Use of The Bromocresol Purple Index (BCPI) in Measuring The Efficiency of Soybean Heat Treatment

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Abstract

A number of the most common methods used in assessing the efficiency of soybean heat treatment were compared in this study. All the methods proved to be useful in assessing the efficiency of heating soybean seeds and soybean products. However, considering the sensitivity, precision, time consumed, and the effectiveness of determination of the characteristics of the samples, the use of purple bromocresol index (BCPI) appears to be justified. The BCPI method turned out to be universal, allowing distinguishing unheated ($BCPI_{BSM}$ <70 $mg \cdot g^{-1}$), under-heated (70 $mg \cdot g^{-1}$ <$BCPI_{BSM}$ <130 $mg \cdot g^{-1}$), properly heated ($BCPI_{BSM}$ = 130-140 $mg \cdot g^{-1}$), and over-heated samples ($BCPI_{BSM}$ > 140 $mg \cdot g^{-1}$).

Nomenclature

T – sorption of the active substance on the seed surface (per unit weight of protein in the seeds dry weight ($mg \cdot g_{BSM}^{-1}$),

E_o – absorbance of the solution after addition of 1cm^3 of working solution to 20 cm^3 of 0.02 $mol \cdot dm^{-3}$ NaOH solution,

E_b – absorbance of the solution after addition of 1 cm^3 of the extract (after centrifugation) to 20 cm^3 of 0.02 $mol \cdot dm^{-3}$ NaOH solution,

D - concentration of the working solution ($mg \cdot cm^3$),

F - volume of the working solution added to the seed sample (cm^3),

m — weight of seeds sample (g),

h — seeds dry matter content (%),

z — protein content in the seeds dry matter (%).

UA - urease activity,

TIA - trypsin inhibitor activity,

CRI - cresol red indicator,

LA - lysine absorption,

BOAA - activity of grass pea neurotoxin,

AV - acid value,

PV - peroxide value of oils and a tocopherol content,

BCGI - bromocresol green index,

p - high precision,

c - sensitivity,

r - high discernability,

$BCPI_{BSM}$ - bromocresol purple index (per unit weight of protein in the seeds dry weight - $mg \cdot g_{BSM}^{-1}$),

A_o - absorbance of the solution obtained after adding $1cm^3$ of working solution (0.13 $mg \cdot cm^{-3}$) to 20 cm^3 of 0.02 $mol \cdot dm^{-3}$ solution of NaOH,

A_b - absorbance of the solution obtained after adding $1cm^3$ of the extract (after centrifugation) to 20 cm^3 of 0.02 $mol \cdot dm^{-3}$ solution of NaOH,

C - concentration of the working solution (0.13 $mg \cdot cm^{-3}$),

V - volume of the working solution (50 cm^3),

M - weight of the seeds test sample,

h - seeds dry matter content (%),

z - protein content in the seeds dry matter (%),

$(^h/_{100}) * (^z/_{100}) /h$ - % of seeds dry matter content,

Introduction

The high utility value of soybean results from their nutritional value, in particular: the high content of well-digestible and balanced proteins that are rich in exogenous amino acids, the abundance of oil with a significant share of polyunsaturated fatty acids (PUFAs), and the vitamins and minerals they contain [1,2].

The development of optimal technology for converting soybeans into food or fodder continues to drive much research [3-9]. However, the use of soybean and soybean products as components of food or fodder involves various forms and methods of their heat treatment, which is applied to reduce the activity of numerous thermolabile antinutritional factors [10, 11], and to develop rheological and organoleptic properties typical of soybean-based products, while maintaining the digestibility of nutrients [12]. In the context of modern agro-food soybean processing, characterized by a high rate of dynamism and large scale of production, combining these mutually opposed objectives, viz. preserving the highest possible nutritional value, while, at the same time, reducing the activity of the antinutritional factors, determines

the application of adequate methods of assessing heat treatment efficiency, which are characterized by high sensitivity and precision combined with low price and simplicity of implementation.

The quality of soybean products obtained in the heating process can serve as a criterion of the form, method, temperature, and time of raw material processing. Assessment of the effectiveness of heating soybean or other soybean products, as well as other food or feed products, can be based on any of their properties that can change as a result of heating. The most common assessment methods can be methodically divided into five basic groups:

- nutritional tests,

- methods based on determination of the content of products of thermal transformations,

- assessment of non-protein properties and thermolabile quality traits,

- microbiological assays,

- analytical methods based on alterations of the properties of product proteins [13].

1.1 Nutritional tests

These are one of the simplest natural methods for evaluation of raw materials or food or feed products. A controlled factor is the impact of analysed mixtures or their components on livestock health via such indicators as the protein efficiency ratio (PER), biological value (BV) of proteins, net protein utilisation (NPU), and true digestibility (TD) [13].

The quality of food and feed components depends on the process of heating thereof, hence thermal treatment has a significant effect on the results of nutritional tests. Yet, their results may be distorted due to the high complexity of metabolic processes occurring in experimental animals and the interpretation may be difficult. Therefore, agro-food processing aims to minimise the extent of nutritional experiments in favour of other less time-consuming methods with a possibly a high correlation with their results [13].

1.2 Determination of the heating efficiency using non-protein components

The efficiency of heating of soybean seeds can be assessed by investigation of the activity of thermolabile vitamins, e.g. thiamine, riboflavin, tocopherols, and tocotrienols [14].

Thermal treatment, especially in the presence of oxygen, may induce many transformations in food or feed components, which result in an increase in the content of characteristic products of these transformations (furosine – [15], e-pyrrolysine – [16], or lysinoalanine – [17]).

A disadvantage of these determinations affecting their usefulness in the current assessment of the effectiveness of thermal treatment is the substantial length of time and labour consumption as well as the high complication of the analytical procedure [13].

1.3 Microbiological assay

Heating is a method for reduction of microbial contamination, whose level can be a measure of the heating length of a product at a given lethal temperature and results in a certain limitation of the product shelf life [13]. A drawback of this type of determinations associated with the microbiological assays is the prolonged time of the determinations and susceptibility to the shortcomings of the analytical procedure [13].

1.4 Methods based on alterations in the properties of product proteins

Three categories are most frequently distinguished in this group:

- methods based on denaturing changes in the solubility or other functional properties of proteins (water absorption, emulsion stability),

- methods based on measurement of the activity of enzymatic proteins or some anti-nutritional factors,

- determinations carried out with the use of indicator compounds characterised by specific reactions with certain protein groups [13].

1.5 Determination of changes in the solubility or differences in the functional properties of proteins

One of the effects of protein heating is the change in their solubility resulting in coagulation. Hence, the degree of protein heating can be determined from solubility in water (NSI) or water solutions [18-19].

Thermal denaturation of proteins, including soybean proteins, can be carried out while analysing their properties in oil or water determined as the water absorption coefficient -WHC, emulsifying capacity - FAC, emulsifying activity index - EAI, and emulsion stability index - ESI

A shortcoming of these methods is the high labour intensity and time consumption as well as the complexity and multistage character of the analytical procedure [13].

1.6 Analysis of changes in enzymatic activity

The activity of enzymes contained in soybean seeds depends on the thermal denaturation of enzymatic proteins. In practice, such assays are based on the activity of urease (UA) or lipoxygenases [20-24].

Their practical application is problematic due to the time-consuming and labour-intensive character of the analyses and the possibility of using varieties with genetically lowered or reduced activity of these enzymes [13].

1.7 Biochemical assays

This group of methods is based on determination of the activity of thermolabile anti-nutritional compounds present in soybean seeds, primarily the antitrypsin inhibitory activity (TIA) and inhibition of

the activity of hemagglutinins, called lectins.

The basis for TIA determinations is the reaction of trypsin with substrates (casein or the synthetic substrate BAPA N-a-benzoyl-DL-arginine-p-nitroanilide) [24,25].

The results of these determinations are calculated in TUI units per mg dry weight of the sample or per unit of protein mass in dry matter. Raw soybean seeds of traditional varieties are usually characterised by an anti-trypsin activity level of ca. 50 TUI/$mg_{d.w.}$ [26]. Thermal denaturation of inhibitory proteins may result in reduction of the TIA to a residual level; at the same time, 10% of the activity of raw seeds is regarded as a safe level of trypsin inhibitor activity that permits their application in feeds.

Proteins with hemagglutinating properties undergo thermal denaturation similarly to trypsin inhibitors. The hemagglutination activity is most frequently determined using the immunosorption method with the use of monoclonal [29,30] or polyclonal antibodies [31,35]. Immunosorption methods can be applied for determination of other soy proteins (b-conglycinin) as well [29].

1.8 Use of indicator substances

These types of methods are based on diagnostic reactions carried out with selected chemical reagents that specifically bind to certain groups of the heated product, facilitating assessment of the heating degree. Such reagents as orange-G, safranin [32], and coomassie blue [33] have long been used. The action of coomassie blue as a diagnostic reagent is based on the colour reaction with proteins dissolved in the test solution. Thermal denaturation of proteins reduces their solubility in the solution, which is reflected in the intensity of the colour of the blue-protein complex and can be a measure of the product heating intensity [34-36]. A disadvantage of the application of coomassie blue is the temporal instability of the staining intensity in the protein-blue complex, which may yield errors in the determinations [13,36].

The cresol red indicator (CRI) is one of the most important and often used methods for investigating the efficiency of heating soybean or its products - soybean meal. The application of cresol red in studies of the intensity of heating food and feed products was proposed already by Frolich [37], and the investigations were continued by Olomucki and Bornstein [40]. With time, a standard was established based on these studies [39] to unify the procedure of application of cresol red as a diagnostic compound in assessment of the intensity of soybean product heating.

The CRI value increases along the thermal treatment of soybean seeds and soybean products, thus facilitating the assessment of the heating intensity [13].

The index of lysine absorption (LA), which is determined based on the reaction of the sample with the FDNB (1-fluoro-2,4-dinitrobenzene) reagent, in accordance with the methodology proposed by Booth [40], can be adopted as an approach for assessment of the efficiency of heating food or feed, including soybean seeds and their products.

A difficulty in the implementation of the FDNB-based determinations of CRI and LA is the necessity to defat samples before the determination process, which lengthens and complicates the analytical procedure [13].

Significant progress in simplification and shortening of analytical procedures in CRI- and FDNB-based studies on the efficiency of heating soybeans and soybean products was provided by the use of bromocresol purple as an active compound, which eliminated the necessity of the laborious process of sample defatting and significantly enhanced the sensitivity of the designed method referred to as the bromocresol purple index (BCPI).

Methods were developed to test the efficiency of heating of selected pulses and oilseeds, such as: soybean, chickpea. The studies were preceded by an initial selection of the possible indicator substances, of which acidic solutions of bromocresol purple (5', 5"- dibromo - 3', 3" - dimethyl phenolsulfonphthalein) turned out to be the most effective ones, while in the analysis of soybean and soybean products also bromocresol green solutions (3,3', 5, 5' — tetrabromo-m — cresolsulfonphthalein) were effective. The threshold of concentration value for the two indicator substances in their solutions resulted from the linear association between their absorbency and their concentration, which was verified experimentally. It was also the condition for the correctness of the studies carried out, the results of which were calculated according to formula 1 [13].

The sensitivity of the method (c) was the basic criterion for comparing the test variants. It was calculated as the absolute value of the direction coefficient of linear regression formula (the result of sorption of the active substance – T, calculated in accordance with formula 1, as the function of the heating time of the seeds), taking as the basis of matching the results for raw seeds samples and seeds autoclaved at the temperature of 121^{o}C for 120 minutes [13]. The sensitivity of the method increases with the higher value of the index c.

For each of the tested variants of the working solution, i.e. bromocresol purple and bromocresol green, the sorption of the active substance – T was calculated, separately for raw and autoclaved (at 121^{o}C for 120 minutes) seeds, as the difference in the solution content before and after the contact with the ground, for sieving through a 0.20 mm mesh, seed sample, measured using absorbance E_b and E_o (formula 1). For each of the tested variants of working solution, the sorption of active substance – T was measured for raw seeds (T_s) as well as autoclaved seeds (T_a) and calculated according to formula (1). For each of the tested variants of working solution, the sensitivity of the analytical method (c) was calculated on the basis of T_a and T_s values (for autoclaved and raw seeds respectively).

$$T = (E_o - E_b)*D*F*E_o^{-1}m^{-1}*(h/100)^{-1}(z/100)^{-1} \quad [1]$$

For the variant of working solution with the greatest sensitivity in the seed testing, the name of bromocresol purple index (BCPI) and bromocresol green index (BCGI) was used, respectively. Additionally,

the lower index in $BCPI_{BSM}$ and $BCGI_{BSM}$ indicates that the calculations were made per unit weight of protein in the seeds dry weight.

Both the bromocresol purple index (BCPI) and the bromocresol green index (BCGI) were characterized by high precision (π), sensitivity (χ) and high discernability (ρ) of the determinations [13], making it possible to accurately assess the characteristics of the samples, in the case of which enzyme analysis (UA) and biochemical assay (TIA) was difficult to perform (Table 1).

Moreover, it was also demonstrated that the results of the tests carried out using BCPI and BCGI methods were highly interdependent with the selected quality discriminants, i.e. urease activity - UA, trypsin inhibitor activity - TIA, cresol red indicator - CRI, lysine absorption – LA, activity of grass pea neurotoxin - BOAA, as well as acid value (AV) and peroxide value (PV) of oils and a tocopherol content — AE, of soybean, grass pea, bean, and rapeseed. The high and significant correlation factors enabled to formulate regression equations correlating the soybean, grass pea, chickpea, soybean oil, and rapeseed oil quality discriminants with the values of the developed BCPI and BCGI test as a dependent variable [13]. The usefulness of some of the developed regression equations was confirmed in the studies on selected characteristics of soybean meal and chickpea meal carried out on a semi-technical scale [13].

By providing the possibility to convert the results obtained the use of these regression equations may replace some of the labour-intensive and time-consuming analytical procedures used so far, for testing quality discriminants by fast and effective BCPI and BCGI tests.

The practical use of the developed assessment methods (BCPI and BCGI) may include:

- complementing the existing methods for the evaluation of quality discriminants of seeds or products derived from them,

- replacement of the existing evaluation methods with the possibility of converting the results using the regression equations developed,

- using them as a replacement for the evaluation methods used so far in testing selected quality discriminants of soybean, grass pea, bean, chickpea, and rapeseed seed and their products.

So far, the most commonly used traditional methods of analysing soybean seeds and products, based on the activity of selected enzymes (urease activity – UA) or thermolabile antinutritional factors (trypsin inhibitor activity – TIA) [43-46], often supplemented by methods based on protein solubility [18,43,45,46], despite their labour intensity and time-consuming nature, as well as complicated analytical procedures and the necessity to use expensive, specialized laboratory equipment operated by highly qualified personnel, are still widely used in practice, even though thus obtained results are sometimes inaccurate and difficult to interpret. It should be noted that the UA and TIA test methods have been well verified in soybean meal studies and the obtained results of these parameters in this type of products were sufficiently correlated [47].

It appears that under the conditions of industrial soybean processing the bromocresol purple index (BCPI) method should meet the demands of practical implementation. This method is the answer to the approach postulating increased sensitivity of analytical methods with simultaneous shortening and simplification of the methodology [13], as it offers high sensitivity and precision results that allow for distinguishing wide range of samples with subtle differences in properties.

The study attempts to compare the usefulness of the BCPI method with the two evaluation methods, i.e. TIA and UA, which are currently most frequently used in the assessment of the effectiveness of soybean heating.

Materials And Methods

The study used traditional varieties of soybean, i.e. not genetically modified ones, which had been purchased at the agri-food market in Elizówka near Lublin (Poland, Lublin Province). The use of traditional varieties of soybean is in line with the strategy of the European Union food processing industry as an alternative to the genetically modified soybean, the presence of which in the amount higher than 0.9% requires a declaration on the product label (food, fodder).

Fully mature soybean seeds were used in the study, with chemical composition and physical characteristics typical for this species. The dry matter content of the seeds constituted around 93.88% of the fresh weight. In the dry matter of the seeds, there was 36.03% of total protein, 19.75% of fat, and 5.23% of ash. The dry matter studies of total protein, fat, and ash were made in accordance with the standards recommended in the EU and in Poland [48-51].

Soybean samples used in the research were subject to heat treatment, in pursuance to intensity variants described in Table 1. The heat treatment consisted of initial humidification of the samples (to the total moisture content of 15, 17, and 20%). Afterwards, the samples were evenly distributed over the area of 1000 cm^2 and heated for 150 or 180 seconds using six ceramic infrared heaters, each with the power of 400 W, located at the distance of 5cm from the heated seeds. The testing stand is presented on the figure 1. After heating with the infrared radiators the samples were conditioned at room temperature or in a glass dewar for the time of 5 minutes and then at ambient temperature (Table 2).

Each of the described heating variants was tested, per unit weight of the protein of the seeds dry matter, for urease activity (AU) according to standard [42], and for trypsin inhibitor activity (TIA) pursuant to standard [44].

For the studies using the bromocresol purple index (BCPI) method samples of soybean seeds or soybean products were ground to pass through a sieve with 0.2 mm mesh. Samples weighing precisely 0.1g were taken from the ground soybean and transferred to a 100 cm^3 conical flask. Next, 50cm^3 of the working solution was added and the mixture was stirred on a magnetic stirrer for 30 minutes. After this time, the contents of the conical flask was transferred to a centrifuge tube and centrifuged for 5 minutes (at 3000 rpm). After the centrifugation, 1 cm^3 was taken from the clear extract and transferred into a test tube

containing 20 cm^3 of 0.02 $mol \cdot dm^{-3}$ solution of NaOH. The obtained mixture was stirred for 10 minutes in a test tube, and afterwards, the absorbance at 589 nm was measured using distilled water as the reference solution (A_b - formula 1). Analogously, the absorbance of the working solution was measured (A_o - formula 1), wherein in the place of the centrifuged extract, 1 cm^3 of bromocresol purple working solution (0.13 $mg \cdot cm^{-3}$) was added to the test tube containing 20 cm^3 of 0.02 $mol \cdot dm^{-3}$ solution of NaOH.

The working solution was obtained by dissolving 130 mg of bromocresol purple (5', 5"- dibromo - 3', 3" - dimethyl phenolsulfonphthalein) in 40 cm^3 of NaOH solution (0.1 $mol \cdot dm^{-3}$) and the addition of 0.1 $mol \cdot dm^{-3}$ solution of HCl to obtain 1 dm^3 of the final product.

The amount of adsorbed active substance (bromocresol purple) was calculated using the formula (1) as the difference between its content in the solution before and after contact with ground seed sample. The test result obtained according to formula 1 takes into account the conversion into the protein content in soybean dry matter, $BCPI_{BSM}$, which was possible thanks to the introduction of conversion factors

$$BCPI_{BSM} = (A_o - A_b) * C * V * A_o^{-1} * M^{-1} * (h/100)^{-1} * (z/100)^{-1} \quad [2]$$

Time consumption (t) of the analytical methods (BCPI, UA TIA) was estimated as the time required to complete one repetition of analysis per one seed sample in accordance with the established analytical procedures [13].

2.1 Analysis of the raw material hardness

The analysis of the raw material hardness was carried out using a TA.XT plus texture analyser made by Stable Micro Systems. The measurements were performed using a compression test on a TA.XT Plus device with a 500N head. The whole seeds, laid horizontally in a position perpendicular to the base of cotyledon split surface, were compressed to 50% of their original height with the head travel speed of 0.83 mm/s. The hardness was defined as the maximum value of the force recorded during the test and it was read from the force-displacement graph. The measurements were made at the ambient temperature of 20±1°C. The tests were done in 5 repetitions.

2.2 Statistical analysis of the research results

The test results for soybean samples, prepared according to Table 1, obtained using the BCPI, UA, and TIA methods were statistically analysed by calculating the average values (S) and standard deviation (SD) of the measurements as well as the accuracy of determination (p - that is numerically equal to the coefficient of variation - (CV) of these determinations) [49]. The precision of the determinations increases with the decreasing value of the coefficient of variation of the measurements carried out.

Taking into account the average values (S) and standard deviations (SD) the significance of differences between average values of the measurements for each of the methods (BCPI, UA TIA) were assessed (for

5% significance level). The significance of differences between average values obtained using each of the methods, i.e. BCPI, UA, and TIA, for the samples prepared in accordance with Table 1, formed the basis for distinguishing the results of measurements (ρ), expressed as the percentage of relevant relations in respect to the total verified relations. The higher the value of ρ (maximally 100%), the greater the distinguishability of the research results.

Results And Discussion

Antitrypsin activity of the soybean seeds reference sample, i.e. not heat-treated, was at the level typical for this kind of material (Table 2), which was also in line with the data from literature of the subject [2,52-55].

Heating the seeds in accordance with the methodology described in Table 2 resulted in a significant reduction of antitrypsin activity in the samples. Except for the reference sample and sample 1, the remaining ones were characterized by a trace level of antitrypsin activity (TIA), viz. the properties indicating their fitness for consumption.

It is assumed that the acceptable level of antitrypsin activity in food or fodder (expressed as mg of trypsin inhibitor per gram of protein) is typically estimated at the level of the tenth part of the protein percentage of the product [1]. Generally, antitrypsin activity of heat-treated soybean, determined according to the accepted methodology, is never below a certain trace level, which is most often explained as the result of a varied thermal resistance of trypsin inhibitors present in soybean seeds (Kunitz-type Inhibitor - KTI, and Bowman-Birck Inhibitor - BBI) [10]. Most of the soybean samples were characterised by a similar level of antitrypsin activity. This resulted in low distinguishability (ρ = 58.33% - Table 3), despite the differences in the method of sample preparation, i.e. varied length and temperature of the treatment. A characteristic feature of soybean subject to heat treatment is that sometimes the precision of assessment carried out using TIA method is lowered. This is particularly common in the case of samples qualified as suitable for consumption, for which sometimes the measured value is comparable with the standard deviation of the measurement (Table 3). Decreased distinguishability and reduced precision of TIA determinations can make it difficult to qualify soybean products correctly, particularly those the quality of which might be sometimes reduced due to excessive heat treatment [12]. Analogous results were obtained in the course of studies on soybean seeds and soybean meal treated with different heating methods [13], and those on microwave-heated soybean seeds [47]. The results of studies carried out on soybean flour samples obtained from Nigerian soybean varieties [57] may also be interpreted similarly.

Raw soybean seeds, constituting the reference sample, were characterized by levels of urease activity (UA - Table 3) typical for this material, which is analogous to the data from the literature of the subject [13]. Subjecting soybean seeds to heat treatment in accordance with the method described in detail in Table 2 led to a change in the characteristics of these samples, including a significant decrease of the urease enzyme activity. Most samples of tested soybean seeds were characterised by zero or trace amounts of

AU, thus achieving consumption usefulness according to the guidelines in the literature of the subject [1]. However, the reduction of urease activity to zero or trace levels was accompanied by a decreased precision of the determinations as well as by similarity of physicochemical properties, observed as a similar or identical value of urease activity (UA), which resulted in a limited distinguishability of the samples (ρ = 58.33% — Table 2). The results obtained indicate limited usefulness of the UA method for evaluating soybean seeds or soybean products characterised by a significant reduction of the urease activity, which may lead to an incorrect classification of over-heated material with reduced digestibility of proteins [12]. Analogous conclusions were formulated based on the previous studies by Szmigielski [13], or can be drawn by analysing the results of studies done by Anozie et al., Caprita et al., Varga -Visi et al. and Purushotam et al. [55-58].

The value of bromocresol purple index ($BCPI_{BSM}$) for raw, unheated soybean seeds was at a typical level, similar to the literature data, viz. it did not exceed 70 $mg \cdot g^{-1}$ [13].

The seed samples varied significantly when it comes to the measured values, indicating a large range of $BCPI_{BSM}$ variations, which often exceeded the double value for the raw seeds. Moreover, the results obtained are characterised by high and consistent accuracy (p factor did not exceed 3% of the measured value and remained at a similar level regardless of the thermal treatment of samples - Table 3).

The results of the study by Szmigielski [13], obtained for soybean seeds, soybean meal, and soybean exudates, were also characterised by a similar, or even greater, range of change of $BCPI_{BSM}$ as well as high and consistent accuracy within a few per cent. Broad range and high precision of the $BCPI_{BSM}$ measurements implied high distinguish ability of the samples (ρ = 91.17%), which indicates that the sensitivity of the method applied is sufficient to accentuate the subtle differences in physicochemical properties of most of the samples prepared (Table 3). A similar, or even higher, value of distinguish ability (ρ) was obtained in the aforementioned work by Szmigielski [13] as well. For most of the heat-treated soybean seeds, the result obtained exceeded 130 $mg \cdot g^{-1}$, which, based on the comparative studies by Szmigielski [13], was accepted as the $BCPI_{BSM}$ level corresponding to consumer suitability of soybean seeds. The results obtained so far (Table 3) indicate that the range of the changes in the $BCPI_{BSM}$ may also be an argument for the application of this method for evaluating over-heated samples of soybean and soybean products, the usefulness of which is reduced by limited digestibility of nutrients [12].

Moreover, considering the time needed for the analysis, the implementation of the BCPI method is also the most favourable option in assessing the effectiveness of heat treatment of soybean seeds and products (Table 3).

The measurement method using BCPI index can be applied to various agricultural materials. In the food industry, soybean is processed in various forms. The conditioning process, i.e. retaining soybean at a higher temperature, resulted in decreased seed hardness as compared to the control test and non-conditioned seed (Fig. 2 and Fig. 3). This is due to prolonged exposure to temperature without removing the water from the product at the same time. This process also affected positively the decrease of

antinutritional compounds in soybean. In the research by Lara et al. [2019] soybean seeds were subject to an IR treatment in order to perform the scalding process that resulted in the reduction of weight and hardness. After 100 seconds of heating, the lowest hardness was obtained at the level of the scalding process. Heating for less than 100 seconds did not produce the expected softening results. Andrejko et al. [59] conducted studies on the effects of microwave processing on soybean. These studies determined the stress values inside the seed during humidification in the case of seeds micronized at 180°C for 120 seconds and those without heat treatment. The results show a significant impact of micronization on the seed structure, as the stress values were ten times lower than those of the control samples. Infrared radiation treatment was also used in the case of African legumes [60]. As reported by the study, water is absorbed faster, which resulted in better viscosity of the pastes obtained. The resulting structural changes caused by infrared heating of various grain legumes lead to the interactions of the seeds biomolecules that depend on the moisture content.

Conclusions

All the methods used, i.e. BCPI, UA, and TIA, proved useful in assessing the efficiency of soybean seeds heat treatment. However, considering the sensitivity, precision, the time necessary for performing the analysis, and the efficiency in distinguishing the characteristics of the samples, the use of bromocresol purple index (BCPI) method seems to be the most rational.

Implementation of the bromocresol purple index (BCPI) method in soybean processing technologies may be carried out as:

- complementation to the previously used methods of assessment, viz. UA, TIA, and PDI

- replacement of the previous methods (UA, TIA PDI), with the possibility of recalculating the results obtained in accordance with developed algorithms.

The BCPI method proved to be universal, making it possible to distinguish between: unheated samples (BCPI < 70 $mg \cdot g^{-1}$), under-heated samples (70 $mg \cdot g^{-1}$ <BCPI< 130 $mg \cdot g^{-1}$), properly heated ones (BCPI = 130-140 $mg \cdot g^{-1}$), and over-heated ones (BCPI > 140 $mg \cdot g^{-1}$) (Table 2).

Declarations

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Authorship contribution statement:

Marek Szmigielski: Conceptualization, Data curation, Investigation, Methodology. **Paweł Sobczak**: Writing-Original draft, Investigation. **Kazimierz Zawiślak**: Visualization, Supervision, Conceptualization. **Dariusz Andrejko**: Visualization, Supervision. **Grażyna Bielecka**: Investigation. **Jolanta Rubaj**: Investigation. Jacek Mazur: Data curation, Formal analysis

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Tables

Table 1. Analytical methods developed until now that use bromocresol purple and bromocresol green [13].

Seed species	Name of the active substance	Active substance content ($mg_{*}cm^{-3}$)	HCl concentration ($mol_{*}dm^{-3}$)
soybean	bromocresol purple	0.13	0.10
soybean	bromocresol green	0.25	0.07
chickpea	bromocresol purple	0.14	0.10

Table 2. Sample preparation method

Sample number	Moisture content (%)	Heating with infrared radiator (s)	Conditioning in a dewar (for 5 minutes Yes / No)
0	-	-	-
1	15	150	N
2	17	150	Y
3	20	150	N
4	20	150	Y
5	15	180	N
6	17	180	Y
7	20	180	N
8	20	180	Y

Table 3. The results obtained by implementing bromocresol purple index (BCPI), urease activity (UA), and antitrypsin activity (TIA) methods in the assessment of soybean seeds samples.

Sample	$BCPI_{BSM}$ $[mg \cdot g_{BSM}^{-1}]$	p [%]	UA $[mg_N \cdot g^{-1} \cdot min^{-1}](30^oC)$	p [%]	TIA $[mg \cdot g^{-1}]$	p [%]
0	$72.85^{\pm}2.09^{a}$	2.87	$7.22^{\pm}0.55^{a}$	12.44	$23.10^{\pm}0.76^{a}$	3.29
1	$120.22^{\pm}1.32^{b}$	1.10	$1.70^{\pm}0.17^{b}$	10.00	$6.40^{\pm}0.50^{b}$	7.81
2	$135.50^{\pm}1.54^{c}$	1.14	$0.14^{\pm}0.03^{c}$	21.43	$3.60^{\pm}0.30^{c}$	8.33
3	$139.26^{\pm}1.27^{d}$	0.91	$0.05^{\pm}0.02^{d}$	40.00	$3.40^{\pm}0.30^{c}$	8.82
4	$143.11^{\pm}1.22^{e}$	0.85	$0.05^{\pm}0.02^{d}$	40.00	$3.40^{\pm}0.30^{c}$	8.82
5	$145.67^{\pm}1.54^{e,f}$	1.06	$0.05^{\pm}0.02^{d}$	40.00	$3.40^{\pm}0.20^{c}$	6.25
6	$146.22^{\pm}1.66^{f}$	1.14	$0.05^{\pm}0.02^{d}$	40.00	$3.20^{\pm}0.20^{c}$	6.25
7	$156.69^{\pm}1.72^{g}$	1.10	$0.05^{\pm}0.02^{d}$	40.00	$3.20^{\pm}0.20^{c}$	6.25
8	$158.19^{\pm}2.20^{g}$	1.35	$0.05^{\pm}0.02^{d}$	40.00	$2.80^{\pm}0.20^{d}$	7.14
τ [h]	1.5		3.0		4.5	
ρ [%]	91.17%		58.33%		58.33%	

Values marked in the columns with the same letter are not significantly different (p <0.05)

Figures

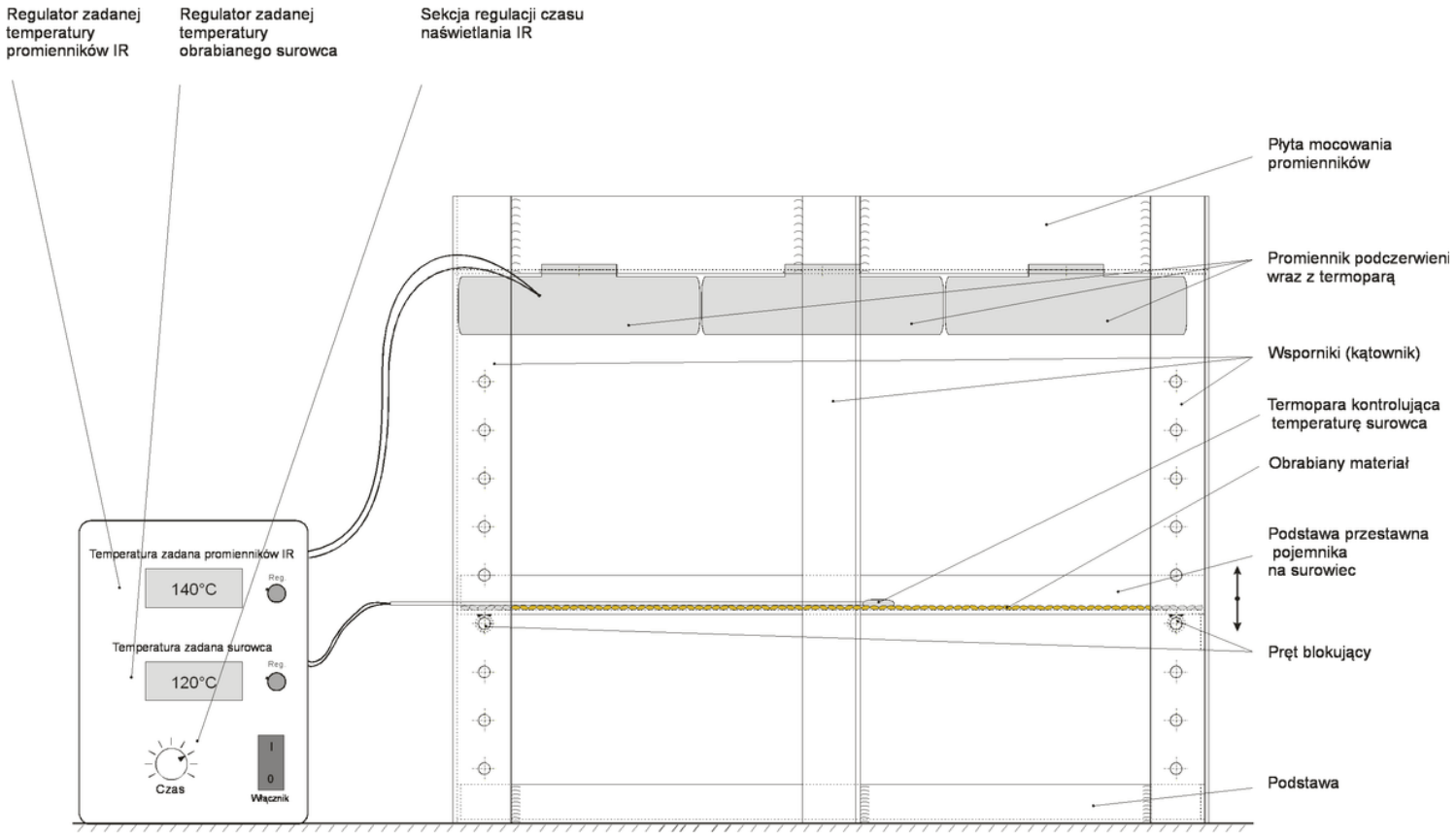

Figure 1

Laboratory chamber for irradiating granular materials with IR rays.

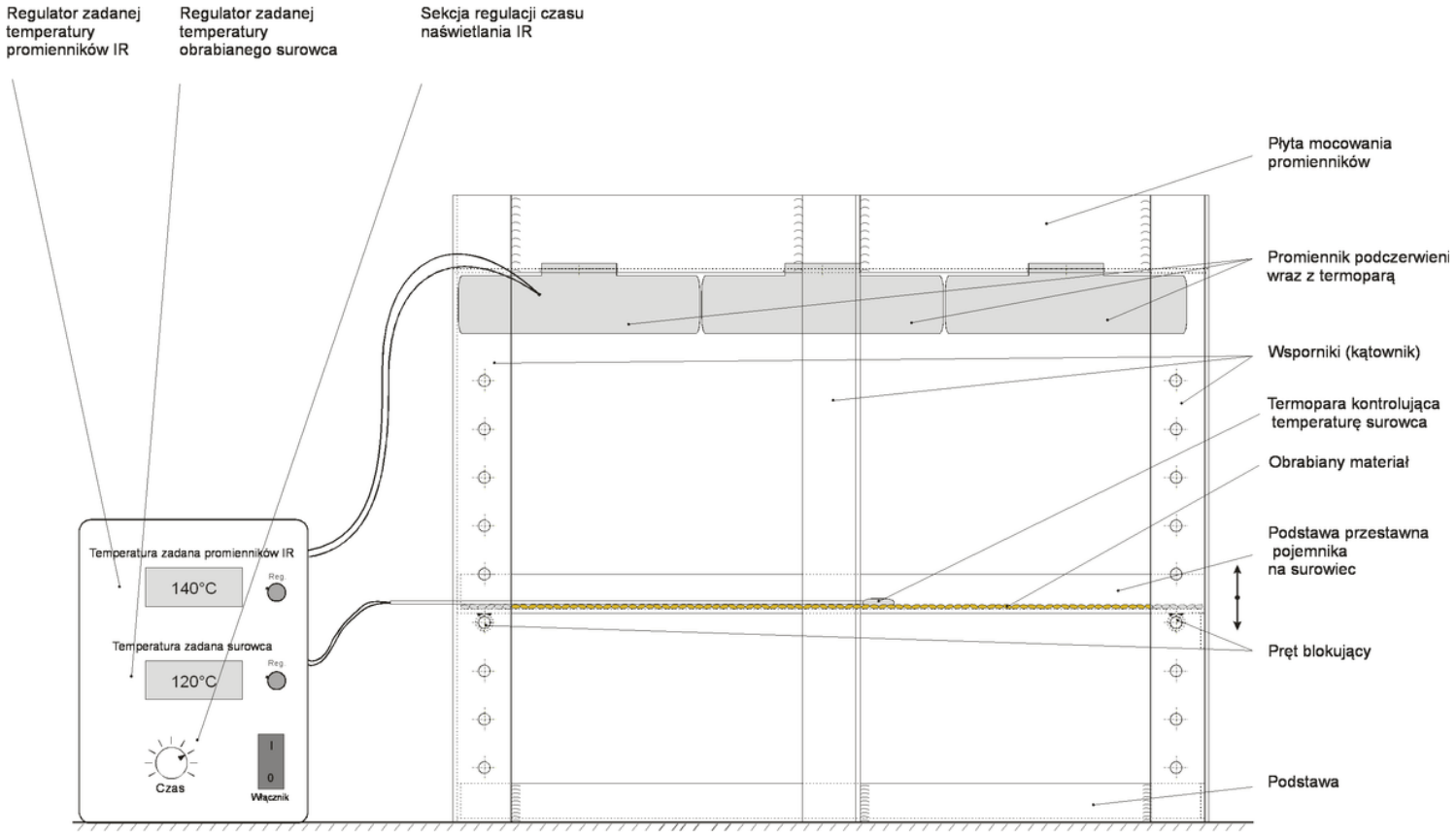

Figure 1

Laboratory chamber for irradiating granular materials with IR rays.

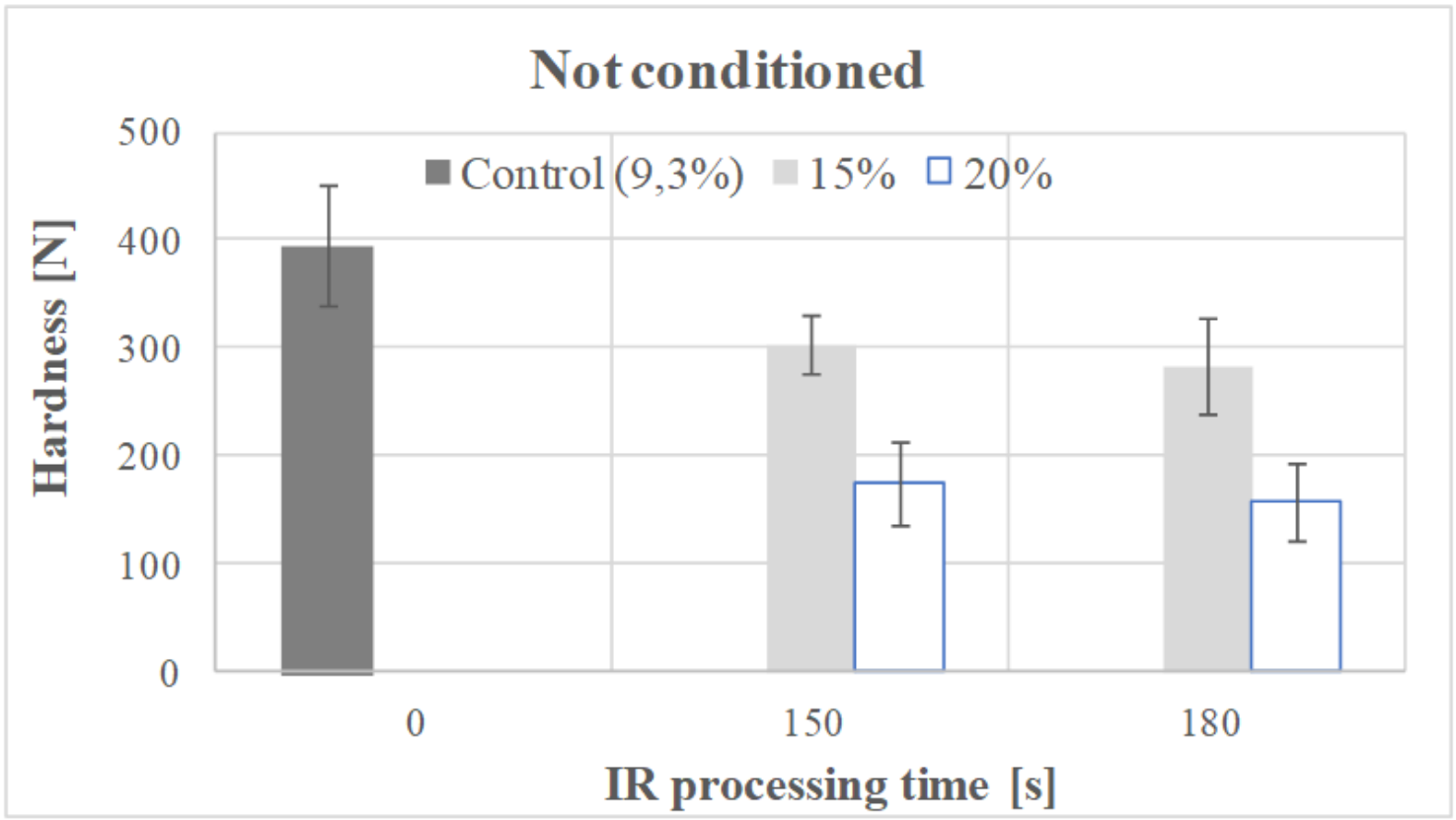

Figure 2

Results of the soybean hardness measurements for not pre-conditioned seeds after micro-wave treatment

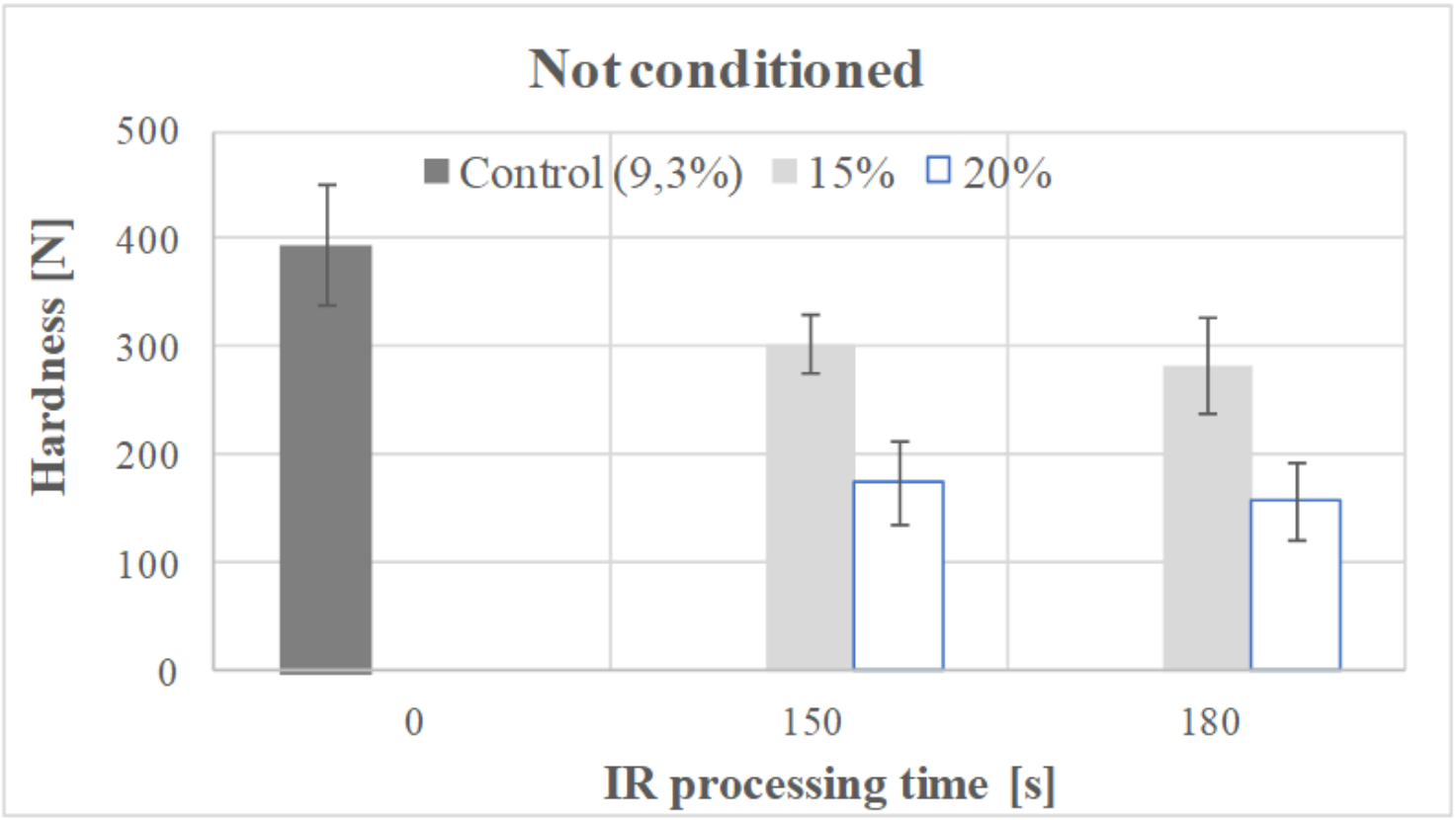

Figure 2

Results of the soybean hardness measurements for not pre-conditioned seeds after micro-wave treatment

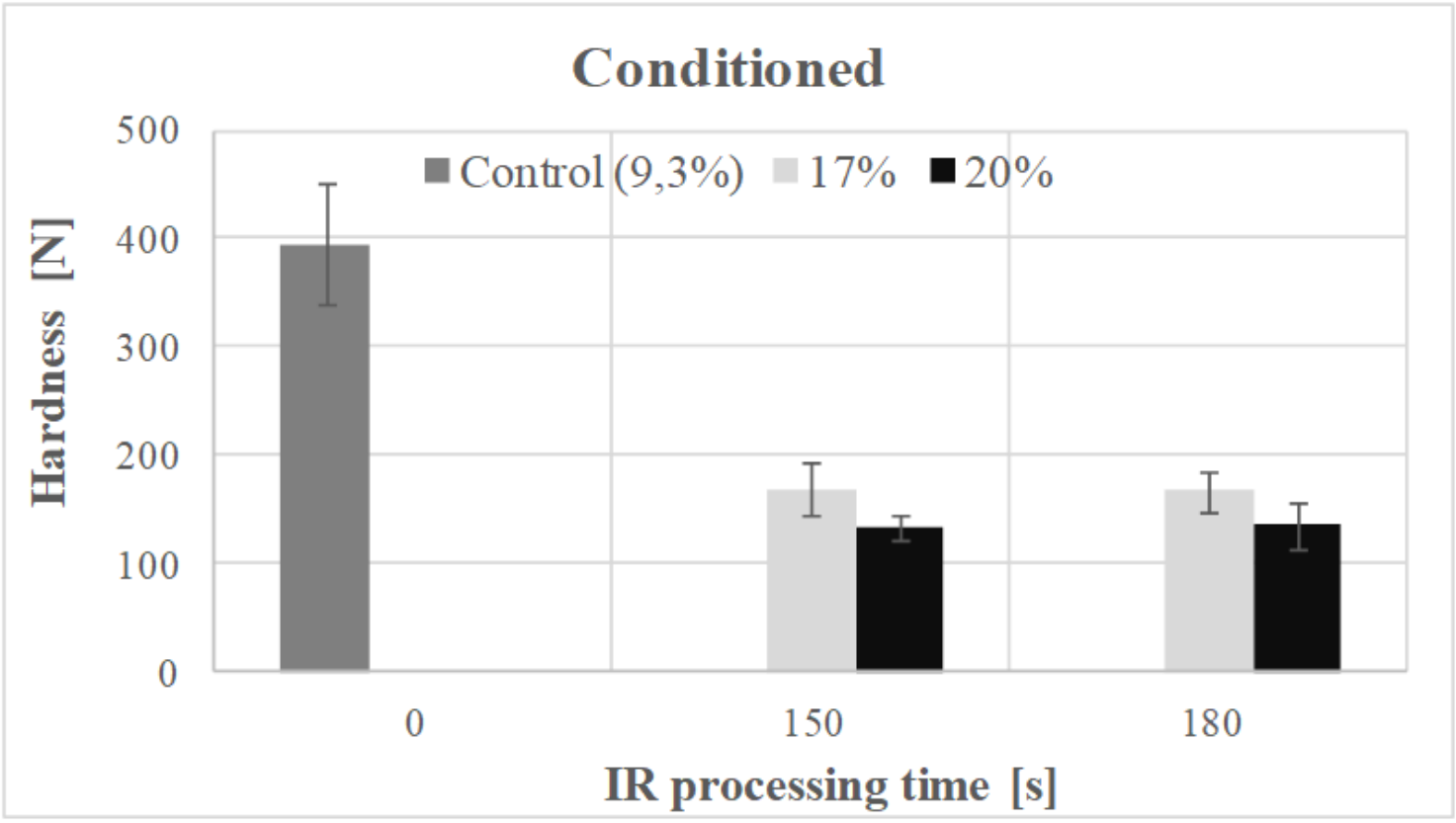

Figure 3

Results of the soybean hardness measurements for pre-conditioned seeds

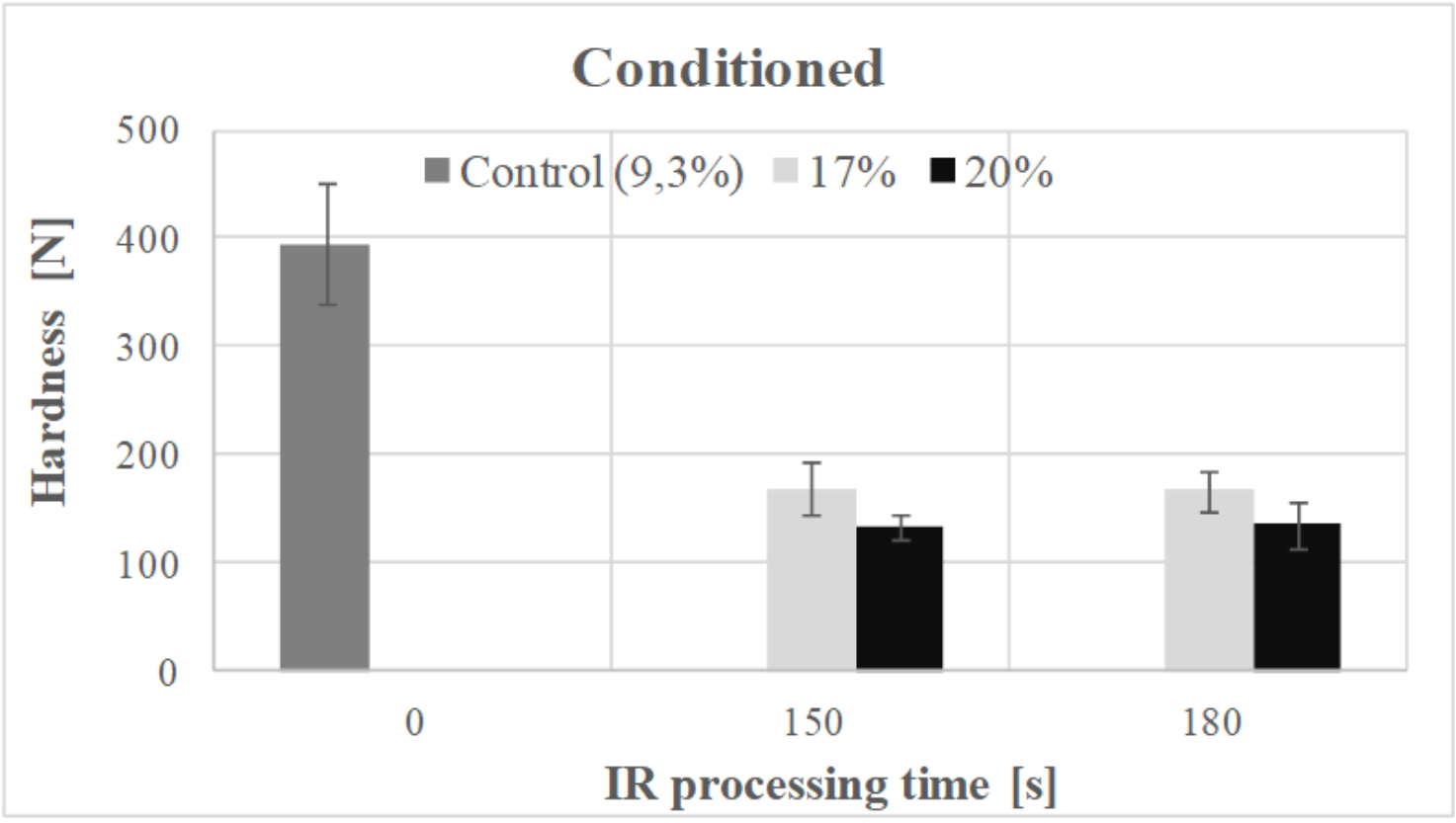

Figure 3

Results of the soybean hardness measurements for pre-conditioned seeds