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***ORIGINAL RESEARCH PAPER***

# EFFECT OF COMBINED CELL WALL DEGRADING ENZYME TREATMENT ON THE TOTAL DISSOLVED SOLIDS AND SUGARS OF SOYMILK

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Soymilk from different varieties of soybean was treated with combinations of cell wall hydrolyzing enzymes (glucanase, cellulose, arabanase, hemicellulase and xylanase). Treated samples and control were evaluated for Total Dissolved Solids (TDS) and different sugars (glucose, raffinose, sucrose, fructose, xylose, maltose, lactose, stachyose, starch, galactose, cellulose) using HPLC. Mean TDS of all enzyme-treated soymilk samples (235.8-268.3 ppm) was significantly ($p \leq 0.05$) higher than the control (167.8 ppm), it also increased significantly ($p \leq 0.05$) after sterilization. Sugars present in the enzyme-hydrolyzed soymilk were significantly ($p \leq 0.05$) different from the control. Sucrose content was depleted after enzyme treatment. The change in content of glucose, xylose, fructose, maltose, raffinose, starchyose had high correlation with TDS. Possible chemical modification of sugars impaired their detection despite increases in TDS. Use of TDS for rapid monitoring of enzyme hydrolyses of soymilk cell-wall sugars is feasible.

**Keywords**: cell wall enzymes, HPLC, soybean sugars, total dissolved solids, soymilk

## Introduction

Soybean (*Glycine max*) consumption is known to have provided good nutrition and health to some of the most populous regions of the world in the Orient where it originated (Wilkens and Hackler, 1969). It has served as a major source of protein for several centuries (Liu, 1997; Iwe, 2003). The use of soybean and its derivatives in various food products have become popular; it is known to reduce some adverse health conditions among its consumers for which the United States Food and Drug Administration (USFDA) approved its "health food" status. Soybean consumption is known to improve heart health by reducing adverse cardiovascular condition, reduce the risk of cancer and tumors (Ohr, 2004). One major product of soybean is soymilk, the aqueous extract from soybean cotyledons. Unfortunately, its

consumption has been retarded by its poor shelf stability during storage at ambient conditions. It is cumbersome to produce fresh samples all the time unless electricity-dependent refrigeration is available. Since the late 50's, considerable research efforts have been made to develop the shelf - stabilized beverage. Researchers found that the beverage could not be concentrated due to the development of high viscosity (Lo *et al*., 1968). It could not be heat sterilized without coagulation (Wei *et al.*, 1985). Pasteurization treatment was insufficient for its preservation. Acidification caused iso-electric point precipitation of soy protein and pH variation generally caused coagulation (Nsofor and Anyanwu, 1992). Combinations of the above treatments still failed to preserve the product. Later, the sprouting of the beans was used to develop a shelf stable beverage (Nsofor and Osuji, 1997). The success was attributed to the hydrolysis of macromolecules and limiting of cross-linkage of soy molecules. The inconsistency of soybean germination ability after drying and storage makes this process difficult for industrial application. The use of exogenous β–D-glucanase (Osuji and Ubbaonu, 2003) also produced a shelf stable beverage. It successfully hydrolyzed soybean cell wall components. It then became important to apply multiple enzymes for hydrolysis of cell wall materials. This is expected to enhance hydrolytic activity and help to simulate the level of hydrolyses that occur during soybean sprouting where multiple enzymes are known to be in operation (Lee and Karunanithy, 1989).

The soybean cell wall macro molecules have been considered as a major factor that causes increase in the viscosity of soy systems which could result in its shelf instability (Urbanski, 1982). If they must be hydrolyzed to achieve shelf stability, it means that there is the need to develop a quality parameter for the evaluation of the degree of hydrolysis. There is no apparent method of evaluating the effect of enzyme hydrolysis of cell-wall sugars that can rapidly quantify the degree of hydrolysis as a critical factor in industrial operations. It will require the determination and measurement of the appropriate sugars of soymilk. Unfortunately for many years, attempts to develop precise and rapid methods for quantification of soybean sugars especially when enzymes are applied have remained difficult (Wang *et al.*, 2008). This is probably as a result of transformation of soybean sugars and occurrence of cross–linkages with some molecules. When sugars are transformed or cross linked, they can escape detection because their physical characteristics are altered. Their wavelengths of light absorption may change and pure preparations of their new forms which can serve as standard preparations may not be available for calibration of analytical equipments. The use of Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and UV/Visible Spectrophotometer may not be sufficiently precise for soy sugars. Besides, they will be too difficult to use in rapid industrial process control. The industry will need a rapid method to ensure that the proper degree of hydrolysis is achieved. This will be similar to the use of iodine test for rapid detection of starch during mashing in breweries. Thus there still exists the need for a rapid and reliable method for the evaluation of soybean sugars. The use of total dissolved solids (TDS) is an apparently viable solution because it is

derived from the change in composition of the medium due to the release of hydrolyzates with higher solubility, which are dissolved into the aqueous medium during enzyme activity. However for TDS to be useful as a quality assurance tool, it has to be correlated with the performance of standard analytical methods. The objective of this study was to investigate the effect of applying multiple cell wall degrading enzymes on the TDS and on the composition of some sugars in soymilk from three different soybean varieties.

## Materials and Methods

### *Production of soymilk from soybeans*

*Materials*

Three different varieties of soybeans (Samsoy 1, Samsoy 2 and TGX) used for this study were procured from the Crop Science Department of the Michael Okpara University of Agriculture, Umudike, Nigeria. The enzyme products used contain a range of cell wall degrading enzymes. Each enzyme product consists of a combination of cell wall enzyme degrading enzymes as follows: Cerflo$^{TM}$ (bacterial beta glucanase and hemicellulase with activity of 200 Beta Glucanase Units per gram (BGU/g) at 30$^{o}$C); Finizym$^{TM}$ (a fungal beta – glucanase preparation produced from *Trichoderma resei* and a selected strain of *Aspergillus niger*. The manufacturer's declared enzyme activity was 200 FBG/g (FBG = Fungal Beta-Glucanase unit) – the amount of enzyme which degrades beta-glucan to reducing carbohydrates with a reduction power corresponding to 1 µmol glucose per minute), Ultraflo Max$^{TM}$ (mixed enzyme preparation containing β-glucanase, xylanase, cellulase, arabinase and enzymes produced by *Humicola insolens* with declared activity of 250 Fungal Xylanase Unit (FXU) per gram and 700 Endoglucanse Unit (EGU) per gram) and Viscozyme L$^{TM}$ (cellulase from *Aspergillu*s sp., cellulase from *Trichoderma reesei,* Hemicellulase from *Aspergillus niger and* Xylanase from *Thermomyces lanuginosus* with declared activity of 100 FBG/g) all produced by Novozymes A/S of Denmark. Other chemicals used were of analytical grade.

*Preparation of soymilk*

Different varieties of soybean were separately used to prepare soymilk in batches by following an adaption of the process described by Nsofor and Osuji (1997). Each batch was processed by blanching 150grams of cleaned soybean in a cooking pot with 2.0 liters of tap water for 15 minutes. The blanched soybeans were hand dehulled and the hulls were removed by flotation. The blanched dehulled coytledons were then used for soymilk extraction by placing them, in a Somimax$^{TM}$ soymilk-making machine (Model No.Ns-360D NewBrook Corporation, USA) using 1.2 liters of tap water. The soymilk was poured into glass bottles, corked and sterilized by heating in an autoclave to 121$^{o}$C in 25 min and held at this temperature for 15min. Sterilized bottles and contents were allowed to cool in an airconditioned room (at 18$^{o}$C) for about 4h and stored at ambient conditions for further analysis.

***Production of enzyme hydrolyzed soymilk***

The production of the enzyme hydrolyzed soymilk followed a similar process as described above except that the water for extraction contained pH buffer 7.0 (Sigma Chemicals, USA) and 0.lml of each of the following enzymes and enzyme combinations were separately used to treat different batches of soymilk: Filterase, Finizym, Ultraflo max, Viscozyme and the combinations were (Filtrase + Ultraflo Max + Viscozyme L) ; (Filtrase + Finizym + Ultraflo Max) ; (Filtrase + Finizym + Viscozyme L) ; (Finizym + Ultraflo Max + Viscozyme L) and (Filtrase + Finizym + Ultraflo Max + Viscozyme L). These were separately added to the soybean during milling and extraction in the Somimax machine. The milk was allowed to stand for 1h at 50$^{o}$C in a water bath before it was corked and sterilized as described above. Enzyme hydrolyzed soymilk samples from each of the ten treatments for three varieties of soybean were separately used for evaluations. The untreated sample was used as the control sample.

***Evaluation of sugars using high performance liquid chromatography***

The evaluation of sugars using HPLC generally followed the process described in AOAC, 2006.

*Sample Preparation and HPLC Analysis*

A 5g portion of each sample was placed in a separate 200-ml beaker with the addition of 40ml deionized water. It was stirred on a magnetic stirrer for one hour and 10ml of 0.3M copper sulfate were added while stirring was on. After stirring, the pH was adjusted to 6.4 using 50% sodium hydroxide and a pH meter. The sample was carefully transferred to a 200ml volumetric flask and it was made up to the 200ml mark with deionized water. It was thoroughly mixed. The sample was filtered through Whatman 2V filter paper overlaid with 0.5g acid-washed celit (to aid filtration) into a 5-oz plastic cup with cap. It was placed on a Sonicator for 2.5h for vortexing. Vortexing of the sample vials for every 10 to 15 min was performed until no residue was found on the wall of the vials. Filtration into a 2-ml injection vial using syringe and 0.2 µm nylon filters was done to get the clear solution ready to be analyzed against reference standards using HPLC.

The use of the HPLC system to identify and quantify the sugars involved the comparison of each peak retention time and area with those of the standards. A standard curve for each sugar was prepared by injecting different sugar standards (glucose, starch, raffinose, sucrose, dextrose, lactose, stachyose, galactose, fructose, xylose and maltose). The HPLC system was conditioned by flushing with deionized water for 3h, flushing with pure acetonitrile (or HPLC grade isopropanol or propan-2-ol when it became difficult) for 3h and deionized water until it was cleared of detectable materials before chromatography was performed.

Calibration standards and samples were analyzed by HPLC with refractive index detector using the following conditions: Mobile phase: 7:3(v/v) acetonitrile/water; Flow rate: 1.0ml/min.; Column temperature: ambient; Elution mode: isocratic, Run time: 25 min.; Injection volume: 75µl. A run is composed of 55 to 60 injections, including replicate samples, standards and a minimum of 10% quality assurance samples, validated control samples, or recoveries. The analysis was done in

duplicates. The HPLC analysis was done based on AOAC Official Method 982.14 (2006).

***Determination of total dissolved solids (TDS)***

The total dissolved solids of the samples were determined using a total dissolved solids meter (ATP Instrumentation –TDS- 5031- Meter High range. ATP Instrumentation, UK.). The instrument probe was inserted into a beaker containing the sample and allowed for a few minutes until the reading equilibrated.

***Statistical analysis***

Data were obtained from duplicate results and were analyzed by calculating the variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) version 16. Significant differences between means were separated by the least significant differences (LSD) between the sample parameters.

## Results and discussion

***Sugar composition of soymilk after different enzyme treatments***

The mean sugar content of soymilk after different enzyme treatments is shown in Table 1. Sucrose is the dominant sugar among the soybean varieties tested.

**Table 1.** Composition (mg/100ml) of some sugars in enzyme-treated soymilk from different soybean varieties and total dissolved solids and pH

| Sugars | Soybean Varieties | | |
|---|---|---|---|
| | **Samsoy 1** | **Samsoy 2** | **Samsoy 2** |
| Glucose | $5.5 \pm 1.13^{a}$ | $5.0 \pm 1.13^{b}$ | $4.4 \pm .97^{c}$ |
| Starch | $1.1 \pm 0.23^{a}$ | $1.1 \pm 0.21^{a}$ | $0.92 \pm 0.27^{b}$ |
| Raffinose | $2.1 \pm 0.97$ | $2.5 \pm 0.30^{a}$ | $2.3 \pm 0.31^{a}$ |
| Sucrose | $15.3 \pm 4.59^{a}$ | $14.5 \pm 4.60^{a}$ | $14.6 \pm 3.82^{a}$ |
| Fructose | $2.8 \pm 2.66^{a}$ | $2.5 \pm 2.43^{a}$ | $2.5 \pm 2.43^{a}$ |
| Xylose | $0.12 \pm 0.139^{a}$ | $0.07 \pm 0.725^{a}$ | $0.06 \pm 0.08^{a}$ |
| Maltose | $0.06 \pm 0.119^{a}$ | $0.02 \pm 0.008^{a}$ | $0.02 \pm 0.008^{a}$ |
| Lactose | $0.58 \pm .214^{a}$ | $0.46 \pm 0.171^{b}$ | $0.46 \pm 0.167^{b}$ |
| Stachyose | $5.2 \pm 0.96^{a}$ | $5.1 \pm 0.76^{a}$ | $4.2 \pm 0.88^{b}$ |
| **Total Dissolved Solids** | $269.8 \pm 32.63^{a}$ | $219.4 \pm 32.97^{c}$ | $237.6 \pm 35.6^{b}$ |
| **pH** | $6.4 \pm 0.56^{a}$ | $5.8 \pm 0.29^{b}$ | $6.6 \pm 0.17^{a}$ |

The values are means of triplicate determinations ± SD
Means with different superscripts along the same row are significantly different

The Samsoy 1 variety had the highest sucrose content (15.3mg/100ml) compared to Samsoy 2 (14.5 mg/100ml) and TGX (14.6 mg/100ml) varieties. This corroborates the findings of Wang et al. (2008) which reported that sucrose was the most abundant sugar in soybean. The TGX variety had a significantly lower ($p \leq 0.05$)

glucose and starch content compared to all the varieties. The mean composition of sugars in soymilk from soybean slurry treated with different plant cell wall degrading enzymes and their combinations is shown in Table 2. Most enzyme treatments produced significantly different results compared to the control except for raffinose. Stachyose was affected more by higher combinations of enzymes. This probably implies that raffinose may be fairly stable and generally unaffected by the enzyme treatments applied. The concentration of the sugars was apparently generally reduced after enzyme treatment which suggests that a transformation of the chemical forms of the hydrolyzate derivatives compared to the pure forms used as standards for calibration of the HPLC. Wang *et al*. (2008) described the difficulty of using normal analytical tools including HPLC for soybean sugar analysis. Cross linkages are known to occur among soybean components (Lo *et al*., 1968). Nsofor and Osuji (1997) also reported the occurrence of cross linkages of soy solutes in soymilk from sprouted soybeans. Alais and Linden (1999) explained that plant oligosaccharides such as ajucose, verbascose, stachyose and raffinose which usually occur in legumes such as soybeans are usually tied to one glucose unit through the 1 - 6 bonding and have the affinity to be cross linked to sucrose. It is possible that the new α-galactosides derived from the hydrolysis of soy oligosaccharides might have been engaged in linkages with sucrose by this described affinity (Alais and Linden, 1999). This also provides an explanation for the depletion of sucrose (in the treated samples compared to the control) as enzyme activity progressed (Table 2). The sucrose (a non cell wall material) ordinarily may not be affected by the activity of the enzymes used in this work. But they were depleted after the enzymes activity and when combinations of three or more enzymes were applied they caused greater depletion of sucrose than when one or two enzymes were used for hydrolysis. It is important for future work to be done on the identification of the transformed varieties of the molecules especially the α-galactosides and for the development of pure samples of such which could be used for the calibration of analytical equipment.

***Effect of variety on the tds and pH of soymilk after treatment with cell wall degrading enzymes***

The results of the total dissolved solids (TDS) and pH of enzyme-treated soymilk samples from different soybean varieties are shown in Table 1. The soymilk from the Samsoy 1 soybean variety had the significantly ($p \leq 0.05$) highest mean TDS (269.8 ppm) compared to TGX (237.6 ppm) and Samsoy 2 (219.4 ppm). This is attributable to the inherent differences among the varieties. Different soybean varieties are known to have differences in their composition (Arshad *et al*., 1980 and Wang *et al*., 2011). Osuji and Anyaiwe (2010) reported that the Samsoy 1 soybean variety produced more soymilk whey after acid precipitation and attributed it to its inherent genetic characteristic differences compared to the soybean varieties studied. It is possible that the cell wall composition of the Samsoy 1 variety as a substrate contained more glucans and hemicelluloses and was more susceptible to the hydrolytic activity of the enzymes applied. It is also possible that the peculiar composition of the Samsoy 1 may predispose it to greater

release of solutes. The Samsoy 2 had the least TDS and pH. Its pH was significantly different from others. A relationship probably exists between the release of solutes and pH. Nsofor and Osuji (1997) reported the differences in Total Solids and Visible Coagulation Time (VCT) for soymilk with different pH made from sprouted soybeans.

***Fffect of cell wall degrading enzyme application on the total dissolved solids and pH of soymilk***

The mean results of the TDS and pH of soymilk from the three soybean varieties after hydrolysis with different cell wall degrading enzymes and their various combinations are shown in Table 3. Single and multiple enzyme treatments caused a significantly ($p \leq 0.05$) higher TDS than the control. Cereflo (it contains mostly glucanase) produced the highest TDS by a single enzyme treatment. Osuji and Ubbaonu (2003) used glucanase treatment alone to improve the shelf stability of predigested soymilk. Osuji and Nwosu (2011) reported an increase in the soluble sugar content of soymilk after hydrolysis of soybean glucans with glucanase.

***Effect of sterilization on the TDS and pH of soymilk after enzyme hydrolyses of soybean cell wall materials***

The mean TDS and pH of soymilk from enzyme treated soybean slurry before and after heat sterilization are shown in Table 4. The TDS significantly ($p \leq 0.05$) increased after sterilization. The increase in TDS could have been as a result of increased hydrolytic activity of the enzymes as heating progressed before they were inactivated by heat. Nsofor and Osuji (1997) reported the increase in soluble proteins and carbohydrates in soymilk after hydrolyses of soybean components during sprouting. The increase in pH might have been as a result of the reactions leading to rearrangement of functional groups in the macromolecules of soybean and causing ionic changes in the beverage system. The increase in pH could also be directly linked to the production of more soluble hydrolyzates during heat treatment and enzyme activity. This could have altered the ionic strength and native chemical balance of the system.

**Table 4.** The total dissolved solids and ph of enzyme-treated soymilk before and after heat sterilization (121°C, 15min)

| Sterilization | TDS(ppm) | pH |
|---|---|---|
| Before | $227.9 \pm 34.35^{b}$ | $6.0 \pm 0.39^{b}$ |
| After | $256.6 \pm 39.19^{a}$ | $6.3 \pm 0.59^{a}$ |
| **LSD** | **5.1725** | **0.1408** |

The values are means of triplicate determinations ± SD.
Means with different superscripts along the same column are significantly different

The correlation coefficient ($R^2$) for the regressed sugar content vs TDS (before and after sterilization) for the different sugars and separately for the different soybean varieties is shown in Table 5. There was a generally high R between some of the sugars and the TDS for all soybean varieties.

**Table 2.** Composition (mg/100ml) of some sugars in soymilk from enzyme-treated three soybean varieties

| Treatment | Glucose | Starch | Raffinose | Sucrose | Fructose | Xylose | Maltose | Lactose | Stachyose |
|---|---|---|---|---|---|---|---|---|---|
| Ct | 7.7±0.97$^{a}$ | 1.3±0.15$^{a}$ | 2.6±1.30$^{a}$ | 22.6±2.52$^{a}$ | 9.5±0.86$^{a}$ | 0.23±0.05$^{b}$ | 0.14±0.20$^{b}$ | 0.84±0.140$^{a}$ | 6.2±0.60$^{a}$ |
| Cf | 4.7± 0.83$^{cd}$ | 1.2± 0.10$^{ab}$ | 2.1 ±1.03$^{a}$ | 16.1± 0.41$^{b}$ | 2.0±0.40$^{cde}$ | 0.04±0.014$^{b}$ | 0.02± 0.01$^{c}$ | 0.59± 0.14$^{bc}$ | 5.3±0.81$^{b}$ |
| Fn | 5.3±0.62$^{b}$ | 1.0±0.2.0$^{cd}$ | 2.1± 1.04$^{a}$ | 16.7±0.78$^{b}$ | 2.1±0.45$^{cd}$ | 0.12±0.21$^{b}$ | 0.02±0.002$^{c}$ | 0.52±0.14$^{bcd}$ | 5.3±0.55$^{b}$ |
| Um | 4.8± 0.54$^{c}$ | 1.1±0.58$^{bc}$ | 2.5± 0.32$^{a}$ | 15.9±0.71$^{b}$ | 2.2±0.20$^{c}$ | 0.05±0.36$^{b}$ | 0.27±0.019 $^{a}$ | 0.52±0.14$^{bcd}$ | 5.3±0.61$^{b}$ |
| Vz | 4.9± 0.53$^{c}$ | 1.2±0.04$^{ab}$ | 2.4±0.16$^{a}$ | 17.1±1.12$^{b}$ | 2.2± 0.09$^{c}$ | 0.04±0.013$^{b}$ | 0.02 ± 0.01$^{c}$ | 0.64 ± 0.80$^{ab}$ | 5.4 ±0.61$^{b}$ |
| Cf+Fn+ Um | 4.4±0.34$^{de}$ | 0.8±0.12$^{e}$ | 2.2±0.083$^{a}$ | 10.9±0.47$^{cd}$ | 1.3±0.30$^{de}$ | 0.03±0.08$^{b}$ | 0.02±0.01$^{c}$ | 0.36±0.100$^{cd}$ | 3.9±0.69$^{c}$ |
| Cf+Fn+ Vz | 3.9±0.63$^{f}$ | 0.7±0.086$^{e}$ | 2.1±0.08$^{a}$ | 11.0±0.50$^{cd}$ | 1.2±0.49$^{e}$ | 0.73±0.87$^{a}$ | 0.02±0.01$^{c}$ | 0.33±0.063$^{d}$ | 3.8±0.37$^{c}$ |
| Fn+Um+ Vz | 4.0±0.49$^{ef}$ | 0.7±0.06$^{e}$ | 2.2±0.08$^{a}$ | 11.3±0.12$^{c}$ | 1.2±0.07$^{e}$ | 0.67±0.90$^{a}$ | 0.21±0.01$^{ab}$ | 0.31±0.450$^{d}$ | 4.1±0.67$^{c}$ |
| Cf+Um+Vz | 4.3±0.17$^{de}$ | 0.9±0.26$^{d}$ | 2.2±0.09$^{a}$ | 9.2±4.0$^{d}$ | 1.2±0.04$^{e}$ | 0.02±0.01$^{b}$ | 0.01±0.01$^{c}$ | 0.29±0.590 $^{d}$ | 3.8±0.63$^{c}$ |
| Cf+Fn+ Um+Vz | 6.0±0.87$^{b}$ | 1.2±0.018$^{ab}$ | 2.7±0.19$^{a}$ | 16.5±2.59$^{b}$ | 3.3±0.19$^{b}$ | 0.18±0.09$^{b}$ | 0.024±0.014$^{c}$ | 0.36±0.096$^{cd}$ | 5.1±0.06$^{b}$ |
| L.S.D | 0.429904 | 0.127234 | 0.709358 | 2.0604 | 0.8590 | 0.2845 | 0.0735 | 0.2323 | 0.3817 |

The values are means of triplicate determinations ± SD. Means with different superscripts along the same column are significantly different
Ct=Control, Cf=Cereflow, Fn=Finizym, Um=Ultraflow max, Vz=Viscozyme

**Table 3.** Total dissolved solids and ph of soymilk from slurry of three varieties of soybean treated with different cell-wall degrading enzymes

| | Ct | Cf | Fn | Um | Vz | Cf+Fn+Um | Cf+Fn+Vz | Cf+Um+Vz | Cf+Fn+Um+Vz |
|---|---|---|---|---|---|---|---|---|---|
| TDS (ppm) | 167.8 ±13$^{e}$ | 265.8 ±29.23$^{ab}$ | 240.8 ±36.71$^{cd}$ | 242.3 ±28.76$^{cd}$ | 254 ±34.81$^{abc}$ | 251.8 ±28.42$^{bcd}$ | 254.0 ±36.38$^{abc}$ | 235.8 ±24.47$^{d}$ | 268.3 ±36.7$^{a}$ |
| pH | 6.3±0.55$^{a}$ | 6.2±0.48$^{a}$ | 6.3±0.55$^{a}$ | 6.2±0.59$^{a}$ | 6.1±0.43$^{a}$ | 6.5±1.23$^{a}$ | 6.4±0.50$^{a}$ | 6.2±0.52$^{d}$ | 6.2±0.44$^{a}$ |

The values are means triplicate determinations ± SD. Means with different superscripts along the same row are significantly different
Ct=Control, Cf=Cereflow, Fn=Finizym, Um=Ultraflow max, Vz=Viscozyme

**Table 5**. Correlation coefficient ($r^2$) of the regression of content of different sugars and the corresponding total dissolved solids of soymilk from different soybean varieties

| Variety | | Glucose | Raffinose | Sucrose | Fructose | Xylose | Maltose | Lactose | Stachyose | Starch | Dextrose | Total sugar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S1 | BS | 0.62 | 0.62 | 0.74 | 0.84 | 0.60 | 0.90 | 0.53 | 0.54 | 0.36 | 0.03 | 0.62 |
| | AS | 0.71 | 0.70 | 0.76 | 0.90 | 0.78 | 0.95 | 0.51 | 0.56 | 0.17 | 0.06 | 0.71 |
| S2 | BS | 0.71 | 0.62 | 0.31 | 0.83 | 0.24 | 0.78 | 0.55 | 0.14 | 0.25 | 0.38 | 0.63 |
| | AS | 0.60 | 0.49 | 0.14 | 0.65 | 0.14 | 0.56 | 0.38 | 0.18 | 0.77 | 0.35 | 0.48 |
| TG | BS | 0.75 | 0.74 | 0.63 | 0.73 | 0.62 | 0.45 | 0.51 | 0.45 | 0.10 | 0.05 | 0.70 |
| | AS | 0.70 | 0.70 | 0.47 | 0.75 | 0.56 | 0.39 | 0.35 | 0.22 | 0.07 | 0.05 | 0.58 |

The values are means of triplicate determinations ± SD
S1=Samsoy 1, S2=Samsoy 2, TG=TGX, AS=After Sterilization, BS=Before Sterilization

This implies that the effect of the same factor(s) was responsible for the observed changes after the treatment. The R was higher after sterilization for Samsoy 1 but this was not the same for the other two varieties. This follows the trend of a significantly higher TDS for Samsoy 1and further indicates the predisposition of Samsoy 1 to enzyme breakdown of its cell wall carbohydrates. The R was consistently high for the relation between TDS and glucose, raffinose and fructose. It was consistently low for lactose, starch, stachyose, and dextrose. The R for sucrose and xylose was high only for Samsoy 1 and TGX varieties but low for Samsoy 2. This is a trend observed in Table 3 which shows that the TDS of enzyme treated soymilk from the different varieties was significantly different with both Samsoy 1 (269.8ppm) and TGX (237ppm) being higher than Samsoy 2 (219.4ppm). It also suggests that the reasons for the lower TDS values for Samsoy 2 could be related to the two sugars. Similarly glucose, raffinose and fructose with consistently high TDS might have been the most affected by the enzyme treatment. Glucose and fructose are the major sugars that make up the oligosaccharides of soybeans. Galactose could not be detected by the equipment used.

## Conclusions

Total Dissolved Solids (TDS) can be used as a tool for rapid evaluation of the degree of hydrolysis of soybean macro-molecules in soymilk. TDS correlates with the results of HPLC measurement of sugars in enzyme hydrolyzed soymilk. Heat sterilization of enzyme hydrolyzed soymilk causes a significant increase in its pH. Application of cell-wall degrading enzyme to soymilk results in chemical transformation of soy sugars which impairs detection by HPLC. The use of combination of cell-wall degrading enzymes for soymilk shelf stability is feasible.

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