation, instead of a point measurement. This is because functional doughs are very often characterized by irregularities due to the mixing of flours. To both threshold the samples from the background and retrieve their reflection spectra, a specific algorithm based on the Otsu's method and coded in MATLAB® R2022b (The Mathworks Inc., Natick, USA) was written using the Image processing toolbox. The spectrum of reflectance of each sample was determined by measuring the average grey level value for each of the 121 images in the corresponding hyperspectral image component. This operation allowed to collect 30 reflection spectra per each of the 11 types of samples. Considering the ten baches for each type, a total of 3300 hyperspectral images were collected.

Mathematical modeling

In classification problems based on hyperspectral data, the selection of wavelengths that are useful for the problem is necessary to reduce the size of the data to be processed and, above all, is very useful for the subsequent engineering of the system, as it defines a limited number of wavelengths that can be managed by commercial hardware. To reduce the calculus complexity, some chemometric tools are used, such as partial least squares and principal component analysis methods (Beghi *et al.*, 2013; Piazzolla *et al.*, 2017; Matera *et al.*, 2021; Altieri *et al.*, 2022; Benelli *et al.*, 2022). These techniques have the advantage of reducing computational complexity by generating linear combinations of predictors. In this way, the calculation algorithm uses the combinations that explain the highest percentage of variance. In contrast, models do not directly give the possibility of defining which variables are the most influential for the classification/prediction problem. In these cases, it is necessary to use methods based on feature selection, to define the actual useful wavelengths and then implement them on multispectral systems during industrial scale-up.

The selected variables are then used to create a mathematical model. This model is then optimized for the required feature classification problem. Reducing the complexity of hyperspectral data leads to benefits in the form of improved prediction performance. Using too many features can degrade prediction performance even when all features are relevant and contain information about the response variable (Ding and Peng, 2005).

The most influent wavelengths were identified to model the percentage of soy flour based on reflectance data using a method for wavelength selection. In particular, the 121 wavelengths obtained from the hyperspectral imaging system were subjected to the Feature Selection Minimum Redundancy Maximum Relevance (FS-MRMR) Algorithm. The method is based on a forward addition scheme to insert data into an empty set S containing the best wavelength to be used for the mathematical model. To this purpose, the algorithm uses a function of interdependence (I) (Darbellay and Vajda, 1999), indicating the interdependence or not of a couple of λ, and a mutual information quotient (MIQ) value to rank features (wavelengths). MIQ (Eq. 2) includes two indexes, and indicating the relevance and redundance of with respect to the response variable (% of soy flour).

where:

$$MIQ = \frac{V_\lambda}{W_\lambda}$$

$$V_\lambda = I(\lambda, y); \quad W_\lambda = \frac{1}{|S|} = \sum_{z \epsilon S} I(\lambda, z) \tag{2}$$

Once the set S was defined, a mathematical model was implemented to predict the percentage of soybean meal based on the selected wavelengths. Based on the assumption that the aim of the research was to find a system that could be easily implemented on the production line, it was decided to test the selected wavelengths one by one, to assess which of the selected wavelengths gave the best results in predicting the percentage of soya meal. From preliminary tests performed (data not shown), the two-terms Gaussian prediction model was chosen (Eq. 3).

$$f(x) = a_1 \cdot e^{-\left(\frac{R_\lambda - b_1}{c_1}\right)^2} + a_2 \cdot e^{-\left(\frac{R_\lambda - b_2}{c_2}\right)^2} \tag{3}$$

The calibration and the validation of the model were performed on randomly selected 50% and 25% of the whole data set, respectively. The remaining 25% was used for the testing phase of the determined model.

Results and Discussion

Visible and near infra-red spectra of acquired samples

The relative reflectance spectra extracted from the hyperspectral images of the 11 dough samples at increasing concentrations of soya flour, from 0 to 50%, were obtained downstream of the processing performed with MATLAB and displayed in Figure 1.

A preliminary analysis of the hyperspectral images (hypercubes) shows that the pasta surface didn't suffer from inhomogeneity (data not shown). Therefore, a punctual hyperspectral method could be used and this is an advantage for the industrial implementation. The analysis in Figure 1 shows an interesting aspect, namely that the addition of only a small percentage of soya flour to semolina flour totally changes the spectrum profile. In fact, the 100% semolina flour shows a significantly higher relative reflectance between 400 and 650 nm, and with a drop in intensity between 800 and 840 nm compared to the mixtures with soya flour. In the latter region of the spectrum, the 100% semolina spectrum and the spectra of the mixtures even appear to be in countertrend. In the near infra-red region, the 100% semolina spectrum shows an increase in relative reflectance values, compared to the mixtures with soybean meal, especially in the band between 930 and 1000 nm. The mixtures with increasing concentrations of soybean meal, on the other hand, show a constancy in the spectral profile, and, in particular, a decreasing relative reflectance trend is visible as the percentage of soybean meal increases. The increases are not constant throughout the spectral range but are greatest in the visible region between 600 and 750 nm.

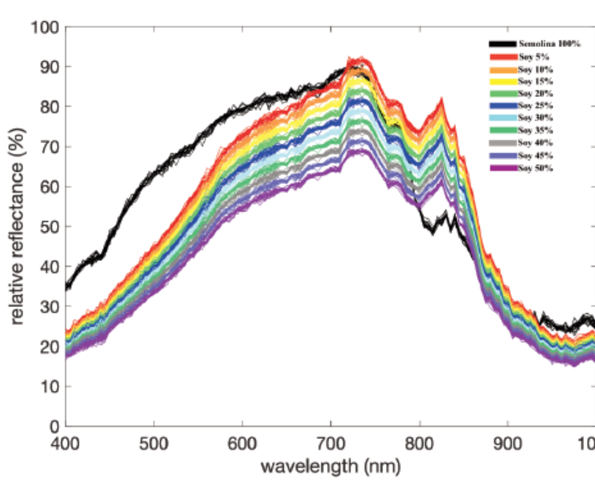

Figure 1. Relative reflectance spectra of the 11 samples considered.

Choice, calibration, and validation of the mathematical modeling

The Curve fitting toolbox of MATLAB® offers a wide choice of methods to fit experimental data and allowing the evaluation of fitting accuracy. The first step was to check the functions that better fit the reflectance data. Analysis of the most influential wavelengths using the MRMR algorithm indicated five variables. The influential wavelengths were tested with the 6-term Gaussian prediction model and the performance of the model was evaluated. On each selected wavelength, the mathematical model was calibrated using 50% of data and then validated on 25% of data. In Tables 1 and 2, performance parameters ($R^{R}_{adjusted}$ and Root Mean Square Error, RMSE) and equations terms, based on calibration and validation dataset are reported.

The results shown in Tables 1 and 2 show how the variables (wavelengths) are ordered with respect to both R^2_{adj} and RMSE.

The model calibration (Table 1) showed high R^2_{adj} values with low RMSE values. The biggest differences between the calibration and validation phase lie in the fifth most influential wavelength, namely that at 855 nm. The lower R^2_{adj} value compared to the other wavelengths is found for both the calibration and validation phases. In fact, in Table 2, the R^2_{adj} value drops to below 90%. The 655 nm wavelength alone can estimate the percentage of soya flour in the dough, with an RMSE of 1.2 and an R^2_{adj} of 0.99, based on the validation set. The transfer function is as follows (Eq. 4).

$$f(x) = 51.96 \cdot e^{-\left(\frac{R_{655}-48.99}{10.62}\right)^2} + 11.44 \cdot e^{-\left(\frac{R_{655}-62.00}{6.61}\right)^2} \tag{4}$$

Once the transfer function was identified, the remaining 25% of the data was used for the testing phase of the mathematical model. Thus, Equation 4 was used to predict the percentages of soya present from spectral data (at 655 nm) unknown to the mathematical model, *i.e.*, not used in the training and validation phase.

The performance of the model in the test phase was comparable to that found in the validation phase. In particular, the R^2_{adj} value was 0.98, and the RMSE value was 1.31 (Figure 2). The model showed a good ability to generalize the data, a sign of the closeness of the validation and test data.

Figure 2 highlights that the considered wavelength and the other 5 selected wavelengths by MRMR algorithm (data not shown), the relative reflectance decreased as percentage of semolina replaced by soy flour increased. On the x-axis the central value of prediction is shown. As depicted, each true value of soy percentage (y-axis) is related to a range of percentage varying between the central value. This variation represents the prediction error. Based on the relationship Absorbance = log(1/Reflectance), at increasing percentage of soy flour in the samples, absorbance increased. It was supposed that those differences should be due to qualitative-quantitative differences in composition between semolina and soy flour (Workman, 2016). More in depth, the wavelength (655 nm) most effective in predicting the percentage of soya flour in the dough corresponded to the chlorophyll absorbance in green light reflectance region (Masithoh *et al.*, 2023; Pahlawan *et al.*, 2022).

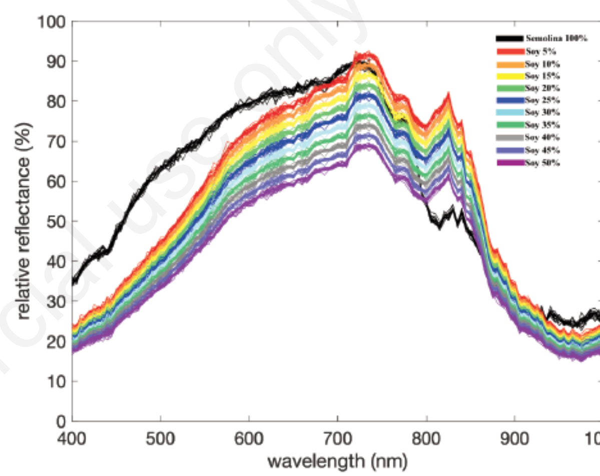

Figure 2. Six-terms Gauss function (Eq. 4) fitting reflectance @655nm using data from the test set. x-axis: central value of predicted class of soy percentage; y-axis: true value of soy percentage.

Table 1. Performance parameters of six-terms Gauss equations on the best 5 wavelengths (calibration dataset).

Wavelength (nm)	$R^2_{adjusted}$	RMSE	a^1	b^1	c^1	a^2	b^2	c^2
655	0.99	1.11	50.15	49.18	10.10	14.21	60.00	3.32
755	0.99	1.41	51.62	36.11	7.22	3.12	43.06	3.98
535	0.99	1.24	0.54	-0.43	1.14	44.54	-1.00	2.32
785	0.96	2.20	-3.13	61.22	3.05	50.99	61.20	11.08
580	0.94	2.96	2.22	51.29	0.01	41.55	50.77	10.16

Table 2. Performance parameters of six-terms Gauss equations on the best 5 wavelengths (validation dataset).

Wavelength (nm)	$R^2_{adjusted}$	RMSE	a^1	b^1	c^1	a^2	b^2	c^2
655	0.99	1.28	51.96	48.99	10.62	11.44	62.00	6.61
755	0.99	1.64	52.6	38.21	9.52	7.65	49.06	4.80
535	0.99	1.65	0.00	-3.77	0.25	49.84	-1.89	2.19
785	0.92	4.50	-6.28	67.79	4.06	52.49	63.70	13.19
580	0.88	5.32	3.56	57.05	1.07	47.26	56.43	12.77

Moreover, another consideration that can be made is that the small RMSE value obtained certainly allows one to discern deviations of soybean meal percentages with deviations between 5 and 10%, as can also be seen in Figure 1. In an industrial context, having a feedback control based on this technique can certainly be very useful to ensure quality production, despite the small deviations found.

Conclusions

A simple hyperspectral imaging-based system in the reflectance mode was used to predict the percentage of soy flour in pasta made with semolina-soy flour mixtures. The elaboration of raw data shows the effectiveness of the FS-RMRM algorithm to individuate the most influent wavelengths in a prediction modeling problem. Moreover, after the validation process, the identified transfer function was able, with an acceptable accuracy, to identify the percentage of substituted soybean flour.

Given today's availability of both hardware and software, the system represents an opportunity to improve pasta production lines, with affordable investment costs and high system reliability. This technology also fits very well into the 4.0 technology perspective, as it can be remotely controlled and generate continuous reports on the analysis of the pasta throughout the production phase. Further research will be conducted to evaluate the efficiency of the model developed in this work on other replacement ingredients.

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