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Title: Analytical Approach to the Quantitative Analysis of Silicon in Plants: Its Application to Plant Silica Extraction.

Abstract approved:

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The literature available on the silica content of herbaceous biomass, and its relative mobility in leaching/extraction systems, includes studies that have employed several different methods for silica quantification. This makes comparison of the data difficult. The objective of this thesis research was to determine the quantitative relationships between the measured silica contents of biomass-relevant straws as determined by routine, published, silica quantification methods. Four representative straws were analyzed (wheat, Kentucky Bluegrass, Tall Fescue, and Perennial Ryegrass) using five methods. The methods included one gravimetric (G) and four colorimetric assays; the colorimetric assays differed with respect to the combination of digestion (two evaluated, D1 and D2) and color-development (two evaluated, C1 and C2) protocols. All of the methods tested were taken from the literature. The gravimetric method-determined silica contents of the straws were, in general, the

highest. The exception being the values for tall fescue, for which all methods gave values that were not significantly different. All four of the colorimetric assays gave similar values for silica content, although in some cases these values were significantly different ($P>0.05$). The major difference in the colorimetric methods was found to be associated with the precision of the digestion protocols. A colorimetric assay, based on alkali digestion and subsequent reaction with ammonium molybdate for color development, was used to illustrate the potential of using moderately hot water ($60 – 90^{o}C$) for the extraction of silica from wheat straw.

Analytical Approach to the Quantitative Analysis of Silicon in Plants: Its Application to Plant Silica Extraction.

By

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Carmen E. Boone, Author

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1. INTRODUCTION

Utilizable energy may be obtained from straws and grasses through thermochemical processes, including pyrolysis, gasification, and combustion (1). Selected inorganic components of straws and grasses are recognized to be detrimental to such processes (2-4); silica being one such component. Silica is known to be associated with fouling, slagging, and agglomeration. Thus, knowledge of a straw's silica content is useful in predicting the performance of that straw in thermochemical processes. There is considerable interest in aqueous leaching-based pre-thermochemical processing treatments that may be used to lower the inorganics content, including silica, of plant-derived biomass (5-13) thus upgrading these materials for thermochemical processing. A useful tool for assessing the impact of such treatments is a readily applicable assay for the measurement of silica.

Several methods have been used to determine the silica content of straw, including gravimetric (14-17), colorimetric (18, 19), energy dispersive spectrometry (20), atomic absorption spectroscopy (21) and atomic emission spectroscopy (22). Inductively coupled plasma atomic emission spectroscopy (ICP-AES) is often considered a reasonable standard against which to compare other methods (23, 24). ICP-AES methods are based on the use of a specialized, expensive spectrometers and the reagents required for sample preparation are rather harsh (*21, 23*). Hence, many

laboratories report silica values for grasses and straws that were obtained using non-ICP-AES methods (see references cited above). Comparative information on the performance of these methods with herbaceous biomass is lacking. Hence, an objective of the research described herein was to determine the relative merits of previously published colorimetric and gravimetric methods for the quantitative determination of silica in straws.

A second objective of the presented research was to demonstrate the application of the autoclave/alkali digestion-colorimetric method in a study evaluating the efficacy of aqueous, moderate temperature, leaching for the removal of silica from wheat straw. The application focused on wheat straw due to the large amount of information available on the leaching of silica from this particular straw (*5, 7, 8, 10, 13, 17, 20, 25-29*) and on moderate temperatures, 30 – 90°C, due to the lack of information available on leaching in this temperature range.

2. LITERATURE REVIEW

2.1. SILICA IN PLANTS

Silica (SiO_2) from rocks is slowly dissolved to form monosilicic acid (H_4SiO_4 or $Si(OH)_4$) referred also as orthosilicic acid; weathering contributes to this process. Monosilicic acid from rocks and biologically deposited silica are both sources of silicon that are available for organisms (*30*). There are several terms used to refer to silica, in order to avoid confusion a definition of terms was cited by Owen 1975 (*31*) and it is partially presented on Table 1.

Plant silicon content is influenced by the availability of silicon in soil. Deposition of silicon in the plant is affected by the transpiration stream. Roots absorb silicic acid, an uncharged molecule, and transport it to the shoots. Then, it is deposited as opal, a type of amorphous silica, and after this it can not be redistributed. (*32-35*). More silica is deposited when more water is absorbed (*32*). Silica has been reported in sorghum, wheat, corn, sunflower, bamboo (*32*) and rice (*36*) in the form of opal. Deposits of silica in plants are called phytoliths; they are deposited throughout the straw stem (Parry in (*29*)) and on the surface of leaves and other parts of the plant (*37*). The excessive accumulation of silica has no harmful effects on the plant (*37*). Silica can be found in plants from 0.1 to 10% on a dry wet basis, with a high coefficient of variation (*34*), but silica is not often found in large amounts (*38*). In most cases, the content of silica in the plant varies among species, age, season and location of the portion studied.

Silicon in plants exists in a variety of forms. In the early nineteen sixties it was supposed that silicon in plants existed as polymeric silicic acid (aqueous form) (*39*). Further research revealed the existence of crystalline silica in *Fragaria* leaves and *Equisetum* shoots, along with amorphous silica (in the form of opal) (*40*). *Equisetum* and *Gramineae* appeared to contain a high proportion of amorphous silica, and this was attributed to its location in the surface: If silica in solution moves quickly through the plant and it gets to where it is deposited by rapid dehydration, the SiO_2 molecule will not be able to crystallize symmetrically (*40*). Studies, on infrared absorption and rates of dissolution, show that silica gel, was the form of amorphous silica present in higher plants and in diatoms; silica in gel form accounts for 90-95% of the silica content in the rice plant (*41*). It was found that silica gel accumulated in the plant can not be used in periods of silicon deficiency because it is immobilized when it solidifies and deposits (*41*). In general, silica in plants could be classified as a type of amorphous silica, and more accurately as opal (*42*).

2.1.1. Transport Mechanism, Deposition and Accumulation

Silica can be taken by plants from soils in the form of monosilicic acid, which has no charge over physiological pH. Its concentration in soils ranges from 0.1mM to 0.6mM. (*34, 43, 44*) Silicon in the form of monosilicic acid is absorbed by roots and it is deposited as hydrated amorphous silica ($SiO_2.nH_2O$)(*36, 45, 46*). This deposition occurs by polymerization of monosilicic acid, $Si(OH)_4$ (Jones and Handreck in (*36*)). The transpiration stream, which is the transport of water to the leaves, affects silica deposition, where water evaporates leading to the accumulation of silica (*34*). Once

silica polymerizes it can not enter the cell membranes and remains in colloidal form (*35*).

It has been reported that silica is deposited in the cell wall, the cell lumen and other extra cellular areas. A relation between biogenic silica and an organic matrix (proteins and carbohydrates) has been documented, and also the existence of a regulatory mechanism by proteinaceous material (*47*). Not much is known about higher plants silicic acid active transport (*48*).

2.1.2. Physiological Role

It has been indicated that silicification is induced as a defense against herbivores. It has been found that plants exposed to herbivores had more silica in their leaves than those exposed to fewer herbivores (49, 50). Silica provides protection against abiotic and biotic stresses and it has also been stated that it enhances plant growth (49, 50). As silica is deposited as solid amorphous silica in the cell walls it provides mechanical strength (34, 51). Silica also provides weather resistance and enhances photosynthetic ability (47). It is utilized by the plant as resistance to fungus diseases (52, 53). Silica has been held responsible for the elimination of toxic agents in plants (Clements in (34), (*34, 52, 54, 55*). It has also shown to promote biochemical defense mechanisms (34). Even though silica is not considered an essential mineral for plants there are clearly several important functions it serves in plants.

2.2. SILICA CHEMISTRY

The chemistry behind silica solubility can be summarized as follows. Silica in solution exists as several forms depending on its pH and concentration. Silica exists as

monosilicic acid, H_4SiO_4 or $Si(OH)_4$, a stable monomeric form, when pH is between 1 to 8 and when the concentration is less than 110-140 ppm (*56*),

$$SiO_2 + 2\ H_2O \rightarrow Si(OH)_4 = (H_4SiO_4)$$

At pH higher than 9 silicate ion forms and solubility increases (*56*),

$$Si(OH)_4 + OH^- \rightarrow SiO(OH)_3^- + H_2O$$

Hydrofluoric acid may increase silica solubility at low pH due to the formation of silicofluoride ions. This is explained by the following equation (*57*),

$$Si(OH)_4 + 6\ HF \rightarrow SiF_6^{2-} + 4\ H_2O + 2\ H^+$$

At concentrations higher than 110-140 ppm of SiO_2 polymerization takes place yielding polysilicic acids and a colloid, gel or precipitate (*56*). Monosilicic acid appears to be stable at pH 1-3, being the most stable at pH 3.2, and least stable at pH 5 and 6, polymerizing instantly at pH 6 (*58, 59*).

2.3. SILICA SOLUBILITY

Lenher and Merril (*60*) reported that even quartz is soluble in water to some extent. This article cited reference solubility values from different authors that range between 0.13-0.21gSiO_2/L at room temperature. Their experiments were carried out at 90° and 25°C consisting of gelatinous silica in contact with solution until equilibrium and then filtered through filter paper equivalent to Whatman 451. A saturation time of 24 hours was reported at 90°C with fairly strong acids (hydrochloric and sulfuric at different percentages). Solubility for gelatinous silica was reported higher in water than in acids, between 0.0212 – 0.0216 gSiO_2/ 50 ml H_2O at 90°C at 24 hours (~0.4%). The independence of solubility from the physical form of silica was discussed, and it was reported that solubility depends on temperature (*60*).

More studies revealed that silica gel solubility is a function of pH and, it is influenced by surface area and gel time (*61*).

Fournier (*62*) investigated amorphous silica solubility in water at high temperatures and pressures. They proposed that "the solubility of amorphous silica at the vapor pressure of the solution, from 0 to 250°C, is given by the equation log C = -731/T + 4.52, where C is the silica concentration in mg/kg and T is absolute temperature".

Amorphous silica has shown to have higher solubility at pH values higher than 9, and solubility appears to be almost unaffected at pH from 0 – 9. Solubilization at room temperature seems to occur at a very slow rate, but it increases at boiling-point. At 0°C it is soluble up to 60-80 ppm, at 25°C up to 100-140 ppm, and at 90°C up to 300-380ppm. Amorphous silica, including opal, dissolves in water but its rate of dissolution is slow even in hot water, the process is so slow that a large amount of solid can exist for indefinite time in water (*63*).

Alexander, Heston and Iler (64) did experiments to clarify if equilibrium was reached when silica was dissolved. They attributed the high solubility of silica above pH 9 to the formation of silicate ion. They demonstrated that the form of silica had an effect on solubility; they noted that amorphous silica is known to be more soluble than crystalline silica (quartz). A lower solubility was reported, 0.01 – 0.012% and also the fact that the solubility of amorphous silica in water at 25°C was influenced by the relationship between the solid phase and a monomeric form of silica in solution, most probably $Si(OH)_4$. Finely divided amorphous silica powder and sols of colloidal

particles of silica were used in these experiments and their solubility's were found to be identical (*64*).

Greenberg and Price (*65*) studied the influence of solutions with concentration of salts, such as sodium chloride and sodium sulfate, one normal and above on silica solubility; these were found to have a negative effect on silica solubility (*65*).

Silica solubility is dependant on the properties of the solution where it is immersed. The concentration of hydrogen ions has shown to be a key to silica solubility, showing a higher solubility above pH 9. Other factors such as temperature, pressure and ionic strength had also shown their effect. Higher salinities have been shown to have a negative effect in silica solubility. The crystallization network is another factor. Amorphous silica is 20 times more soluble than crystalline silica (quartz) (Robie and Waldbaum in (*66*)). A study about diatom solubility reported higher dissolution in Na_2CO_3 due to its effect on increasing the pH, consequently affecting the equilibrium $Si(OH)_4 \rightarrow SiO(OH)_3^- + H^+$ (*66*). Solubility seemed to be promoted also in KNO_3 and $LiNO_3$ solutions, which also have an effect on increasing the pH slightly; on the other hand it's solubility in $MgCl_2$ and $CaCl_2$ solutions was lower than in water (*66*). This study agreed in finding an effect of ionic strength in decreasing solubility, which was tested from 0.6 M to 3M NaCl (*66*).

2.4. METHODS FOR QUANTIFYING SILICA

Several methods can be used to determine silica in plant material, such as: gravimetric, colorimetric, spectrophotometric, x-ray, among others. They can be classified as gravimetric, non-destructive spectrophotometric (i.e.: x-ray fluorescence, near infra-red) and spectrophotometric after solubilization (i.e.: atomic absorption

(AA), inductively coupled plasma (ICP) and colorimetric). These methods can also be classified by their complexity, equipment costs, training cost and time to get the results. This comparison is presented in Table 2.

Table 3 shows different methods used in different studies to quantify silica in plant material. To date there is a need to have a uniform method for the determination in plant material, a method that is time effective and affordable.

2.4.1. Determination of Silica by Gravimetry

A gravimetric method after ashing was published in the late 1970s to determine silicon in plants (*15*). This method consisted in oxidizing the organic compounds of the plant sample, and solubilizing the residual non-silica material with an acid, the remaining precipitate was assumed to be silica or “crude silica”, removed by filtration and weighed (*15*). Later a micro gravimetric method was proposed (*67*).

A modification of the gravimetric method was suggested by Elliot and Snyder (*14*). This rapid gravimetric method consisted in oxidizing, washing, and weighing the sample in one single Gooch crucible. They also added an acetone extraction step in order to “solubilize various organometallic components”.

2.4.2. Solubilization of Plant Silica and Colorimetry

Solubilization of plant silica is required prior to spectrometric analysis. There are several ways to achieve this purpose, the most typical being 1) fusion with strong alkali (i.e. NaOH, Na_2CO_3) to convert silica into sodium silicate and dissolve it in water, 2) digestion with HNO_3/HCl, 3) autoclave induced digestion with NaOH and H_2O_2, or 4) extraction at room temperature with HCl and HF (shaking overnight) (*21*).

Other solubilization techniques have been applied, such as a digestion process that involves a reaction with sulfuric acid, anhydrous sodium carbonate and hydrochloric acid (*68*). Nayar et al (*69*) reported a direct method of estimation of silica in rice plant tissues without ashing and fusion, where silica was taken into solution after digesting the plant sample under heat with concentrated nitric acid followed by anhydrous sodium carbonate in suspension. The outcome showed that results with nitric acid digestion were comparable to the one with the fusion method (69).

After silicon is taken into solution, it can then be determined by colorimetric methods such as the molybdosilicate and heteropoly blue method (adding a reducing agent to the molybdosilicate complex) (*70*). The molybdosilicate based colorimetric method for sea water was adapted to rice leaves with the aim of finding a method of high sensitivity to determine silica in rice other than the gravimetric or micro gravimetric methods. This method involves the production of yellow molybdosilicic acid ($H_4SiMo_{12}O_{40}$) or its reduction product, molybdenum blue (*68*). The latter is also called heteropoly blue method, and its preferred because the formation of the blue compound enhances the method's sensitivity (*57*).

Details of the colorimetric method are as follows: An aliquot of the solution is adjusted to pH 1.6 with HCl (*39*). Lower pH was shown to slow down the color development reaction (*71*). The sample first reacts with ammonium molybdate 5% and after 10 minutes tartaric acid is added to destroy any phosphomolybdate complex (also known as molybdophosphoric acid) (*39*). Tartaric, citric and oxalic acids can be used to prevent the formation of molybdophosphoric acid (*72*). Reducing solution (containing 1-amino-2-naphthol-4-sulfonic acid, sodium sulfite, sodium bisulfite

dissolved in water) is added to produce the blue silicomolybdic complex and the sample is diluted. The absorbance of the sample is read at 815nm (*39*).

The basis of the colorimetric method for determination of silica is that only soluble silica forms a yellow silicomolybdate complex with molybdic acid and colloidal silica does not. A review of several articles regarding the conditions that have an effect in this methodology revealed that pH is an important factor, the form of silicomolybdic acid has an effect in the absorbance, and that silica concentrations should not be greater than 100ppm (*56*). Only monomeric silicic acid reacts with ammonium molybdate, therefore other polymeric forms can not be detected by this methodology (*21*).

Electrolytes present in water samples have an effect in the spectrophotometric determination of silica. The first step of this methodology involves the formation of molybdosilicic acid, which exists as two isomers, α and ß (*73*). The isomers' formation depends on the pH of the solutions, the ß isomer decays faster and its decay is increased by the presence of electrolytes (*73*).

Other modifications of the molybdosilicate method have been proposed (*21*) such as the use of 20% acetic acid instead of 1+1 HCl.

The feasibility of silica detection methods has found limitations due to the lack of reference materials among others. The application of the molybdenum blue method in biological samples is still questionable (*74*). Despite of all, the colorimetric method is one of the most used due to its lower detection limits, and low cost in comparison to induced plasma and atomic absorption (*21*).

2.4.3. Other Detection Options

Solubilized samples could be measured by other techniques such as Atomic Absorption Spectrophotometry (AAS) and Inductively Coupled Plasma-Atomic Emission Spectrophotometry (ICP-AES), tests on HCl and HF extracts have been done finding no difference between these two methods (*22*). The determination of silica by ICP-AES has been studied and adopted as a direct method in seawater (*55*).

Silicon has been measured by direct current plasma emission in urine samples, best results have been documented when using calibration curves with standards in a similar matrix. The method gave reliable results in the range 0-50 mg per L (*75*).

Solubilization of silica by means of pyridine N-oxides has been proposed by Ranganathan et al (*76*) but further research is needed.

2.5. METHODS OF SILICA EXTRACTION

First attempts on silica extraction were reported in 1961 by Copa and Wallace (*39*), where ground oat straw in a small chromatographic tube was extracted with water, and diluted HCl, passing through at 1ml/min. The eluate was collected for analysis. It was cited by Copa and Wallace that organic reagents tend to reduce the rate of extracted silica, but if plants were extracted initially with acetone, methanol or ethanol, and then with water the silica extraction rate was increased. This was attributed to plant silica being enclosed by acetone soluble materials. Primary extraction with benzene and methyl alcohol followed by hot water extraction or hot methyl alcohol resulted in an extraction of 82% silica (*39*).

In the 1970s, a group of studies on extraction of silicon from plant material was done in Poland at the Institute of Chemistry and Analytics (*77-81*). Extraction of

silica from *E. arvense* and *Urtica dioica*, was done at an optimum herb to water ratio of 1:100 (w/w) and resulted in a extraction of 16% silica and less than 50% respectively (*81*). Sixty five percent silica was extracted from *C. acanthoides* at a herb water ratio of 3:200, at 90°C for 6hr (*77*). *Agropyron repens* 3:200 was extracted 24 hours at 90°C achieving an extraction of 54% silica (*79*). Further research was done in order to find a way to preserve the plant silica water extracts, 0.01% formic acid gave maximum stability lowering the pH and preventing polymerization (*78*).

A further silica extraction experiment was done using NaOH. The methodology consisted on pre-desilication of wheat straw with 1% NaOH, 80°C for 30 min (*28*), 73% silica was removed. More alkali extraction studies were reported (*82*) where alkali soluble wheat straw lignins are extracted with no report on silica; treatments of 1.5% KOH at 20°C for 6hr, 1.5% LiOH at 20°C for 6 h, and 1.5% NaOH at 20°C for 0.5 to 144 h. Samples were neutralized afterwards with glacial acetic acid (*82*). Deashing/desilication of 78.2% from wheat straw was accomplished with sodium carbonate (10% Na_2O) at 60°C for 30 min (*29*).

Five leaching methods were applied for rice straw by Jenkins (*10*), wheat straw and switch-grass (wood and sugar cane too). Leaching with room temperature (20-25°C) water by 1) spraying tap water over 100g whole straw (30 mm) bed on an extended mesh for 1 min, 2) 100g milled straw (19mm) flushed with 20 l tap water, 3) milled straw (19mm) flushed with 20 l distilled water, 4) 50 g milled straw (20 mesh) flushed with 7 l distilled water, 5) 100 g whole straw submerged in 7 liters distilled water for 24 h, 6-8) natural rain washing treatments. Amount of silica in ash increased after the extraction treatments, silica appear to be inert. Moreover, silica was proposed

as a tracer for predicting ash content assuming that it is not lost by washing (*10*). This finding agrees with the results obtained after leaching wheat straws, with tap water at room temperature where silicon content remained the same (*83*).

More studies related leaching of inorganic materials showed improvement of combustion properties of biomass. Leaching of rice straw, wheat straw, switch grass, and banagrass (20mesh) with water reduced about 80% of potassium and sodium and 90% of chlorine. Leaching processes involved soaking samples in water overnight at room temperature followed by washings (*27*).

More water extraction research has been done recently but mainly focused on combustion properties, and elements such as potassium, sodium, chlorine and sulfur (*84*). Leaching process was done using tap water at room temperature, samples were put in a 200 mesh plastic grid and were submerged into tap water for 24hrs. Water mass ratios were 45, 200 and 120 g/g (*84*). Removal of 71% of K, 72% of Cl and 98% of Na from chars was achieved by washing with water at 82°C (*8*).

3. MATERIALS AND METHODS

3.1 PLANT SAMPLES

Samples of Kentucky Bluegrass (*Poa pratensis*) were collected on an eastern Washington farm, milled to pass a 20-mesh screen and dried to a moisture content of <10%. The sample was provided by the USDA-ARS Forage Laboratory in Corvallis. Perennial Ryegrass (*Lolium perenne*), wheat straw (*Triticum aestivum*) and tall Fescue (*Festuca arundinacea*), were obtained from local farms (mid-Willamette Valley, Oregon), dried at 40^{o}C in accordance with NREL 9/21/05 method B (Item 10.3) 2005 (*85*) to < 7% moisture and milled to pass a 20-mesh screen.

3.2 EXPERIMENTAL DESIGN FOR COMPARISON OF QUANTITATIVE METHODS

A gravimetric and four colorimetric assays were compared. The four colorimetric assays were based on the possible combinations obtained when grouping two distinct digestion methods and two distinct color-development methods (details of each procedure below). A pictorial diagram of the treatment structure (experimental design) is presented in Figure 1. The gravimetric method was done in triplicate on three separate occasions (days) for each of the straws tested. The colorimetric methods were also done on three separate occasions for each straw. On each of those occasions, the test digestion method was done in triplicate and the subsequent color-development assays of each resulting digesta-containing solution were done in duplicate.

3.2.1 Digestion

3.2.1.1 Acid/Alkali Digestion

Digestion method 1 (D1) was adapted from that of Nayar et al. 1975 (*69*). To 100 mg straw, dry weight basis, in a 50 ml glass conical flask was added 5 ml concentrated nitric acid and the resulting suspension was digested on a hot plate, at boiling in an appropriate fume hood, until complete as indicated by the ceasing of brown fumes and a volume reduction to 2 ml. The resulting suspension was transferred with several washings into a 150 ml stainless-steel beaker containing 1-1.5 g anhydrous sodium carbonate in suspension and boiled 2-5 minutes (*69*). The resulting solution was transferred to a polypropylene bottle and then diluted with deionized/distilled water to 50 g and subsequently analyzed for silica content using one of the color-development methods described below.

3.2.1.2 Autoclave Induced Digestion

Digestion method 2 (D2) was that of Elliott and Snyder (1991) (*18*), as modified by Bell and Simmons (*86*). To 100 mg of straw, dry weight basis, in a 250 ml polypropylene screw closure centrifuge bottle (Nalgene 3120) was added 2 ml hydrogen peroxide 50% and 4.5 g NaOH 50% (w/w) sodium hydroxide. The resulting suspension was autoclaved at 138 kPa for 1 hour (*18*). The digested sample was diluted to 50 g with deionized/distilled water and subsequently analyzed for silica content using one of the color-development methods described below.

3.2.2 Colorimetric Quantification

3.2.2.1 Colorimetric Method 1

In color development method 1 (APHA, 1995 (*70*)), silica in solution is acidified with hydrochloric acid, reacts with ammonium molybdate, oxalic acid is added to remove phosphate interferences and finally a reducing agent is added to develop a blue complex, measured at 650nm (*70*). Silicon standards were prepared from VWR Silica standard No VW3461-2, 1.00 ± 0.010 mg SiO_2 ml^{-1} solution. Digests were analyzed in duplicate on the same day.

3.2.2.1 Colorimetric Method 2

In color development method 2 (*18*), silica in solution is acidified with acetic acid, then reacts with ammonium molybdate, tartaric acid is added to remove phosphate interferences and finally a reducing agent is added to develop a blue complex, measured at 650nm (*18*). Silicon standards were prepared from VWR Silica standard No VW3461-2, 1.00 ± 0.010 mg SiO_2 ml^{-1} solution. Digests were analyzed in duplicate on the same day.

3.3 SILICA DETERMINATION BY GRAVIMETRY

Gravimetric silica analyses were conducted as recently described by Morikawa and Saigusa 2004 (*16*). Approximately 3 g of straw, weighed to the nearest 0.1 mg, were ashed in a platinum crucible at 575°C for 24 hr. The resulting ash was washed 6 times with 5 ml dilute acid mixture (1.5 M HNO3: 3.71 M HCl), pouring off the supernatant following gravity settling of the undissolved ash. The suspension resulting from the final wash was filtered using an acid hardened ash-less filter paper (Whatman 541), and rinsed with distilled water until chloride ion could not be

detected in the rinse (detected as AgCl precipitate). The acid-insoluble ash-containing filter paper was then ashed as above and weighed – the ash residue was taken as silica (*16*).

3.4 TOTAL SOLIDS/ MOISTURE

Total solids were determined gravimetrically as described by NREL LAP-012 1994 using a forced-air convection oven at 105 ± 2°C (*87*), drying to constant weight. Moisture determinations were done in triplicate.

3.5 ASH

Ash was determined gravimetrically as described by NREL LAP-005 2005 using a muffle furnace at 575°C ± 25°C (*88*), drying to constant weight. Ash determinations were done in triplicate.

3.6 STATISTICAL ANALYSYS

SAS 9.1.3 software for Windows was used to run Chi-Square tests to determine the homogeneity of day-to-day variances within methods; this test being done to objectively test the appropriateness of pairing the color development methods (C1 and C2) and the digestion methods (D1 and D2) across days. The test was not significant at 95% confidence level for day-to-day dependence, thus allowing pairing of the data and its analysis as a nested model, with 5 treatments and 9 replicates per treatment.

The nested data, with replicates within days and days within treatments, was analyzed for statistically significant differences between methods using a general

linear model and the sources of observed difference were identified by Tukey's post hoc test (SPSS 14.0.2 for Windows).

3.7 SENSITIVITY AND PRECISION

Calibration sensitivities are herein taken as the slopes of the corresponding calibration curves; analytical sensitivities are taken as the slopes of the calibration curves divided by the standard deviations of the signals at the concentrations of interest (*89*). Precisions are herein based on the standard deviations of the corresponding assays.

3.8 EXTRACTION TECHNIQUE

Wheat straw was used for extraction experiments. Extractions were done with deionized/distilled water at three temperatures (30°C, 60°C and 90°C); four time points (0.5, 1.5, 3 and 6 hours) were chosen to ascertain the time-course of extraction at each of the temperatures. Each time/temperature combination was analyzed in triplicate. To initiate the extraction, 1 gram of straw was added to 99 g temperature-equilibrated water in a 125 ml screw capped polymethylpentene Erlenmeyer flask. Flasks containing straw and water at the appropriate temperature were agitated at 150 rpm for the duration of the extraction period.

After the extraction period, test solutions were filtered using Whatman No. 41 (pre-dried and weighed, retention size 20-25 μm) into a pre-weighed filtration flask, the filtrate was then filtered again using Whatman No. 42 paper (pre-dried and weighed, retention size 2.5 μm), the filtrate being collected in the same pre-weighed filtration flask. Filter papers containing the moist retentate and the filtration flask

containing the combined filtrate were weighed. The moisture, ash, and silica (gravimetric) content of the retentate were determined gravimetrically (methods as described above). The total solids, ash and silica (colorimetric) content of the filtrate were also determined. The experimental design for this extraction experiment is depicted in Figure 2.

4. RESULTS AND DISCUSSION

4.1 COMPOSITION OF STRAWS USED IN THIS STUDY

The organic macrocomponent compositions of the straws used in this work are reported in Table 4, ash and moisture composition are reported in Table 5. Published ash contents for comparable straws are presented in Table 6. The range of ash and silica values observed for the straws may be attributed, among other things, to differences in growth conditions, parts of the plants analyzed, age, and extents of extraneous contamination. The moisture content of the straws, as stored throughout the study, was approximately 7%; moisture was monitored throughout the study for purposes of reporting results on a dry-weight basis. The differences in the ash values presented in Table 4 reflected the fact that the ash content determined by the present study is based in the original plant material.

4.2 COMPARISON OF METHODS

A comparison of the mean values obtained by the different analytical methods is presented in Table 9. The imbedded superscripts indicate that there was a statistically significant difference in the measured values, within a straw, depending on the method used. In general, the gravimetric method recorded the highest silica contents, the exception being the value obtained for Tall Fescue (the straw with the overall lowest silica content), in which case the gravimetric value was not significantly different from each of the colorimetric-derived values. Comparing the values for the colorimetric assays, there was a general trend for the autoclave/alkali digestion technique to provide higher silica values than the corresponding acid/alkali

digestion method. The table shows no evidence for a difference in the two color-development methods tested. Published values for the silica content of comparable straws are provided in Table 8, illustrating that the values obtained in this study, regardless of the method used, fall within the range of those reported in the literature.

The data summarized in Table 9 is broken out in Table 7 and Figures 3 to 6. Table 7 gives the day-to-day values obtained with each method; Figures 3 to 6 illustrates the dispersion of the complete data set taken together per plant sample. Differences in the precision of the methods are evident in these presentations. Figures 7 and 8 are included to illustrate the striking difference in the within-day precision of the digestion methods. Together, these data indicate that the autoclave/alkali digestion method is superior with respect to the precision of the method. For this reason the stability of the silica solution obtained by the autoclave/alkali digestion method was tested by assaying the solution for silica content over a 15 day storage period (solution resulting from wheat straw digestion was stored at room temperature in screw capped high-density polyethylene bottle). The results from this experiment are presented in Figure 11 demonstrating that the solution was stable over this time period, meaning digested solution could be analyzed any time within two weeks of digestion without quantitative consequences.

Silica content of the straws is presented in Table 7. Published silica content for comparable straws is presented in Table 8. The data depicted in Table 7 and Figures 3 to 6 illustrates that the differences in the two color-development methods are small. An experiment in which 10 replicates of the C2 colorimetric assay was done using silica standards (0.0025, 0.02, and 0.01 mg SiO_2 per assay) to get an

indication of the precision of this color-development method; the coefficients of variation at the three concentrations were 0.025, 0.037, and 0.031, respectively. The calibration curves of Figure 9 were used to determine the sensitivities of the two color-development methods. The calibration sensitivity of method C1 (with fresh reagent) was found to be 47 % higher than that of C2. The mean analytical sensitivity of the C2 assay, based on the standard deviations obtained in the experiment described just above, was 0.02. The stability of the reagents used in the two color-development assays was also tested. The results summarized in Figure 10 A show a decrease in the calibration sensitivity (slope) of the C1 assay over the eight-day test period. In contrast, there was no change in this parameter for the C2 assay over the same test period (Figure 10 B). The implication being that the C2 reagents, but not the C1 reagents, would generate reproducible data over the course of a week of use. The sensitivity data for C1 should be viewed as preliminary, as further testing is needed to verify the lability of these reagents.

In most of the plant samples, silica content values obtained with the gravimetric method (G) were higher. Higher values could be attributed to an incomplete dissolution of the acid soluble ash. Some soluble ash could remain and could be measured as silica rendering higher silica values, which is in agreement with King et al (*90*).

On the other hand, digestion protocol D1 and D2 could represent under-digested samples, giving lower silica content. Acid/alkali digestion protocol (D1) goes though boiling and washings steps which could include potential errors. As explained by Van Dyck et al (*74*), sample loss on hot plate digestion and improper

dilution, could introduce errors on digestion protocols. Boiling could result in projecting droplets of the analyte out of the reacting flask; on the other hand if washings are not done properly some silica can be left behind, both situation would result in lower values. One more error source in the acid/alkali digestion process is the addition of anhydrous sodium carbonate in an approximate amount of 1 – 1.5 g, which could result in different pH of the final solution, and pH is closely related to silica solubility. All these factors could contribute to have lower silica contents. Acid/alkali digestion protocol consistently showed some values above the average, the presence of this high values could be attributed to outliers or to the presence of samples that have been fully digested.

Acid/alkali digestion protocol (D1) figures appear more scattered than the ones with autoclave induced digestion protocol (D2). Protocol D2 digests the plant sample under same alkali conditions; pH is kept always high favoring silica solubility. Autoclave induced digestion rendered values that appear more precise. A closer comparison between digestion protocols is revealed in Figures 7 and 8, Perennial Ryegrass, Tall Fescue and Wheat straw confirm the tendency affirmed above, autoclave induced method provides data with lower spread than those obtained with acid/alkali digestion. Silica values appear closer for AID, independently from the color development protocol.

Color development protocols showed no evident difference on silica content, but the values were lower than G values. Independently from the digestion protocol color development protocols consist in the formation of a blue complex, which is formed by reactive silica with molybdic acid. Reactive silica is a term that refers to

silica in the form of monosilicic acid, it does not include polymeric silica, which is un-reactive and would not be measured by the color development protocol (*56, 57, 91*) resulting in lower values.

4.3 METHOD APPLICATION IN THE EVALUATION OF HOT-WATER LEACHING OF SILICA FROM STRAW

The data presented in Figure 12 illustrates the application of the C2 method in a study evaluating the efficacy of using hot water to leach silica from wheat straw. Experiments of this type are important with respect to upgrading the quality of straws for thermochemical processing. The object of the leaching, in this case, is to remove silica, along with the alkali and alkaline-earth metals, from the straw prior to thermochemical processing (*9, 25, 92*). The evaluation is done by measuring the silica content of the starting material and the silica content of the leachate (see methods for details). The data demonstrate the importance of temperature in removing silica. Less than 5% of the original straw silica could be detected in the leachate following the 30^{o}C treatment. The 90^{o}C treatment, however, resulted in a removal of greater then 40% of the original silica from the straw. Correspondingly, this same treatment resulted in the removal of approximately 12% of the total solids from the original straw. These results are in general agreement with those of Thompson et al. (*20*); a study in which energy dispersive spectrometry and inductively coupled plasma – atomic emission spectrometry were employed. To maintain maximum heating values for the leached straw, one would want to minimize the extraction of organics while maximizing the extraction of deleterious inorganics.

Effect of extraction temperature over silica content by Gravimetry in retente (R) and extract (E) are given on Figure 13. Mass balance was very hard to achieve. Numbers evidence a higher extraction percentage of 60% silica at 90°C after 6 hours of extraction. Plotting silica extraction based on the color development protocol, Figure 12, gave a lower fraction, 45% at 90°C after 6 hours of extraction. For comparison Copa et al 1961 (*39*) could extract 82% of the silica from oat straw with hot water (boiling), straw was previously acetone rinsed. Piekos et al 1976 (*78*) extracted silica up to 65% at 90°C after 6 hours from herbs. In contrast Thompson et al 2003 (*20*) reported the extraction of little silica from wheat straw at 25, 37 and 50°C and Jenkins et al 1995 (*93*) noted that silica increases in concentration after leaching and appears inert. In this study treatment at 30°C also removed little silica.

According to literature values, Lenher and Merrill 1917 (*60*), a solubility of 0.02 g silica per 100 ml was expected, our results based on gravimetric method were lower 0.001 g /100 ml at 30°C for 6 hours. On the other hand at 90°C for 6 hours, 0.04g/100ml was expected and our results almost agreed, approximately 0.03 g / 100 ml.

Figure 1. Diagram of Treatment Structure for Method Evaluation, D1_C2, acid/alkali digestion (*69*) paired with colorimetric method 2 (*18*); D2_C2, autoclave induced digestion paired with colorimetric method 2 (*18*); G, gravimetric method (*16*); D1_C1; acid/alkali digestion (*69*) paired with colorimetric method 1 (*70*); D2_C1, autoclave induced digestion (*18*) paired with colorimetric method 1 (*70*).

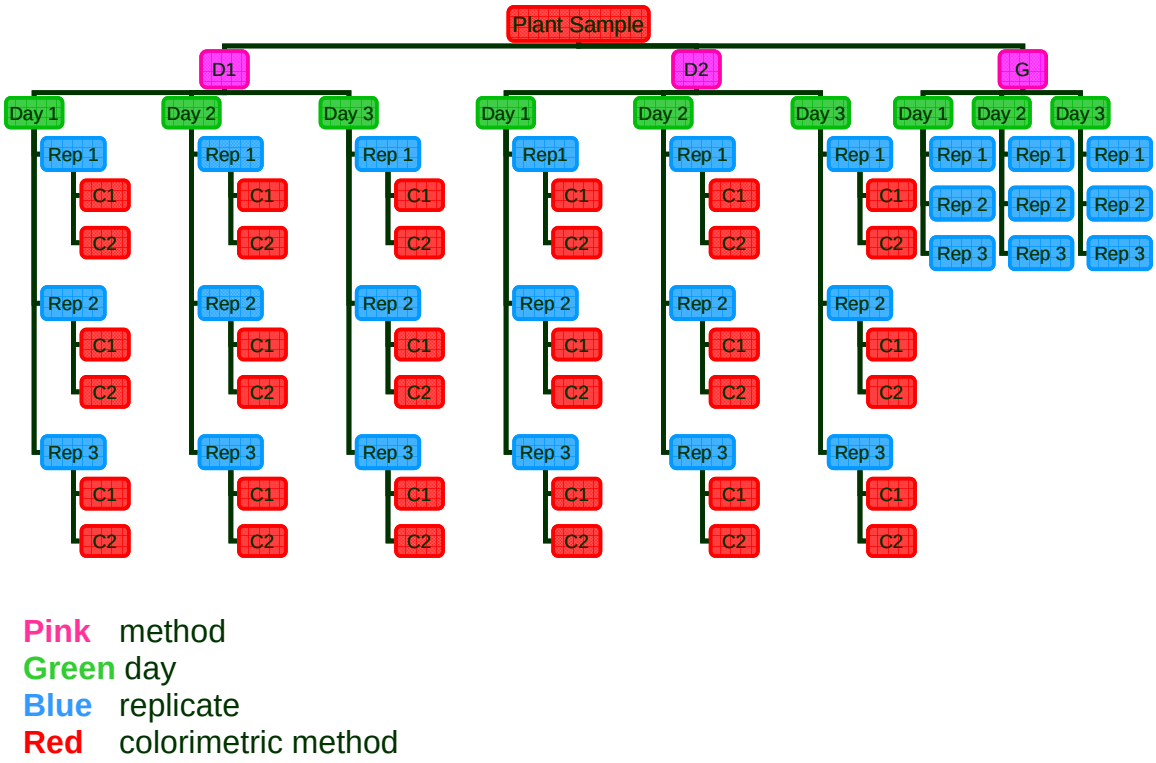


Figure 2. Diagram of Treatment Structure for Extraction - all treatments done in triplicate.

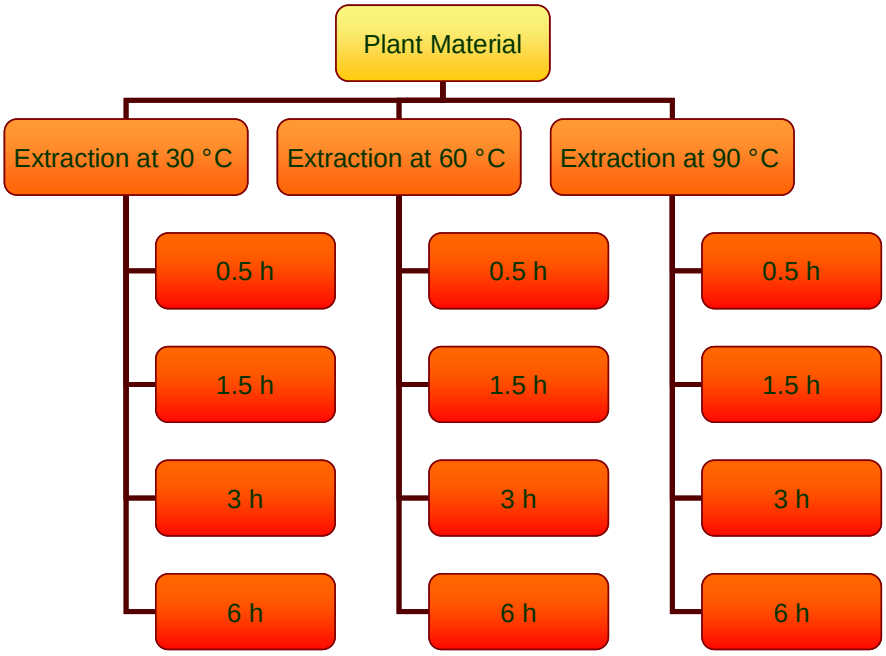


Figure 3. Data dispersion by method: Kentucky Bluegrass. D1_C2, acid/alkali digestion (*69*) paired with colorimetric method 2 (*18*); D2_C2, autoclave induced digestion paired with colorimetric method 2 (*18*); G, gravimetric method (*16*); D1_C1; acid/alkali digestion (*69*) paired with colorimetric method 1 (*70*); D2_C1, autoclave induced digestion (*18*) paired with colorimetric method 1 (*70*).

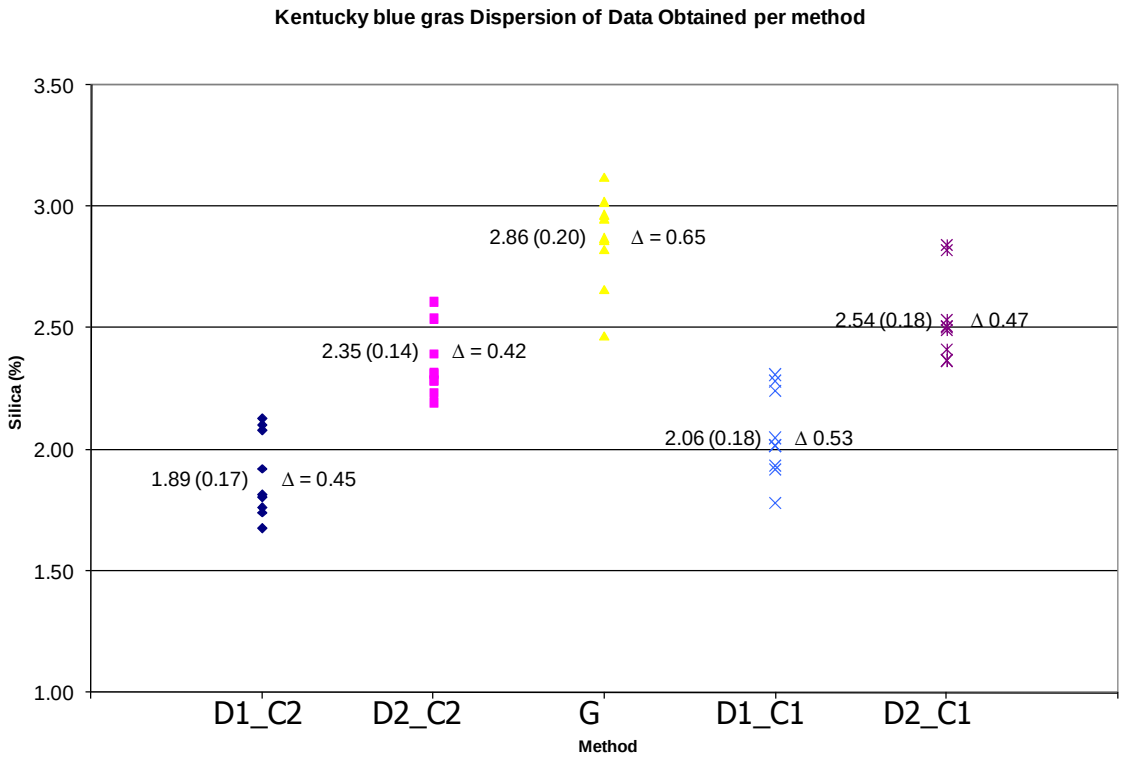


Figure 4. Data dispersion by method: Perennial Ryegrass. D1_C2, acid/alkali digestion (*69*) paired with colorimetric method 2 (*18*); D2_C2, autoclave induced digestion paired with colorimetric method 2 (*18*); G, gravimetric method (*16*); D1_C1; acid/alkali digestion (*69*) paired with colorimetric method 1 (*70*); D2_C1, autoclave induced digestion (*18*) paired with colorimetric method 1 (*70*).

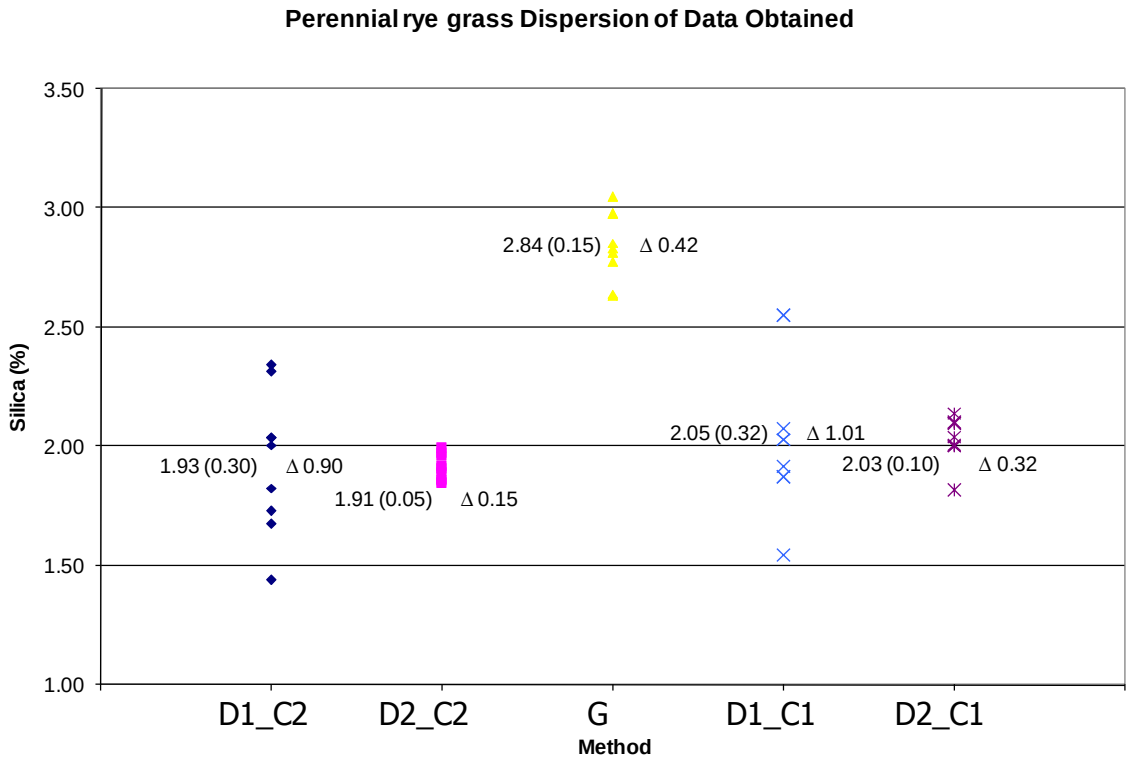


Figure 5. Data dispersion by method: Tall Fescue. D1_C2, acid/alkali digestion (*69*) paired with colorimetric method 2 (*18*); D2_C2, autoclave induced digestion paired with colorimetric method 2 (*18*); G, gravimetric method (*16*); D1_C1; acid/alkali digestion (*69*) paired with colorimetric method 1 (*70*); D2_C1, autoclave induced digestion (*18*) paired with colorimetric method 1 (*70*).

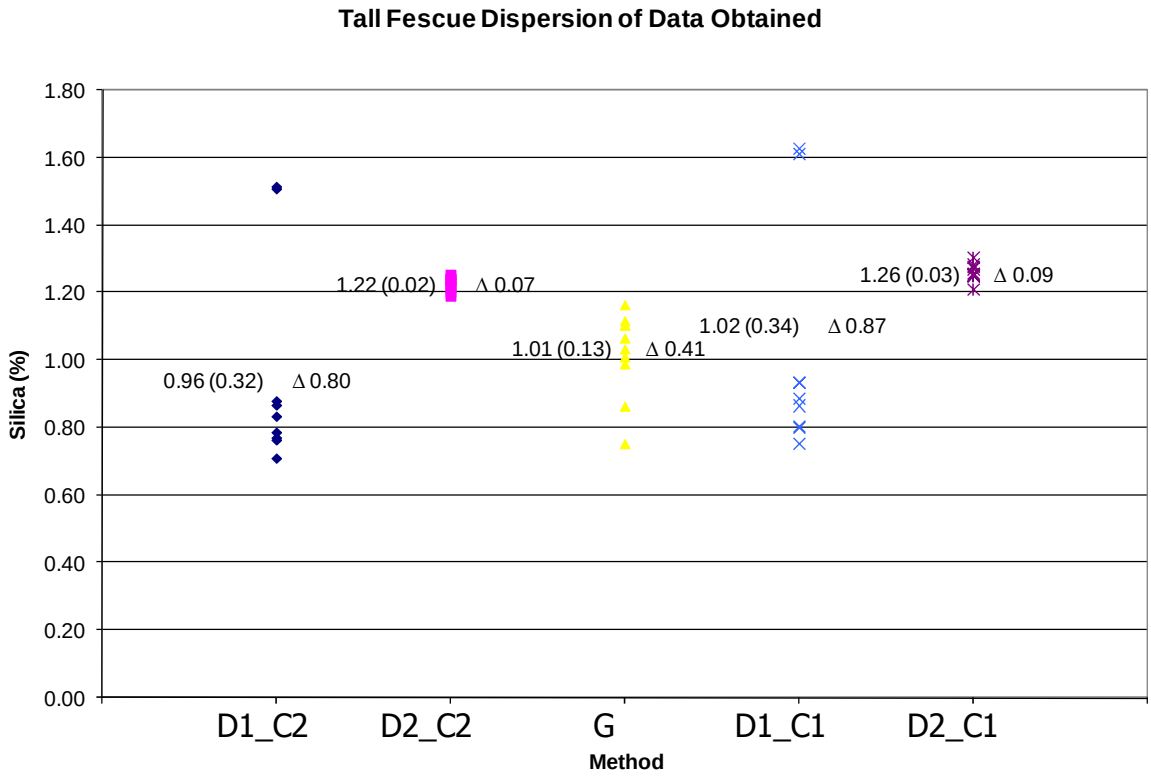


Figure 6. Data dispersion by method: Wheat straw. D1_C2, acid/alkali digestion (*69*) paired with colorimetric method 2 (*18*); D2_C2, autoclave induced digestion paired with colorimetric method 2 (*18*); G, gravimetric method (*16*); D1_C1; acid/alkali digestion (*69*) paired with colorimetric method 1 (*70*); D2_C1, autoclave induced digestion (*18*) paired with colorimetric method 1 (*70*).

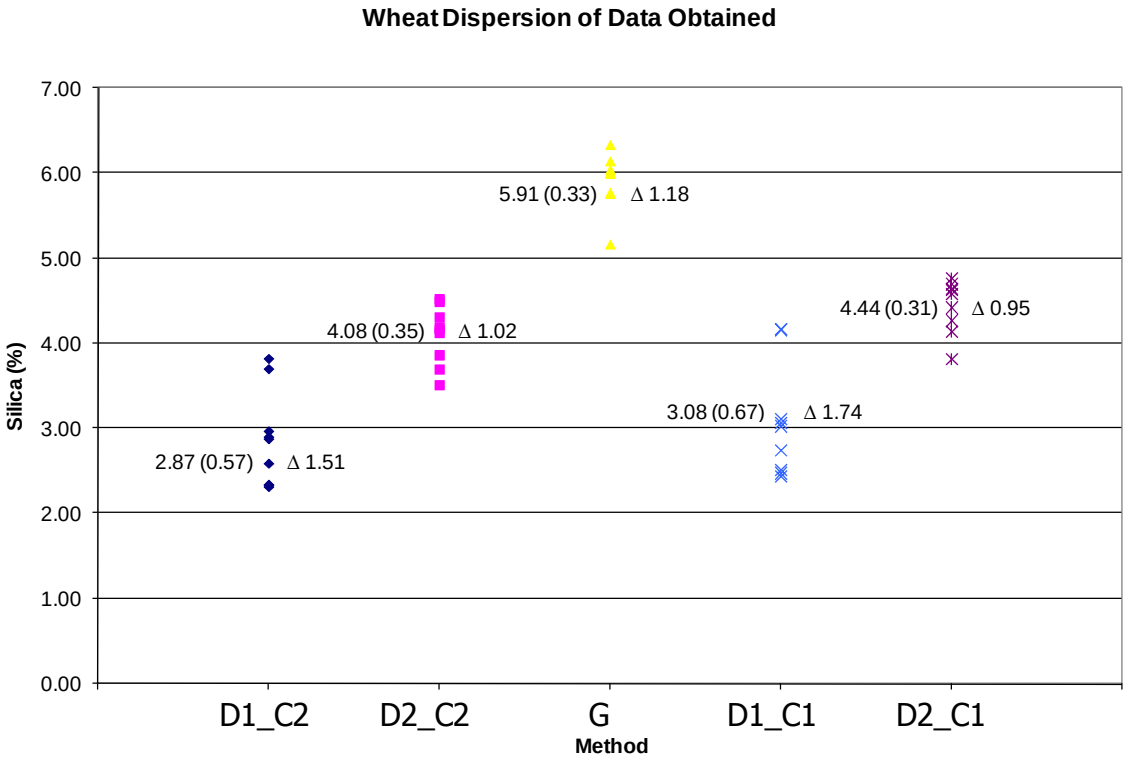


Figure 7. Digestion method comparison per plant sample: Kentucky Bluegrass (top) and Perennial Ryegrass (bottom). D1_C1; acid/alkali digestion (*69*), with colorimetric 1 (*70*) versus D1_C2, acid/alkali digestion (*69*), with colorimetric 2 (*18*); and D2_C1, autoclave induced digestion (*18*), with colorimetric 1 (*70*)D2_C2, versus autoclave induced digestion with colorimetric 2 (*18*).

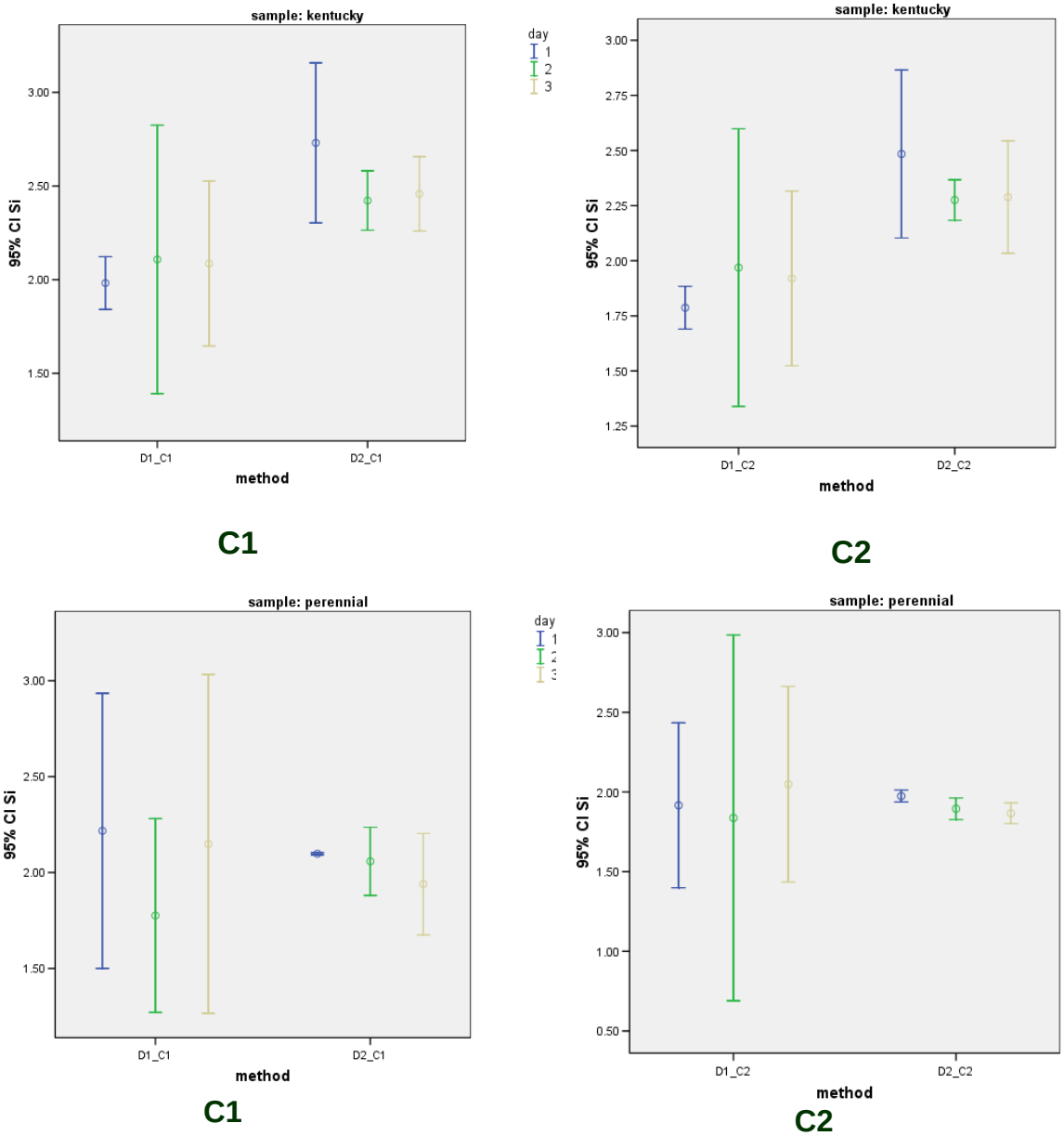


Figure 8. Digestion method comparison per plant sample: Tall Fescue (top) and wheat straw (bottom). D1_C1; acid/alkali digestion (*69*), with colorimetric 1 (*70*) versus D1_C2, acid/alkali digestion (*69*), with colorimetric 2 (*18*); and D2_C1, autoclave induced digestion (*18*), with colorimetric 1 (*70*)D2_C2, versus autoclave induced digestion with colorimetric 2 (*18*).

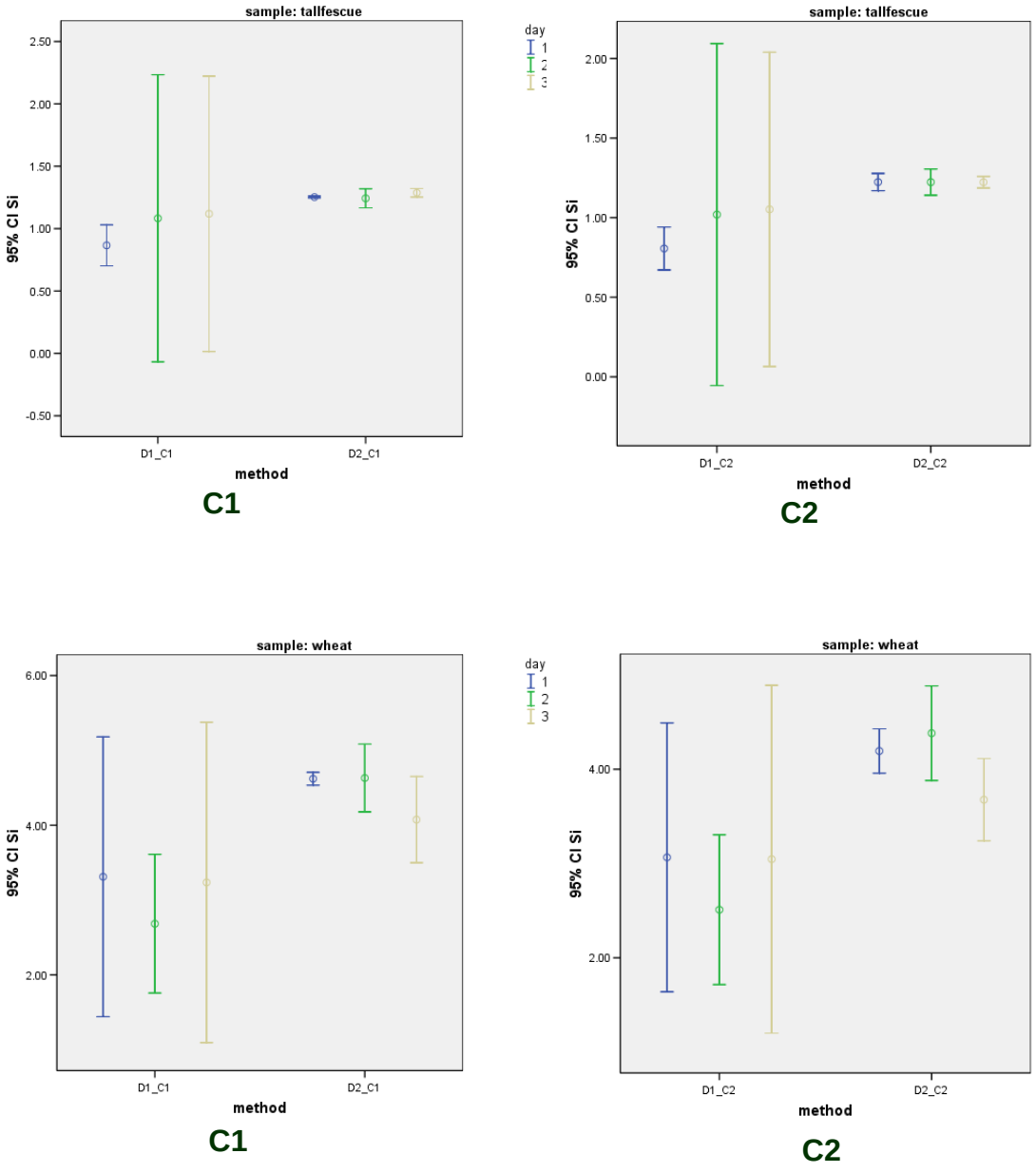


Figure 9. Calibration curves for colorimetric methods (C1 and C2) for evaluating sensitivity.

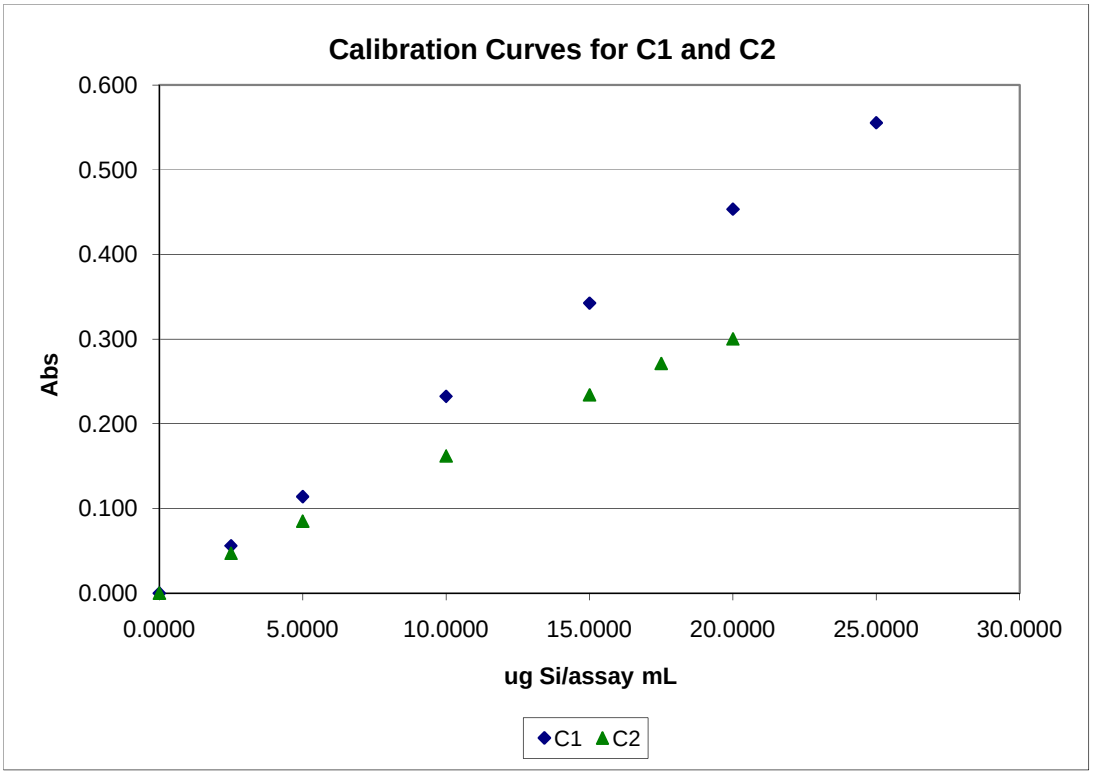
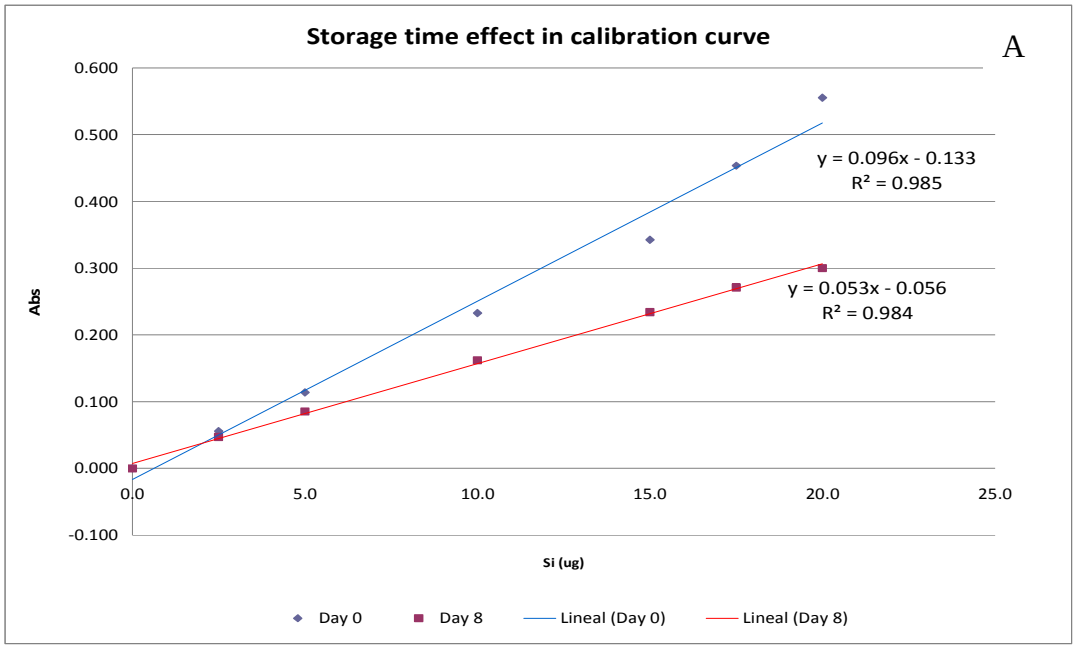


Figure 10. Effect of reducing agent storage time over the calibration curve. A) Reducing agent for colorimetric method 1, B) Reducing agent for colorimetric method 2.



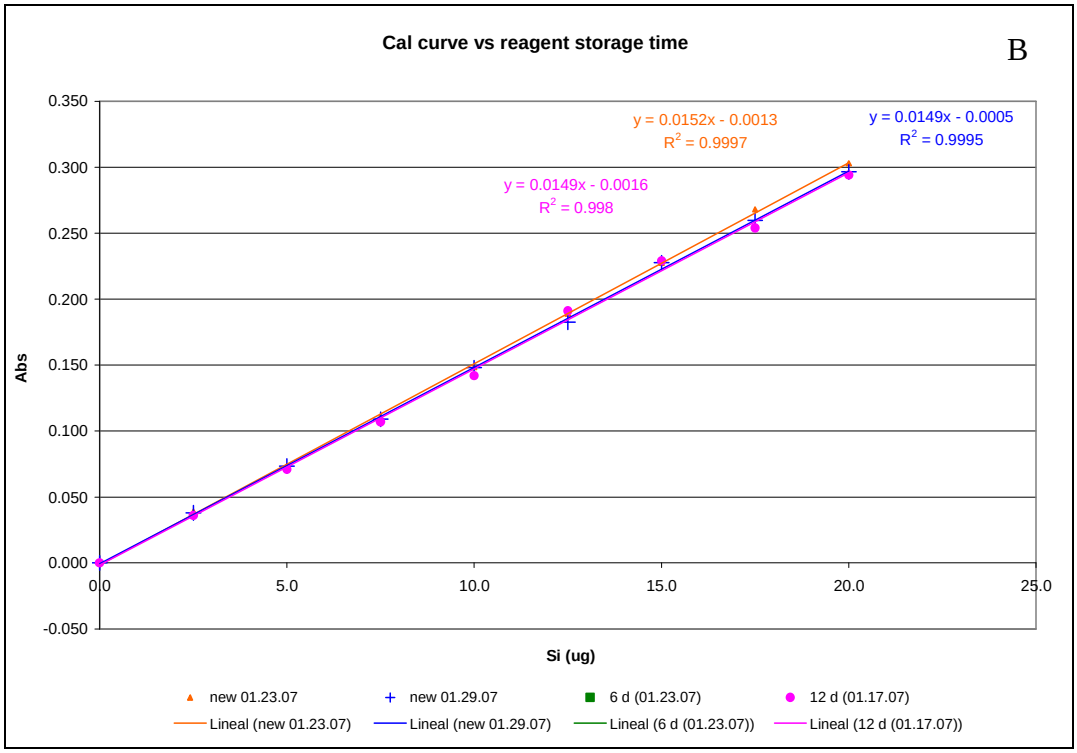


Figure 11. Effect of digested samples storage time on silica determination by colorimetric method 2. Sample: Wheat, n = 6.

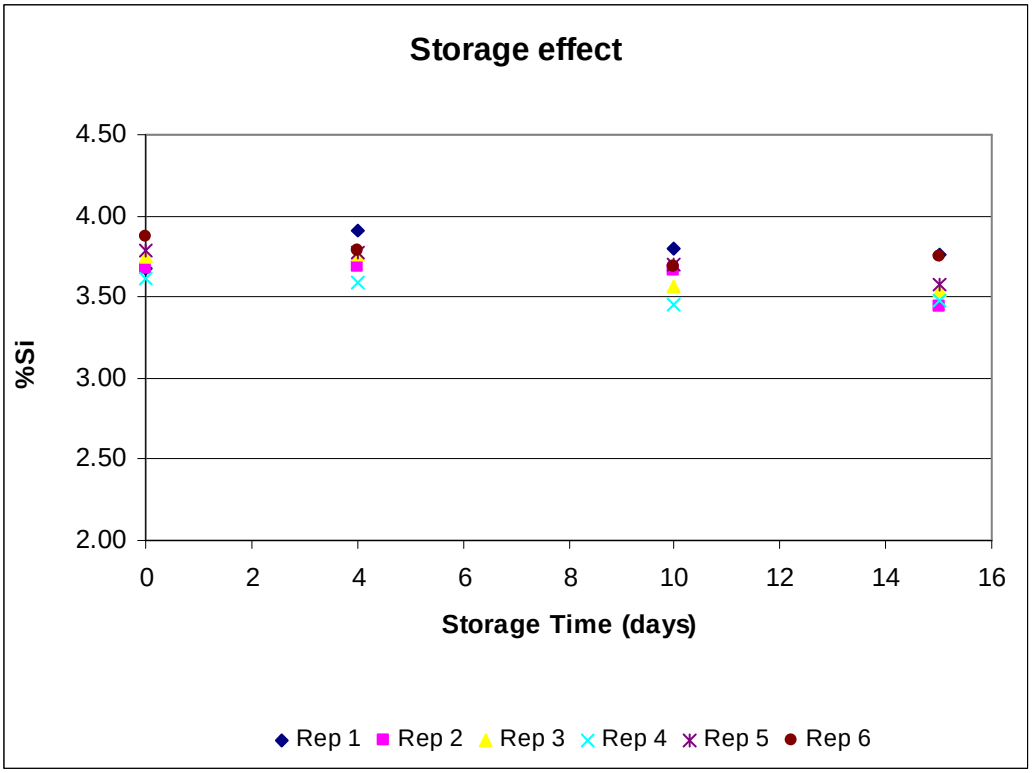


Figure 12. Temperature effect on silica extraction by colorimetric analysis of extract.

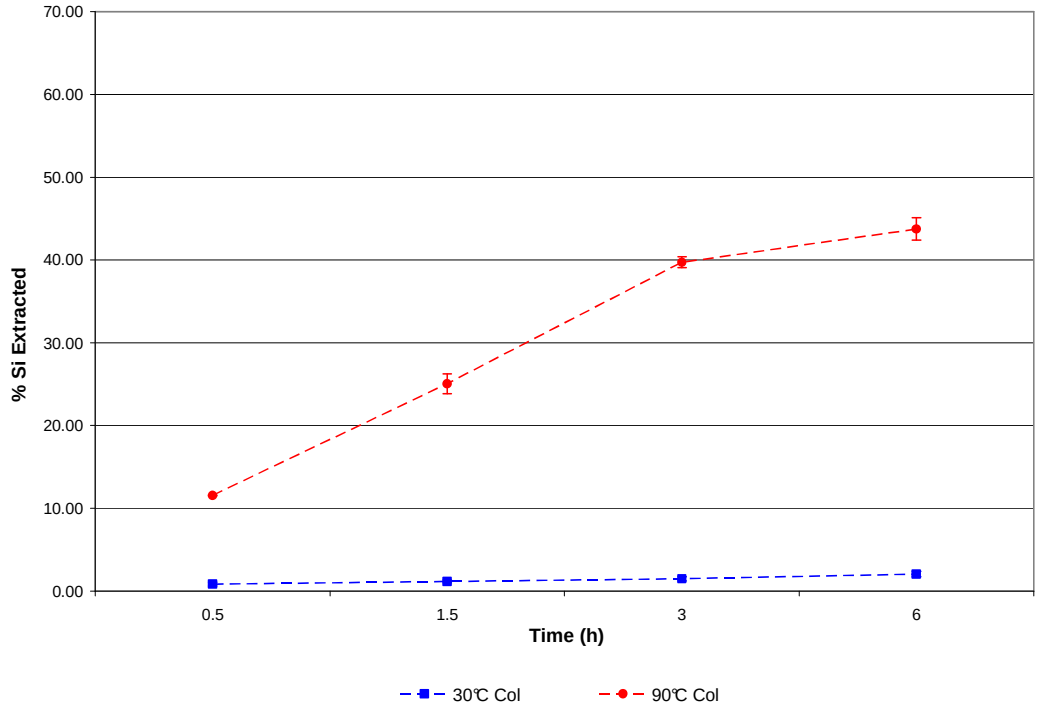


Figure 13. Temperature effect on silica extraction by gravimetric analysis of extract (E) and retente (R).

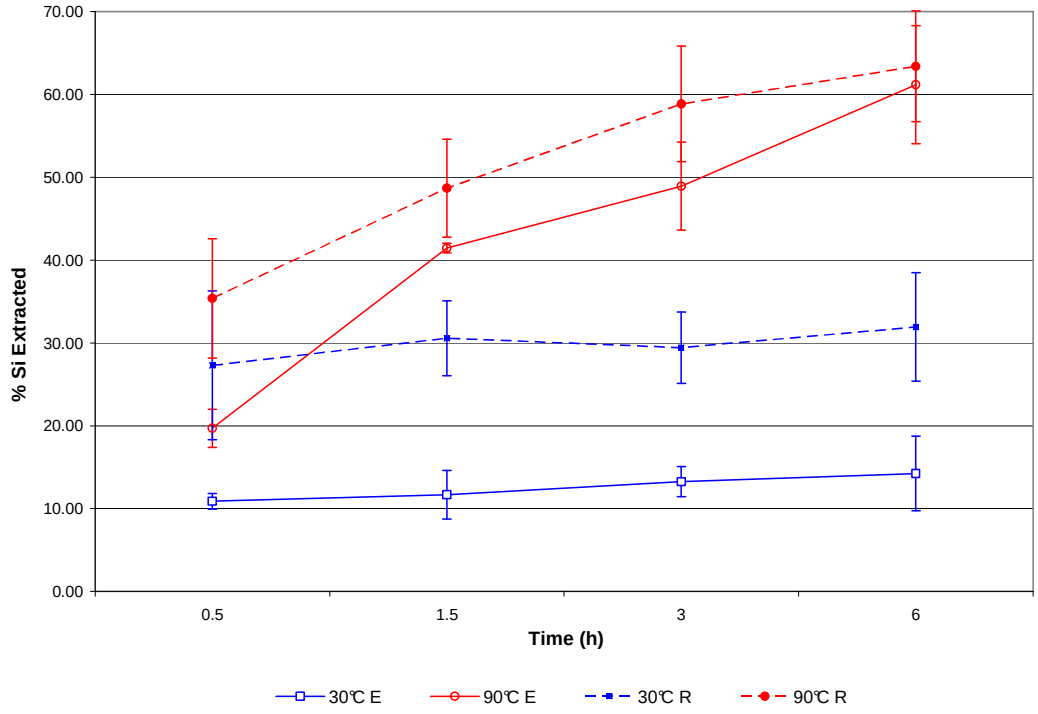


Table 1. Crystalline, amorphous, and aqueous forms of silica (SiO_2). (Published by Owen 1975 (*31*))

Crystalline silica:	**Species of silica with crystal structure**
a) Macrocrystalline	Quartz, tridymite, or cristobalite
b) Cryptocrystalline	Consists of fibrous crystallites with submicroscopic pores; general term is chalcedony
Amorphous silica:	**Forms of silica lacking crystal structure**
a) Silica gel	Hard amorphous silica containing 20 to 30% water, prepared commercially either as a chemical reagent or as a desiccant
b) Gelatinous silica	Appears in solution as gelatinous flocs or as a continuous gel, formed either by evaporation of silica solution, by allowing a supersaturated solution to stand, or by acidifying a fairly concentrated solution of an alkali silicate
c) Silica sol or colloidal silica	Silica dispersed in water in particles of colloidal dimensions (10^{-3} to 10^{-6} mm)
d) Opal	Naturally occurring silica, including the silica of diatomite and radiolarite, generally with less than 12% water. Some varieties appear to be transitional to crystalline material (cristobalite)
e) Silica glass	Prepared by the quenching of a silica melt
Aqueous silica:	**Silica species in solution**
a) Orthosilicic acid	The principal from of silica in saturated solutions with pH less than 9 is the monomer H_4SiO_4. Above pH 9, orthosilicic acid dissociates ($K1=10^{-9.8}$, (*64*))
b) Dissolved or colorimetric silica	The silica in true solution (H_4SiO_4) that reacts with ammonium molybdate within 2 minutes after the solutions are mixed
c) Polymerized silica	Silicic acids containing two (disilicic), three (trisilicic) or more atoms of silicon per molecule (including colloidal suspensions). At room temperatures noncyclic silicic acids react with ammonium molybdate within 5 min, but cyclic polysilicic acids require several hours to react (*94*). High order polymers do not react al all. In unsaturated solutions, poly silicic acids depolymerize to the monomer (*64*).
d) Total silica	All silica species in solution can be determined colorimetrically following conversion of polymerized silica to the monomer by treatment with NaOH, or by gravimetric techniques. In most natural waters gravimetric and colorimetric silica are identical, indicating the prevalence of the monomer H_4SiO_4.

Table 2. Frequently cited quantification methods for silica determination

Method	Equipment cost	Training needed	Reagents materials
Gravimetric	low	low	medium
XRFS	high	high	low
Colorimetric	medium	medium	medium
AA	high	high	medium
EDS	high	high	medium
ICP	high	high	medium

XRFS: X-ray fluorescence spectroscopy
AA: atomic absorption
EDS Energy dispersive x-ray spectroscopy
ICP: Inductively Coupled Plasma

Table 3. Silica determination methods used in plant and biomass research

Method	**Sample**	**Study**
Acid/alkali digestion and colorimetric	Rice straw	Nayar et al 1975 (*69*)
Atomic absorption (AA) ASTM D 3682	Wheat straw	Demirbas 2003 (*8, 95*)
AA, atomic emission spectrometry (AES)	Plant materials	Novozamsky et al 1984 (*22*)
Autoclave induced digestion (AID) and colorimetric	Rice straw	Bell and Simmons 1997 (*86*)
AID colorimetric vs. Na Fusion	Rice straw	Elliot and Snyder 1991 (*18*)
Colorimetric	Oat straw	Copa and Wallace 1961 (*39*)
Colorimetric vs. Gravimetric	Horsetail, Plumeless Thistle, Quackgrass, Stinging nettle	Piekos and Paslawska 1975 (*77-81*)
Combustion coupled to molecular beam mass spectrometer (MS)	Wheat straw	Dayton et al 1999 (*27*)
Energy-dispersive spectrometry (EDS)	Wheat straw	Hess et al 2003 (*96*)
EDS	Wheat straw	Hess et al 2003 (*96*)
EDS	Wheat straw	Thompson et al 2003 (*20*)
Gravimetric	Blueberry cuttings	Morikawa and Saigusa 2004 (*16*)
HCl-HF digestion with inductively coupled plasma (ICP) vs. AID colorimetric	Plant material	Taber et al 2002 (*23*)
Not specified	Wheat straw	Jenkins et al 1996 (*10*)
Not specified	Banagrass	Turn et al 1997 (*97*)
Plasma emission spectrometry	Bleach plant filtrates	Välttilä et al 1996 (*98*)
Rapid gravimetric	Rice straw	Elliot et al 1988 (*14*)
Scanning Electron Microscopy (SEM)	Rice and wheat straw	Jenkins et al 1995 (*93*)
SEM / Energy Dispersive X-ray (EDX)	Wheat straw and olive residue	Arvelakis et al (*5*)
SEM / EDX	Wheat straw and Olive kernels	Koukios et al 1999 (*13*)
Acid insoluble ash (gravimetric) TAPPI T244	Wheat straw	Pekarovic et al 2001 (*29, 99*)

Table 4. Composition of Extractive Free Residue following Sequential Soxhlet (H_2O and EtOH) Extractions of Commercial Grass Species. All values are percent of original (unextracted) solids (from Masrungson (2006) (*100*)).

Name	**Glycans**	**Acid Insoluble Lignin**	**Acid Soluble Ligning**	**Ash**	**Extractives**
Kentucky Bluegrass	45.4	11.1	1.77	1.76	29.2
Perennial Ryegrass	45.8	11.8	1.76	1.31	28.1
Tall Fescue	41.0	10.7	1.95	0.84	29.4
Wheat straw	53.8	14.0	1.62	2.19	16.3

Table 5. Moisture and ash content of plant samples, n=9

Sample	**Scientific name**	**% Moisture**	**% Ash (d.w)**
Kentucky Bluegrass	*Poa pratensis*	7.71 (0.18)	5.28 (0.06)
Perennial Ryegrass	*Lolium perenne*	7.19 (0.26)	5.54 (0.05)
Tall Fescue	*Festuca arundinacea*	7.24 (0.05)	5.40 (0.00)
Wheat straw	*Triticum aestivum*	7.10 (0.08)	7.82 (0.07)

Table 6. Ash content of plant samples reported in other studies

Sample	Part of plant	Scientific name	Ash %	Study
Kentucky Bluegrass, Newport	NA	*Poa pratensis*	0.13	Han et al 1975 (*101*)
Kentucky Bluegrass	NA	*Poa pratensis*	5.27	Masrungson 2006 (*100*)
Perennial Ryegrass	Leaves	*Lolium perenne*	0.12	Han et al 1975 (*101*)
Perennial Ryegrass	Stem	*Lolium perenne*	1.29	Han et al 1975 (*101*)
Perennial Ryegrass	NA	*Lolium perenne*	5.92	Masrungson(2006) (*100*)
Tall Fescue	Straw	*Festuca arundinacea*	6.48	Masrungson(2006) (*100*)
Wheat straw – Boundary	Straw	*Triticum aestivum*	4.1	Hess et al 2003 (*96*)
Wheat straw – Stephens	Straw	*Triticum aestivum*	5.4	Hess et al 2003 (*96*)
Wheat straw leached	Straw	*Triticum aestivum*	5.56	Demirbas and Fatih 2003 (*95*)
Wheat straw leached	Straw	*Triticum aestivum*	6.45	Dayton et al 1999 (*27*)
Wheat straw soaked	Straw milled	*Triticum aestivum*	6.45	Jenkins et al 1996 (*10*)
Wheat straw	Straw	*Triticum aestivum*	7.24	Masrungson(2006) (*100*)
Wheat straw	Straw	*Triticum aestivum*	7.5	Arvelakis, et al 1999 (*83*)
Wheat straw	Stem fraction	*Triticum aestivum*	8.7	Thompson et al 2003 (*20*)
Wheat straw – Westbred 936	Straw	*Triticum aestivum*	9.0	Hess et al 2003 (*96*)
Wheat straw	Whole straw	*Triticum aestivum*	11.2	Thompson et al 2003 (*20*)
Wheat straw	Straw	*Triticum aestivum*	12.78	Dayton et al 1999 (*27*)
Wheat straw	Straw	*Triticum aestivum*	12.78	Jenkins et al 1996 (*10*)
Wheat straw	Straw	*Triticum aestivum*	19.7	Demirbas 2003 (*8*)

Table 7. Silica content (%) for plant samples

Straw	Day	Replica	D1_C2	D2_C2	G	D1_C1	D2_C1
Kentucky Bluegrass	1	1	1.80	2.31	2.47	2.01	2.53
		2	1.81	2.61	3.02	2.01	2.82
		3	1.74	2.53	3.12	1.92	2.84
	2	1	1.68	2.24	2.86	1.78	2.36
		2	2.10	2.31	2.66	2.24	2.49
		3	2.13	2.28	2.83	2.31	2.41
	3	1	1.92	2.40	2.87	2.05	2.50
		2	1.76	2.19	2.95	1.93	2.37
		3	2.08	2.28	2.97	2.28	2.50
Perennial Ryegrass	1	1	2.03	1.99	2.98	2.55	2.10
		2	1.68	1.97	2.64	2.07	2.10
		3	2.04	1.96	2.63	2.03	2.10
	2	1	1.73	1.92	2.98	1.91	2.00
		2	1.44	1.90	2.85	1.87	2.14
		3	2.34	1.86	3.05	1.54	2.04
	3	1	1.83	1.86	2.78	2.55	1.82
		2	2.01	1.85	2.83	2.03	2.00
		3	2.31	1.90	2.81	1.87	2.00
Tall Fescue	1	1	0.87	1.20	0.99	0.93	1.26
		2	0.79	1.24	1.03	0.86	1.25
		3	0.76	1.23	1.06	0.80	1.25
	2	1	0.83	1.25	0.86	0.88	1.21
		2	1.51	1.19	1.16	1.61	1.25
		3	0.71	1.23	0.75	0.75	1.27
	3	1	0.88	1.24	1.01	0.93	1.30
		2	0.77	1.22	1.10	0.80	1.28
		3	1.51	1.21	1.11	1.63	1.28
Wheat	1	1	2.90	4.30	5.16	3.03	4.65
		2	2.59	4.11	6.01	2.74	4.58
		3	3.70	4.17	5.77	4.17	4.63
	2	1	2.33	4.52	6.00	2.50	4.70
		2	2.31	4.48	6.04	2.43	4.77
		3	2.88	4.15	5.75	3.11	4.42
	3	1	2.97	3.85	5.99	3.07	4.27
		2	3.82	3.50	6.14	4.17	4.13
		3	2.34	3.68	6.33	2.47	3.82

D1_C2, acid/alkali digestion (*69*) paired with colorimetric method 2 (*18*)
D2_C2, autoclave induced digestion paired with colorimetric method 2 (*18*)
G, gravimetric method (*16*)
D1_C1; acid/alkali digestion (*69*) paired with colorimetric method 1 (*70*)
D2_C1, autoclave induced digestion (*18*) paired with colorimetric method 1 (*70*).

Table 8. Silica content for plant samples reported in other studies, data under brackets were calculated from reported silica % and ash content

Sample	**Scientific name**	**Part of plant**	**SiO_2 %**	**Study**
Wheat straw	*Triticum aestivum*	Straw	(2.9)	Arvelakis, et al 1999 (*83*)
Wheat straw leached	*Triticum aestivum*	Straw	(2.7)	Arvelakis, et al 1999 (*83*) Demirbas and Fatih 2003 (*95*)
Wheat straw	*Triticum aestivum*	Straw	4.580	Dayton et al 1999 (*27*)
Wheat straw leached	*Triticum aestivum*	Straw	3.985	Dayton et al 1999 (*27*)
Wheat straw	*Triticum aestivum*	Straw	(9.6)	Demirbas 2003 (*8*)
Wheat straw – Westbred 936	*Triticum aestivum*	Straw	2.3	Hess et al 2003 (*96*)
Wheat straw – Boundary	*Triticum aestivum*	Straw	2.8	Hess et al 2003 (*96*)
Wheat straw – Stephens	*Triticum aestivum*	Straw	3.2	Hess et al 2003 (*96*)
Wheat straw	*Triticum aestivum*	Straw	(4.6)	Jenkins et al 1996 (*10*)
Wheat	*Triticum aestivum*	Shoot	2.455	Hodson et at 2005 (*102*)
Wheat straw	*Triticum aestivum*	Whole straw	2.6	Thompson et al 2003 (*20*)
Wheat straw	*Triticum aestivum*	Stem fraction	1.3	Thompson et al 2003 (*20*)
Tall Fescue	*Festuca arundinacea*	Shoot	1.308	Hodson et at 2005 (*102*)
Perennial Ryegrass	*Lolium perenne*	Shoot	3.644	Hodson et at 2005 (*102*)
Kentucky Bluegrass	*Poa pratensis*	Shoot	1.543	Hodson et at 2005 (*102*)

Table 9. Silica content (%) for plant samples, SUMMARY: D1_C2, acid/alkali digestion (*69*), with colorimetric 2 (*18*); D2_C2, autoclave induced digestion with colorimetric (*18*); G, gravimetric method (*16*); D1_C1; acid/alkali digestion (*69*), with colorimetric 1 (*70*); and D2_C1, autoclave induced digestion (*18*), colorimetric 1 (*70*).

Grass		**D1_C1**	**D1_C2**	**D2_C1**	**D2_C2**	**G**
Kentucky Bluegrass* **	Mean	2.06[a]	1.89[a]	2.54[b]	2.35[b]	2.86[c]
	SD	(0.18)	(0.17)	(0.18)	(0.14)	(0.20)
	CV	8.80	9.07	6.97	5.88	6.86
Perennial Ryegrass*	Mean	2.05[a]	1.93[a]	2.03[a]	1.91[a]	2.84[b]
	SD	(0.32)	(0.30)	(0.10)	(0.05)	(0.15)
	CV	15.84	15.34	4.72	2.75	5.11
Tall Fescue* **	Mean	1.02[a]	0.96[a]	1.26[b]	1.22[a,b]	1.01[a,b]
	SD	(0.34)	(0.32)	(0.03)	(0.02)	(0.13)
	CV	33.61	33.03	2.08	1.73	12.79
Wheat* **	Mean	3.08[a]	2.87[a]	4.44[b]	4.08[b]	5.91[c]
	SD	(0.67)	(0.57)	(0.31)	(0.35)	(0.33)
	CV	21.80	19.70	7.05	8.49	5.63

*, **, *** Attributes are significant at $p<0.05$, $p<0.01$, and $p<0.001$

a, b, c Treatments means with different superscripts within a row are significantly different from one another

5. CONCLUSION

Commonly employed methods for the quantification of silica, which avoid the use of specialized equipment, have been compared. The methods were applied to the analysis of Pacific Northwest-relevant straws. In general, the methods give silica contents that are similar. However, significant differences were observed. The gravimetric method tended to give higher silica values than the corresponding colorimetric assays. Significant differences between the colorimetric assays were observed. The colorimetric assays may be divided into two protocols, a digestion protocol and a color-development protocol. It was determined that the significant differences observed between the colorimetric methods could be attributed to the digestion protocols; the precisions of the digestion protocols were markedly different. The tested color-development protocols gave similar results when applied to silica-containing solutions resulting from a single digestion protocol. The color development protocols differed somewhat in their calibration sensitivities and in the stability of their reagents. Considering the above information, it appears that the alkali digestion protocol, combined with the tartaric-acid based color-development protocol, as proposed by Elliot and Snyder (*18*), is a reasonable colorimetric method for assessing the silica content of straws. The application of this method was demonstrated in an experiment showing that moderately hot water may be used to leach a significant fraction of the silica contained in wheat straw.

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