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A non-destructive technique for estimation of amylose, resistant starch and starch in potato using NIR spectroscopy

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Abstract

The NIRMaster is a bench top Near Infrared Spectroscopy (NIRS) for quantification and identification of the biochemical components at ≥0.1% level. NIRS generates a characteristic spectrum that is distinct for a given sample and allows identification as well as quantification of its components and it is a non-destructive, rapid method for the determination of biochemical parameters. In this current study about forty-eight potato cultivars were studied for amylose, resistant starch and starch contents respectively to develop calibrated models using NIRMaster. Training and calibration part was done using the fresh produce of potatoes harvested from the Modipuram campus of ICAR-CPRI and validation was done using fresh produce from the same location as well as advance-colored hybrids from Patna center of ICAR-CPRI. The reference predicted values for amylose, resistant starch and starch were in the range of 15.81 to 32.83%, 24.38 to 48.49% and 41.15 to 56.21% respectively. The developed methods showed accuracy in the range of approximately 60-70% for all the targeted parameters. However, accuracy can be further enhanced by expanding the training/ calibration sample sets. The results obtained in this study have shown high applicability of NIR spectroscopy for the measurement of amylose, resistant starch, and starch content for potato samples for quicker estimation. This is the first report being presented on NIR spectroscopy about the nutritional composition of Indigenous potato varieties.

Keywords: Potato, Near-infrared spectroscopy, Amylose, Resistant starch, Starch, Validation

Introduction

Potato (*Solanum tuberosum*) is a starchy, tuberous crop belonging to the family Solanaceae and growing in different parts of the world with a total production of 376 million tonnes, sharing China and India as the first and second largest potato producers on the planet (FAOSTAT, 2022) indicating Asia as the center of global potato production. Potato is the third most important food crop worldwide followed by rice and wheat in terms of consumption. It is a good source of proteins, vitamins (C, B_1, B_3, B_6, folate, pantothenic acid, and riboflavin), minerals and many bioactive compounds (Dalamu et al., 2014). Potatoes are the most abundant form of

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storage polysaccharide having specific structures and compositions (amylose/amylopectin ratio). Amylose is mostly a linear chain polymer containing α-1,4 glycosidic linkages and amylopectin is a large, branched polymer with linkages of α-1,4 that serve as the backbone and α-1,6 bridges that serve as branching points. Amylose is located in between amylopectin clusters and randomly dispersed. The straight chain nature limits the surface area exposed for digestion which gives resistant power to amylose to digestion than other starch molecules and is, therefore, an important form of resistant starch. Resistant starch refers to the undigested starch which is resistant to the amylolytic enzymes in the small intestine as they pass through the upper part of the gastrointestinal tract and stimulate the growth or activity of advantageous bacteria (Englyst et al., 1992) and this can be divided into four different types of resistant starch (hereafter RS) RSI, RSII, RSIII and RSIV (Topping, 2003). Resistant starch is present profoundly in cereal grains, seeds and in heated starch or starch containing foods (Charalampopoulos et al., 2002). Resistant starch has received greater attention for both its potential health benefits and functional properties and these can also be incorporated into starchy foods as healthy food additives (Sajilata et al., 2006).

Traditional methods for determining amylose, resistant starch and starch content in larger samples are laborious, time consuming, and chemically consuming lengthy operations. In this context, infrared spectroscopy has emerged as an analytical technique for numerous varieties of applications. In general, each molecule has a unique fingerprint of vibrational frequencies, which makes FTIR (Fourier transform infrared spectroscopy) a specific technique for molecular identification (Bunaciu et al., 2015). Near-infrared spectroscopy is a fast, simple, and non-destructive advanced analytic tool, that does not use any chemicals and large sample preparations, it can determine a huge number of constituents (Huang et al. 2008) and it has been widely used for qualitative and quantitative analysis of major constituents in vegetable products (Sanchez and Marin, 2011). NIRS is a technique which is especially suitable for the major constituents, which means the concentration of constituents in the sample should be about 0.5% and higher (Brunt and Drost 2010). Due to differences in the vibrational and rotational energies of specific bonds in the spectral region, typically C–H, O–H and N–H, it is possible to detect the different compounds present in food samples (Osborne and Fearn, 1986). In addition to that NIRS is not a stand-alone technology; it needs reference data, which is being produced by the accuracy of the chemical method or wet lab method (Lebot, 2012). Hartmann and Buning (1998), studied the NIR diffuse reflectance (1100-2500 nm) mode to measure important constituents of peeled potato tubers such as fructose, glucose, sucrose, total reducing sugars, starch, and crude protein and NIRS spectra from potato samples used to relate the dry matter content and texture of the potatoes (Van et al., 2002). Dull et al. (1989) successfully used the wavelength range of 800 nm to 1000 nm to measure the percent dry matter in sliced and intact potato tubers by near-infrared spectroscopy in the transmittance mode. Earlier, Joshi et al. (2017) also optimized NIRS based dry matter evaluation methodology. The training and calibration of the instrument were done using fresh produce of potatoes in the year 2014 harvested from Modipuram Campus of ICAR-CPRI, India whereas, validation of the application was done from fresh produce of same location and 13 advance coloured hybrids from Patna centre of ICAR-CPRI, India in 2015. In addition, NIRS was studied to develop calibration models for carbohydrates (Chen et al., 2009) as well as dry matter content and reducing sugar content to manage the quality evaluation of processing products developed from stored potatoes (Scanlon et al., 1999). Also, NIRS-calibrated models were developed to determine the quality characteristics of potato samples (Van et al., 2002; Brunt and Drost, 2010). Therefore, the main aim of this study was to develop calibrated models for amylose, resistant starch, and starch content in different potato cultivars using NIR Master and their validation for wider applications.

Materials and Methods

Potato samples

Research material for the studies viz., 48 Indian potato cultivars were procured from ICAR-CPRIC, Modipuram, India grown using the standard package of practices (Kumar et al., 2007). After skin curing, the potatoes were stored at elevated temperature (10-12°C) with sprout suppressant CIPC or Chloropropham treatment (Singh and Ezekiel, 2010) till analyzed. Four healthy tubers per cultivar were selected, washed thoroughly, and left overnight. They were than peeled and chopped into the chips. These chips were oven-dried (Make: REICO) at 70°C for 16 h and then ground into a fine powder using a small multipurpose mixer grinder (Lumix International, India). These samples were stored in airtight vials and were divided into two sets; one set was used for wet lab chemical analysis of amylose, starch and resistant starch for reference values and the other set to obtain the spectral data using NIRMaster (Buchi, Switzerland).

Reference analysis

Amylose content

The amylose content in the flour samples was estimated using a methodology standardized by Juliano et al. (1971). To do this, 100 mg of dry sample was mixed with 1 ml of ethanol (95%) and 9 ml of 1N NaOH. It was then heated for 10 min in a boiling water bath and immediately cooled it to room temperature and made up to 100 ml. An aliquot of 5 ml was taken into a 100 ml volumetric flask followed by the addition of 1N acetic acid and 2 ml iodine solution and making the volume to 100 ml. Before taking absorbance, it was shaken properly for 15 min and covered with black cloth and the absorbance was read at 620nm against a blank solution. Amylose was calculated as OD × 80.

Starch concentration

A modified protocol was used to measure starch content in the samples based on the method of Ranganna (1986). In this method, 100 mg of dry sample was mixed with 5 ml of warm distilled water followed by incubation for 15 min in a hot water bath at 60°C. Five ml (95% pure) ethanol was added followed by centrifugation three times at 10,000 × g rpm for 5 min, to remove the sugars in the sample. During centrifugation, 50% pure ethanol was used for frequent washing of sugars. After the third wash, the supernatant was discarded, and 2 ml warm distilled water and 1 ml concentrated HCL were added. Tubes were covered with an aluminum foil and incubated in a hot air oven for 2½ hours at 85°C with constant shaking for proper reaction between conc. HCl and the sample. After incubation, centrifuge tubes were cooled down to room temperature. Then, two drops of phenolphthalein indicator were added to the sample and a neutralization reaction was carried out by adding conc. NaOH drop wise until the development of a light pink color. After neutralization, centrifugation was carried out at 10000 rpm for 5 min and the filtrate and residue volumes were noted down. An aliquot of 100 μl of filtrate was taken into the other set of test tubes and 900 μl of distilled water was added to the filtrate. One milliliter of Nelson's reagent (Nelson A & Nelson B) was added and incubated by covering it with the aluminum foil in a hot water bath at 60°C for 20 min. Then, 1 ml of arsenomolybdate was added to the filtrate after proper cooling. The solution was diluted to volume (10 ml) with distilled water and the absorbance read at 620 nm against a blank solution containing 100 μl distilled water, 1 ml Nelson reagent, 1 ml arsenomolybdate solution and 7 ml distilled water. The starch calculation part is as follows:

$$Factor\ (A) = \frac{0.0668 \times W}{\text{Volume make up (ml)}} \quad \ldots Eqn.(1)$$

$$X = OD \times A \quad \ldots Eqn.(2)$$

$$Percent\ of\ Starch = \frac{X\ (mg) \times Volume\ makeup \times 100}{\text{Weight of sample (mg)} \times 0.1\ \text{(aliquot taken)}} \ldots Eqn.(3)$$

Resistant Starch Content

The determination of resistant starch in potato samples (dry samples) was done according to the method given by Goni et al. (1996) with slight modifications (Raigond et al., 2016). To do this, a 100 mg sample was mixed with pepsin (pepsin in KCl-HCl buffer pH 1.5, to make the sample protein-free) and incubation was carried out at 40°C for 60 min in a hot water bath. After proper cooling, second incubation was done with α-amylase (37°C, 16 h, α-amylase in Tris-maleate buffer at pH 6.9) to hydrolyze the digestible starch. Reaction mixture was centrifuged (30 min, 10,000 g) and washed at least thrice to make the residue reducing sugar free. To this 3 ml of distilled water and 3 ml of 4M potassium hydroxide was added and incubated the mixture for 30 min at room temperature with constant shaking. Then 600 μl of amyloglucosidase added to the sample and incubated at 60°C for 45 min with constant shaking in acidic media created by chemical mixture (5.5 ml of 2M HCl and 3 ml of 0.4M acetate buffer, pH 4.75). The glucose content of this supernatant was determined using glucose-peroxidase assay kit (Sigma chemicals) through absorbance at 450 nm. From this OD using following expression resistant starch [mg (100 mg)$^{-1}$] was calculated as glucose (0.833 × OD) × 0.9.

Instrument and Acquisition of NIR Spectra

The analyses were performed on a NIRMaster (IP54/ Pro IP65 FT-NIR spectroscopy). NIRMaster is a Fourier transformation Near Infrared Spectrometer (FT-NIR) to identify the components of interest and generates an invisible interferogram beam, which interacts with the molecules of the sample and generates characteristic feedback. The measurement cell of the detector takes this feedback and processes it mathematically via Fourier transformation into a spectrum. This spectrum gives the characteristics of the given sample (Buchi, NIRMaster, Technical data sheet). NIR scanning was conducted on each sample in a quartz sampling cup (87 mm diameter and 87.5 mm height) in a rotary diffuse reflectance holder for the estimation of amylose, resistant starch, and starch. Reflection mode was used to investigate the NIR absorption in the range 12500 to 4000 cm^{-1}(800 to 2500 nm). In reflectance mode, the incident light penetration is limited by the sample surface and a part of such light passes through the sample and is refracted and then diffusely reflected into the sensor. The reflected portion contains the spectral information of the sample which is known as diffuse reflectance. By this mode we can detect the internal composition of the tubers, and this technique is widely used for the quantitative analysis of many biological products (Burns and Ciurczak, 2007). The scan results were averaged and recorded in absorption mode (log 1/R).

Samples were selected from 2015 fresh produce reference data by wet lab chemistry methods and scanned 12 times at a resolution of 8 cm^{-1} by NIRS scanned data, which was used for the preparation of the calibration model. This calibration data was imported and given for the development of an application for amylose, resistant starch and starch content. The Partial Least Square method has been used for the development of a prediction model for amylose, resistant starch, and starch. The spectral and reference data were subjected to the NIRCal, which is an optional software package for NIRMaster, for the development of qualitative and quantitative calibration models (Buchi, Switzerland). The produced application's sufficiency was determined in terms of total randomization versus the horizontal axis.

Results and Discussion

Results of wet lab methodologies for amylose, resistant starch, and starch are presented in Tables 1, 2 & 3, the NIRS data (predicted data) and wet chemistry (reference data) experiments were compared for 48 varieties of fresh harvest of potatoes for establishment of equations of calibration for the prediction of amylose, resistant starch, and starch. Diffuse reflection mode was used to investigate the NIR absorption in the range 12500 to 4000 cm^{-1}(800 to 2500 nm) for training, calibration and validation was carried out for amylose, resistant starch, and starch content. Training and calibration part was done using the fresh produce of potatoes harvested from

Table 1. Validation data set for amylose content (%) on dry weight basis

Sl. No.	Variety	Amylose content, % (wet lab analysis)	Amylose content, % (NIRS predicted)	Variation	% Variation	High 30%	Low 30%
1	K. Satlej	15.96	16.36	0.40	2.53	20.74	11.17
2	K. Deva	16.98	21.74	4.76	28.06	22.07	11.88
3	K. Anand	17.62	22.29	4.67	26.48	22.91	12.34
4	K. Sherpa	18.09	19.56	1.47	8.11	23.52	12.67
5	K. Safed	18.70	17.00	1.70	9.09	24.31	13.09
6	K. Shailja	19.41	15.81	3.60	18.53	25.23	13.58
7	K. Sadabhar	19.43	22.64	3.21	16.50	25.26	13.60
8	K. Arun	20.00	24.76	4.76	23.78	26.00	14.00
9	K. Girdhari	20.89	24.69	3.80	18.19	27.16	14.62
10	K. Giriraj	21.19	18.25	2.94	13.88	27.55	14.83
11	K. Neela	21.21	18.05	3.16	14.89	27.57	14.84
12	K. Kundan	21.58	24.18	2.60	12.05	28.05	15.11
13	K. Lalima	21.60	24.63	3.03	14.05	28.08	15.12
14	K. Alankar	21.76	19.86	1.90	8.73	28.29	15.23
15	K. Surya	22.63	28.45	5.82	25.70	29.42	15.84
16	K. Muthu	23.33	28.03	4.70	20.15	30.33	16.33
17	K. Ashoka	23.40	26.08	2.68	11.47	30.42	16.38
18	K. Badshah	23.45	30.06	6.61	28.21	30.48	16.41
19	K. Megha	23.53	25.18	1.65	7.01	30.59	16.47
20	K. Himalini	23.94	27.65	3.71	15.48	31.13	16.76
21	K. Swarna	24.61	28.57	3.96	16.09	31.99	17.23
22	K. Chandramukhi	25.05	32.42	7.37	29.42	32.57	17.54
23	K. Frysona	25.39	22.43	2.96	11.66	33.01	17.77
24	K. Jyoti	27.05	26.57	0.48	1.77	35.17	18.94
25	K. Kuber	28.77	27.82	0.95	3.30	37.40	20.14
26	K. Chipsona-3	29.71	29.02	0.69	2.32	38.62	20.80
27	K. Naveen	31.33	24.41	6.92	22.09	40.73	21.93

Table 2. Validation data set for resistant starch content (%) on dry weight basis

Sl. No.	Variety	Resistant starch content, % (wet lab analysis)	Resistant starch content, % (NIRS predicted)	Variation	% Variation	High 30%	Low 30%
1	K. Jyoti	24.42	29.75	5.33	21.83	31.75	17.09
2	K. Megha	24.92	32.13	7.21	28.93	32.40	17.44
3	PS/6-24	26.75	34.24	7.49	28.00	34.78	18.73
4	K. Lalima	26.75	25.83	0.92	3.44	34.78	18.73
5	PS/5-75	27.44	34.63	7.19	26.20	35.67	19.21
6	P-7	27.83	32.41	4.58	16.46	36.18	19.48
7	K. Chipsona-2	27.89	30.10	2.21	7.92	36.26	19.52
8	K. Badshah	28.61	36.54	7.93	27.72	37.19	20.03
9	K. Kundan	29.11	29.60	0.49	1.68	37.84	20.38
10	K. Sadabhar	35.53	34.50	1.03	2.90	46.19	24.87

11	K. Chamatkar	35.94	32.25	3.69	10.27	46.72	25.16
12	K. Kumar	36.08	34.44	1.64	4.55	46.90	25.26
13	K. Bahar	36.33	35.83	0.50	1.38	47.23	25.43
14	K. Lauvker	36.78	24.38	12.40	33.71	47.81	25.75
15	K. Neela	37.19	48.29	11.10	29.85	48.35	26.03
16	K. Muthu	37.75	34.17	3.58	9.48	49.08	26.43
17	K. Deva	40.14	34.59	5.55	13.83	52.18	28.10
18	K. Anand	38.83	48.24	9.41	24.23	50.48	27.18
19	PS/6-88	39.55	31.85	7.70	19.47	51.42	27.69
20	K. Ashoka	45.16	35.48	9.68	21.43	58.71	31.61
21	Kufri Alankar	45.61	48.49	2.88	6.31	59.29	31.93

Table 3. Validation data set for starch content (%) on dry weight basis

Sl. No.	Variety	starch content, % (wet lab analysis)	starch content, % (NIRS predicted)	Variation	% Variation	High 30%	Low30%
1	K. Chandramukhi	36.31	41.21	4.90	13.51	47.20	21.78
2	K. Chipsona-2	53.48	41.96	11.52	21.54	69.52	32.09
3	K. Chipsona-3	57.32	47.25	10.07	17.56	74.51	34.39
4	K. Chamatkar	64.76	52.21	12.55	19.38	84.19	38.86
5	K. Deva	65.69	52.31	13.38	20.37	85.40	39.41
6	K. Frysona	65.94	42.17	23.77	36.04	85.72	39.56
7	K. Himalini	66.58	50.06	16.52	24.81	86.55	39.95
8	K. Jyoti	67.69	56.21	11.48	16.96	87.99	40.61
9	K. Kuber	67.87	47.95	19.92	29.35	88.23	40.72
10	K. Kumar	68.06	42.09	25.97	38.15	88.47	40.83
11	K. Kundan	68.17	41.15	27.02	39.64	88.62	40.90
12	K. Lauvker	70.71	45.07	25.64	36.26	91.92	42.43
13	K. Megha	70.73	41.27	29.46	41.65	91.94	42.44
14	K. Muthu	71.08	42.87	28.21	39.69	92.40	42.65
15	K. Naveen	71.28	41.54	29.74	41.72	92.66	42.77
16	K. Neela	72.34	44.62	27.72	38.32	94.04	43.40
17	K. Pushkar	73.61	51.75	21.86	29.70	95.69	44.17
18	K. Red	76.45	46.34	30.11	39.39	99.39	45.87
19	K. Sadabhar	76.90	50.02	26.88	34.95	99.97	46.14

the Modipuram campus of ICAR-CPRI, India, whereas validation of methods was done using produce from the same location as well as advance-colored hybrids from the Patna center of ICAR-CPRI. Totally 48 powdered potato samples were scanned 16 to 32 times by giving the desirable input (no. of measurements) in the operator configured in the management console. After scanning of samples, a calibration curve was developed using the NIRCal software. After the development of calibration curves, the same application was used for result analysis after assigning the calibration/s to the application. The residual plot technique represents the accuracy of the prediction, which has been shown by the adequacy of the developed application as complete randomization against the horizontal axis.

Amylose content, resistant starch and starch content of potato cultivars

After the development of the calibration model, the same model was used to estimate the amylose, resistant starch and starch for the dried potato samples. Validation graphs were developed by using reference values from the wet lab method and NIRS-predicted values to get precise validation results (Figures 1, 2 & 3). Thepredicted values for amylose, resistant starch and starch content using NIRS were in the range from 15.81 to32.83%, 24.38 to 48.49% and 41.15 to 56.21% against 15.44 to 39.92%, 19.47 to 45.61% and 45.95 to 78.82% of wet lab experimental value, respectively. This showed that percent error in amylose, resistant starch and starch prediction varied from 0.40 to 6.61%, 0.49 to 12.40%

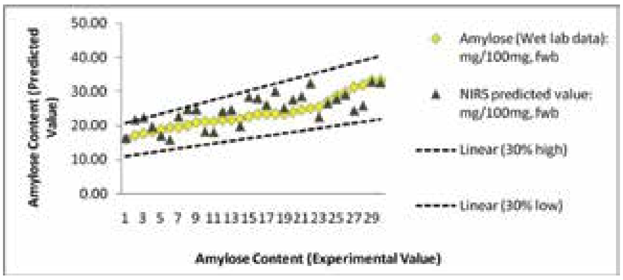

Fig. 1. Performance of Near Infrared for amylose content estimation in dried Indian potato cultivars

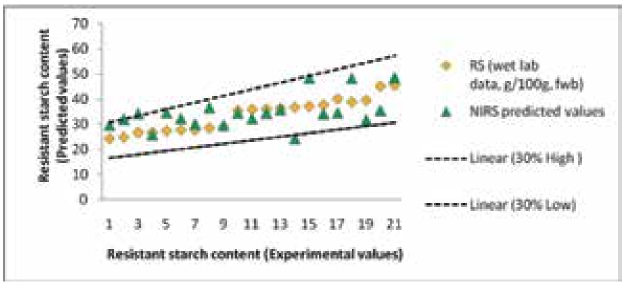

Fig. 2. Performance of Near Infrared for resistant starch estimation in dried Indian potato cultivars

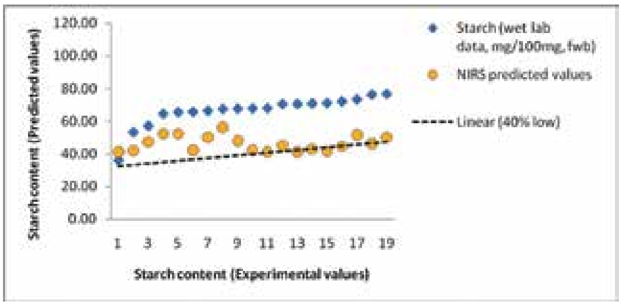

Fig. 3. Performance of Near Infrared for starch estimation in dried Indian potato cultivars

and 4.90 to 29.46%, respectively. The performance of spectral data from near-infrared spectroscopy for amylose content and resistant starch was shown in Fig. 1 and 2. The predicted values for amylose, resistant starch, and starch were in the range of 15.81 to 32.83%, 24.38 to 48.49% and 41.15 to 56.21% however the wet lab data was 15.96 to 33.25%, 24.92 to 45.61% and 36.31 to 76.90% for amylose, resistant starch, and starch respectively. The figures indicate that all the predicted values from the calibrated application fell between 30% higher and lower than the actual wet lab data and are reflected in the graphs by a dotted line. It showed 70 percent reliability in the developed method by NIRMaster for the prediction of amylose and resistant starch content in the oven-dried potato samples. However, all the predicted values of starch were in the range, which was approximately 40% higher and lower than the actual wet lab data and reflected in the graphs by dotted lines. These results reflected approximately 60 percent reliability of the developed method in NIRMaster for the prediction of starch content in oven-dried potato samples. However, various research studies showed 90 percent to 95 percent prediction confidence expected with a larger number of potato samples using NIR spectroscopy (Haase, 2003; Haase, 2006). Validation dataset for amylose, resistant starch, and starch from reference values of wet lab methods and spectral data from NIRS predicted values are reported in this study. Higher prediction accuracy could be achieved by increasing sample spectra for making calibration for selected constituents.

Prediction adequacy

Residual plots represent the accuracy of the prediction, which was made by plotting residuals on the vertical axis and the predicted values of amylose, resistant starch, and starch of advance-colored hybrids on the horizontal axis for each model. The fitness of the model is shown by the randomization against the horizontal axis. Fig. 4, 5 & 6 showed the prediction models for complete randomization of amylose, resistant starch and starch, this figure showed the predictability of developed applications for amylose, resistant starch, and starch

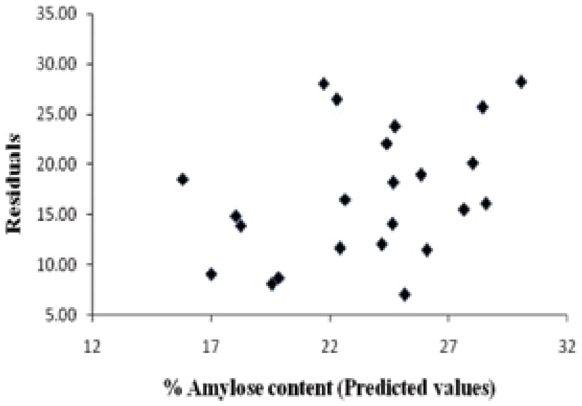

Fig. 4. Residual plot for amylose content validation (advance-coloured hybrids)

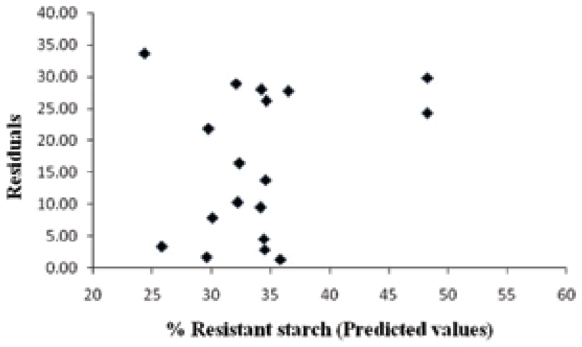

Fig. 5. Residual plot for resistant starch validation (advance-coloured hybrids)

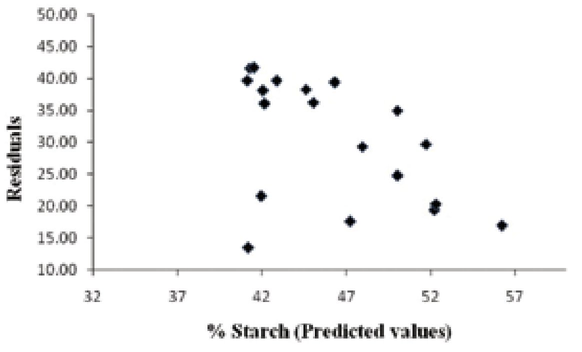

Fig. 6. Residual plot for starch validation (advance-coloured hybrids)

content in unknown samples, which could be further enhanced by expanding training/calibration sample sets i.e., providing a greater number of the sample (spectra) along with reference data while developing an application. Except starch residual plot, the remaining two residual plots for amylose and resistant starch prediction showed more randomization. Results showed more accuracy in the range of approximately 60-70% for the amylose and resistant starch prediction model compared to the starch prediction from the developed application. Pedreschi et al. (2010) reported high accuracy for online NIR interactance to predict the fat and dry matter of potato chips.

Conclusion

Application of NIRS for the estimation of amylose, resistant starch and starch content is a better alternative in favor of fast estimation. As a rapid, and/or non-invasive method, NIRS is less time-consuming, more robust, more reproducible, and more cost-effective than human labor or other laboratory destructive methods used for quality assurance purposes. It provides accurate and reliable results for multiple parameters simultaneously. The results obtained in this study have shown a high applicability of NIR spectroscopy for the measurement of amylose, resistant starch, and starch contents in potato samples. NIRMaster can be used for the screening of tuber crops for developing new varieties with high nutrient values, and analysis of nutrients in fortified foods and functional foods. Findings can also help to understand the complex nature of different potato varieties.

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