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LEMONGRASS PUREES: INFLUENCE OF PRETREATMENTS ON THE BIOACTIVE COMPOUNDS AND PHYSIOCHEMICAL PROPERTIES

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ABSTRACT

Lemongrass puree with 3 different pretreatments were evaluated for flavonoid content, antioxidant capacity, total chlorophyll, ascorbic acid, titrable acidity and pH and compared with the corresponding values of control puree and fresh lemongrass. The pretreatments given were T1- potassium metabisulphite, T2- citric acid and T3- mixture of Magnesium Chloride, Sodium Bicarbonate and Potassium metabisulphite followed by hot water blanching. Puree was made with three different total soluble solid (TSS) levels i.e. 2.5, 3.5 and 4.5°Brix. Results obtained showed that the maximum quality retention was in case of T1 treatment (potassium metabisulphite) with TSS 2.5, followed by T3 (mixture of Magnesium Chloride, Sodium Bicarbonate and Potassium metabisulphite). T2 (citric acid) treatment showed maximum antioxidants capacity.

Keywords: Chemical Pretreatments, Hot Water Blanching, Puree, TSS, Quality

I. INTRODUCTION

Herbs and spices are used in the diet [1,2,3]. Herbs are mainly used as flavoring agents. These are also known for their nutritional, antioxidant, medicinal, insect repellent, antimicrobial and medicinal properties [4]. Herbs and spices have been acknowledged as means of various phytochemical compounds with strong antioxidant activity [5,6,7,8].

Lemon grass belongs to the section of Andropogan called cymbopogam species of the family Germineae. It acts as cholesterol lowering agent and effective in curing headaches, digestive problems, congestion, coughing, diarrhea, gas, bowel spasms, vomiting, flu symptoms, as a mild sedative. Herbal medicines and their identified active constituents also possess anti-acid/anti-peptic, gastro-protective and/or anti-ulcer [9]. Lemongrass leaves are about 100-125 cm long and 1.0 to 1.5 cm broad and linear. Lemongrass generally grows 1 to 1.5 m high and 3 m high at flowering. Lemongrass has panicle Inflorescence and produce small blue flowers. Because of its protogynous nature, Lemongrass is adapted for cross-pollination [10,11].

Herbs possess essential oils that are used mainly in perfumes, deodorants and skin products; India is traditionally the largest producer of lemongrass oil. However, the production has declined steadily over the years and export failed from 1800 tonnes in 1961-62 to 65 tonnes in 1994- 95 and today it is 400 tonnes [12].

Blanching in water has the advantage of a homogenous treatment of food and the possibility of modulating the temperature of blanching. Water blanching usually results in a more uniform treatment, allowing processing at lower temperatures. The conditions of carrot blanching are a temperature of 95°C for 1 minute to inactivate polyphenol oxidase and peroxidase [13]. For Salak blanching, temperatures below 70°C must be used during 5 minutes [14]. Water blanching results in increased leaching of minerals and nutrients [15,16].

To deal with this problem, chemical agents are added which helps to prevent nutritional losses. For aonla, water and potassium metabisulphite (0.3%) at 80°C for 3 min resulted in prevention of leaching of nutrients due to addition of potassium metabisulphite and blanching inactivated enzymes [17].

During storage of lemongrass, red pigment (anthocyanin) formation occurs on the outer, exposed layer of the pseudostem [18]. This affected outer leaf sheath layer has to be removed, which leads to reduction of the diameter of the pseudo stem along with loss in total weight. Also, this process affects the aroma of the lemongrass, leading to customers' dissatisfaction and a loss of market value [19].

Puree is the form, which is most easy to handle and preserve with minimum loss of important chemical and physical constituents or characters of herb. Pureeing, or making an herbal paste, is the more suitable method for culinary purposes.

Shelf life of herbs is short due to fast discoloration of cut surfaces and loss of flavors enzymatic and physiological reactions after harvesting. The work done on herb purees and documented literature is not available. Keeping in view these aspects, the present study has been planned with the objective to study influence of pretreatments on the bioactive compounds and physiochemical properties of lemongrass puree.

II. MATERIALS AND METHODS

The experiments to fulfill the objective were conducted in the Department of Processing and Food Engineering, College of Agricultural Engineering and Technology, PAU Ludhiana.

2.1 Procurement of material

Fresh lemongrass was procured from Field Fresh Foods Private Limited Ladhowal, Ludhiana.

2.2 Preparation of lemon grass

Lemongrass was selected with no sign of infection and damaged part was removed off and washed thoroughly in running water. The main bulb part of lemon grass was cut using a stainless steel knife. Bulb was further cut by slicing into small pieces to make it easy for handling and making its puree.

2.3 Pre-treatments

To avoid undesirable changes various pre-treatments are given following cleaning, slicing and cutting of main parts. Lemongrass was dipped for 5 min in case of all the treatments; T1- potassium metabisulphite (0.25% (w/v)), T2- citric acid (0.5% (w/v)) and T3- mixture of magnesium chloride (0.1% (w/v)), sodium bicarbonate (0.1%(w/v)), potassium metabisulphite (2.0% (w/v)) following hot water blanching at 90-100°C for 15 min (tied in muslin cloth). Lemongrass was immediately cold down under running tap water.

2.4 Processing of lemongrass puree

The chopped lemongrass subjected to processing as shown in Fig. 1. Total soluble solids were fixed at three levels – 2.5, 3.5 and 4.5 °Brix by addition or removal of water.

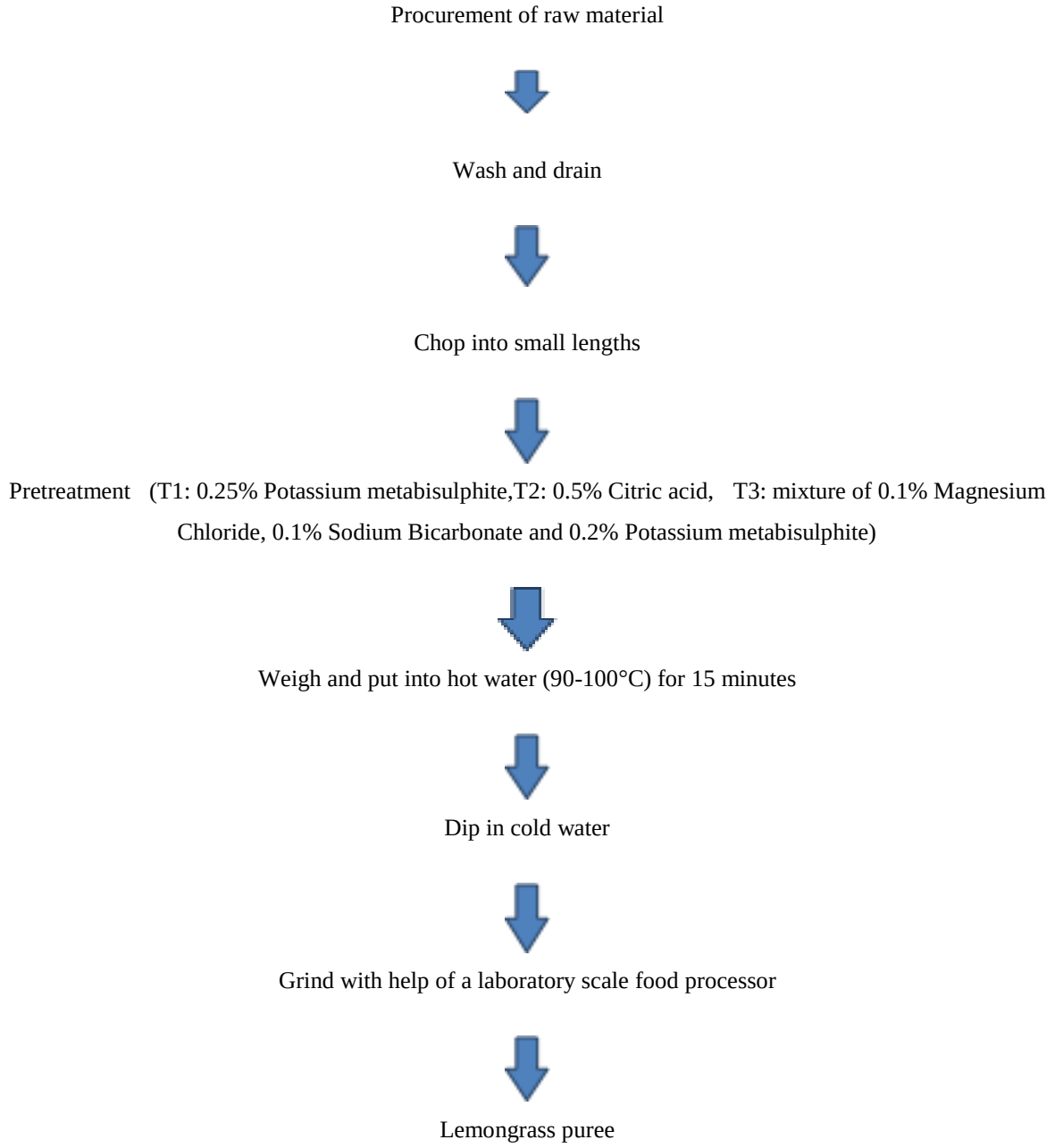

Fig. 1 Flow chart showing processing of Lemongrass puree with pre-treatments

III. QUALITY ANALYSIS

3.1 Ascorbic acid

Lemongrass was analyzed for ascorbic acid using a reported method [20]. 1 g of sample was shredded in and mortar using 20 ml of metaphosphoric aced-acetic acid solution and was filtered. 5 ml of filtered extract was

titrated against dye till the appearance of a pink color and volume of dye used to oxidize vitamin C in sample was noted. Content was calculated by filtering the standard ascorbic acid (0.2mg/ml) with dye. Ascorbic acid content can be calculated using these formulas: $$Dye\ factor = \frac{0.5}{titre\ value}$$ (1)

$$\text{mg of ascorbic acid per 100 g or ml} = \frac{\text{titre} \cdot \text{dye factor} \cdot \text{volume made up} \cdot 100}{\text{aliquot of extract taken for estimation} \cdot \text{weight or volume of sample taken for estimation}}$$

. .

3.2 Titrable acidity

In order to determine the acidity of lemon grass puree, a proposed method [20] was used. 5g of sample were diluted to 50 ml in volume in a flask. 10 mL of diluted sample was taken in a conical flask and 2-3 drops of phenolphthalein indicator were added. This solution was then titrated against 0.1N NaOH. Findings were applied in formula given in (3):

$$\text{Total acid}(\%) = \frac{\text{titre} \cdot \text{normality of alkali} \cdot \text{volume made up} \cdot \text{equivalent weight of acid} \cdot 100}{\text{volume of samole taken for estimation} \cdot \text{weight or volume of sample taken} \cdot 1000} \quad (3)$$

3.3 pH Measurement

pH was measured using pH analyzer (ELCO LI 614) available in Quality Control Laboratory. The pH analyzer (was first calibrated manually at 20 °C with the help of buffer of pH 7 by dipping sensor in the buffer. Further the sample whose pH is to be determined is taken and temperature is again set to 20°C and the sensor is dipped into the sample and the pH of sample is displayed on the display of pH analyzer.

3.4 Flavonoids content

One gram of sample was homogenized with 10 ml of methanol and 0.5 ml of supernatant was diluted with 1.5 ml of methanol as per reported method [21]. To this add 1 ml of 1% AlCl3 and 1 ml of 1 % potassium acetate. The resulting solution was diluted with 2.8 ml distilled water. The mixtures were allowed to stand for 30 minutes and the optical density of the mixtures was measured against the blank (2 ml of methanol, 1 ml potassium acetate, and 2.8 ml distilled water) at 415 nm with the help of a UV-Vis spectrophotometer on fresh weight basis. A standard curve was run simultaneously using Rutein (40-200µg) From this curve; the values of flavonoids were obtained.

3.5 Pigments

The pigments (chlorophyll, β-carotene and lycopene) were determined and quantified using a proposed procedure [22]. 1 g sample was homogenized with 10 mL of acetone and n-hexane (4:6) using a tissue homogenizer (Labco, India) for 30 s over ice. The homogenized solution was allowed to settle down. Then, 1 mL of the supernatant was taken and was diluted with 9 mL of acetone and n-hexane (4:6), more dilution can be done if required. The resulting solution was analyzed spectro-photometrically with the help of and UV-Vis spectrophotometer (Model Spectroscan 80DV, Biotech Engineering management Company Limited, UK). The optical density was measure at 663, 645, 505 and 453nm using acetone and n-hexane (4:6) as a blank.

Chlorophyll, (µg ml-1) were quantified using the following equations and then expressed as mg/g fresh weight of sample,

Chlorophyll a = 0.999A663 – 0.989A645 (4)

Chlorophyll b = 0.328A663 – 1.77A645 (5)

Total chlorophyll = Chlorophyll a+ Chlorophyll b (6)

Where A663, A645, A505 and A453 are the absorbances at 663, 645, 505 and 453 nm, respectively.

3.6 Antioxidants capacity

Antioxidant capacity was determined through assessment of free radical-scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (HiMedia Laboratories, India) as per the method [23]. 1 g of lettuce was extracted with 5 mL of methanol. The extract solution was centrifuged at 600 rpm for 15 min at 4°C, using a cold centrifuge (MP 400-R, Eltec Limited, India) Aliquots of 0.01 mL of supernatant so obtained were mixed with 3.9 mL of methanolic DPPH (0.025g/l) and 0.090 mL of distilled water. The resulting mixture was shaken with the help of a vortex shaker (Labco, New Delhi, India) and was then uncubated in dark for 30 min. Absorbance of the mixture was measured against the blank at 515 nm with the help of UV-Vis spectrophotometer (Spectroscan 80DV, Biotech Engineering Management Company Limited, UK). The results were obtained as the percentage decrease with respect to the absorbance of a reference DPPH solution. Free radical inhibition by DPPH was calculated in following way,

Scavenging activity (%) = $[(A_{blank}-A_{sample}) / A_{blank}] \times 100$, (7)

Where: A_{blank} is the absorbance of the Methanolic DPPH, A_{sample} is the absorbance of the test sample.

3.7 Statistical analysis

The analysis of effect of different pretreatments on quality of lemongrass puree was done using two-way factorial ANOVA technique (SAS software) and GraphPad PRISM Version 7 software, Inc. USA. The results of analysis were used to evaluate the significant difference among the various parameters at $p < 0.05$.

IV. RESULTS AND DISCUSSION

Influence of pretreatments on the bioactive compounds and physiochemical properties of lemongrass puree is presented as follow:

4.1 pH

The decrease in pH during pretreatments and processing is shown in Fig. 2 for lemongrass puree. pH was found to be maximum (5.92) in control samples followed by T1 treated lemongrass puree (TSS 4.5°Brix) with 5.66 pH with increase of 0.28% and decrease of 4.23% respectively. Minimum value of 4.10 (30.63% decrease) was observed in T2 (TSS 2.5°Brix) treated puree. The effect of pretreatments and TSS levels was statistically analyzed (ANOVA) and was found to be highly significant at $p<0.05$ for lemongrass puree (TABLE 1).

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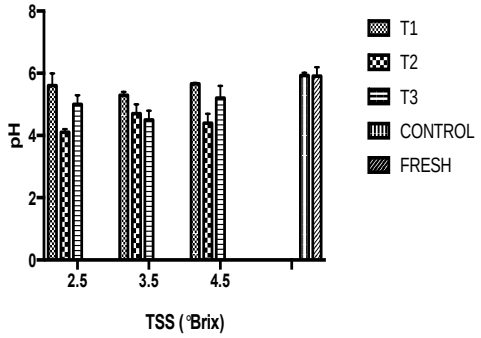

Fig 2: pH of lemongrass

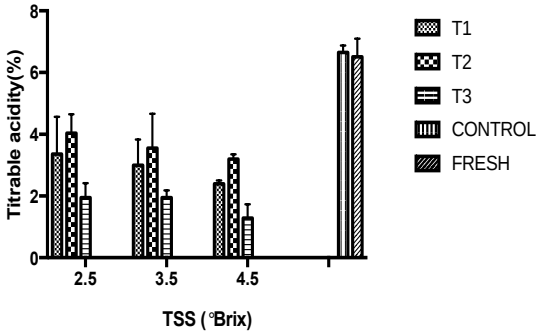

Fig. 3: titrable acidity of lemongrass

4.2 Titrable acidity

The change in titrable acidity of Lemongrass puree during processing is presented in Fig. 3. Minimum and maximum titrable acidity were observed in T3 treated lemongrass puree with TSS 4.5°Brix with 80.34%(1.28) decrease and 5.78 in control samples with decrease of 11.16% respectively. Similar results have been reported indicating that pH was decreased immediately after treatment of fresh cut sweet potato [24]. When lemongrass puree was analyzed statistically using ANOVA, highly significant results were obtained for TSS levels, pretreatments and their interaction (TABLE 1).

4.3 Total chlorophyll

Total chlorophyll of processed lemongrass puree after pretreatments is shown in Fig. 4. A decrease was observed in chlorophyll content after pretreatments. Similar results were observed for watercress [25]. Lemongrass control puree was observed with maximum retention of total chlorophyll (14.05 mg/g) with the decrease of 9.74% from total chlorophyll of fresh sample, followed by T1 treated puree (TSS 2.5°Brix) with 13.16 mg/g. Minimum value 7.33 mg/g (52.92% decrease) was observed in T2 treatment with TSS 2.5°Brix. When examined experimentally, it is clear that the results were highly significant for effect of pretreatments and TSS levels and also for their combinations when LS means were compared (TABLE 1).

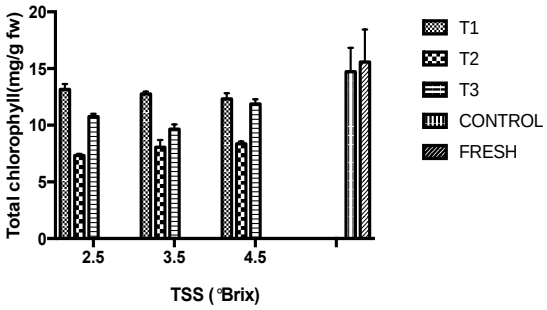

Fig. 4 total chlorophyll of lemongrass

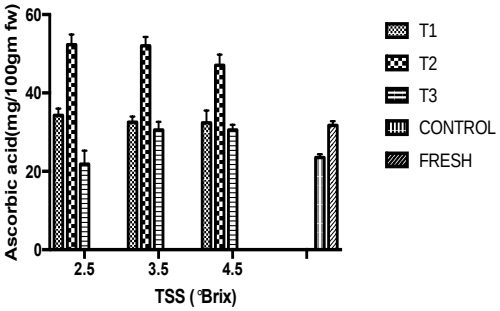

Fig. 5 ascorbic acid of lemongrass

4.4 Ascorbic acid

The change in ascorbic acid content of lemongrass puree with different pretreatments is as shown in Fig. 5. Pretreatments caused little decrease in ascorbic acid of purees with minimum decrease of 2.92% followed by 7.81% in T1 treated lemongrass puree with TSS 4.5°Brix and TSS 2.5°Brix respectively. A study reported that

decrease of 70% was observed in New Zealand spinach after blanching when compared with raw spinach [26]. An increase (48.30%) to the value of 52.33 mg/100g ascorbic acid content was found in T2 treated lemongrass puree with TSS 2.5°Brix. Statistically it was observed that pretreatments cause significant effect on ascorbic acid of lemongrass puree (TABLE 1).

4.5 Flavonoid content

The variation in flavonoids content of lemongrass puree is shown in Fig. 6. In a previous study it was observed that blanching increase phenolic contents due to the enhanced liberation and thus better extraction of phenolic compounds after disruption of cell walls [27]. But according to anothher research, leaching effect of blanching is assumed to be more decisive than the enhanced release of phenolic compounds when highly water soluble compounds taken into account [28]. T2 treated lemongrass puree with TSS 3.5°Brix had 0.51 mg/g flavonoids with decrease of 80.38%, and maximum flavonoids content of 1.93 mg/g was observed in control samples followed by T1 treated puree (TSS 2.5°Brix) with 1.78 mg/g flavonoids content and decrease of 31.95%. Statistical analysis by using ANOVA shows that results were highly significant at $p<0.05$(TABLE 1).

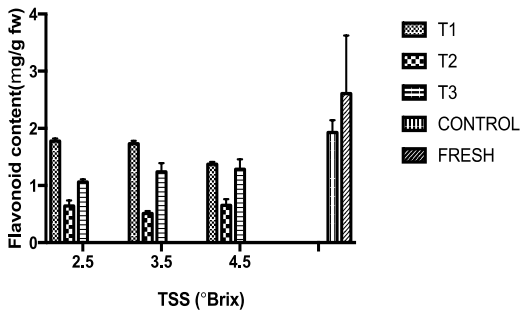

Fig 6 flavonoid content of lemongrass

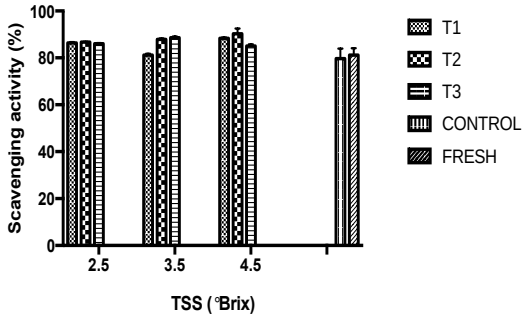

Fig.7 antioxidant capacity of lemongrass

4.6 Antioxidant capacity

Effect of various pretreatments and processing resulted in an increase in antioxidant capacity as compared to control puree as presented in Fig. 7. For lemongrass maximum antioxidant capacity (90.27%) was found in puree with T2 treatment and TSS 4.5°Brix with increase of 11.13%. In a study on turnip greens, observations suggest that use of ascorbic acid during the blanching process gave rise to a high antioxidant capacity of turnip greens [29]. Minimum increase (0.04%) was observed in T1 treated puree with TSS 3.5°Brix. Statistical analysis of observed data to find the effect of pretreatments showed that effect was highly significant. While considering effect of TSS and combined effect of pretreatment and TSS, these were also highly significant for antioxidant

capacity of lemongrass puree (TABLE 1).

V. CONCLUSION

Pretreatments had significant effect on titrable acidity of lemongrass puree, having minimum titrable acidity in T1 at TSS 4.5. Also, pretreatments affected pH of lemongrass puree significantly at $p<0.05$ having maximum value for puree at 4.5°Brix with T1. Ascorbic acid was found to be maximum in T2 followed by T1 at TSS 4.5°Brix and T2 at TSS 2.5°Brix. Statistically, results were found significant within both pretreatments and TSS

levels for total chlorophyll. Differences for interaction of pretreatments and TSS levels were non-significant. Variation of flavonoids in lemongrass puree with different pretreatments was little for T1 and T2 at all TSS levels. Comparing the pretreatments, TSS levels and interaction of both, all were found to have significant effect on flavonoid content of lemongrass puree. For antioxidant capacity of lemongrass puree, it was observed that T1, T2, T3 have significant affect. Also, combined effect of TSS levels and T1, T2 and T3, was found to be significant. But keeping in view all the quality parameters, lemongrass puree with pretreatment T1 and TSS 2.5°

Table 1. ANOVA table for effects of pretreatments on quality of lemongrass puree

Parameter	pH	Titrable acidity (%)	Total chlorophyll (mg/g fw)	Ascorbic acid (mg/100g fw)	Flavonoid content (mg/g fw)	Antioxidant capacity (Scavenging activity (%))
F1- PRETREATMENTS						
T1	5.52^{a}	2.91^{b}	12.74^{a}	33.04^{b}	1.62^{b}	85.33^{c}
T2	4.40^{c}	3.59^{a}	7.92^{c}	50.46^{a}	0.60^{c}	88.28^{a}
T3	4.90^{b}	1.72^{c}	10.76^{b}	27.65^{c}	1.94^{a}	86.59^{b}
F2- TSS						
2.5	4.96^{a}	3.06^{a}	10.39^{a}	38.91^{b}	1.106^{a}	86.69^{c}
3.5	4.80^{b}	2.86^{a}	9.89^{b}	38.38^{a}	1.029^{b}	87.02^{b}
4.5	4.78^{c}	2.62^{c}	9.76^{b}	38.09^{a}	0.96^{c}	87.57^{a}
F1	*	*	*	*	*	*
F2	*	*	*	*	*	*
Interaction	*	*	*	*	*	*

Notes: Pretreatments (T1: hot water blanching+potassium metabisulphite; T2: hot water blanching+citric acid; T3: hot water blanching+mixture of magnesium Chloride, sodium Bicarbonate and potassium metabisulphite); the experimental data were subjected to ANOVA; means with different letters in the same column differ significantly ($p < 0.05$) *

significance, ns non-significance; the values represent mean of three replication.

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