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Silicon supplementation improves the nutritional and sensory characteristics of lentil seeds obtained from drought-stressed plants

Running title: Silicon supplementation and nutritional profile of lentil seeds

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ABSTRACT

BACKGROUND

Lentil is an important nutritionally rich pulse crop in the world. Despite having a prominent role in human health and nutrition, it is very unfortunate that global lentil production is adversely limited by drought stress, causing a huge decline in yield and productivity. Drought stress can also affect the nutritional profile of seeds. Silicon (Si) is an essential element for plants and a general component of the human diet found mainly in plant-based foods. This study investigated the effects of Si on nutritional and sensory properties of seeds obtained from lentil plants grown in Si-supplied drought-stressed environment.

RESULTS

Significant enhancements in the concentration of nutrients (protein, carbohydrate, dietary fibre, Si) and antioxidants (ascorbate, phenol, flavonoids, total antioxidants) were found in seeds.

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Significant reductions in antinutrients (trypsin inhibitor, phytic acid, tannin) were also recorded. A novel sensory analysis was implemented in this study to evaluate the unconscious and conscious responses of consumers. Biometrics were integrated with a traditional sensory questionnaire to gather consumers responses. Significant positive correlations ($R=0.6-1$) were observed between sensory responses and nutritional properties of seeds. Seeds from Si-treated drought-stressed plants showed higher acceptability scores among consumers.

CONCLUSION

The results demonstrated that Si supplementation can improve the nutritional and sensory properties of seeds. This study offers an innovative approach in sensory analysis coupled with biometrics to accurately assess consumer's preference towards tested samples. In the future, the results of the current study would help in making a predictive model for sensory traits and nutritional components in seeds using machine learning modelling techniques.

Keywords: *Antioxidants; Antinutrients; Sensory analysis; Biometrics; Tannin; Nutrients*

INTRODUCTION

Lentil (*Lens culinaris* Medikus) is one of the most important pulse crops in the world which plays a significant role in agricultural sustainability and nutritional food security. Lentil seeds contain 20 - 36.4 % proteins, 35 - 53 % starch, 2 - 4 % fibres and 2.4 - 3.1 % minerals with high levels of Magnesium (Mg), Phosphorous (P), Calcium (Ca) and Sulphur (S) on a dry matter basis¹. Lentil seeds are a valuable source of vitamins including ascorbic acid, thiamine, folic acid and riboflavin. The high protein content of lentil seeds has made them a nutritious

substitute for meat and are often regarded as “poor man’s meat” especially in developing countries as an affordable plant protein source to feed people¹. Lentil protein contains high content of lysine (63 - 73 g / Kg protein), one of the limiting but essential amino acids in cereals¹. Thus, the consumption of lentil seeds with rice or wheat ensures a balanced diet and provides adequate amounts of essential amino acids such as methionine, cysteine and tryptophan for human nutrition. Lentil seeds are an excellent source of phytochemical antioxidants such as carotenoids, tocopherols, and phenolics; thus, offering varied health benefits to humans such as protection against cardiovascular diseases, diabetes and cancer².

Despite having a prominent role in human health and nutrition, it is very unfortunate that global lentil production is adversely limited by various biotic and abiotic stresses. Among them, drought stress is the most important abiotic stress especially in Mediterranean and subtropical countries, causing decline in yield up to 24 % during reproductive stage and 70 % during the pod development stage³. Drought stress also affects the nutritional profile of lentil seeds and leads to severe reduction in protein, starch and mineral⁴. Climate change scenarios require the development of drought-resilient lentil genotypes and/or management practices to mitigate stress for improved productivity and nutritional status of the crop.

Among various drought stress mitigation strategies, silicon (Si) application can promote lentil plant growth under drought stress through regulation of hydrolytic enzymes, osmolytes and antioxidant metabolism⁵. The mineral Si is an essential element for plants (especially under stresses) and a general component of the human diet found mainly in plant-based food^{6,7}. Positive effects of Si on lentil growth and development under drought stress are well established⁵. However, seeds obtained from Si applied and drought-stressed lentil have

not been evaluated for Si effect on the nutritional quality (concentrations of nutrients, antinutrients and antioxidants) and on the sensory acceptability of cooked seeds. To unravel this research gap, the nutritional quality of (boiled and stir-fried) seeds was assessed in terms of protein, carbohydrate, total dietary fibre, ash, moisture, fat and antioxidants, namely ascorbate (ASC), phenol and flavonoid. Since lentil seeds also contain some antinutritional components such as trypsin inhibitors, phytic acid and tannins that could inhibit their carbohydrate and protein utilization⁸, the analysis of antinutrients was also carried out in this study.

Sensory attribute intensities and acceptance scores are commonly used to assess the acceptability of food and beverage products. This type of sensory analysis commonly uses Quantitative Descriptive Analysis (QDA), descriptive test or 15-cm non-structured scale for assessing food quality. However, it has always been known that consumer sensory tests are subjective as it is based on the participants previous experience⁹⁻¹¹. From previous studies, differences between cultures in the use of hedonic scales have been found, with Asians being more polite and avoiding extremes even though they liked or disliked the product extremely¹²⁻¹⁶. To avoid this situation, biometric responses of the panellists can be measured and used to obtain accurate food sensory perception data. Biometric data is more reliable and accurate than intensity and hedonic scale tests for sensory analysis as it captures responses from the autonomic nervous system (ANS) such as emotions, facial features, and the physiological changes in the body while tasting a product, these may not be altered or controlled by consumers (unconscious response). The advantages of using both intensity and hedonic scale tests as well as biometric responses are to obtain the first reaction that consumers have, when

evaluating the product and how they change their responses once they process the information (thinking process or conscious response). Furthermore, some responses (conscious) are driven by cultural background and may not reflect actual hedonic or sensorial responses^{17,18}.

Biometrics measures physiological characteristics of individuals, which in turn aids in their automated recognition. Recently, biometric techniques have been widely used for personal authentication purposes, such as fingerprinting, face scanners and voice recognition¹⁹. More recently, research has been focused on these biometric techniques to obtain responses from the autonomic nervous system (ANS)^{20,21}. The ANS is related to the subconscious biological features of the human body such as heart rate (HR), body temperature and respiration levels, which are associated to other emotional responses like arousal and stress. The perception of different tastes can produce responses from the ANS, causing physiological changes such as variations in body/skin temperature²².

In the current study, sensory analysis of seeds was performed using a Biosensory application (App; The University of Melbourne, Parkville, Australia), which capture sensory attributes intensities and acceptance scores and non-invasive biometric responses from the participants based on remote sensing techniques²³. Biometric techniques are used to capture unconscious responses such as facial expressions, skin temperature and HR non-invasively, based on video and infrared thermal imagery analysis. The integration of these techniques would be an efficient strategy to gather more information from consumers to correlate with sensory characteristics, while tasting a product. Recently, this method was validated²⁴ and used successfully as reliable tool to assess acceptability of beer^{15,16,25}, insect-based snacks¹⁸ and chocolates by consumers^{17,26}.

Compared to previous studies^{15-18,24-27}, the current study assessed whether the supplementation of Si would affect the sensory attributes of the seeds obtained from drought-stressed lentil plants, taking into consideration of the nutritional aspects of seeds as well. No previous research has investigated the emotional or the physiological responses of consumers associated with sensory evaluation of drought stressed seeds. When choosing foods, nutritional value along with sensory factors, such as appearance, taste, smell and texture, can play a critical role in a consumer's food preferences and eating habits. Many consumers eat with two crucial elements in mind: nutrition value and sensory quality. There are various chemical compounds that have effects on neurotransmitters that trigger emotions as well as nerves that cause different stimuli. For example, tannins cause the irritation of trigeminal nerves, and this is the cause of the astringent sensation in the mouth^{28,29}. On the other hand, there are compounds such as alcohol, which causes depression and alkaloids, which stimulate the dopamine release that, at the same time, cause happiness³⁰. Greater understanding of sensory traits and nutritional aspects of seeds could lead to tailoring of our diet to meet the required standard as well as to evaluate the food products, to provide a detailed information aimed at increasing consumer acceptability. The findings from the current study would be of interest to the agricultural scientists, product developers and potential marketers, because nutritional information combined with tasting is assessed and integrated by the consumer's responses to rank the samples, that can be associated with product acceptance.

The hypothesis set for this study was that Si supplementation to drought-stressed lentil will enhance the nutritional potential as well as the sensory traits of seeds. Hence, the objectives of the present study were to analyse the effect of Si on the nutritional, antinutritional,

antioxidant potential as well as the sensory acceptability of cooked lentil seeds from plants grown in Si supplied, drought-stressed environment. The sensory acceptability of these seeds was evaluated using both sensory attribute intensities and acceptance scores as well as the non-invasive biometric indices. The Biosensory App has been developed previous to this study to serve as a tool to obtain biometrics from sensory panellists assessing different food and beverage/packaging products as described in the Biosensory App papers^{15-18,23-27,30}. The Biosensory App was used in this study as a tool to evaluate several biometric measurements when compared to other methods. The focus of this paper does not merit an in-depth comparison of the Biosensory App with other methods since it will be outside the scientific scope of this study.

MATERIALS AND METHODS

Plant materials and drought stress treatments

Two lentil genotypes, ILL 6002 (drought-tolerant-G1) and ILL 7537 (drought-sensitive-G2) were grown under drought stress with Si (DSi) and a control (non-stressed without Si-C) in a growth room facility at Parkville campus at The University of Melbourne. The environmental conditions in the growth room were as follows: temperature: $23 \pm 2^{\circ}\text{C}$, relative humidity: 45-50 %, photoperiod: 12 h and light radiation: $300 - 325 \mu\text{mol m}^{-2} \text{s}^{-1}$ ³¹. Sodium metasilicate (Na_2SiO_3) was used as the source of Si, which is the most commonly used soluble form of Si in animal supplemental studies⁶. Si supplementation and drought stress treatments were according to methods described in Biju et al.⁵

Sample preparation and cooking treatments

The sample preparation and cooking methods were based on the method given by Hefnawy³². Lentil seeds (200 g) were soaked in tap water (1:10 w/v) for 1 h at room temperature (25 °C). The soaked seeds were drained and rinsed three times with 500 mL tap water, then cooked by methods described below:

i) Boiling (B)

The rinsed soaked seeds (100 g) were cooked in tap water (100 °C) in the ratio of 1:10 (w/v) on a hot plate until they became soft when felt between the fingers (15 min).

ii) Stir-frying (SF)

The soaked seeds (100 g) were fried without oil in a non-stick pan for 5 minutes until the sample became crisp-tender and stirred after roughly every minute during cooking³².

The cooked (boiled/stir-fried) seeds were used for the analysis of nutrients, antinutrients, antioxidants and were given to the consumers for sensory evaluation. The samples used in this study were (i) G1 Control Boiled (**G1C-B**), (ii) G1 Control Stir-fried (**G1C-SF**), (iii) G1 Drought +Si Boiled (**G1DSi-B**), (iv) G1 Drought +Si Stir-fried (**G1DSi-SF**), (v) G2 Control Boiled (**G2C-B**), (vi) G2 Control Stir-fried (**G2C-SF**), (vii) G2 Drought + Si boiled (**G2DSi-B**), and (viii) G2 Drought +Si Stir-fried (**G2DSi-SF**).

Proximate composition and Si analysis

Analyses of cooked (boiled/stir fried) lentil seed samples for protein, total dietary fibre,

ash, moisture and fat were carried out according to the standard methods of the Association of Official Analytical Chemists (AOAC)³³.

Total carbohydrate content in the samples was estimated by the method proposed by Dubois et al.³⁴. One gram of cooked (boiled/stir-fried) seeds was taken and homogenized followed by the addition of 2 mL of 1 M sulfuric acid (H₂SO₄). The samples were transferred to the hot water bath for 1 h at 90 °C followed by centrifugation for 10 min at 3388 g. One mL of supernatant was taken and pipetted into a boiling tube, followed by the addition of 0.5 mL of 80 % phenol, and 5 mL of 95 % H₂SO₄. After 10 min, the tubes were placed in a boiling water bath for 20 min at 30 °C. Subsequently, the tubes were held under running water for 5 min to bring them to room temperature and then left for 30 min to cool down. Absorbance was taken at 490 nm against blank using a UV/visible spectrophotometer. The concentration of total carbohydrates was estimated from the standard curve prepared using glucose.

Silicon content in cooked (boiled/stir-fried) seeds was estimated using the modified Autoclave-induced digestion method proposed by Elliot and Snyder³⁵ with some modifications in amount of seed sample taken and concentration of hydrogen peroxide (H₂O₂) used. Samples were ground in a Retsch centrifugal mill ZM-200 (Retsch, Haan, Germany) into powder. The powder (0.1 g) was gently mixed with 30 % H₂O₂ (3 mL) and 50 % sodium hydroxide (NaOH - 3.25 mL) in polyethylene tubes using vortex. The tubes were placed in an autoclave (SABAC, model T62) at 138 kPa for 1 h at 126 °C. Digested samples were brought to a final volume of 50 mL with distilled water. Nine mL of 20 % acetic acid (CH₃COOH) and 2.5 mL ammonium molybdate ((NH₄)₂MoO₄-54g/L, pH 7.5) were added to 1.0 mL of digested sample taken in a 50 mL of polypropylene volumetric flask. Subsequently, 20 % tartaric acid (1.25 mL) and

reducing solution containing 8 g L⁻¹ sodium sulphite (Na₂SO₃-0.25 mL), 1-amino-2-naphthol-4-sulfonic acid (C₁₀H₉NO₄S-1.6 g/L) and sodium bisulphite (NaHSO₃-100 g/L) were added immediately. The absorbance was measured at 650 nm after 30 min using a UV/visible spectrophotometer. The concentration of total Si was estimated from the standard curve prepared from Si standard solutions (Si1000, Kanto Chemical Co. Inc., Japan).

Estimation of antinutrients

Determination of trypsin inhibitor

Trypsin inhibitor activity is measured indirectly by inhibiting the activity of trypsin as per the method proposed by Kakade et al.³⁶. A crude trypsin inhibitor preparation was obtained by extraction of cooked (boiled/stir fried) lentil seeds (0.5 g) with 25 mL distilled water for 2 h in a refrigerator with subsequent centrifugation at 16128 g for 20 min at 4-6 °C and the supernatant was collected. Trypsin inhibitory activity was determined by measuring the residual hydrolytic activity of trypsin towards the substrates BAPNA (N-benzoyl-L-arginine-p-nitroanilide), at pH 8.2 after pre-incubation for 45 min with inhibitor. One inhibitor unit was defined as the amount of trypsin inhibitor in µg protein required to inhibit 50 % of the corresponding enzyme activity³⁷. Trypsin inhibitor activity (units/mg protein), was calculated from the absorbance read at 410 nm in a UV-visible spectrophotometer against the blank. Protein concentration was determined by Bradford dye-binding assay³⁸, using bovine serum albumin (BSA) as standard.

Determination of phytic acid content

Phytic acid content was determined according to the method proposed by Wheeler and Ferrel³⁹. Powdered samples from the cooked (boiled/stir-fried) seeds (1 g) were extracted with 50 mL of 3 % trichloro acetic acid (TCA) using a stirrer for 30 min. Following centrifugation at 1008 g for 10 min, the supernatants were removed and diluted with deionized water. The aliquot (1 mL) was mixed with 4 mL ferric chloride (FeCl₃) solution and heated in a boiling water bath (100 °C) for 45 min. After cooling, the mixture was again subjected to centrifugation at 1008 g for 10 min. The precipitate was collected and washed twice with 25 mL 3 % TCA. The resulting precipitate was dissolved in 2 mL deionised water (DI water) and mixed with 3 mL 1.5 N sodium hydroxide (NaOH). The final volume was made up to 50 mL with water and heated in a boiling water bath for 30 min. Hot reaction mixture was filtered through Whatman No. 2 filter paper (GE Healthcare, New South Wales, Australia) and the precipitate was washed with 60 mL hot water. The precipitate from the paper was dissolved with 40 mL hot and concentrated 3.2 N nitric acid (HNO₃) into a 100 mL volumetric flask. Subsequently, the paper was washed several times using water (50 mL) and the residues were collected in the same flask. After cooling the contents in the flask to room temperature, 5 mL aliquot was transferred to a 100 mL volumetric flask and diluted to 75 mL with water. After adding 20 mL of 1.5 N potassium thiocyanate (KSCN), the absorbance was recorded immediately at 480 nm using a UV-visible spectrophotometer. The concentration of phytic acid was estimated from the standard curve prepared using ferric nitrate [Fe(NO₃)₃] as the standard.

Determination of tannin content

Tannin content was estimated using Folin-Denis reagent following the method proposed by Schanderi⁴⁰. The cooked (boiled/stir-fried) seed samples (0.5 g) were mixed with 75 mL distilled water in a 250 mL conical flask and boiled for 30 min at 100 °C. The cooled mixture was subjected to centrifugation at 448 g for 20 min and the collected supernatant was made up to 100 mL in volumetric flask. An aliquot (0.1 mL) was transferred to 100 mL in volumetric flask containing 75 mL distilled water and to this mixture, 5 mL of Folin-Denis reagent and 10 mL of sodium carbonate solution (Na₂CO₃) was added followed by dilution to 100 mL with distilled water. The resulting reaction mixture was mixed well, and the absorbance was recorded at 700 nm after 30 min using a UV-visible spectrophotometer. The concentration of tannin was estimated from the standard curve prepared using tannic acid as the standard.

Antioxidants (Ascorbate, Phenol, Flavonoids, Total antioxidant activity)

Estimation of ascorbate (ASC) content

Ascorbate (ASC) was estimated according to the method proposed by Mukherji and Chaudhari⁴¹. Cooked (boiled/stir-fried) seeds (one g) were homogenized in 6 % TCA, and the homogenate was centrifuged at 2800 g for 15 min to collect the supernatant. Two mL of 2 % Dinitrophenylhydrazine (DNPH) was added to 4 mL of the supernatant, followed by the addition of one drop of 10 % thiourea. The resulting mixture was boiled for 15 min in a boiling water bath (100 °C) and then cooled to room temperature, followed by the addition of 5 mL of chilled H₂SO₄ at 0 °C. The absorbance was read at 530 nm using a UV-visible spectrophotometer. The concentration of total ASC was estimated from the standard curve prepared using known concentration of ASC.

Estimation of phenol content

Total phenol content in cooked (boiled/stir-fried) seed samples was estimated by the method of Mayr et al.⁴². Samples (1 g) were refluxed in 80 % methanol for 10 min. After cooling, the samples were homogenized using a mortar and pestle. The homogenate was then filtered and centrifuged at 11200 g for 10 min. An aliquot of the supernatant (0.1 mL) was pipetted out and made up to 3 mL using 80 % methanol. Subsequently, Folin-Ciocalteu reagent (0.5 mL) was added and kept for 3 min. 2 mL 20 % Na₂CO₃ was added to the mixture and kept in a boiling water bath (100 °C) for 1 min. The absorbance was recorded at 650 nm against the blank using a UV-visible spectrophotometer. The concentration of total phenols was estimated from the standard curve prepared using pyrocatechol.

Determination of flavonoid content

Flavonoid content of cooked (boiled/stir-fried) seed samples was estimated using the aluminium chloride colorimetric method described by Zhishen et al.⁴³. Seed extracts (0.5 mL of 1:10 g/mL methanol) were transferred to a test tube and mixed with 1.5 mL of methanol, 0.1 mL of 10 % aluminium chloride (AlCl₃), 0.1 mL of 1 M potassium acetate (C₂H₃O₂K) and 2.8 mL of distilled water. After keeping the mixture for 30 min at room temperature, the absorbance was measured at 415 nm using a UV/visible spectrophotometer. The total flavonoid content (mg catechin (CAE)/g DW) was calculated from the standard curve prepared using catechin.

Determination of total antioxidant activity

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Estimation of total antioxidant activity from the cooked (boiled/stir-fried) seeds were performed following the ABTS (2,2- azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) method⁴⁴. The reaction mixture contained 2 mM ABTS, 15 mM H₂O₂ and 0.25 mM horseradish peroxidase in 50 mM phosphate buffer, pH 7.5. The reactions were monitored at 730 nm using a UV/visible spectrophotometer at 25 °C until a stable absorbance was obtained due to ABTS radical formation. Subsequently, different concentrations (0.1–0.8 mM) of ASC were added for a standard curve set-up. Adding of methanol extracts of powdered seeds in reaction mixture resulted in absorbance decreasing due to ABTS radical depletion. Absorbance alterations were read from standard curve and results were expressed as mg of ASC equivalents per g of fresh weight (mg eq ASC/ g FW).

Sensory evaluation and biometrics

A total of N = 51 participants (35 females and 16 males) ranging from 20 to 50 years old and recruited from the staff and students of The University of Melbourne, Australia participated in the sensory analysis (Ethics ID 1545786.2). A sensory session was performed in individual sensory booths, illuminated with uniform lighting using white LED lights and controlled temperature, 24–26 °C, in the sensory laboratory at The University of Melbourne. Each booth was equipped with an integrated camera system that consists of an infrared thermal camera FLIR AX8™ (FLIR systems, Wilsonville, OR, USA), and a Samsung Galaxy View 18" tablet (Samsung group, Seoul, South Korea) coupled with a newly developed Biosensory application (The University of Melbourne, Melbourne, Vic, Australia)²³. This system was used to show the questionnaire designed for this study, record videos and infrared thermal images

from tastings and capture the conscious responses from the participants. For each participant, 3 g of each sample were served in small plastic cups labelled with 3-digit random numbers. Participants were instructed to taste the cooked seed samples with a plastic tablespoon, read and respond to the questions related to the session. The Biosensory app advised to cleanse their palate with crackers and water in between different samples. Additionally, samples order was randomized to minimize any potential carryover effects due to tasting of several samples in one session and to avoid bias. The participants were asked to rate their liking and the intensity of the attributes of each sample (Table 4) using a 15-cm non-structured scale. The anchor points in the scale were 'dislike extremely ' on the left-hand side and 'like extremely ' on the right-hand side⁴⁵.

Biometric measurements (unconscious responses) were acquired from video data acquisition and infrared thermography to obtain a series of physiological (HR and skin temperature changes, respectively), emotional and behavioural responses from facial expression analysis (neutral, happy, sad, angry, surprised, scared, disgusted, and contempt, gaze directions and head orientations) from participants during the sample assessments. Specifically, peripheral skin temperature (IR) of the face was recorded using the infrared thermal camera. The infrared thermal images (IRTIs) were taken automatically every two seconds while the participant was tasting the samples to capture the immediate response and temperature changes. The captured IRTIs were processed using the FLIR Tools software (FLIR Systems, Wilsonville, OR. USA) to acquire the radiometric data files and the RGB images (Red Green Blue images and are also referred as a true colour images) separately. Subsequently, the data files (.csv/comma-separated values), together with the RGB images

(.jpg/joint photographic experts group), were automatically analysed in batch using an algorithm written in Matlab® R2019a (Mathworks Inc., Matick, MA. USA). This algorithm is based on the cascade object detector to enable the automatic identification of the eye section of each participant in every jpg image as a region of interest and to capture the maximum temperature of that region, while tasting each sample⁴⁶.

Heart rate of the participants in beats per minute (BPM) was assessed from videos captured during the tasting session. The videos were processed using a customised algorithm developed in Matlab® ver. R2019a (Mathworks Inc., Matick, MA, USA) based on luminosity changes of the face in the green colour component using the principle of photoplethysmography and machine learning modelling to obtain HR in BPM²⁴. Facial expressions and head movements from the videos were assessed using the FaceReader™ 7.1 software (Noldus Information Technology, Wageningen, The Netherlands), which used face-detection algorithms to detect movements from distinct facial areas, which were in turn linked to a database combined in the program to relate them with distinct expressions. The intensity of eight different emotions (neutral, happy, sad, angry, surprised, scared, disgusted, and contempt), two emotional dimensions related to positive and negative responses of the participants for the samples (valence and arousal), and the head orientation in x-, y- and z- axes were collected. Furthermore, parameters such as gaze direction (forward/left/right), mouth (open/closed), left eye and right eye (open/closed), left eyebrow and right eyebrow (lowered/raised/neutral), were also obtained to assess the consumers acceptability of samples.

Experimental design and statistical data analysis

A completely randomized design with a factorial treatment arrangement ($2 \times 2 \times 2$) was used with five replicates for each treatment. Two lentil genotypes (ILL 6002 and ILL 7537) were used in this study. The treatments involved drought stress with Si and non-stressed without Si, and the cooking methods employed in this study were boiling and stir-frying. The results are expressed as mean and standard deviation (SD) for each treatment. Furthermore, all nutritional (five replicates) and sensory data in this study were assessed for significant differences using analysis of variance (ANOVA), followed by a Tukey pairwise *post-hoc* comparison test ($P \leq 0.05$) using Minitab® v17 (Minitab Inc., Pennsylvania, USA).

Multivariate data analysis

A multivariate data analysis based on principal components analysis (PCA), cluster analysis and correlation matrix were performed on the data obtained from nutritional, antinutritional, antioxidant and sensory evaluation (conscious and unconscious responses) by using customized codes written in Matlab® R2019b. The PCA was used to find relationships between all the studied parameters and the cluster analysis was used to classify the samples. The correlation matrix was developed to assess the statistically significant correlations ($P \leq 0.05$) between the nutritional parameters of the samples and the unconscious (biometrics)/conscious responses of the participants. The factor loadings of PCA are given as supplementary material (Table 1).

RESULTS AND DISCUSSION

Nutritional, antinutritional and antioxidant analysis of cooked lentil seeds

Proximate composition (protein, carbohydrates, total dietary fibre, ash, moisture, fat) and Si analysis

Proximate compositions of cooked (boiled/stir-fried) seed samples of two lentil genotypes under different treatments are presented in Table 2. Significant differences ($P \leq 0.05$) were observed for total crude protein content in seeds under different treatments and cooking methods; however, genotypes did not reflect significant differences ($P \geq 0.05$). Seeds from Si-treated drought-stressed plants had significantly higher amount of protein (1.6 -fold in drought tolerant genotype-G1 and 1.9 - fold in drought sensitive genotype - G2) as compared to seeds obtained from control ($P \leq 0.05$). Increase in protein with Si supply could be related to the activation of enzymes associated with storage protein/amino acids (located mainly in the cotyledons) synthesis in seeds. There is evidence for Si mediated upregulated accumulation of proteins as well as for Si modulated expression of vital proteins involved in several metabolic pathways in plants under abiotic stresses⁴⁷. The higher protein in stir-fried seeds compared to boiled seeds, irrespective of the stress treatments, could be due to the less time (5 min) taken for stir-frying when compared to the time taken for boiling (15 min), which is not enough for breaking the peptide bonds to cause protein denaturation⁴⁸.

The cooked seeds from Si supplied drought-stressed plants (DSi) showed 40 % increase in carbohydrate content in comparison to their control plants for both the genotypes. An increase of 1.3-fold and 1.4-fold was noticed in boiled and stir-fried seeds of drought tolerant genotypes, respectively. Similarly, 1.2 - fold and 1.3 - fold increase was observed in boiled seeds and stir-fried seeds of drought sensitive genotypes, respectively. However, seed carbohydrate content of the two genotypes remained almost same (200 - 210 mg/Kg) for both

the cooking methods. The increased carbohydrate content in cooked seeds from Si-treated drought-stressed plants can be attributed to the increased activity of amylase enzyme in seeds of drought-stressed plants with Si supplementation⁵. Additionally, Si might have helped in formation of links between lignin and carbohydrates, together with phenolic acids to produce organo-silicon compounds such as silicon-galactose complex to protect plants under stress⁴⁹.

A similar increased trend was found in seed fibre levels of Si supplied drought-stressed plants as compared to control plants. (G1 plants - 1.3-fold and G2 plants - 1.2 - fold) with non-significant differences between cooking methods. The increase in total dietary fibre in the studied seed samples could be due to (i) protein–fibre complex formation after likely chemical modification induced by Si treatment or (ii) Si might have helped in restoring the major total dietary fibre components in seeds such as cellulose, hemicellulose, pectin and lignin under stressed environment⁵⁰.

The fat content of cooked seeds (6 - 12 g/Kg) remained the same for all the treatments, cooking methods and the genotypes. However, seed ash content differed significantly with Si treatment ($P \leq 0.05$). In the present study, Si treatment seemed to have no influence on the fat content of cooked seeds from drought-stressed plants, even though Si is known to play a key role in the saturation of fatty acids in plants under stressed environments⁵¹.

As expected, boiling method retained more moisture in seeds compared to stir-frying. The water restoration in drought-stressed lentil plants with Si deposition in leaf cells is evident from the previous study³¹. However, the non-significant difference in seed moisture content of seeds from Si supplied drought-stressed plants compared to control highlights the need to explore apoplast environment of seed tissues to unravel the role of Si in regulating seed

moisture. Previous reports also confirmed the non-significant differences for moisture content of wheat and oat seeds obtained from Si-treated plants⁵².

Lentil seeds contained Si in the range of 2.2 - 2.5 mg/100 g dry weight and no significant changes in Si content were noticed with boiling or stir-frying of seeds. Si content contributes to more than 50 % of ash in seeds⁵³ thus, the increased ash content in seeds from Si-treated plants, compared to control was expected. High levels of Si found in Si-treated seed samples might be explained by the deposition/accumulation of orthosilicic acid (plant available silicon) in the epidermal cells of seeds.

Si is considered necessary for various vital functions related to blood vessels, brain and bones in animals and humans. Inadequate Si intake can lead to cardiovascular diseases, abnormal skeletal development, and other diseases related to brain in animals and humans⁵⁴. It has been reported that plants with relatively high levels of Si in other parts, such as stem and leaf, can also have high Si levels in their seed⁵². Thus, high Si content of seeds in this study can be explained by high Si content in the leaves of the same genotypes³¹. The daily recommended dietary intake of Si in humans is about 20-50 mg/day⁵⁵. Thus, including cooked lentil seeds in food will help in supplementing human diets with required Si contents.

Analysis of antinutrients and antioxidants in lentil seeds

The biological utilization and digestibility of legumes is affected by the presence of antinutritional factors such as trypsin inhibitors, phytates and tannins⁵⁶. The results of this study revealed that Si application to drought-stressed plants significantly reduced the content of antinutrients (Trypsin inhibitor, phytic acid and tannin) in seeds compared to control ($P \leq 0.05$). However, cooking methods did not show significant differences in seed anti-nutritional

levels (Fig. 1a). Silicon might have played a role in dissociating the phytate-protein/mineral complexes and tannin-protein complexes along with some inhibitory action on chelating properties of trypsin inhibitors⁵⁷. In depth investigation on the role of Si involved in elimination of antinutrients is important for the development of seeds with high nutritional quality under drought stress.

The present study showed that levels of antioxidants analysed were significantly ($P \leq 0.05$) higher in the cooked seeds obtained from Si supplied drought-stressed plants compared to seeds from control (Fig. 1b). Boiled seeds from Si supplied drought-stressed plants showed 31 - 56.2 % more ASC content than control plants, whereas stir fried seeds had 8.6 -12.5 % increase compared to control. Total phenol content of cooked seeds from Si supplied drought-stressed plants varied from 30 – 33 % in drought tolerant (G1) and 22 – 25 % in drought sensitive (G2) plants respectively, compared to non-stressed controls. Even though seeds from both genotypes under control with different cooking methods showed almost same flavonoid content, seeds from Si supplied drought-stressed plants showed significant increase compared to control. Boiled seeds from Si supplied drought-stressed G1 plants (G1DSi - B) showed 3.3 - fold increase in total antioxidant activity and the stir-fried seeds (G1Si - SF) showed 2.6 - fold increase compared to respective controls. Similarly, boiled and stir-fried seeds from Si supplied drought-stressed G2 plants (G2DSi - B and G2DSi - SF) displayed 3.6 - fold and 3 - fold increase in total antioxidants activity, respectively, relative to controls.

The results of this study showed that total antioxidant activity along with ASC, phenol and flavonoid content in seeds from Si supplied drought-stressed plants are enhanced as a function of Si supplementation, which supports the previous findings about interferences

between Si treatment and antioxidant metabolism in lentil under similar environment^{5,31}. Si supply under stress in this study resulted in increased accumulation of flavonoids and triggered total antioxidant activity in seeds. Additionally, as the antioxidant compounds may undergo physical or chemical changes during cooking, both boiling and stir-frying had the same impact on seed antioxidant levels.

Sensory evaluation of seeds using conscious and unconscious responses

The results of sensory attributes from consumers (intensity of aroma, texture, softness, flavour and overall liking) for G1 and G2 seed samples showed significant differences for various treatments and cooking methods. However, non-significant differences were found among genotypes (Table 3). Other sensory attributes (appearance, colour, discolouration, peppery smell, off odour, hardness, juiciness, beany taste, aftertaste and unusual flavour) of the samples provided in the questionnaire displayed non-significant differences ($P \geq 0.05$) for cooking methods as well as for genotypes. Moreover, seeds from Si supplied drought-stressed plants were the highest liked in terms of aroma, flavour, softness and texture. For overall liking, the stir-fried seeds from Si supplied drought-stressed plants were the most preferred, followed by boiled seeds from Si supplied drought-stressed plants [G1DSi – SF (9.78 ± 0.27) and G2DSi – SF (9.91 ± 0.29)] [G1DSi – B (8.10 ± 0.23) and G2DSi – B (8.14 ± 0.12)]. The stir-fried seeds from control plants were the second least preferred and boiled seeds from control plants, the least preferred [(G1C - SF (7.80 ± 0.07) and G2C – SF(7.73 ± 0.12))] [(G1C - B (5.03 ± 0.27) and G2C – B (5.16 ± 0.29))].

The exact mechanism of Si in enhancing aroma of cooked seeds from Si-treated plants is uncertain; however, it could be due to electrostatic interaction, such as formation of hydrogen

bonds between Si and the aroma molecules⁵⁸. Silicon might have also altered the composition of chemical compounds by forming complexes⁴⁹ resulting in desired smell, texture and taste of seeds and thus, the consumers liked the flavour of seeds from Si supplied drought-stressed plants more compared to control. It is also expected that the cooking methods will change the texture, aroma and flavour of the seeds. Stir-frying method changed the texture of the seeds to be crispy with a distinct aroma compared to boiling, which might have accounted for the high rating of stir-fried samples over boiled ones by the consumers. While the mechanism of these sensory effects of Si, including the present results, remains to be uncovered, it is assumed that the promotive effects of Si are due to its ability to conjugate with organic compounds.

The results of biometric indices obtained from (Table 4) the FaceReader™ showed that there were significant differences ($P \leq 0.05$) in emotional parameters (happy, angry, surprised, scared and disgusted) along with the facial features (valence, arousal, head orientation and gaze direction) for treatments and cooking methods, whereas non-significant differences found across the genotypes. Heart rate (HR) and temperature (Table 4) had significant differences ($P \leq 0.05$) for treatments, cooking methods and genotypes. Boiled and stir-fried seeds from G2 plant under control elicited significantly higher HR compared to seeds from Si supplied drought - stressed G2 plants (G2DSi). A similar trend was noticed for G1 plant with higher values compared to G2 plants. For skin temperature, boiled and stir-fried seeds from Si supplied drought-stressed plants showed significantly lesser values compared to their controls, irrespective of the genotypes.

The sensory acceptability results revealed that the participants accepted and liked seeds from Si supplied drought-stressed plants. That was re-confirmed from their unconscious responses (emotions and facial expressions). The stir-fried seeds from Si supplied drought-stressed plants showed higher intensities for happy, surprised, valence, arousal, left eye, right eye, left eyebrow and right eyebrow compared to control. On the other hand, boiled and stir-fried seeds from control plants had highest intensities for angry, scared and disgusted. Emotions play an important role in food choice and liking. Food liking is mainly related to positive emotions such as happiness and surprised, and responses to disliked foods to negative emotions such as anger and scared⁴⁹. Moreover, these results also validate findings obtained from conscious responses.

Emotions influence nearly every type of cognitive activity in subtle yet crucial ways and are managed by hear-brain neurodynamics⁶⁰. Emotional state of humans can also be affected by changes in HR and facial temperature. Changes in HR (elevated /lowered) can affect emotions of individuals in different ways, as stress can increase blood pressure (BP), systolic pressure (SP) and diastolic pressure (DP). There are different views on HR towards emotional experiences. It has been shown that increased the HR was positively correlated with the emotions like anger, fear, and sadness more than happiness and surprise did, while increased HR negatively correlated with disgust^{61,62}. Granero⁶³ concluded that that HR decreases with fear, sadness, and happiness. Research conducted by de Wijk et al.⁶⁴ concluded that liking scores were positively correlated with increases in HR. In this study, anger showed positive correlation with HR and was negatively correlated with positive emotions like happy and surprised (Fig. 2a, 2b). In addition, overall liking of the samples was also was negatively

correlated with HR. Thus, the lower anger intensities and HR values found for the highly preferred samples clearly shows that liking is linked to a decrease in HR. However, this result contradicts the findings of a recent study by Gonzalez et al.²⁵, where they found a positive correlation between HR and perceived quality in beer. This contradiction may be due to the effects caused by different stimuli.

In this study, the body temperature of the consumers was positively correlated to the emotions such as scared, angry and disgusted (Fig. 2a). The lower skin temperature readings from consumers for cooked seeds from Si supplied drought-stressed plants could be related with higher liking. A similar trend was observed by above-mentioned authors while assessing the sensory acceptability of beer samples²⁶. However, our results do not coincide with the findings of De Wijk et al.⁵⁴ who showed that the liked samples induced higher temperatures than disliked samples. This shows that different stimuli or types of food may elicit different trends in the physiological reactions. Several other studies also have established that temperature has a strong effect on some individual emotions in different ways^{16,17}. Different studies and discussions on how temperature affects emotions of humans show contradicting results. In one previous study, increased temperature was found to be associated with happiness and sadness, while decreased temperature was related with fear, surprise, and disgust⁵¹. Specifically, some research has observed that decreased peripheral temperature tend to be associated with fear-related response and increased vascular resistance^{61,65,66,67}. It has also been reported that temperature is increased to emotions such as anger and fear^{61,62}. Thus, the results from the current study have demonstrated that a specific ANS pattern may be associated with

each basic emotion and this emotional perception might strongly influence cognitive abilities, favouring decision making.

Correlations between nutritional potential and sensory attributes of seeds using multivariate data analysis

Results from the multivariate data analysis showing nutrients, antinutrients and antioxidants along with sensory and biometrics descriptors of seed samples are displayed in the PCA (Fig. 2a). In the PCA biplot, x-axis represents principal component one (PC1) and y-axis represents principal component two (PC2). PC1 and PC2 explained 68.06 % and 12.39 % of data variability respectively, with a total of 80.45 % of data variability in PCA biplot (Fig. 2a). Seed samples are separated according to the treatments and cooking methods used in this study. From the PCA results, all the studied samples were ordered according to their overall liking and preferences by the consumers, based on all the studied parameters (nutritional, antinutritional, conscious and unconscious responses) as given in the PCA biplot (Fig. 2a) and correlation diagram (Fig. 2b). As shown in PCA (Fig. 2a), stir-fried seeds from Si supplied drought-stressed plants (G1DSi - SF and G2DSi - SF) were the highest in liking of protein, overall liking, softness, flavour and texture, followed by boiled seeds from the same treatment (G1DSi - B and G2DSi - B) being liked for nutrients like Si, carbohydrate, dietary fibre and moisture. Whereas, stir-fried seeds from control plants were ranked third in liking with antinutrients. Boiled seeds from control plants were the least liked samples with total antioxidants along with increased temperature and HR.

As per the factor loadings (Table 1), PC1 was mainly represented by the nutrient protein, biometric responses such as the movements of left and right eyes, right eyebrow along

with the conscious responses such as the intensity of softness, aroma, texture and overall liking in the positive side. The negative side of the PC1 axis was represented by y-head orientation, left eyebrow movement, temperature, gaze direction and flavonoids. On the other hand, PC2 was primarily represented by the nutrients like fat, moisture and carbohydrates on the positive side and by antinutrients such as phytic acid and tannin along with total antioxidants and Si on the negative side of the axis.

Cluster analysis separated seed samples into three clusters according to their type of cooking methods and treatments (Fig. 3). The correlation matrix (Fig. 2b), showed significant and positive correlations ($p \leq 0.05$) between nutrients (carbohydrate, protein, dietary fibre) and the sensory attribute intensity scores (overall liking, texture, softness, aroma). Interestingly, the conscious responses showed significant and positive correlations with unconscious responses (happy, surprised, Y and Z head orientation, left and right eyes, left eyebrow and gaze direction). Furthermore, nutrients displayed significant negative correlations to antioxidants and antinutrients.

The PCA biplot, cluster diagram and the correlation matrix (Figs 2-3) in the present study showed significant differences in the nutrients, antinutrients and antioxidants profile of seeds and the sensory perception and preference of consumers towards different seed samples. PCA results showed that the texture, softness, aroma and flavour along with protein accounted for the highest liking of stir-fried seeds from Si supplied drought-stressed plants (G1DSi - SF and G2DSi - SF). Additionally, Si content, carbohydrate, dietary fibre and moisture also played a role in ranking the samples, as revealed from the second ranking assigned to boiled seeds from Si supplied drought-stressed plants (G1DSi - B and G2DSi - B). The lowered liking of

boiled and stir-fried seeds from control plants could be explained by the bitter or astringent taste due to antioxidants and antinutrients.

The separation of samples in cluster diagram (Fig. 3) also showed that the studied samples were significantly different for the various variables studied. The separate clustering of boiled and stir-fried seeds based on the studied parameters reveal that various cooking methods can affect the nutritional composition and sensory quality of seeds. Furthermore, the separate clustering of seeds from Si supplied drought-stressed plants from control validate the modulations in nutrients, antinutrients, antioxidants and the sensory acceptability of seeds in response to Si supplementation as seen in this study.

The significant positive correlations found for overall liking of the samples to nutrients (protein, carbohydrate and dietary fibre), and the negative correlations to antinutrients (tannin, phytic acid, trypsin inhibitor) and total antioxidants show consumers' preference to seeds from Si supplied drought-stressed plants (Fig. 2b). Thus, these results validate that the acceptability of cooked seeds from Si-treated plants is based on the enhanced nutritional quality due to Si supplementation. Moreover, the unconscious responses such as happy, surprised, eye movements and eyebrows, Z-head orientation and gaze direction showed significant positive correlations with the conscious responses (overall liking, texture, softness, aroma and flavour) (Fig. 2b). These results showed that strength and valence of unconscious responses can correlate consumers perception and hence the liking of food. Additionally, the above findings validated the other objective of this study that sensory acceptability or the higher preference for seeds from Si supplied drought-stressed plants were not biased on sensory attribute

intensities and acceptance scores. These results also reveal that the sensory acceptability of a product can be assessed based on the unconscious responses from the consumers.

CONCLUSION

Silicon supplementation to drought-stressed lentil genotypes significantly improved the nutritional and antioxidant potential of seeds along with notable reductions in antinutritional factors. Higher acceptance scores were found for seeds from Si-treated drought-stressed plants, confirmed via descriptors such as higher flavour, softness, aroma and good texture along with stir-frying as a better cooking option compared to boiling. The emotional and physiological responses captured using non-invasive biometrics also lead to similar findings and supported the results from conscious responses. Furthermore, the overall liking based on the conscious and unconscious responses was correlated with seed nutrients. Thus, this study offers an innovative approach in sensory analysis coupled with biometrics techniques to accurately assess consumer's preference and liking towards tested samples. In the future, the results of the current study would help in making a predictive model for sensory traits and nutritional components in lentil seeds using machine learning modelling techniques. Most importantly, these results highlight that Si is not only involved in mitigation of drought stress but also in improving the nutritional and sensory acceptability of seeds under drought stress. However, the underlying mechanism(s) of improving seed nutritional quality with Si supplementation in drought-stressed environment needs further elucidation, particularly at the molecular level. The current results support the idea that while the potential use of Si application provides drought stress alleviation for the lentil crop, growers can be assured of enhanced nutrient potential and

sensory quality of the harvested seeds. The methodology and results of this study can further be employed to understand the interactions of other major crops/stresses/elemental supplementations on nutritional/sensory qualities of seeds for consumer acceptability.

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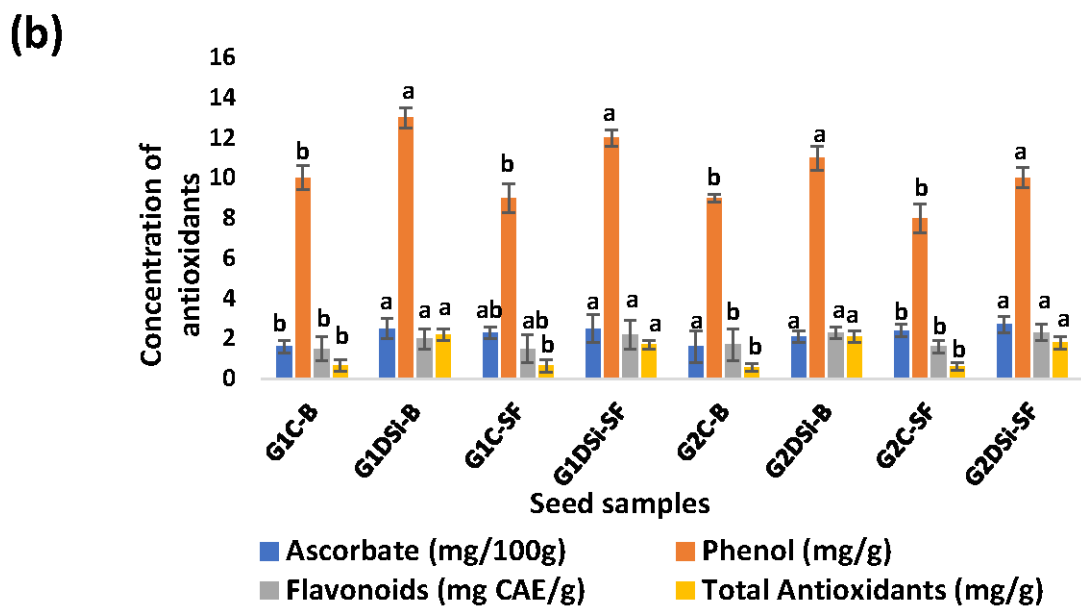
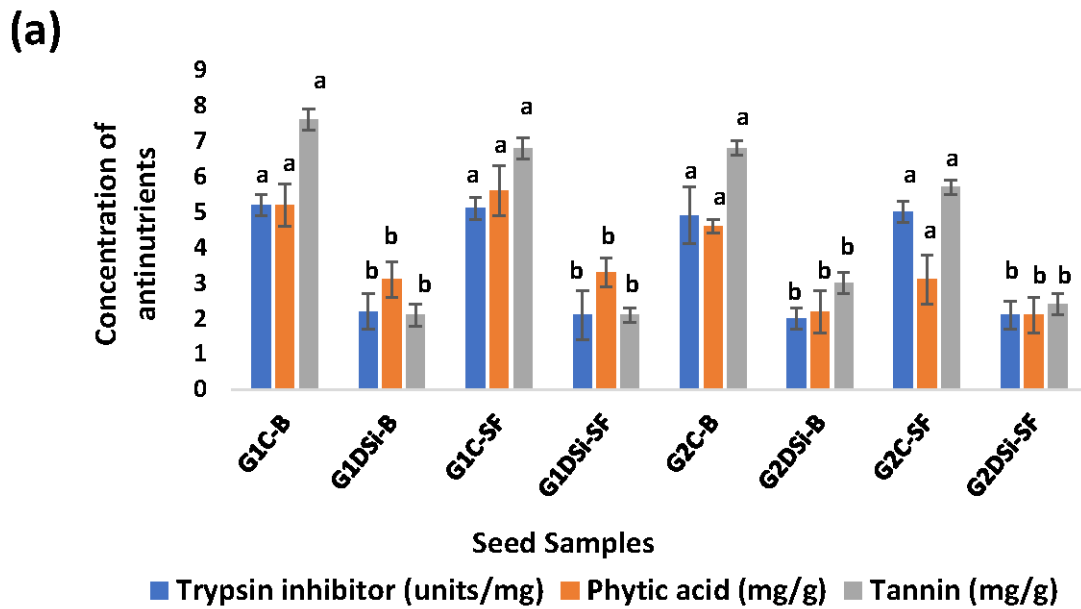
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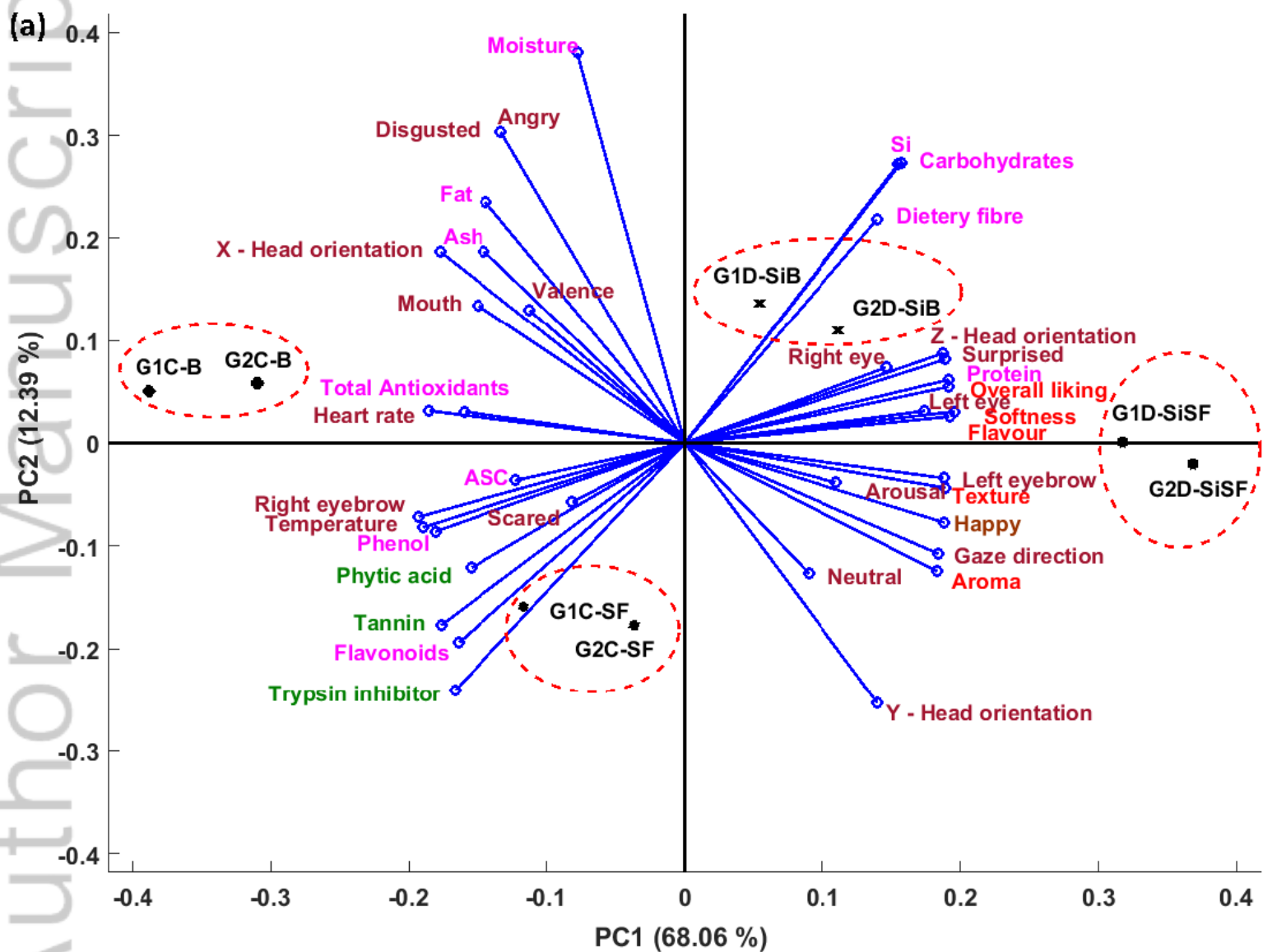
Figure legends

1. Figure 1 (a) Different antinutrients concentrations (trypsin inhibitor, phytic acid and tannin) (mg/g) in G1 and G2 seed samples (b) Concentration of ascorbate (mg/100g), phenol (mg/g), flavonoids (mg CAE/g) and total antioxidants (mg/g) in G1 and G2 seed samples. The results are expressed as mean \pm SD (n = 5). Different letters in bars (with same colours) for a compound indicate significant differences ($P \leq 0.05$) between the samples. Abbreviations for seed samples used in this figure are (i) G1 Control Boiled (**G1C-B**), (ii) G1 Control Stir-fried (**G1C-SF**), (iii) G1 Drought +Si Boiled (**G1DSi-B**), (iv) G1 Drought +Si Stir-fried (**G1DSi-SF**), (v) G2 Control Boiled (**G2C-B**), (vi) G2 Control Stir-fried (**G2C-SF**), (vii) G2 Drought + Si boiled (**G2DSi-B**) and (viii) G2 Drought +Si Stir-fried (**G2DSi-SF**).

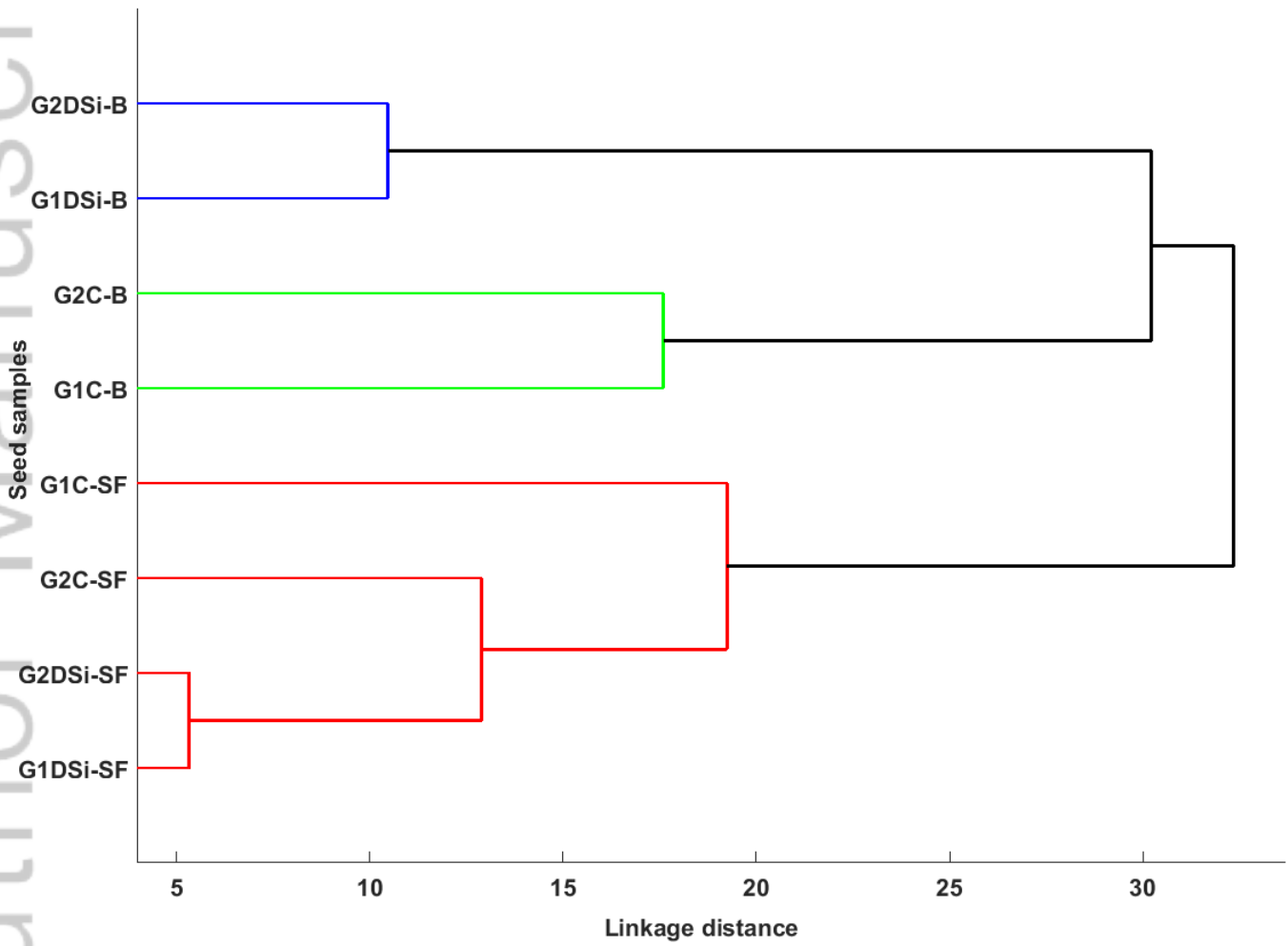
2. Figure 2. Results from the multivariate data analysis ($p \leq 0.05$) showing (a) principal components analysis (PCA) biplot for all the studied parameters including nutrients, antinutrients and antioxidants along with the sensory and biometric indices. The pink descriptors in vectors corresponds to the proximate composition, nutrients and antioxidants, green descriptors represent antinutrients, brown descriptors depict the unconscious responses and red descriptors represent the conscious responses and (b) correlation matrix with the nutrients, antinutrients, antioxidants, conscious and unconscious responses. Abbreviations for seed samples used in the PCA biplot are (i) G1 Control Boiled (G1C-B), (ii) G1 Control Stir-fried (G1C-SF), (iii) G1 Drought +Si boiled (G1DSi-B) , (iv) G1 Drought +Si Stir-fried (G1DSi-SF), (v) G2 Control Boiled (G2C-B), (vi) G2 Control Stir-fried (G2C-SF), (vii) G2 Drought + Si boiled (G2DSi-B) and (viii) G2 Drought +Si Stir-fried (G2DSi-SF).
3. Figure 3. Cluster diagram showing separation of samples according to the types of treatments. Abbreviations for seed samples with different treatments and cooking methods are given in Table 1.



JSFA_10759_Fig.1a and b.tif

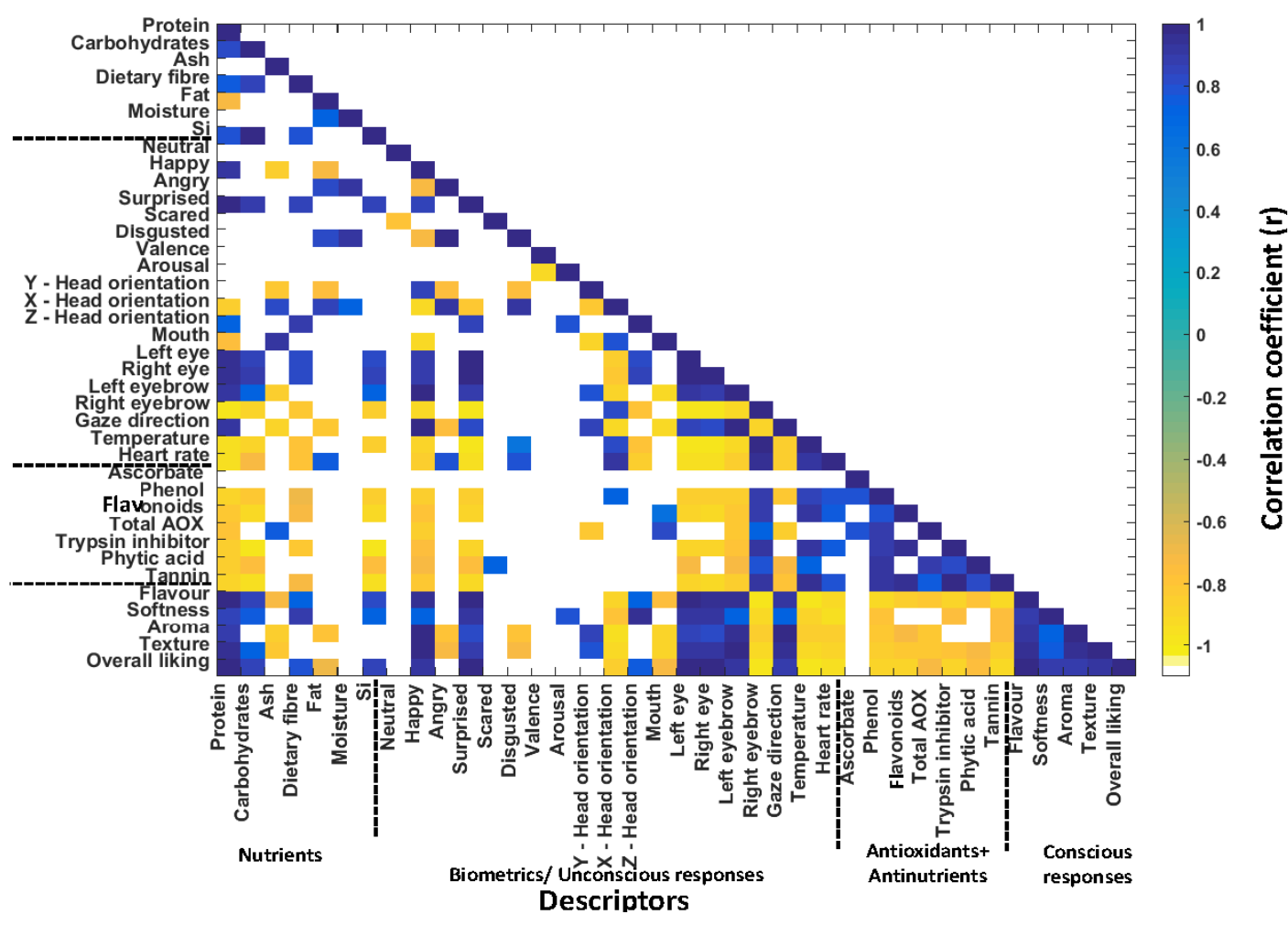


JSFA_10759_Fig.2a.tiff



JSFA_10759_Fig.3.tif

(b)



JSFA_10759_Fig. 2b.tiff

Table 1. Factor loadings of the two principal components for each parameter.

Parameters	Principal component	
	PC1 (68.06%)	PC2 (12.39%)
Nutrients		
Protein	0.19	0.02
Carbohydrates	0.15	0.27
Ash	-0.14	0.18
Dietary fibre	0.13	0.21
Fat	-0.07	0.38
Moisture	0.15	0.27
Si	0.08	-0.12
Biometric/unconscious responses		
Neutral	0.18	-0.07
Happy	-0.13	0.3
Angry	0.18	0.08
Surprised	-0.08	-0.05
Scared	-0.13	0.3 0
Disgusted	-0.11	0.12
Valence	0.1	-0.03
Arousal	0.14	-0.25
Y-Head orientation	-0.17	0.18

X - Head orientation	0.14	0.07
Z - Head orientation	-0.14	0.13
Mouth	0.19	0.05
Left eye	0.18	0.08
Right eye	0.18	-0.03
Left eyebrow	-0.19	-0.07
Right eyebrow	0.18	-0.1
Gaze direction	-0.18	-0.08
Temperature	-0.18	0.03
Heart rate	0.19	0.06
Antioxidants and antinutrients		
Ascorbate	-0.14	0.23
Phenol	-0.12	-0.03
Flavonoids	-0.18	-0.08
Total	-0.16	-0.19
antioxidants		
Trypsin inhibitor	-0.15	0.03
Phytic acid	-0.16	-0.24
Tannin	-0.15	-0.12
Conscious responses		
Flavour	-0.17	-0.17
Softness	0.19	0.03
Aroma	0.17	0.03

Texture	0.18	-0.12
Overall liking	0.18	-0.04

Table 2: Proximate composition analysis of lentil seeds from different treatments.

Seed samples	Total crude protein (g/100g)	Carbohydrates (g/100g)	Total dietary fibre (g/100g)	Ash (g/100g)	Moisture (%)	Fat (g/100g)	Silicon (mg/100g)
G1C-B	09.53±0.03 ^d	20.99±0.44 ^b	8.03±0.44 ^b	0.83±0.03 ^a	12.21±0.51 ^a	1.1±0.04 ^a	2.3±0.02 ^b
G1DSi-B	13.15±0.23 ^b	28.37±0.02 ^a	10.53±0.43 ^a	1.77±0.02 ^b	13.61±0.59 ^a	1.2±0.11 ^a	3.3±0.29 ^a
G1C-SF	11.02±0.34 ^c	19.69±0.12 ^b	08.87±0.78 ^b	0.75±0.04 ^a	04.98±0.98 ^b	0.9±0.01 ^a	2.2±0.49 ^b
G1DSi-SF	15.36±0.66 ^a	28.60±0.21 ^a	10.83±0.48 ^a	1.73±0.06 ^b	04.37±0.29 ^b	0.7±0.01 ^a	3.5±0.67 ^a
G2C-B	08.73±0.54 ^d	21.37±0.55 ^b	08.98±0.82 ^b	0.81±0.06 ^a	14.29±0.91 ^a	1.2±0.04 ^a	2.5±0.23 ^b
G2DSi-B	14.35±0.43 ^b	27.69±0.51 ^a	11.25±0.54 ^a	1.76±0.03 ^b	13.20±0.48 ^a	0.8±0.01 ^a	3.4±0.65 ^a
G2C-SF	12.57±0.43 ^c	20.99±0.59 ^b	08.85±0.92 ^b	0.75±0.06 ^a	3.96±0.72 ^b	0.7±0.04 ^a	2.3±0.32 ^b
G2DSi-SF	17.25±0.45 ^a	28.36±0.03 ^a	11.51±0.65 ^a	1.77±0.07 ^b	3.69±0.51 ^b	0.6±0.04 ^a	3.2±0.13 ^a

Data are expressed as means ± standard deviations (n = 5) and different letters indicate statistically significant differences between samples within the same genotypes (P ≤ 0.05).

NS = means are not significantly (P ≥ 0.05) different.

Abbreviations for seed samples used in this table are (i) G1 Control Boiled (**G1C-B**), (ii) G1 Control Stir-fried (**G1C-SF**), (iii) G1 Drought +Si Boiled (**G1DSi-B**), (iv) G1 Drought +Si Stir-fried (**G1DSi-SF**), (v) G2 Control Boiled (**G2C-B**), (vi) G2 Control Stir-fried (**G2C-SF**), (vii) G2 Drought + Si boiled (**G2DSi-B**) and (viii) G2 Drought +Si Stir-fried (**G2DSi-SF**)

Table 3. Mean and standard deviation* of the intensity scores of sensory attributes from the participants for different samples.

Sensory attributes	G1C-B	G1DSi-B	G1C-SF	G1DSi-SF	G2C-B	G2DSi-B	G2C-SF	G2DSi-SF
Appearance^{NS}	9.68±0.21 ^a	9.13±0.35 ^a	9.54±0.32 ^a	9.36±0.60 ^a	9.40±0.29 ^a	9.41±0.35 ^a	10.13±0.36 ^a	10.01±0.25 ^a
Colour^{NS}	6.27±0.03 ^a	7.12±0.12 ^a	7.19±0.01 ^a	7.88±0.23 ^a	7.81±0.32 ^a	6.99±0.32 ^a	7.06±0.34 ^a	6.45±0.26 ^a
Discoloration^{NS}	7.34±0.23 ^a	6.99±0.33 ^a	7.12±0.25 ^a	6.84±0.14 ^a	6.61±0.35 ^a	7.15±0.03 ^a	7.21±0.32 ^a	6.79 ±0.11 ^a
Aroma	6.33±0.23 ^b	8.33 ±0.31 ^a	6.72±0.23 ^b	9.33±0.65 ^a	6.96±0.31 ^b	8.83 ±0.33 ^a	6.55 ±0.44 ^b	9.76 ±0.24 ^a
Peppery smell^{NS}	3.55±0.32 ^a	3.54±0.23 ^a	4.10±0.38 ^a	4.33±0.37 ^a	2.8±0.11 ^{ab}	4.43±0.26 ^a	4.20±0.24 ^a	4.26±0.32 ^a
Off odour^{NS}	2.70±0.36 ^a	2.89±0.65 ^a	2.72±0.37 ^a	2.59±0.36 ^a	2.80±0.36 ^a	2.97±0.59 ^a	2.76±0.28 ^a	2.06 ±0.33 ^a
Texture	5.71±0.32 ^b	8.92±0.35 ^a	5.33±0.33 ^b	9.54±0.38 ^a	5.82±0.33 ^b	8.50±0.37 ^a	5.01±0.32 ^b	9.83±0.22 ^a
Hardness^{NS}	6.86±0.66 ^a	6.16±0.57 ^a	7.21±0.28 ^a	7.07±0.35 ^a	7.10±0.57 ^a	6.99±0.35 ^a	6.89±0.54 ^a	6.95 ±0.36 ^a
Softness	6.42±0.32 ^b	9.23±0.32 ^a	6.43±0.32 ^b	9.56±0.65 ^a	6.63±0.32 ^b	9.58±0.33 ^a	6.81± 0.64 ^b	9.01±0.34 ^a
Juiciness^{NS}	4.47±0.02 ^a	4.59±0.28 ^a	4.41±0.23 ^a	4.88±0.29 ^a	4.20±0.22 ^a	4.47±0.21 ^a	4.80±0.38 ^a	4.85±0.26 ^a
Flavour	6.31 ±0.06 ^b	8.55 ±0.22 ^a	7.52 ±0.65 ^b	9.74 ±0.22 ^a	6.58±0.28 ^b	8.97 ±0.37 ^a	7.03 ±0.26 ^b	9.98±0.27 ^a
Beany taste^{NS}	9.01±0.32 ^a	8.80±0.27 ^a	9.15±0.66 ^a	9.18 ±0.31 ^a	9.20±0.28 ^a	8.93 ±0.26 ^a	9.09 ±0.23 ^a	9.43±0.23 ^a
Aftertaste^{NS}	5.56±0.37 ^a	5.32±0.31 ^a	5.71±0.25 ^a	5.94±0.28 ^a	5.12±0.27 ^a	5.08±0.28 ^a	5.08±0.26 ^a	4.70±0.33 ^a
Unusual flavour^{NS}	3.86±0.28 ^a	2.96±0.32 ^a	3.54±0.27 ^a	2.99±0.28 ^a	3.28±0.29 ^a	2.95±0.27 ^a	2.80±0.16 ^a	2.98±0.37 ^a
Overall liking	5.03±0.27 ^d	8.10±0.23 ^b	7.80 ±0.07 ^c	9.78±0.27 ^a	5.16±0.29 ^d	8.14±0.12 ^b	7.73±0.12 ^c	9.91±0.29 ^a

*Mean and standard deviation values of n=51 replicates (participants).

NS = Non-significantly different (P ≥ 0.05)

Different letters in a row indicate statistically significant differences between samples ($P \leq 0.05$). Abbreviations for seed samples used in this table are (i) G1 Control Boiled (**G1C-B**), (ii) G1 Control Stir-fried (**G1C-SF**), (iii) G1 Drought +Si Boiled (**G1DSi-B**), (iv) G1 Drought +Si Stir-fried (**G1DSi-SF**), (v) G2 Control Boiled (**G2C-B**), (vi) G2 Control Stir-fried (**G2C-SF**), (vii) G2 Drought + Si boiled (**G2DSi-B**) and (viii) G2 Drought +Si Stir-fried (**G2DSi-SF**).

Table 4. Mean and standard deviation values* of the biometric indices from the participants for different samples.

Biometric indices	G1C-B	G1DSi-B	G1C-SF	G1DSi-SF	G2C-B	G2DSi-B	G2C-SF	G2DSi-SF
Physiological parameters								
Heart rate (BPM)	85.10±0.23 ^b	80.30±0.54 ^d	82.32±0.61 ^c	70.95±0.33 ^e	88.23±0.24 ^a	79.90±0.11 ^d	81.51±0.27 ^{cd}	68.30±0.62 ^e
Temperature (°C)	37.30±0.06 ^a	33.31±0.32 ^b	36.2±0.35 ^a	34.2±0.67 ^{bc}	37.14±0.23 ^a	34.01±0.33 ^b	36.40±0.35 ^a	33.13±0.38 ^{bc}
Emotional parameters								
Neutral^{NS}	0.20±0.01 ^a	0.20±0.02 ^a	0.21±0.03 ^a	0.22±0.01 ^a	0.23±0.03 ^a	0.23 ±0.04 ^a	0.24±0.04 ^a	0.24±0.02 ^a
Sad^{NS}	0.15±0.01 ^a	0.16± 0.01 ^a	0.14±0.03 ^a	0.15±0.04 ^a	0.12±0.01 ^a	0.13 ±0.03 ^a	0.15±0.04 ^a	0.16±0.01 ^a
Happy	0.23±0.03 ^d	0.78±0.01 ^b	0.60 ±0.09 ^c	0.95 ±0.01 ^a	0.31±0.06 ^c	0.78 ±0.01 ^b	0.63±0.06 ^c	0.98± 0.06 ^a
Angry	0.08±0.001 ^a	0.02±0.002 ^b	0.08±0.003 ^a	0.01±0.001 ^b	0.09±0.01 ^a	0.02±0.003 ^b	0.08±0.005 ^a	0.01±0.007 ^b
Surprised	0.01±0.006 ^b	0.07±0.005 ^a	0.02±0.008 ^b	0.08±0.002 ^a	0.01±0.004 ^b	0.08 ±0.008 ^a	0.02±0.006 ^b	0.08±0.006 ^a
Scared^{NS}	0.04±0.001 ^a	0.01±0.003 ^a	0.05±0.008 ^a	0.01±0.005 ^a	0.05±0.007 ^a	0.02±0.006 ^a	0.04±0.002 ^a	0.01±0.003 ^a
Disgusted	0.09±0.001 ^a	0.02±0.009 ^b	0.08±0.002 ^a	0.02±0.005 ^b	0.09±0.003 ^a	0.01±0.002 ^b	0.09 ±0.005 ^a	0.02 ±0.001 ^b
Contempt^{NS}	0.01±0.001 ^a	0.02±0.002 ^a	0.02±0.003 ^a	0.02±0.001 ^a	0.02±0.005 ^a	0.03±0.002 ^a	0.04±0.002 ^a	0.06±0.001 ^a
Facial features								
Valence	-0.23±0.08 ^a	-0.53±0.05 ^b	-0.27±0.03 ^a	-0.51±0.07 ^b	-0.29±0.03 ^a	-0.59±0.06 ^b	-0.27±0.08 ^a	-0.50±0.03 ^b
Arousal	0.36±0.02 ^b	0.88±0.05 ^a	0.19±0.06 ^c	0.81±0.02 ^a	0.30±0.04 ^b	0.90±0.07 ^a	0.39±0.03 ^b	0.81±0.04 ^a
Mouth	0.09±0.001 ^a	0.01±0.005 ^c	0.06±0.003 ^b	0.02±0.006 ^c	0.08±0.003 ^a	0.02±0.003 ^c	0.05 ±0.008 ^b	0.01± 0.008 ^c
Left eye	0.48±0.01 ^c	0.76±0.03 ^b	0.45±0.02 ^c	0.92±0.01 ^a	0.48±0.01 ^c	0.77±0.02 ^b	0.43±0.03 ^c	0.93± 0.02 ^a
Right eye	0.27±0.01 ^d	0.45 ±0.02 ^b	0.35±0.01 ^c	0.56 ±0.02 ^a	0.24±0.05 ^d	0.42±0.03 ^b	0.31±0.08 ^c	0.55±0.04 ^a
Left eyebrow	-0.65±0.01 ^d	-0.23±0.05 ^b	-0.32±0.06 ^c	-0.13±0.03 ^a	-0.63±0.04 ^d	-0.22 ±0.07 ^b	-0.33±0.06 ^c	-0.13±0.05 ^a
Right eyebrow	- 0.14±0.05 ^a	-0.23±0.06 ^b	-0.12±0.07 ^{ca}	-0.34±0.05 ^c	-0.14±0.02 ^a	-0.25± 0.03 ^b	-0.13±0.05 ^a	-0.36±0.03 ^a
Gaze Direction	-0.31±0.02 ^c	0.85±0.02 ^b	0.88±0.01 ^b	1.33±0.01 ^a	-0.33±0.06 ^c	0.82 ±0.01 ^b	0.33±0.002 ^c	1.3±0.003 ^a
Head orientation								
Y-Head orientation	12.33 ±0.22 ^b	13.57±0.31 ^b	17.59±0.32 ^a	17.61±0.31 ^a	12.38±0.22 ^b	16.57±0.32 ^b	13.78± 0.23 ^b	17.69±0.20 ^a

X-Head orientation	-5.47±0.23 ^a	-8.81±0.21 ^b	-5.98±0.33 ^a	-9.23±0.32 ^{bc}	-5.46±0.32 ^a	-8.99± 0.35 ^c	-6.97±0.32 ^{bc}	-9.27±0.11 ^c
Z-Head orientation	-3.02±0.63 ^a	-6.01±0.52 ^b	-3.1±0.63 ^a	-6.43±0.32 ^b	-3.06±0.73 ^a	-6.04±0.02 ^b	-3.19±0.67 ^a	-6.45±0.01 ^b

*Mean and standard deviation values of n=51 replicates (participants).

NS = means are not significantly ($P \geq .05$) different.

Different letters indicate statistically significant differences between samples ($P \leq 0.05$). Abbreviations for seed samples used in this table are

(i) G1 Control Boiled (**G1C-B**), (ii) G1 Control Stir-fried (**G1C-SF**), (iii) G1 Drought +Si Boiled (**G1DSi-B**), (iv) G1 Drought +Si Stir-fried (**G1DSi-SF**), (v) G2 Control Boiled (**G2C-B**), (vi) G2 Control Stir-fried (**G2C-SF**), (vii) G2 Drought + Si boiled (**G2DSi-B**) and (viii) G2 Drought +Si Stir-fried (**G2DSi-SF**). Scales used for the facial expressions were as follows: emotions (neutral, sad, happy, angry, surprised, scared, disgusted, contempt) were 0 to 1, valence and arousal -1 to 1, mouth and eyes 0 to 1, eye brows and gaze directions -1 to 1 and head orientation -67.5 to 67.5.

