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Processing techniques alter resistant starch content, sugar profile and relative bioavailability of iron in groundnut (*Arachis hypogaea* L.) kernels

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ABSTRACT

The effect of processing techniques (soaking, roasting and microwave irradiation) on biochemical parameters of groundnut, especially resistant starch (RS) and non-resistant starch (NRS), along with phytic acid (PA), iron (Fe), PA/Fe molar ratio and sugars were studied. Changes in RS and NRS content after processing have not been reported yet. Roasting for 12 min at 160 °C caused an increase in RS (61.1 %) and NRS (67.2 %) content in the GAUG-10 genotype. Water-soaking of kernels for 8 h reduced the amount of PA and increased the amount of Fe and the molar ratio of PA/Fe. Processing techniques reduced sugar alcohols (inositol and mannitol) and stachyose content in groundnut kernels. Variation in nutritional and antinutritional traits of groundnut influenced by processing technique suggests a degree of genotypic tolerance.

1. Introduction

Groundnut (*Arachis hypogaea* L.), also known as earthnuts, monkey nut and peanut, is the third most important oilseed crop globally and has been used for human consumption for ages as it possesses balanced nutrition (Suchoszek-Lukaniuk et al., 2011). In addition, groundnut is a good source of dietary fiber and provides essential nutrients, including B group vitamins, vitamin E, minerals (Fe, zinc, potassium and magnesium), antioxidant minerals (selenium, manganese and copper), and other antioxidant polyphenolic compounds like flavonoids and resveratrol (Bishi et al., 2015). Groundnut seeds possess a low-glycemic index (14 on a 100 point scale) and have potential health benefits, including reducing the risk of diabetes, cardiovascular disease, and some cancers (Foster-Powell et al., 2002). Resistant starch is an unexplored metabolite in groundnut, which like dietary fibers, possesses multiple health benefits. RS is the undigested portion of total starch, which is later on fermented by natural microbial flora of the colon to produce short-chain fatty acids (Berry, 1986). In contrast, non-resistant starch (NRS) is the starch content digestible by body enzymes. Studies based on digestion kinetics have also confirmed the presence of a starch fraction, which sustains the complete digestion in the small intestine (Peterson et al., 2018). In India, 10–18 g per day of RS is recommended to reap its health benefits. Its daily intake ranges from 30 to 40 g per day in developing countries. The energy value of digestible starch is 15 kJ/g (4.2 kcal g^{-1}), but RS contributes only 8 kJ/g (2 kcal g^{-1}). RS has gained massive attention in health sciences as it is reported to improve colonic health, intestinal Fe and calcium absorption and prevent colonic cancer, gall stone formation and insulinemia (Leu et al., 2002). RS is also important from an industrial point of view, as many food-based industries use it to prepare moisture-free edible products to improve oral-tactile perception, taste, color, and texture. Therefore, RS has gained importance in the recent past with efforts to improve its content through genetic engineering and postharvest processing.

Standard food processing industries practice dry heat treatments to attain characteristic crunchy and crispy texture in the kernels, altering sugar profile, especially in terms of sucrose and raffinose family oligosaccharides (RFOs). High levels of sucrose, oleic acid and low levels of RFOs and oil content are considered desirable traits in peanuts for value-

Abbreviations: MW, Microwave; RS, Resistant starch; NRS, Non-resistant starch; PA, phytic acid; Fe, Iron.

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addition (Bishi et al., 2015). On the other hand, the anti-nutritional component PA either forms complexes with essential amino acids/proteins or binds minerals, thereby reducing its bioavailability (Ajay et al., 2018; Gupta et al., 2015). However, a high proportion of magnesium, potassium, and calcium in kernels also reduces the binding of zinc and iron with phytic acid, thus enhancing the bioavailability of these elements (Singh et al., 2022). In general, raw seeds contain far higher anti-nutritional compounds than their processed forms; hence, the processing is necessary before using them as food or feed. Groundnut kernels are generally processed using various methods, such as water soaking, sprouting, boiling, roasting, frying and steaming. In terms of health benefits, minimally processed foods are considered better than highly processed. Traditional water soaking and dry heat processing methods like roasting utilize minimum inputs and is less time-consuming. Water soaking is an old domestic practice often used to prepare foods at home. This practice improves the palatability and taste of some legume seeds (Mubarak, 2005). High-temperature short-time processing like roasting and MW irradiation causes transfer of heat energy *via* conduction and causes rapid dehydration and chemical changes due to the reduction in water activity of the grains (Verma et al., 2019). Though PA is heat stable, its level decreases considerably during soaking, germination, and fermentation in many legumes (Luo et al., 2009). In contrast, RS content increased at high temperatures (Mahadevamma and Tharanathan, 2004). Therefore, the processing of groundnut seeds improves the nutritional quality to varying degrees, either by improving chemical composition or by reducing anti-nutritional components. According to the literature review, there is no information about the effect of multiple processing techniques on the RS content and nutritional and antinutritional compounds of groundnut. Therefore, an investigation was conducted to study the impact of various processing techniques, like soaking, roasting (dry heating) and MW irradiation, on the nutritional and antinutritional composition of popular groundnut cultivars.

2. Materials and methods

Kernels of the ten popular groundnut cultivars in India were collected from the Germplasm Resource Section, ICAR-Directorate of Groundnut Research (DGR), Junagadh, Gujarat, India. Cultivars selected for the present study are Girnar 2, GG-20, GJG-22, Kadiri-6, TG-37A, HNG-10, GAUG-10, TG-38, JL-776 and TG-26. Fully matured seeds were cleaned by hand to remove the foreign materials and then stored in polyethene bags at a room temperature of 25 °C until further use. All the chemicals and reagents were analytical grade procured from Sigma Aldrich (St. Louis Street, MO, USA) and HiMedia (Delhi, India). All the solutions were prepared in deionized water of resistivity not less than 18.2 M Ω/cm (Heal Force, NW series). Seed treatments were given as depicted in Fig. 1 and were ground to fine seed meal in an electric grinder. Each sample was packed in polyethene bags of 0.08 mm thickness and placed at 4 °C until used for various biochemical analyses. Each experiment is conducted with three replications. The moisture percentage of treated seeds was determined by NIR (Dickey John, Instalab 700).

2.1. Experimental approach

2.1.1. Resistant starch

RS content was determined using an RS assay kit (Megazyme International Ireland, Ltd., Bray, Ireland) with slight modifications. 100 mg fine ground seed meal was digested with pancreatic α-amylase (10 mg/mL) containing 3 U/mL of amyloglucosidase (AMG, diluted in 0.1 M sodium maleate buffer, pH 6.0) with continuous shaking at 200 rpm for 16 h at 37 °C. The reaction was terminated by adding 4 mL of ethanol (99% v/v), and the RS pellet was recovered on centrifugation (3000 rpm for 10 min), whereas supernatant was processed further to estimate non-resistant starch, as described in the next heading. The pellet was rinsed twice with ethanol 50% (v/v) and re-suspended in 2 mL of 2 M KOH with constant stirring on an ice water bath for 20 min. To this, 8 mL of sodium acetate buffer (1.2 M, pH 3.8) was added, followed by the addition of 0.1 mL of AMG (300 U/mL). Tubes were incubated at 50 °C for 30 min with intermittent vortexing, followed by centrifugation at 3000 rpm for 10 min. Aliquots of supernatant (0.1 mL) were mixed well with 3 mL GOPOD reagent and incubated at 50 °C for 20 min. The absorbance was measured at 510 nm against the reagent blank. RS content (g/100 g) was calculated as per the formula of the assay

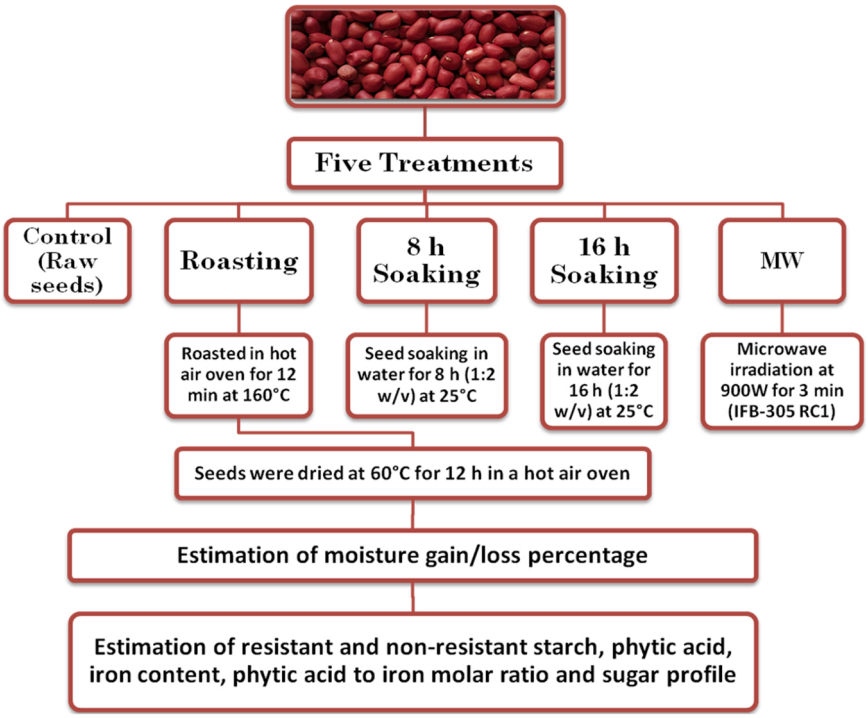

Fig. 1. Experimental approach and methods of seed treatments.

procedure.

2.2. Non-resistant starch

The estimation of NRS is in continuation with an analysis of RS. The supernatant collected after adding ethanol and subsequent rinsing with ethanol 50% (v/v) were pooled together and raised to 100 mL with sodium acetate buffer (100 mM, pH 4.5). To 0.1 mL of aliquot was incubated with 10 μL of AMG (300 U/mL) for 20 min at 50 °C. GOPOD reagent (3 mL) was added to this and again incubated for the next 20 min at 50 °C. The absorbance was measured at 510 nm against the reagent blank. NRS was content (g/100 g) calculated as per the formula of the assay procedure.

2.3. Phytic acid

Phytic acid content was determined using a phytic acid assay kit (Megazyme International Ireland, Ltd., Bray, Ireland) as described by Singh et al. (2022).

2.4. Iron content

The Fe content of groundnut seeds was measured by MP-AES (Agilent 4200 MP-AES, Santa Clara, CA, USA), as described by Sreenivasulu et al. (2017) using multi-element analysis. The sample injection system consisted of solvent-resistant tubing, a double-pass cyclonic chamber, and an inert flow blurring nebulizer (OneNeb). Instrumental parameters such as the viewing position, nebulizer gas pressure and background correction were optimized for Fe to ensure interference-free detection. Its content was monitored at 259.94 nm. The purity of applied chemicals and various equipment parts was verified by running blank samples. The MW-assisted acid digestion method was utilized to prepare samples using the MW assisted acid digestion system. Each sample (0.5 g) was digested with the addition of 15 mL of diacid (nitric acid and perchloric acid) in a 3:1 ratio. Following digestion, the solutions were allowed to cool at room temperature, transferred to 50 mL volumetric flasks and raised to the required volume with deionized water. Each sample was measured in triplicate, and Fe concentration (ppm) was calculated using the external standard calibration method.

2.5. Iron bioavailability

The relative bioavailability of Fe was calculated by the molar ratio of phytic acid to iron, that is, PA: Fe ratio. The respective moles were determined by dividing the weight of PA and Fe by their atomic weight (PA: 660.3 g mol^{-1}; Fe: 56 g mol^{-1}). Subsequently, PA: Fe molar ratio was obtained after dividing the moles of PA with the moles of Fe.

2.6. Sugar profiling

Seed samples from each cultivar were extracted for sugar profiling as described by Bishi et al. (2015). Mannitol, myo-inositol, trehalose,

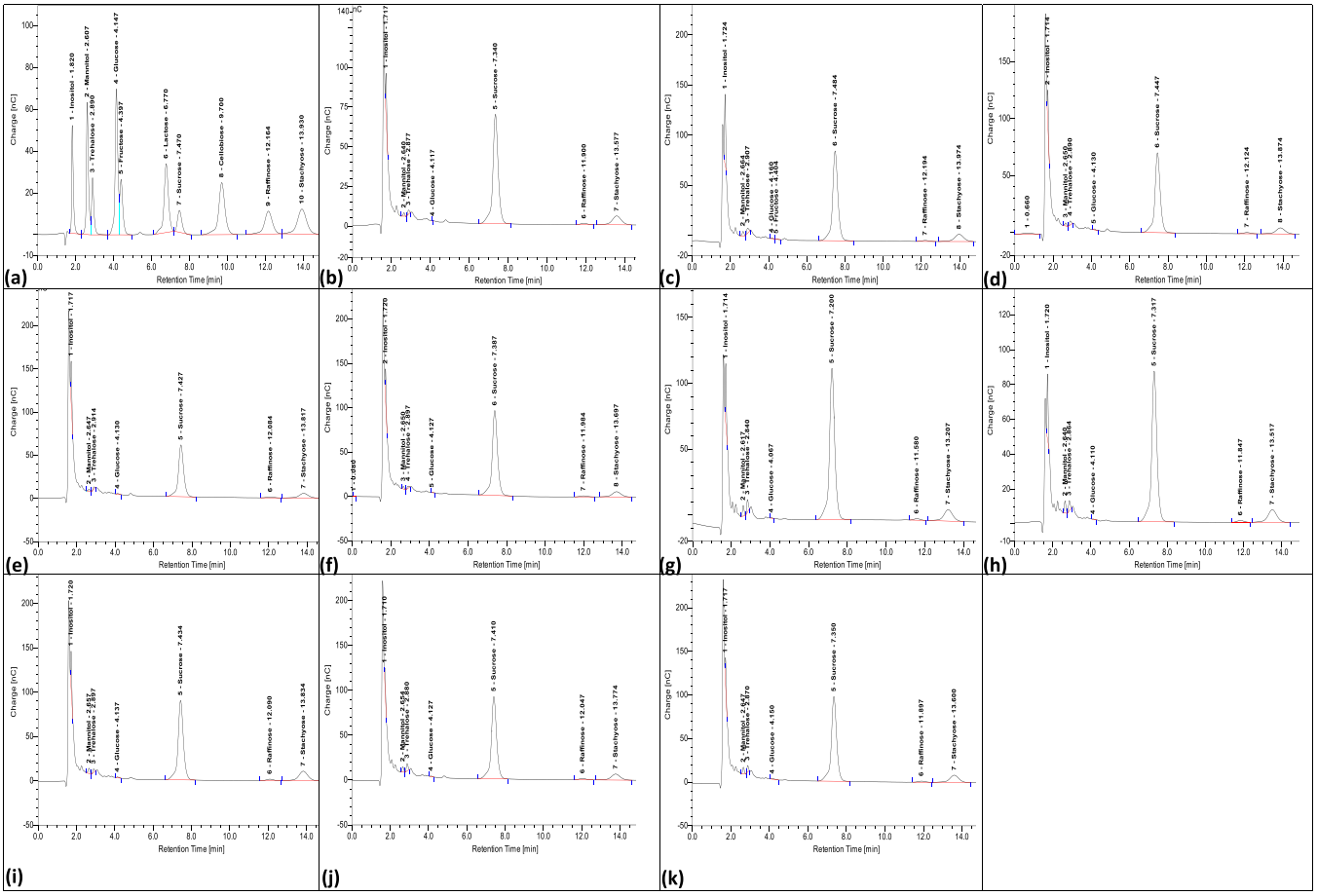

Fig. 2. Chromatograms of sugars obtained through ion chromatography. A mixture of sugars in seed samples and standards were separated in an ion chromatograph (Dionex, ICS 3000) equipped with CarboPac PA10 analytical column, where (a) is the standard sugar mixture with elution order of myoinositol (1), mannitol (2), trehalose (3), glucose (4), fructose (5), lactose (6), sucrose (7), cellobiose (8), raffinose (9) and stachyose (10); (b-f) are the chromatograms of JL-776 and (g-k) are of Kadiri-6 obtained from raw, roasted, 8 h soaking, 16 h soaking and MW irradiated samples. Lactose was used as an internal standard and concentrations are in ppm, used for respective sugars in parenthesis.

glucose, fructose, sucrose, raffinose, stachyose, lactose, cellobiose and verbascose were used as standard sugars. Lactose and/or cellobiose were used as internal standards. The concentrations of all the saccharides in the standard master mixture were adjusted to have a distinct peak for each saccharide in the chromatogram (Fig. 2a). Saccharides were extracted in 80% ethanol, and 25 μL of the sample was injected through a membrane filter in the ion chromatograph (ICS 3000 Dionex, USA) equipped with an amino trap column, CarboPac PA10 guard column, followed by CarboPac PA10 analytical column. NaOH (150 mM) was run as an elution buffer with a flow rate of 1 mL min^{-1}. Chromeleon software provided with the equipment was used for data integration.

2.7. Statistical analysis

The analysis of variance was performed for moisture content, Fe content, PA content, molar ratios, and resistant and non-resistant starch

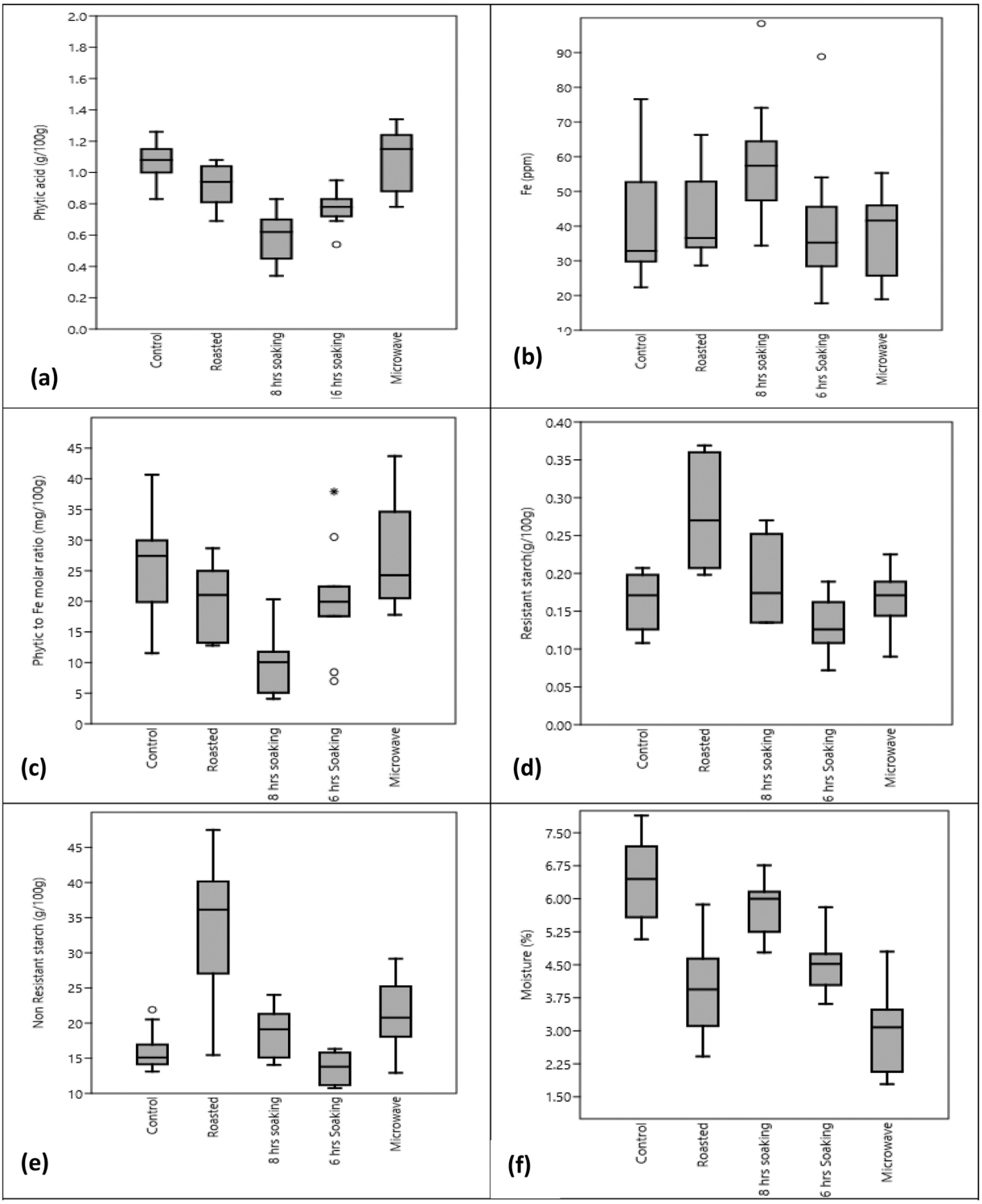

Fig. 3. Boxplots showing the variability between the treatments and biochemical parameters in ten groundnut varieties, where (a) phytic acid (g/100 g); (b) Fe content (ppm); (c) phytic acid to iron molar ratio; (d) Resistant starch (g/100 g); (e) Non-resistant starch (g/100 g); (f) Moisture (%).

using the mixed and general linear model procedures of SAS. Means separation was done using Fisher's protected least significant difference (LSD) at 0.05 level of significance. Boxplots were plotted using the PAST package. Data is represented in the form of Boxplots because they give an indication of how the values in the data are spread out. They have the advantage of taking up less space, which is useful when comparing distributions between many treatments, or datasets. They basically provide a visual summary of the data enabling readers to understand results in terms of identifying mean values, dispersion of the data set and best treatment (Williamson et al., 1989). We also added whiskers to the boxplot to show the extent of variability outside the upper and lower quartiles. Principal component analysis (PCA) was performed, and its biplot was prepared using the library FactoMineR and factoextra using R studio.

3. Results and discussion

3.1. Phytic acid

The effect of processing methods on PA in raw and treated groundnut kernels is shown in Fig. 3a. Raw seeds had PA content from 0.83 to 1.26 g per 100 g. Compared with their raw counterpart, soaked kernels for 8 h had a maximum reduction in PA from 23 % to 68 %, with an average of 45.5 %. (Oloffs et al., 2000) also reported that processing techniques like soaking, roasting and heat reduced PA content, especially water soaking, due to leaching out of PA in water. According to Food and Agriculture Organization (FAO), International Network of Food Data Systems (INFOODS) and the International Zinc Nutrition Consultative Group (IZiNCG) database, a significant amount of PA is degraded to its lower forms in processed foods (Dahdouha et al., 2019). In our study, out of ten genotypes, Kadiri-6 has shown the highest reduction after 8 h soaking, followed by GG-20 (62 %) and GJG-22 (57 %). Since PA is a water-soluble analyte, a significant decrease in its content can be realized by discarding the soaked water, as its content has leached out from the seeds into the water during treatments. Complete elimination of PA was not observed in any of the treatments, that might be due to the strong electrostatic interactions between the oxygen atoms of attached phosphate radicals within the PA structure which might impart heat resistance (Egli et al., 2002). Besides soaking for 8 h, other heat-based treatments (roasting and MW irradiation) were also effective in reducing PA content in groundnut, but to a lesser extent, that could be attributed to decreased water extractability during heating processes. Our observations are in agreement with previous findings that report PA reduction after water soaking of various cereals and legumes for 6–24 h (Bishnoi et al., 1994; Liang et al., 2008). Another study based on PA reported 20–30 % reductions in soaked cereals (*e.g.* rice, rye, and wheat) and up to 14% dephytinization in soaked legumes (Egli et al., 2002). Soaking preheated rice at 10 °C resulted in a 42–59 % reduction (Liang et al., 2008), a 54 % reduction in water-soaked black beans (Greiner and Konietzny, 1999) and 67–83 % reduction in pea seeds (Bishnoi et al., 1994). Here, the extent of reduction varies among treatments and cultivars, suggesting that different cultivars have distinctive capacities to hydrolyze PA based on the endogenous phytase activity and seed matrix interactions (Egli et al., 2002). Groundnut seed matrix is a bit complex where phytase and other hydrolyzing enzymes required for germination are located in the embryo, while food storage compounds (like starch, and lipids), are contained in the cotyledons. High oil content in groundnut seed cotyledons (about 52 %) may interfere with the passive diffusion of PA or activation of phytases by impeding water migration during soaking, roasting and MW irradiation.

3.2. Iron content

Mp-AES is a powerful and popular analytical tool for analysing Fe and other minerals as it uses nitrogen plasma instead of argon plasma. Fe concentration of raw and processed groundnut kernels of ten groundnut genotypes is shown in Fig. 3b. Soaking for 8 h and 16 h was able to retain more Fe content than control. The significant reduction (Table S1) in Fe content after dry heat processing might be due to its leaching from the seeds during roasting and MW treatments. For the soaked groundnut kernels of ten genotypes for 8 h, the Fe content ranged from 34.4 to 98.3 ppm, while the raw kernels contained 22.4–76.6 ppm. Raw kernels of the Kadiri-6 genotype have high Fe content (76.6 ppm). The highest percentage increase was observed in Girnar-2 after soaking for 8 h (66%), followed by 16 h (53.3 %). A similar effect of soaking treatment on Fe content in processed Kabuli chickpeas was observed by Xu et al. (2016). An increase of Fe content after water soaking may be due to activation of phytase enzyme and resulting dissociation from PA chelates. As discussed earlier that 8 h and 16 h soaking had decreased PA content; the similar, interplay between Fe content and Phy to Fe molar ratio has been reviewed by many scientific groups and found that processing methods like soaking have a beneficial impact on Fe content and its bioavailability (Gupta et al., 2015).

3.3. Molar ratio

The PA to mineral molar ratio is widely used to anticipate the inhibitory effect of PA on mineral bioavailability. According to FAO and INFOODS, if Fe's bioavailability in foods is affected by a ratio above 1 or even above 0.4, it significantly affects its absorption (Hurrell and Egli, 2010). The molar ratio of Phy/Fe in raw kernels was in the range of 11.5–40.6. Soaking treatment for 8 h was the best among the treatments to reduce the Phy/Fe molar ratio. GG-20 genotype showed the maximum reduction in Phy/Fe molar ratio (4.07) followed by Girnar-2 (4.36) after 8 h soaking treatment (Fig. 3c). The said molar ratio of MW irradiated samples was at par with raw samples. This is because the extent of reduction in PA content was less in microwave treated kernels as compared to other treatments; the same trend is reflected in PA: Fe molar ratio also. For 8 h soaking treatment, Phy/Fe molar ratios were all below 14 except for TG-37A genotype, which may have an acceptable level of Fe bioavailability. Our results are in line with the findings of Gupta et al. (2015) and Xu et al. (2016). Luo et al. (2009) studied the impact of processing on PA, *in vitro* soluble Fe and Phy/Fe molar ratio in *Vicia faba* and showed that soaking and fermentation processing methods had reduced PA and improved Fe content. Also, phytase enzymes dissociate inositol Hexa- and Penta-phosphates, inhibiting iron absorption to smaller inositol phosphates (inositol tetra-, tri- and di-phosphates) inorganic phosphate, which do not hamper iron absorption. Therefore, by soaking, the main inhibitory factor of Fe bioaccessibility and bioavailability, PA, gets partially degraded and may not remain a strong chelator of Fe.

3.4. Resistant starch

There is a wide variance in the content of RS in seeds of leguminous plants (up to 80 %). This can be attributed to the presence of intact cellular structures encapsulating starch granules, high levels of amylase and the presence of PA. Food processing can enhance or reduce RS content depending on varietal performance and adopted processing methods. The effect of simple food processing technique on RS content in groundnut is shown in Fig. 3d RS content in selected genotypes ranged from 0.11 to 0.21 g per 100 g, where it was least in TG-26 and highest in Kadiri-6. There was a marked increase in RS after roasting (33–61 %), followed by soaking in water for 8 h. All ten genotypes showed an increase in RS content after roasting as compared to their corresponding raw equivalents. Except for GJG-22, 8 h soaking treatment also increased RS content, but the increasing percentage was less than roasting. GAUG-10 showed a consistent increase in RS content after roasting (61 %), 8 h soaking (46 %) and MW irradiation (17.6 %). Vaidya and Sheth (2011) and Nigudkar (2014) reported an increase in RS content of wheat and maize after roasting and water soaking treatment. Nigudkar (2014) explained that dry heat methods (roasting,

frying and MW) used in routine cooking have a detrimental impact on the starch content and its digestibility due to trans-glycosidation reactions. During roasting, complex seed starch matrix experiences chemical alterations resulting in the formation of atypical glycosidic bonds and subsequent reduction in amylolytic susceptibility, forming RS. Surprisingly, extended water soaking for 16 h decreased RS content as compared to raw seeds. Nigudkar (2014) found a similar decrease in processed and freshly cooked legumes after soaking and germination. Soaking and germination of legumes can activate certain dormant enzymes, thereby reducing RS content and improving the digestibility of these legumes. Garcia et al. (2007) reported an enhancement in total starch and RS content in *Phaseolus vulgaris* samples soaked in water. This pattern might be associated with lixiviation of other bean components, which leads to increased RS content. Heating methods like roasting and MW irradiation have become more efficient than soaking. This is because dry heating restricts the retrogradation of starch components. At the same time, in the case of soaking, RS might have been converted into rapidly digested starch or slowly digested starch either by chemical modifications and/or by gelatinization of starches, thereby altering RS content (Chung et al., 2008).

In addition to a processing technique, other potential causes of variability in RS content may include the presence of anti-nutrients like PA and oil content. Muir and Kerin (1992) have shown that adding oil to rice samples resulted in a non-significant difference in RS content. However, it could be possible that starch-oil interactions may interfere with the estimation of RS content as groundnut naturally contains high amounts of fats. Because RS has a lower calorific value than fully digestible starch. It is manipulated or incorporated into a wide range of mainstream foods such as roasted, baked, soaked products to add value to its physio-chemical properties and, appearance and taste.

3.5. Non-resistant starch

NRS is one of the healthy food components, found to be beneficial in the stabilization of glucose metabolism, diabetes, satiety and mental health (Peterson et al., 2018). To understand the influence of processing methods on RS content, it is necessary to study its soluble or digestible counterpart, *i.e.*, non-resistant starch, as both attributes to total starch content of the seed. Fig. 3e shows NRS content of raw and processed groundnut kernels of ten popular varieties. Roasted samples showed significantly higher NRS content than their raw counterparts, with a 15–67 % increase. Roasted kernels of GAUG-10 showed a 67 % increase while JL-776 showed the least increase of 15 %. NRS content of GAUG-10 is in line with RS content, thereby exhibiting a maximum increase percentage of total starch content. The next effective treatment was MW irradiation, though the increasing percentage was less pronounced than roasting. Except for GG-20 and JL-776 genotypes, a 15 – 42 % increase was observed in MW treated kernels. Both the wet heat methods (roasting and MW irradiation) were more effective than soaking. However, GAUG-10 genotype showed a consistent increase in RS and NRS content in all treatments except for the extended soaking of 16 h. Various factors influence starch content and its digestibility, such as varietal characteristics, physicochemical properties, moisture content, and microstructural composition of the seed and processing methods adopted. Mittal et al. (2012) reported that moisture content and heating time considerably influence digestible starch quality, nutritional value and digestion. Similarly, studies on wet and dry heat treatments on processed flour of legumes showed better digestibility and palatability (Mahadevamma and Tharanathan, 2004; Rehman and Shah, 2005).

3.6. Moisture

Moisture content is an important factor affecting the flavor, texture, quality, infestation rate and shelf life of oilseeds and nuts. The initial moisture content of fresh groundnut kernel ranged from 5.1 % to 7.9 %, with a mean value of 6.4%. As in the case of dry heat methods of roasting and MW treatment, there was a decrease in moisture content (16–57 % and 28–71 %, respectively). The maximum drop in moisture percentage was observed in MW irradiation treatment followed by roasting and soaking treatments (Fig. 3f). GJG-22 genotype showed maximum reduction (71 %) in moisture content after MW treatment, whereas, TG-26 showed minimum reduction (28 %). In both wet heat treatments of MW and roasting, TG-26 genotype showed a lesser reduction in moisture percentage (28 % and 16 %, respectively). Increased temperature reduces moisture content and generates aroma which stimulates mortality of insects/pests. Das et al. (2014) reported that MW treated cashew nuts were free from infestation and rancidity even after 6 months of storage. Our results are in agreement with Luo et al. (2009) in faba beans and Mubarak (2005) in *P. aureus*. Cämmerer and Kroh (2009) showed that with the increasing roasting temperature and time, oxidative stability of groundnut was improved, and shelf life was prolonged.

3.7. Sugar profile

Food processing alters the sugar profile of the groundnut seeds, which play a crucial role in osmotic balance, membrane stability and energy metabolism. It is vital for the development of groundnut flavor during processing techniques, as sugars, especially monosaccharides, and amino acids, catalyzes Maillard reactions (Baker et al., 2003). A total of eight sugars were identified in raw and treated kernels. Sucrose being major sugar, showed an 88–93 % increase over the control, followed by stachyose (4–7 %). Inositol, mannitol, trehalose, glucose, fructose and raffinose were present in smaller quantities. Significant changes in all the sugars were observed in response to processing techniques (Table 1). The stachyose content of nearly all varieties decreased significantly after 8 and 16 h of soaking. Stachyose and Raffinose are members of RFOs known to cause flatulence, gastric discomfort, and diarrhea in humans and some monogastric animals (Bishi et al., 2015). Various processing methods such as soaking, roasting, germination and fermentation have been suggested to reduce RFOs and other anti-nutritionals (Samtiya et al., 2020). Reduced sucrose content was seen by extended soaking for 16 h in all the varieties except TG-38 and GJG-22. An increase in glucose content after 16 h soaking was well correlated with the decrease in sucrose levels. Interestingly, fructose has appeared in all varieties upon roasting, except in Kadiri-6. Additionally, it has also appeared in GAUG-10 after soaking (8 h and 16 h) and MW irradiations. These observations in glucose, fructose and sucrose contents are likely explained by several competing reactions involving sugars during processing techniques. Maillard browning during roasting and MW irradiations, inversion of sucrose into glucose and fructose during soaking and breakdown of glucose from raffinose and stachyose might influence their levels (McDaniel et al., 2012). Sugar content in different genotypes reported in the present study was comparable with the earlier reports (Bishi et al., 2015; Chakraborty et al., 2016; Mahatma et al., 2016). After processing techniques, the least impacted groundnut variety was JL-776 variety (Fig. 2b-f), and the most affected was Kadiri-6 (Fig. 2g-k).

3.8. Principal component analysis

Principal component analysis (PCA) was carried out to observe any possible clustering within the nutritional and anti-nutritional factors among the cultivars under the influence of treatments. The first two principal components account for 68.1% (PC1 = 35.7 % and PC2 = 32.4 %, respectively) of the total variation as seen in scree plot between component dimensions on the x-axis and percentage of explained variances y-axis (Fig. 4). Perhaps, a scree plot is a demographic way to determine the number of principal components exhibiting variations and an elbow (bend) in this signifies the exact number of principal components. This analysis clearly shows the differences among the samples of ten varieties and five treatments, including control. The PCA

Table 1
Sugar profiles (ppm) of groundnut kernels during processing techniques.

Variety	Treatment	Sugars (ppm)							
		Inositol	**Mannitol**	**Trehalose**	**Glucose**	**Fructose**	**Sucrose**	**Raffinose**	**Stachyose**
TG 37 A	Control	519	213	1164	6	ND	49,765	408	3186
TG 38		356	684	16	7	ND	73,810	1056	6742
JL 776		399	97	287	5	ND	41,173	327	2258
TG 26		1006	958	730	13	ND	85,076	639	6035
GAUG 10		895	341	350	76	ND	66,847	815	4870
HNG 10		965	225	348	23	ND	50,705	633	6269
Gir 2		477	418	187	13	ND	53,464	633	6798
GG 20		726	127	642	16	ND	44,840	363	4465
GJG 22		654	214	378	20	ND	48,092	515	4785
Kadiri 6		679	367	1132	8	ND	68,849	401	3492
TG 37 A	Roasted	1454	175	923	17	47	59,533	517	3533
TG 38		453	895	186	17	60	94,437	1037	7466
JL 776		1044	228	813	4	187	54,125	404	3013
TG 26		243	713	940	29	24	82,288	495	4763
GAUG 10		1584	364	684	47	77	70,873	947	5219
HNG 10		851	194	870	22	24	49,470	345	4529
Gir 2		1097	237	433	30	66	42,745	471	5067
GG 20		897	193	870	21	24	49,466	343	4526
GJG 22		1063	245	562	20	37	61,398	532	4685
Kadiri 6		549	317	757	4	ND	51,904	396	2995
TG 37 A	8 hrs Soaking	524	134	605	41	ND	51,675	645	3096
TG 38		629	585	326	58	ND	78,506	1022	5056
JL 776		418	137	482	19	ND	42,383	416	2188
TG 26		293	867	742	22	ND	87,055	649	5455
GAUG 10		754	189	412	522	23	49,526	839	2752
HNG 10		678	349	323	55	ND	52,634	657	5503
Gir 2		389	248	330	24	ND	56,686	709	5221
GG 20		944	181	799	17	ND	49,235	555	4361
GJG 22		596	364	1023	17	ND	57,917	487	3190
Kadiri 6		626	305	534	22	ND	53,743	689	4504
TG 37 A	16 hrs Soaking	559	128	966	10	ND	47,784	617	2710
TG 38		456	650	241	53	ND	76,123	959	5413
JL 776		737	179	304	24	ND	35,732	432	2220
TG 26		999	606	1038	20	ND	78,075	845	4759
GAUG 10		248	108	233	512	145	31,852	370	1595
HNG 10		602	141	265	34	ND	34,715	647	3886
Gir 2		566	266	306	24	ND	51,717	804	5281
GG 20		653	168	666	24	ND	39,695	594	3689
GJG 22		506	249	403	81	ND	49,855	727	4340
Kadiri 6		247	270	1054	13	ND	55,127	567	2756
TG 37 A	Microwave (MW)	277	167	1055	21	ND	53,612	455	3172
TG 38		171	732	395	13	ND	76,093	908	6571
JL 776		496	134	193	5	ND	57,953	445	2322
TG 26		673	151	1507	21	ND	64,542	496	3431
GAUG 10		438	313	437	10	18	69,396	975	5462
HNG 10		483	293	218	22	ND	45,206	524	5215
Gir 2		373	277	285	11	ND	53,672	576	6165
GG 20		559	237	583	31	ND	52,634	412	5116
GJG 22		255	175	280	35	ND	51,336	510	4709
Kadiri 6		416	277	841	35	ND	58,905	492	3434
LSD (P = 0.05)	Variety (V)	2.51	1.50	1.49	0.84	0.43	1.36	1.32	1.72
	Treatment (T)	1.77	1.06	1.06	0.59	0.31	0.96	0.93	1.22
	V x T	5.61	3.35	3.34	1.88	0.96	3.04	2.94	3.86

* All values are mean of three replications; ND: not detected

biplot can be divided into four sections (Fig. 5).

Notably, the Fe content, present in the lower-left section of the PCA biplot, was the variable with negative loadings on PC1 and PC2. Contrastingly, RS and moisture content showed (lower right section) positive loadings on PC1 and negative loadings on PC2. In the upper left section, PA is the variable with negative loadings on PC1 and positive loadings on the PC2 component. Lastly, PA: Fe molar ratio variable and NRS (upper right section) showed positive loadings on PC1 and PC2. Furthermore, the increasing length of the arrows in the PCA biplot represents increasing variability which is the case found with Fe content and PA to Fe molar ratio in the lower left and upper right sections, respectively. In addition, the principal component survey showed that RS content in groundnut varieties was strongly associated with their PA to Fe molar ratio biochemistry (Fig. 5). The same analysis suggested a good agreement between RS and kernel moisture content in varieties like GJG-22, GAUG-10 and Kadiri-6. In line with our initial hypothesis, PA content exhibits low concordance with the nutritional component. Here, it would be interesting to note that how the biochemical composition of the present analyzed groundnut varieties are influenced by their relative heterogeneity.

4. Conclusion

Using the processing techniques, we demonstrated that RS content in groundnut seeds can be increased. Roasting at 160 °C for 12 min has caused a marked increase in RS and NRS content. Further, if the goal is to reduce PA and improve Fe availability, 8 h water soaking would be the best choice. This study provides simple and convenient methods to enhance RS content, reaping the benefits and enhancing groundnut quality by processing.

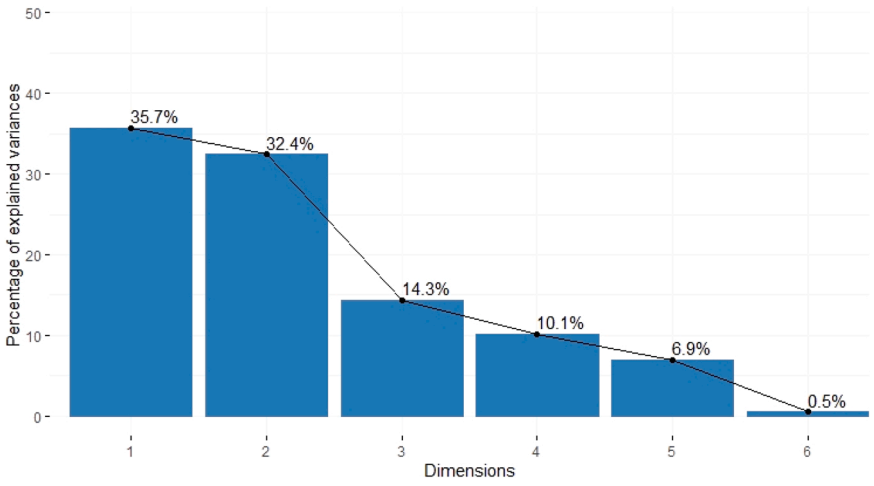

Fig. 4. Scree plot showing % of explained variances *vs* dimensions accounted for variations.

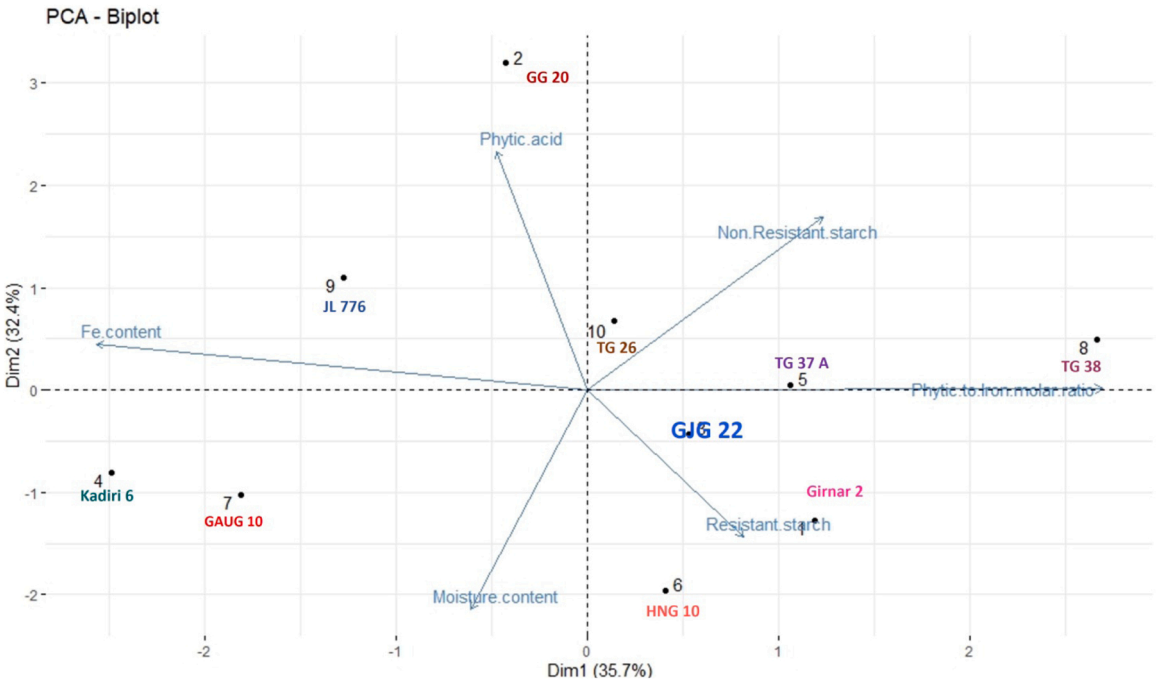

Fig. 5. PCA biplot of scores and loadings of data obtained from PA, Fe content, phytic to iron molar ratio, moisture content, resistant and non-resistant starch analysis for ten varieties and five treatments including control.

CRediT authorship contribution statement

AV: Conceptualized the research work and biochemical analysis and drafted the manuscript; MKM: Conceptualized the research work, project supervision and editing of MS; LKT: Performed lab analysis; SS: review of research work and phytic acid analysis; GK and PK: Data curation and validation, formal analysis and interpretation of biplot results; ALS: Supervision and editing the final draft of MS.

Declaration Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2022.104653.

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