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The Relationship Between Varieties and Acrylamide Formation In Roasted Barley

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Abstract

Attempts being made to increase the awareness for addressing the acrylamide issues among barley breeders and food technologists. Selection from existing hulled barley varieties and genotypes for low free asparagine accumulation and specifying the optimal roasting conditions in coffee-substitutes based on roasted barley offer suitable mitigation interventions and strategies at the agronomic and the industrial scales to avoid excessive acrylamide formation and to generate safe barley products for dietary use. To these interventions, this study aims to compare the acrylamide forming potential of three hulled barley varieties with different genotypic origins grown under normal agronomic conditions during two growing seasons of 2018/2019 and 2019/2020, then used for roasted coffee-substitutes manufacture, and correlate this to different roasting temperatures and acrylamide concentrations. Mean squares of genotypes were highly significant ($P \leq 0.05$) for all agronomic characteristics and free asparagine accumulation (12.96**, 12.65**) in both seasons. Acrylamide concentrations in coffee substitutes showed strong negative or weak correlations with glucose, sucrose, fructose, and maltose and strong positive correlations (r =0.8029& 0.8025) with free asparagine contents (318.2-682.3& 322.3-681.5 mg Kg^{-1}) in the grains in both seasons. Indicating free asparagine is the main determinant of acrylamide-forming potential in different barley genotypes. The acrylamide concentrations in the ground (<10-242.8 µg kg^{-1}) and brewed (0.26-1.70 µg 70 mL^{-1}) coffee substitutes significantly ($P \leq 0.05$) increased as roasting temperatures increased at 180, 200 & 220°C, except for variety Giza133 decreased shortly at 220°C. These levels did not exceed the recommended limit (500 µg kg^{-1}) by the European Commission; however, overconsumption should be of concern. Roasting temperatures significantly ($P \leq 0.05$) influenced the physical properties of the roasted barley grains and the physicochemical properties of coffee substitutes with strong correlations with acrylamide concentrations. This study exhibits an indicator to help risk managers and decision-makers to set priorities for further action for addressing the acrylamide problem.

Keywords:Hulled barley; Genotypic and Agronomic factors; Acrylamide precursors; Roasting process; Barley coffee substitutes

1. Introduction

Barley is one of the most abundantly utilized cereal crops worldwide. It accounts for 12% of total global cereal production, ranking fourth after wheat, rice, and maize [1]. In Egypt, barley grows in the Northern Coastal Regions in 71,000 ha with an average grain yield of 1.77 tons/ha and a total production of 114,000 tons [2].

Diversity in grain composition between hulled barley genotypes potentially facilitates its use in a vast array of products having numerous beneficial and desirable characteristics such as malt, beverages, and coffee substitutes as well [3, 4].

The coffee substitutes based on roasted barley were developed to reduce the potential risks of coffee consumption associated with high caffeine content [5]. The roasting process of barley grains improves the taste, the odor, appearance, and texture of their coffee substitutes, due to the formation of aroma compounds such as pyrazines, ketones, and aldehydes [6, 7]. However, the roasting of barley grains above 120° C resulted in acrylamide formation unintentionally in the Millard reaction as the grains are a rich source of free asparagine— the main precursor in the formation of acrylamide in foods [8, 9].

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Moreover, the concentration of the acrylamide from free asparagine and some reducing sugars is about 80-fold higher than that in free asparagine alone in a model heated system, where sugars have different capacities to produce acrylamide with free asparagine according to their types and the degree of hydrolysis [10].

The European Commission [EC, 11] established a benchmark level of 500 μgkg^{-1} for the presence of acrylamide in coffee substitutes exclusively from roasted barley. Also, the CONTAM Panel Experts of the Food Chain in the European Food Safety Authority [EFSA, 12] selected Benchmark Dose Lower Confidence Limit ($BMDL_{10}$) values of 0.17 and 0.43 mg kg^{-1} BW day^{-1} for neoplastic and neurological effects of acrylamide based on animal evidence, respectively. Onwards, the Panel considered that dietary exposure to acrylamide would be unlikely to be sufficient to cause neurological effects, while potential neoplastic effects were concerning. In these regards, many cited reports indicated the presence of acrylamide in many coffee substitutes based on roasted barley sold in different global markets, and sometimes some of them exceed the benchmark level established by the European Commission, which may imply probable adverse health effects due to the risk exposure of the dietary acrylamide intake [9, 13-15].

The above-mentioned claims highlight the need of reducing dietary acrylamide intake by implementing suitable mitigation strategies at agronomic and industrial scales by applying good practices [16-18]. These strategies should not only exert low influence on the coffee substitute sensory characteristics but also comply with the regulations and be economically sustainable [19].

Agronomic intervention for reducing the acrylamide-forming potential of barley include the evaluation of existing varieties and genotypes for low asparagine accumulation in barley grain that may be adapted into cultivation and breeding programs [20, 21].

Industrial interventions for reducing acrylamide formation include the implementation of a fast detection for free asparagine concentration at the factory platform. This could result in consignments of grain with high free asparagine concentrations being discarded away to find a market other than for roasted products [15]. Besides, specifying the optimal roasting conditions to avoid excessive acrylamide formation and to generate safe barley products [16].

Accordingly, the main objectives of this study were to compare the acrylamide forming potential of three hulled barley varieties with different genotypic origins grown under normal agronomic conditions during two growing seasons of 2018/2019 and 2019/2020, then used for roasted coffee-substitutes manufacture, and correlate this to different roasting parameters and acrylamide concentrations.

2. Experimental

2.1. Materials

Three hulled barley genotypes were selected for this study. These genotypes have genetic and morphological variability. Besides, they cover nearly about 60% of the area of barley cultivated in Egypt. The names and the pedigrees of the genotypes are as follows:

(1) Giza 123: Giza 117/FAO 86
(2) Giza 132: Rihane-05//As46/Aths*2" Aths/ Lignee686
(3) Giza 133: Carbo/ Gustoe

The standards of Acrylamide-1-^{13}C (99%), L-Asparagine ≥98% (HPLC), glucose, fructose, sucrose, and maltose were purchased from Sigma Chemical Co., Saint Louis, Missouri, USA. Acrylamide of 99.9% purity was obtained from Alfa Aesar-Fisher Scientific Co., Sweden. SPE-cleanup Cartridge was obtained from Thermo Scientific™, HyperSep™ Hypercarb™, Inc. Waltham, Massachusetts, U.S. The Millipore water purification system (Bedford, MA, USA) was used for water distillation. All other chemicals and reagents were HPLC-grade.

2.2. Methods

2.2.1.Field trial design

Field experiments were conducted during the two successive seasons 2018/2019 and 2019/2020, at the Experimental Farm of Sakha Agricultural Research Station, Agricultural Research Centre, Kafr El Sheikh Governorate, Egypt. Seeds were hand drilled at the recommended sowing rate of barley in Egypt (52 kg acre) in the first week of December. Each plot was sown in (4.2 m^2) six rows of 3.5 m, with 20 cm between rows. This experiment was designed in a randomized complete block design (RCBD) with three replications. The studied characteristics were days to heading, days to maturity, plant height (cm), spike length (cm), number of grains spike^{-1}, number of spikes m^{-2}, 1000-grain weight (g), biological yield (ton hectare^{-1}), and grain yield (ton hectare^{-1}).

2.2.2.Sample preparation

Barley grains were manually cleaned and freed of impurities and then dehulled using a rotary drum (dimensioned 0.30 m×0.20 m, speed 50 RPM).

The hulled grains were divided into two portions; a portion was used for the roasting process and the formation of coffee substitutes. The other portion was milled into flour in Cyclone Mill, sieved to 200 µm and kept in glass jars (-20º C) for moisture, free asparagine, and sugar analyses.

2.2.3.Analysis of free asparagine

The free asparagine concentration was determined in the three hulled barley samples according to the method described by Žilić et al. [10]. Hulled barley flour (0.5 g) was extracted with distilled water and then agitated for 10 min and filtered through a 0.45 µm membrane filter. Briefly, 1.0 mL water extract was added to 1.0 mL Phthaldialdehyde reagent in borate buffer (100 mM, pH 10.4). A Shimadzu Scientific HPLC system with an RP-18 column (250 mm × 4 mm) and Refractive detector-535 was used to analyze the free asparagine. The injection volume was 20 µl, and the mobile phase was 825 mL potassium phosphate buffer: 145 mL acetonitrile: 30 mL tetrahydrofuran; at a flow rate of 1.0 mL min^{-1} and emission wavelengths of 455 nm. Standard solutions of *L*-asparagine were used for the quantitative calibration and the free asparagine concentration was expressed as mg Kg^{-1} of hulled barley flour.

2.2.4.Analysis of sugars

The concentrations of reducing sugars (glucose, fructose, maltose), and sucrose in the three hulled barley samples were measured according to Curtis et al. [22]. Sample (0.5 g) was added to 10 mL of methanol in water (1:1 v/v). The suspension was stirred for 15 min then settled for 15 min. The produced aliquot was centrifuged at 7200*g* for 15 min, then diluted four times in water, and filtered (0.2 µm filter) into a vial. An HPLC system (YL 9100, YL INSTRUMENT CO., LTD, Korea) with an NH_2 column and a Refractive detector was used to analyze the sugar content. The injection volume was 25 µL, and the mobile phase was 75% ACN and 25% water at a flow rate of 2 mL min^{-1}. Standard solutions of glucose, fructose, sucrose, and maltose were used for the quantitative calibration**.**

2.2.5. Roasting process and formation of coffee substitutes and brews

Hulled barley varieties each of 500 g were conditioned to 10% moisture content to eliminate the differences in moisture content during roasting. The roasting process was performed in a convection oven (Memmert, Cambridge, UK) at 180±20º, 200±50º, and 220±20°C for 60 min. Grain surface temperature was recorded during roasting by placing a digital Pen thermometer with a stainless steel sensor probe (Type WT-1, Elitech® International, USA) into the drilled hole of the grain. After roasting, barley grains were cooled down (37±5°C), then ground and sieved to fine coffee substitute powder (200 µm) in Cyclone Mill. The moisture content of the coffee substitute was determined following the standard Method 3.2 for Barley according to EBC [23] of the European Brewery Convention Analysis Committee. A part of a coffee substitute sample (7 gm) was brewed in 70 mL of boiling water for 5 min. The brew was cooled, centrifuged (2800*g* for 5min), filtered, and kept at 4ºC for acrylamide analysis.

2.2.6.Analysis of acrylamide

The coffee substitutes based on roasted barley (ground and brewed) were extracted using solid-phase extraction (SPE) cleanup for acrylamide analysis, then determined following the validated method of Soares et al. [24]. Ground coffee substitutes (2.0 g) and brewed coffee substitutes (1.25 mL) were weighed in centrifuge tubes (50 mL), then water (10 mL) and EtOH (15 mL) were added, and the samples were mixed for 5 minutes. The samples were spiked with internal standard (25 µL: 10 mg L^{-1}), centrifuged at 15,000*g* for 5 minutes at 4ºC. A total of Carrez I (1 mL) and Carrez II (1 mL) solutions were added. Then samples were centrifuged at 15,000*g* for 15 minutes at 4ºC, filtered through filter disks (0.20 µm). The filtrates were transferred to a preconditioned SPE clean-up column with methanol and water (1:1). The extracts were passed through the cartridge. The first 2 mL were discarded, and 4 mL of filtrates were collected and concentrated to dryness using nitrogen gas. The residues were transferred to GC-MS (Model GC-MS QQQ 7000A, Agilent, USA) for determining the acrylamide. Finally, the acrylamide eluate was transferred to a vial for injection. GC-MS was performed using an Agilent 19091S-433: HP-5MS UI connected to an Agilent 160-7625-5 Inert Fused Silica (R1). The separation was performed using a 30 m x 250 µm x 0.25 µm column and 0.7 m x 150 µm x 0 µm column with Helium Quench Gas with a flow rate of 2.25 mL /min. The acrylamide eluted at around 4 min. Acrylamide standard solutions were prepared in acetone to determine linearity and detection limits. The limit of detection (LOD) was 5 µg kg^{-1}, limit of quantitation (LOQ) was 10 µg L^{-1}, precision was from 2-6%, and the recovery was 89%.

2.2.7.Physical properties of roasted barley varieties and their coffee substitutes

2.2.7.1. The grain dry mass loss (M)

The M of the roasted barley grains was calculated from the total weight of the dried grain before and after the roasting according to equation (1).

$$\mathbf{M}\ (\%) = \left[\frac{Mc - Mr}{Mc}\right] * 100 \qquad (1)$$

Where, M*:* grain mass loss (%) during the roasting,
M_c*:* unroasted grains total mass (Kg)
M_r*:* roasted grains total mass (Kg).

2.2.7.2. The bulk density (BD)

The BD of the coffee substitutes based on roasted barley was calculated by measuring the volume of a known mass of coffee substitute in a graduated cylinder according to equation 2 as follows:

$$\mathbf{BD}\ (\mathbf{Kgm^{-3}}) = \frac{(weight \text{ of coffee substitute})}{(volume\ of\ \text{coffee substitute})} \qquad (2)$$

2.2.7.3. The puffing index (PI)

The puffing index (PI) of the roasted barley grains was calculated according to equation 3 as follows:

$$(\mathbf{PI}) = \frac{(Bulk\ density\ of\ unroasted\ grains)}{(Bulk\ density\ of\ roasted\ grains)} \qquad (3)$$

Where the bulk density (Kg m^{-3}) is the volume of a known mass of the grains in a graduated cylinder.

2.2.7.4. The grain hardness

The hardness of the roasted barley grains was measured using a force gauge instrument model SHIM.FGC-50 Shimpo, Nidec-Shimpo, Kyoto, Japan. Each grain was placed horizontally between two parallel iron plates; the applied force (N) to make the grain rupture was determined by calculating the area under the force-deformation curve. The moisture contents were from 71.5 to 91.5 g Kg^{-1}, and the reading for each treatment is of five replicates.

2.2.7.5. CIE Colour measurements

The CIE colour space parameters of the coffee substitutes based on roasted barley samples were measured according to the CIE-Commission International of Illumination [25]. The coffee substitutes were passed through 200 µm sieved then colour space parameters were measured in five replicates using a colorimeter (CR-400, Konica Minolta Sensing Inc., Japan). The colour values were recorded as L^* =lightness (0 = black, 100 = white), a^* (-a^* = greenness, +a^* = redness) and b* (-b^* = blueness, +b^* = yellowness).

2.2.7.6. The roasting index of coffee substitutes based on roasted barley

The roasting index was assigned by using the SCAA [26] of Roast Colour Standard classifications and the Agtron Scale, ranging from Agtron N0. #95, $L^* \geq 57$ (= very light roast) to Agtron N0. # 25, $L^* \leq 18$ (= very dark roast), at intervals of 10 down.

2.2.8. Statistical analysis

The components of the analysis of variance for the agronomic trial were evaluated for each experiment as described by Kearsey and Pooni [27]. The data of technological trials were analyzed using computer software CoStat 6.303 (CoHort, USA, 1998–2004) for Windows. An analysis of variance (ANOVA) followed by Duncan's multiple range tests at $P \leq 0.05$ was used to compare between means. The correlation coefficient (*r*) was also calculated from the Excel spreadsheet to show the relationship between acrylamide concentrations and the studied characteristics.

3. Results and discussion

3.1. The agronomical trail and yield potential

Table 1 represents the mean squares of all traits of the studied genotypes in two seasons. Results pointed out those mean squares of genotypes were highly significant for all traits in both seasons. **Table 2** shows the mean performances of the three barley genotypes for the different characters under study. Overall mean values for days to maturity showed that the most desirable mean values towards the earliness were exhibited by the Giza 123 in both seasons with average values of (126.67 and 128.33 days) in the first and second seasons, respectively. Concerning plant height and spike length, data in **Table 2** showed highly significant differences among the barley genotypes in both seasons. Giza 132 had the highest mean values for plant height and spike length in both seasons (134.00 and 131.33 cm for plant height and 9.47and 9.53 cm for spike length in the first and second season, respectively. For grains $spike^{-1}$, the number of spikes m^{-2}, 1000-grain weight, biological yield and grain yield results of mean performance as shown in **Table 2** revealed that Giza133 gave the highest mean values for these traits in both seasons (73.00 & 71.00); (592.00 & 546.67); (56.51 & 56.55g); (18.42 &17.94 ton ha^{-1}); (5.65 & 5.43 ton ha^{-1}), respectively. These findings are in agreement with [28, 29].

Also, from the previous results, all traits recorded over the two seasons were significant. Such results indicated that the tested genotypes varied from each other and ranked differently from one season to another. These findings are similar to previous reports [29, 30].

Table 1 Estimated mean squares of different agronomic traits for hulled barley genotypes at 2018/ 2019 and 2019/ 2020.

		Days to heading		Days to maturity		Plant height cm		Spike length cm		Grain spike^{-1}	
SOV	*df*	2018/2019	2019/2020	2018/2019	2019/2020	2018/2019	2019/2020	2018/2019	2019/2020	2018/2019	2019/2020
Rep	2	1.33	0.78*	0.11	0.78	0.11	1.44	0.62*	0.1	0.62*	0.1
Genotypes	2	8.33*	21.78**	6.77*	24.11**	554.77**	299.11**	6.87**	4.22**	6.87**	4.22**
Error	4	0.68	0.11	0.44	0.78	11.61	0.44	0.07	0.17	0.07	0.17
		Number of spikes m^{-2}		1000-grain weight g		Biological yield ton hectare^{-1}		Grain yield ton hectare^{-1}		Free asparagine mg Kg^{-1}	
SOV	*df*	2018/2019	2019/2020	2018/2019	2019/2020	2018/2019	2019/2020	2018/2019	2019/2020	2018/2019	2019/2020
Rep	2	388.78	49.78	0.19	0.37	0.02	0.06	0.01	0.01	1.33	0.01
Genotypes	2	13618.78**	7260.44**	37.52**	48.43**	2.58**	5.03**	0.41**	0.54**	12.96**	12.65**
Error	4	111.44	156.44	0.07	0.32	0.02	0.16	0.01	0.01	1.33	0.01

SOV- Source of variance, *df*- degree of freedom, $^{**}P \leq 0.05$

Table 2 Mean performance estimates of traits for hulled barley genotypes at seasons 2018/ 2019 and 2019/ 2020.

	Days to heading		Days to maturity		Plant height cm		Spike length cm		Grain spike^{-1}	
	2018/2019	2019/2020	2018/2019	2019/2020	2018/2019	2018/2019	2018/2019	2019/2020	2018/2019	2019/2020
Giza123	88.33	91.67	126.67	128.33	123.33	126.00	8.50	9.20	60.33	63.67
Giza132	90.00	93.67	129.67	134.00	134.00	131.33	9.47	9.53	63.33	62.67
Giza133	86.67	88.33	128.33	131.00	107.00	112.00	6.50	7.33	73.00	71.00
LSD 5%	1.85	0.75	1.51	1.99	7.72	1.51	0.59	0.95	4.21	5.82
	Number of spikes m^{-2}		1000-grain weight g		Biological yield ton hectare^{-1}		Grain yield ton hectare^{-1}	
	2018/2019	2019/2020	2018/2019	2019/2020	2018/2019	2019/2020	2018/2019	2019/2020
Giza123	472.63	444.00	49.47	48.90	16.74	15.44	5.04	4.59
Giza132	501.33	450.67	55.61	53.81	17.53	17.28	5.24	5.11
Giza133	592.00	546.67	56.51	56.55	18.42	17.94	5.65	5.43
LSD 5%	21.13	26.21	0.56	1.21	0.38	0.92	0.21	0.27

3.2. Acrylamide precursors in hulled barley varieties

There are many efforts and approaches for reducing free asparagine and sugar accumulations in cereal grains, which in turn lead up to a progressive reduction in acrylamide-forming precursors such as identifying the varieties and the genotypes with low acrylamide potentiality that may be incorporated into cultivation and breeding programs [20].

3.2.1.Free asparagine

Table 3 shows the concentrations (mg Kg^{-1}) of free asparagine in the three hulled barley genotypes of seasons 2018/ 2019 and 2019/ 2020. Free asparagine contents showed insignificant ($P \leq 0.05$) variations in both seasons, however, the significant ($P \leq 0.05$) variations were found in the three hulled barley genotypes with concentrations between 318.2 and 682.3 mg Kg^{-1}. Barley variety of Giza133 has the highest significant ($P \leq 0.05$) concentration of free asparagine, almost 47 percent more free asparagine than Giza132. Mizukami et al. [9] found that asparagine concentration in 18 barley grains varies according to their varieties, and their asparagine concentrations ranged from 110 to 670 mg Kg^{-1}.

3.2.2.Reducing sugars and sucrose

Table 3 shows the concentrations (mg g^{-1}) of the reducing sugars (glucose, fructose, and maltose) and the sucrose in the three hulled barley varieties of seasons 2018/ 2019 and 2019/ 2020. The results showed evidence of significant ($P \leq 0.05$) differences in the type and concentrations of sugars in the three hulled barley genotypes. However, these differences were insignificant ($P \leq 0.05$) between seasons of 2018/ 2019 and 2019/ 2020. The barley variety of Giza123 has moderate amounts of glucose, fructose, and sucrose. However, the barley variety of Giza132 has the highest concentrations of glucose, and sucrose, and traces of fructose.

Table 3 Concentrations of free asparagine (mg Kg^{-1}) and sugars (mg g^{-1}) in the three hulled barley varieties of seasons 2018/ 2019 and 2019/ 2020.

Barley varieties	Free asparagine mg Kg^{-1}		Glucose mg g^{-1}		Fructose mg g^{-1}		Maltose mg g^{-1}		Sucrose mg g^{-1}	
Season	**2018/2019**	**2019/2020**	**2018/2019**	**2019/2020**	**2018/2019**	**2019/2020**	**2018/2019**	**2019/2020**	**2018/2019**	**2019/2020**
Giza123	674.3±0.1[b]	670.1±0.6[b]	3.25±0.17[b]	2.99±0.33[b]	2.22±0.07[a]	3.13±0.42[a]	ND	ND	2.50±0.01[b]	2.67±0.09[b]
Giza132	318.2±0.01[c]	322.3±0.2[c]	6.53±0.2[a]	6.66±0.16[a]	0.77±0.13[b]	1.00±0.11[b]	ND	ND	7.61±0.05[a]	7.61±0.16[a]
Giza133	682.3±0.17[a]	681.5±0.17[a]	ND	ND	ND	ND	55.41±0.37	57.43±0.22	ND	ND

ND: not detected

Data are presented as means ± SDM (n = 3) & means within a column with different letters are significantly different at $P \leq 0.05$

Meanwhile, the barley variety of Giza133 is the sole and superior variety of maltose. These differences in concentrations of sugars can be a consequence of genetic and environmental factors [10].

3.2.3.The correlations between free asparagine, reducing sugars and sucrose, and acrylamide

The reducing sugars have different capacities to produce acrylamide with free asparagine ranked as follows: glucose> fructose> maltose [10]. However, our results showed different potentials as shown in **Table 5** (**a**); glucose and sucrose showed strong negative correlations with free asparagine in the grains and acrylamide in their coffee substitutes at both seasons. Moreover, fructose and maltose showed very weak correlations with free asparagine in the grains and acrylamide in their coffee substitutes at both seasons. However, free asparagine concentrations in barley varieties showed strong positive correlations (r =0.8029, 0.8025) with acrylamide concentrations on their respective coffee substitutes at both seasons, respectively. Showing free asparagine concentration to be the main determinant of acrylamide-forming potential in different hulled barley genotypes. These observations are the same as those obtained [8, 22] in some rye varieties and in wheat grains [15, 31].

According to our results, the lowest significant ($P \leq 0.05$) free asparagine content and potentially the lowest acrylamide forming precursor is exhibited by the barley variety of Giza132.

3.2.4.The correlations between free asparagine and the genotype and the agronomic traits

The analysis of variance for the free asparagine accumulation of the three hulled barley genotypes (**Table 1**) showed that the effect of the genotype was highly significant ($P \leq 0.05$) at 12.96** and 12.65** in both seasons, respectively. Many cited researches indicated the significant influence of the genotype on the variations of free asparagine accumulation [8, 9, 31, 32]. This significant influence shows the potential for genotypic selection in addressing the acrylamide problem [10].

The data in **Table 2** showed that free asparagine accumulation in the three hulled barley genotypes did not vary significantly ($P \leq 0.05$) between the seasons of 2018/2019 and 2019/2020. This result is in contrast to earlier findings [31, 33, 34] who found significant effects between free asparagine accumulation and the year of cultivation. Also, the correlation coefficients (r) analysis in Table 5 (b) indicated that free asparagine accumulation in the hulled barley grains showed strong negative correlations or very weak correlations with all of the agronomic characteristics, except for the grain $spike^{-1}$ in 2019/2020 showed a strong positive correlation (r = 0.6135). A similar observation was reported in wheat [35], who found close relation (R^2 = 0.72) between free asparagine content and the grains per spike.

Besides the main objective of selecting from hulled barley genotypes of low acrylamide-forming potentials, high yield potential also plays an important role in the breeding of these genotypes.

Free asparagine accumulations showed very weak negative correlations and no correlations (**Table 5b**) with biological yield and grain yield at both seasons, respectively.

Accordingly, we combined the hulled barley samples of both seasons to perform all the physical analyses of roasted barley grains and their respective coffee substitutes in these combined samples to narrow the window of the number of samples and avoid repetition in a discussion section.

3.3. The correlations between roasting temperatures, moisture, physical properties of roasted barley grains and their respective coffee substitutes, and acrylamide formation

Table 4 represents the Physical properties of roasted barley grains and physicochemical properties of their respective coffee substitutes at different roasting temperatures. The mass loss (%) of the roasted barley grains significantly ($P \leq 0.05$) different between the varieties and increased from 16.1 to 34.3% as the roasting temperature increased. Our results are in agreement with those of [36], who found 14, 18, and 24% mass loss in light, medium, and deep roasted barley. The correlation results (**Table 5c**) indicated strong positive correlations (r = 0.9994/ 180° C), (r = 0.9559/ 200° C), (r = 0.9301/ 220°C) between acrylamide concentration and grain mass loss. This is mainly because of the loss in sugars taking place in the Millard reaction, pyrolysis, and acrylamide formation [37]. Vargas-Elias et al. [38] found a strong positive correlation (r^2 = 0.98) between the mass loss and the roasting index when the mass loss of coffee beans was above 0.8%.

The puffing index is an indicator of volume expansion in roasted kernels [39]. The puffing index significantly varied among the roasted barley varieties and increased from 1.07 to 1.79 with increasing roasting temperatures, being the highest significant ($P \leq 0.05$) at 220ºC 60 min^{-1} in Giza123. Sharma et al. [40]found that roasting temperature at 280ºC 20 S^{-1} leads to the expansion of different barley grains, because of the disorganization of starchy endosperm and expansion of cavities present in grains. The correlation results (**Table 5c**) showed strong positive correlations (r = 0.9076 at 200°C & r = 0.91427 at 220°C) between acrylamide concentrations and the puffing index, as the volume expansion increased, the pressure internal gases and Millard-reaction products increased [41].

The hardness of the grains is significant because it is an indicator of roasting degree; besides it influences the energy requirement for the milling process [42]. The hardness of the roasted barley grains significantly ($P \leq 0.05$) varied among the varieties (**Table 4**), and the force required to break the grains decreased significantly ($P \leq 0.05$) from 197.4 N to 30.23 N as the roasting temperature increased. Meaning that the medium roasted coffee substitute requires less energy for milling than very light and medium-light roasted coffee substitutes, respectively. A decrease in hardness upon roasting was also reported for wheat [43]; hulled barley [40]; coffee-like palm date seeds [44] and roasted coffee bean [45]. This influence could be ascribed to the fact that the grain expands, gelatinization of starch happens, and cracks develop, resulting in a decrease in hardness [42].

The correlation results (**Table 5c)** showed a weak negative correlation (r = -0.2271) at 200° C and moderate negative correlation (r = - 0.5209) at 220°C between hardness values and acrylamide concentrations, more likely a property of endospermic tissues, not dependent on the outer layers of the roasted barley grains [46].

The bulk density of roasted coffee substitute is an influencing factor for its packing, storage, and shipping; since it determines the volume occupied by a given mass of the coffee substitute [47]. The bulk density values of the roasted coffee substitutes (**Table 4**) are from 697.5 to 407 Kg m^{-3} for the moisture range of 55.3-15.2 g Kg^{-1}. The bulk density significantly ($P \leq 0.05$) decreases as the roasting temperature increases. Similar observation to this study about bulk density change with the degree of the roasting was reported in barley [40] from 591 to 478 Kg m^{-3} and from 830 to 770 Kg m^{-3} in oven-roasted wheat [48]. That is maybe due to the volume increase and simultaneous weight decrease of the grains with increasing roasting degree, determined by the rise in pressure of CO_2 gas, water vapour, and volatile substances [44]. Meaning that the medium-roasted coffee substitute needs more volume packaging and transportation costs than the medium-light coffee substitute and very-light roasted coffee substitute, however, it is less subjected to oxidation risk due to its lower moisture content [41].

Strong negative correlations (r = -0.9840 at 200°C) & (r = -0.9294 at 220°C) were found between the acrylamide concentrations and bulk density values (**Table 5c)**.

That is mainly because, during roasting, the reduction of bulk density is accompanied by volume increase and weight decrease of the grains determined by the increase of the water vapour, CO_2, and Millard- reaction products.

The colour parameters, especially the L^* value, have been adopted as a roasting indicator for roasted barley products in food factories [9].

Table 4 displays the colour values of coffee substitutes based on roasted barley varieties. The L^* and b^* values significantly ($P \leq 0.05$) decreased as the roasting temperature increased. However, the values of a^* were significantly ($P \leq 0.05$) increased up to 200°C and then significantly ($P \leq 0.05$) decreased at 220°C.

The coffee substitute based on Giza133 showed the highest significant darker and yellower colour at 220°C 60 min^{-1}.

Table 4 Physical properties of roasted barley grains and physicochemical properties of their respective coffee substitutes at different roasting temperatures.

Barley varieties	Roasting temperature °C / 60 min	*Roasted Barley Grains*			*Coffee Substitutes*							
		Mass loss %	Puffing Index	Hardness N	Bulk Density Kg m^{-3}	*L**	*a**	*b**	Roasting Index	Moisture g Kg^{-1}	Acrylamide in ground coffee substitutes µg kg^{-1}	Acrylamide in brewed coffee substitutes µg 70 mL^{-1}
Giza123	180±20°	22.21±1.3^{e}	1.10±0.01^{g}	197.4±6.8^{a}	662.5±2.5^{b}	70.61±0.8^{b}	5.63±0.2^{c}	21.34±0.35^{bc}	Very light	51.2±0.11^{a}	213.64±31^{b}	1.44±0.68^{b}
Giza132	180±20°	16.05±0.67^{f}	1.18±0.02^{f}	169.66±2.1^{b}	620.5±5.5^{c}	65.79±0.29^{c}	6.13±0.03^{b}	21.82±0.33^{ab}	Very light	55.3±0.15^{a}	< LOQ ±14^{c}	0.26±0.0^{c}
Giza133	180±20°	22.60±0.49^{e}	1.07±0.02^{g}	173.33±13.4^{b}	697.5±3.5^{a}	74.79±0.49^{a}	5.31±0.12^{d}	22.02±0.33^{a}	Very light	47.0±0.12^{ab}	235.8±17^{a}	1.65±0.15^{a}
Giza123	200±50°	25.38±1.1^{cd}	1.34±0.01^{de}	63.3±5.9^{f}	542.5±1.5^{e}	56.61±0.26^{e}	6.33±0.09^{a}	18.73±0.19^{d}	Medium light	37.9±0.08^{c}	222.57±34..3^{b}	1.56±0.24^{b}
Giza132	200±50°	24.15±0.94^{d}	1.31±7.4^{e}	127.36±7.4^{d}	563±3^{d}	48.5±0.55^{f}	6.23±0.01^{ab}	14.12±0.27^{e}	Medium light	46.7±0.17^{ab}	39.15±15.4^{c}	0.57±0.21^{c}
Giza133	200±50°	26.14±1.3^{c}	1.37±0.03^{d}	144.76±7.8^{c}	544.5±3.5^{e}	60.97±0.1^{d}	6.31±0.09^{ab}	20.90±0.5^{c}	Very light	34.9±0.06^{c}	242.8±12.6^{a}	1.70±0.25^{a}
Giza123	220±20°	34.25±0.3^{a}	1.79±0.06^{a}	30.23±1.1^{h}	407±10^{h}	40.62±0.26^{g}	3.73±0.16^{f}	7.18±0.19^{g}	Medium	16.9±0.1^{d}	239.09±21.6^{a}	1.67±0.2^{a}
Giza132	220±20°	26.80±0.26^{c}	1.42±0.02^{c}	98.37±12.4^{e}	515.3±7.3^{f}	41.28±0.23^{g}	4.41±0.08^{e}	7.98±0.26^{f}	Medium	29.4±0.04^{bc}	192.79±34.2^{b}	1.35±0.2^{b}
Giza133	220±20°	28.32±0.46^{b}	1.51±0.03^{b}	44.9±6.6^{g}	493±3^{g}	39.84±0.54^{h}	4.38±0.12^{e}	7.71±0.09^{f}	Medium	15.2±0.07^{d}	181.8±44^{bc}	1.27±0.23^{c}

*L** =lightness (zero = black, 100 = white), *a** (-*a** = *greenness*, +*a** = redness) and *b** (-*b** = blueness, +*b** = yellowness), LOQ: The Limit of Quantification = 10 µgkg^{-1}, portion size in brews: 7 g of coffee substitute / cup (70 mL).

Data are presented as means ± SDM (n = 4) & means within a column with different letters are significantly different at $P \leq 0.05$.

Table 5 (a) Correlation coefficients (*r*) between free asparagine, sugars, and acrylamide.

	Free asparagine 2018/2019	*Free asparagine 2019/2020*	*Glucose 2018/2019*	*Glucose 2019/2020*	*Fructose 2018/2019*	*Fructose 2019/2020*	*Maltose 2018/2019*	*Maltose 2019/2020*	*Sucrose 2018/2019*	*Sucrose 2019/2020*	*Acrylamide*
Free asparagine 2018/2019	1										
Free asparagine 2019/2020	0.999972	1									
Glucose 2018/2019	-0.87762	-0.88121	1								
Glucose 2019/2020	-0.90323	-0.90644	0.998412	1							
Fructose 2018/2019	0.161721	0.154268	0.331118	0.277443	1						
Fructose 2019/2020	0.189929	0.182513	0.303946	0.249803	0.999589	1					
Maltose 2018/2019	0.516571	0.523019	-0.8638	-0.83405	-0.76143	-0.74255	1				
Maltose 2019/2020	0.516571	0.523019	-0.8638	-0.83405	-0.76143	-0.74255	1	1			
sucrose 2018/2019	-0.95327	-0.95553	0.98143	0.990677	0.14397	0.115558	-0.75111	-0.75111	1		
sucrose 2019/2020	-0.94543	-0.94786	0.985916	0.993771	0.168649	0.140338	-0.76737	-0.76737	0.999688	1	
Acrylamide	0.80286	0.802539	-0.68569	-0.70823	0.16878	0.191228	0.380947	0.380947	-0.75342	-0.74619	1

Table 5 (b) Correlation coefficients (*r*) between agronomic characteristics and free asparagine.

	Days to heading		*Days to maturity*		*Plant height*		*Spike length*		*Grain/ spike*		*Number of spikes/m²*		*1000-grain weight*		*Biological yield*		*Grain yield*		*asparagine*	
	2018/2019	*2019/2020*	*2018/2019*	*2019/2020*	*2018/2019*	*2019/2020*	*2018/2019*	*2019/2020*	*2018/2019*	*2019/2020*	*2018/2019*	*2019/2020*	*2018/2019*	*2019/2020*	*2018/2019*	*2019/2020*	*2018/2019*	*2019/2020*	*2018/2019*	*2019/2020*
Days to heading 2018/2019	1																			
2019/2020	0.9894	1																		
Days to maturity 2018/2019	0.4474	0.3129	1																	
2019/2020	0.5303	0.4016	0.9955	1																
Plant height 2018/2019	0.9925	0.9997	0.3350	0.4230	1															
2019/2020	0.9676	0.9940	0.2072	0.2991	0.9912	1														
Spike length 2018/2019	0.9802	0.9986	0.2614	0.3519	0.9970	0.9984	1													
2019/2020	0.9265	0.9713	0.0779	0.1723	0.9655	0.9915	0.9827	1												
Grain/ spike 2018/2019	-0.7291	-0.8206	0.2860	0.1937	-0.8070	-0.8782	-0.8502	-0.9330	1											
2019/2020	-0.9150	-0.9639	-0.0486	-0.1433	-0.9574	-0.9872	-0.9768	-0.9996	0.9432	1										
Number spikes/m² 2018/2019	-0.7264	-0.8184	0.2897	0.1975	-0.8047	-0.8763	-0.8481	-0.9316	1.0000	0.9419	1									
2019/2020	-0.8346	-0.9057	0.1193	0.0245	-0.8955	-0.9466	-0.9271	-0.9805	0.9855	0.9859	0.9848	1								
1000-grain weight 2018/2019	-0.1157	-0.2586	0.8366	0.7808	-0.2359	-0.3627	-0.3101	-0.4810	0.7642	0.5065	0.7667	0.6438	1							
2019/2020	-0.3518	-0.4839	0.6798	0.6070	-0.4633	-0.5767	-0.5302	-0.6782	0.8972	0.6995	0.8989	0.8093	0.9705	1						
Biological yield 2018/2019	-0.5280	-0.6456	0.5233	0.4401	-0.6275	-0.7252	-0.6857	-0.8088	0.9662	0.8257	0.9672	0.9085	0.9047	0.9807	1					
2019/2020	-0.2530	-0.3907	0.7520	0.6861	-0.3691	-0.4890	-0.4396	-0.5985	0.8467	0.6218	0.8487	0.7441	0.9902	0.9946	0.9552	1				
Grain yield 2018/2019	-0.6579	-0.7602	0.3792	0.2896	-0.7448	-0.8267	-0.7940	-0.8930	0.9951	0.9058	0.9955	0.9640	0.8242	0.9364	0.9869	0.8951	1			
2019/2020	-0.3758	-0.5063	0.6607	0.5864	-0.4859	-0.5975	-0.5519	-0.6969	0.9083	0.7177	0.9099	0.8241	0.9640	0.9997	0.9854	0.9916	0.9451	1		
asparagine 2018/2019	-0.8763	-0.7972	-0.8229	-0.8731	-0.8111	-0.7264	-0.7636	-0.6306	0.3092	0.6076	0.3055	0.4660	-0.377	-0.1426	0.0536	-0.2443	0.2138	-0.1171	1	
2019/2020	-0.8799	-0.8017	-0.8186	-0.8694	-0.8155	-0.7316	-0.7684	-0.6365	0.3163	0.6135	0.3127	0.4727	-0.370	-0.1351	0.0611	-0.2370	0.2211	-0.1096	1	1

Table 5(c) Correlation coefficients (*r*) between acrylamide, moisture, physical properties of roasted barley grains and their respective coffee substitutes.

180º C	*Mass loss*	*Puffing Index*	*Hardness*	*Moisture*	*Bulk Density*	*L**	*a**	*b**	*Acrylamide*
Mass loss	1								
Puffing Index	-0.97722	1							
Hardness	0.558505	-0.36974	1						
Moisture	-0.88817	0.965458	-0.11487	1					
Bulk Density	0.913877	-0.97922	0.173635	-0.99824	1				
*L**	0.909187	-0.97685	0.162392	-0.99885	0.999935	1			
*a**	-0.94127	0.991486	-0.24561	0.991166	-0.99729	-0.99639	1		
*b**	-0.17933	-0.03355	-0.91621	-0.2928	0.235527	0.246596	-0.1634	1	
Acrylamide	0.999434	-0.9838	0.530296	-0.90312	0.927012	0.922675	-0.95209	0.6936	1
200ºC									
Mass loss	1								
Puffing Index	0.990831	1							
Hardness	0.068639	0.2028	1						
Moisture	-0.99008	-0.96201	0.072233	1					
Bulk Density	-0.88853	-0.81839	0.39675	0.944188	1				
*L**	0.999338	0.98526	0.032303	-0.99453	-0.90463	1			
*a**	0.837447	0.755929	-0.48775	-0.90594	-0.99485	0.856773	1		
*b**	0.997597	0.979089	-0.00065	-0.99743	-0.91818	0.999457	0.8733	1	
Acrylamide	0.955985	0.907577	-0.2271	-0.98773	-0.98405	0.966026	-0.960944	0.974017	1
220º C									
Mass loss	1								
Puffing Index	0.999156	1							
Hardness	-0.79793	-0.82202	1						
Moisture	-0.57126	-0.6045	0.950544	1					
Bulk Density	-1	-0.99923	0.799106	0.572862	1				
*L**	-0.14569	-0.18621	0.712564	0.895235	0.147614	1			
*a**	-0.98798	-0.98079	0.695147	0.437495	0.987672	-0.00903	1		
*b**	-0.98967	-0.99473	0.87609	0.683012	0.989951	0.285992	0.955612	1	
Acrylamide	0.930137	0.914265	-0.52085	-0.22996	-0.92942	-0.627786	-0.97573	-0.8679	1

The same observations were obtained for roasted malt at 235, 245, or 255°C for 10, 20, or 30 min [49]; for roasted barley grains sold in the Japanese market [13], and for drum-roasted barley at 180-240°C [9].

The *L** and *b** values showed strong positive correlations with acrylamide concentrations up to 200°C 60 min^{-1}, and then they showed strong negative correlations at 220°C. However, *a** values showed strong negative correlations with acrylamide concentrations at different roasting temperatures (**Table 5c**). That explains why the darker-coffee substitute of Giza133 contains much lower amounts of acrylamide than its respective lighter-colour coffee substitute. A similar correlation to this study was reported previously [9] in roasted barley grains, where the acrylamide level showed a good correlation with the *L** value, wherein the darker roasted barley grains with lower *L** values contained lower amounts of acrylamide as a result of deep roasting.

The Specialty Coffee Association of America (SCAA) [26] created a scale to assign the roasting index in coffee and coffee substitute products. SCAA scale is more often known as the Agtron number, and as a precise standard generally, used by manufacturers to determine the degree of roasting [50].

Table 4 & Figure 1 represent the roasting indexes corresponding to Agtron numbers and *L** values of coffee substitutes based on roasted barley.

There were no differences in roasting indexes between coffee substitutes of different roasted barley varieties at the same roasting temperature, except Giza133 showed a higher *L** value than those of Giza123 and Giza132, at 200ºC 60 min^{-1}. The *L** values of the roasted coffee substitute at 180°C were 65.79-74.79, corresponds with the Agtron *N0.95* and roasting degree of very light according to SCAA classification. Meanwhile, the *L** values of the roasted coffee substitute at 200°C were 48.5-56.61, corresponding with the Agtron *N0.75* and roasting degree of medium-light according to SCAA classification. However, the *L** values of the roasted coffee substitute at 220°C were 39.84-40.62, corresponding with the Agtron *N0.55* and roasting degree of medium according to SCAA classification.

The moisture content is one of the universal criteria used to assess the quality of roasted coffee and coffee substitutes as well [50].

The moisture contents of barley coffee substitutes (**Table 4**) are from 55.3 to 15.2 g Kg^{-1}, and it decreases as the roasting temperature increases. The decrease in the moisture content was significant ($P \leq 0.05$) and higher in barley coffee substitutes of Giza123 (80.5 % loss of moisture content), and Giza133 (68 % loss), while it is much lower in coffee substitutes from Giza132 (46.8 % loss). This decrease in the moisture content could be due to the dehydration of the barley grains during roasting [51]. The results showed strong negative correlations (r = -0.9031 at 180°C), (r = -0.9877 at 200°C) between acrylamide concentrations and the moisture content (**Table 5 c)**. That is because moisture plays a significant role in acrylamide formation in foods during thermal processing. The moisture content regulates the physical characteristics and mobility of chemical components in food matrix. In addition, water alone affects the chemical route and the mechanism pathway for acrylamide formation [52].

Table 4 shows the acrylamide concentrations in different coffee substitutes (both ground and brewed) based on roasted hulled barley varieties. The acrylamide concentrations significantly ($P \leq 0.05$) increased as roasting temperatures increased, except for the coffee substitutes of Giza133 decreased shortly at 220°C (**Figure 2 c**). This observation is the same as that obtained previously [9] that the acrylamide levels in drum-roasted barley grains increased as the surface temperature accelerated, reaching a max of 180 to 240°C. Above this temperature, the acrylamide level decreased with continued roasting, exhibiting a bell-shaped curve. That phenomenon was more likely to occur when the rates of degradations exceed the rates of formations due to the reaction of acrylamide with other reactive species like melanoidins during roasting, or due to the thermal decomposition of acrylamide to simpler compounds, as a result of a deep roasting [53, 54].

The acrylamide concentrations of coffee substitutes showed strong positive correlations with different roasting temperatures (**Figure 2**). The same strong correlations were obtained previously [49], were likely due to that higher roasting temperature promotes pyrolysis and decarboxylation of free asparagine causing the change in the physicochemical structure of barley starch, resulting in the higher formation of Millard reaction products [53].

The acrylamide concentrations of ground and brewed coffee substitutes were from < LOQ to 242.8 µg Kg^{-1} and 0.26 to 1.70 µg 70 mL^{-1}, respectively. The coffee substitutes based on Giza133 have the highest significant ($P \leq 0.05$) acrylamide concentrations at 200°C, followed by Giza123 at 220°C.

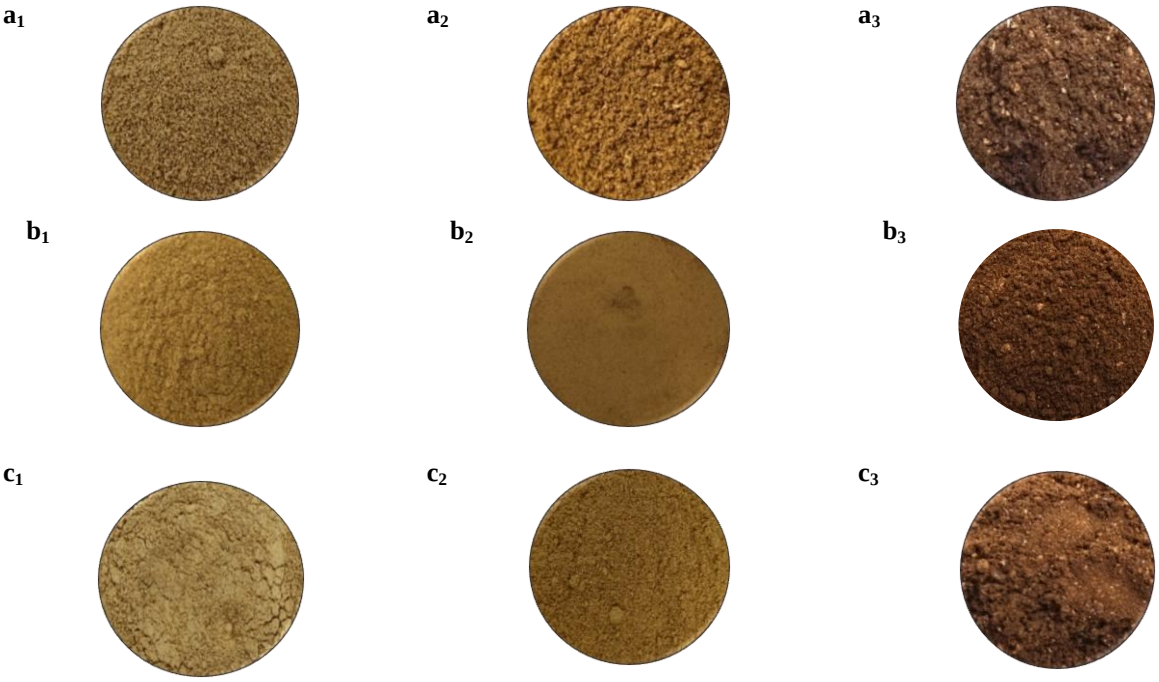

Figure 1 Color of coffee substitutes based on roasted hulled barley varieties with different roasting indexes. **(a)** Coffee substitutes of Giza123, **(b)** coffee substitutes of Giza132, and **(c)** coffee substitutes of Giza133. Subscript 1 denotes roasting temperature of 180±20º/ 60 min, subscript 2 denotes roasting temperature of 200±50º / 60 min, and subscript 3 denotes roasting temperature of 220±20º/ 60 min.

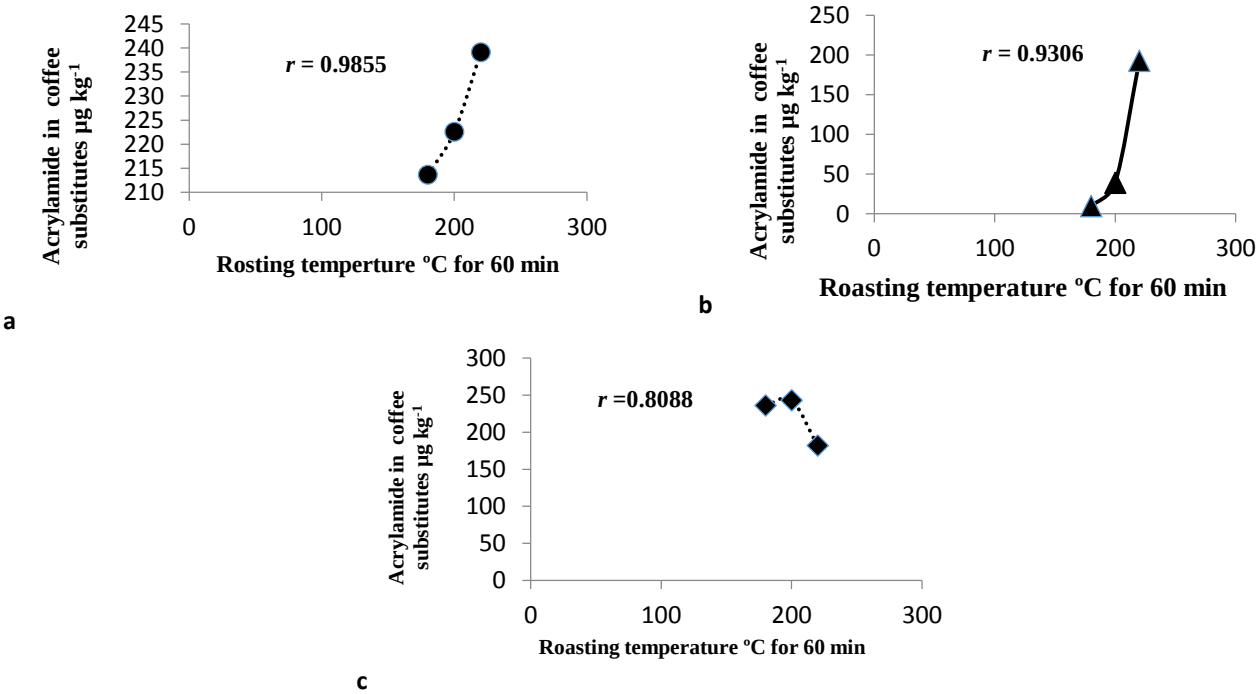

Figure 2 Correlation between roasting temperature (ºC) of hulled barley varieties and acrylamide (µg kg^{-1}) in their respective coffee substitutes. **(a)** hulled barley variety of Giza123, **(b)** hulled barley variety of Giza132, and **(c)** hulled barley variety of Giza133.

Meanwhile, the coffee substitutes based on Giza132 have the lowest significant ($P \leq 0.05$) acrylamide concentrations at 180º & 200°C. This result is because Giza132 has the lowest significant ($P \leq 0.05$) free asparagine content (**Table 3**) as a potential acrylamide-forming precursor. The acrylamide levels in very-light and medium-light roasts of Giza132 are at only 5% and 20% of the maximum level of medium-roasted one. In contrast, the level of acrylamide presents in the medium-roast of G133 corresponds only to 77% and 75% of those presented in its respective very-light roasts (180 and 200ºC).

Similar observations about the concentration of acrylamide are obtained by Mizukami et al. [13], who found a mean of 240±80 µg Kg^{-1} in 45 roasted barley products available at the Japanese market. However, Bertuzzi et al. [14] found a mean of 393±367 µg Kg^{-1} of the acrylamide in 22 coffee substitutes based on roasted barley sold in Italy, and about 27.3% of the samples exceeded the EC limit.

For the brewed coffee substitutes, Mizukami et al. [9] found the acrylamide concentration in a roasted barley product was 170 µg Kg^{-1} and the leaching rates of acrylamide in the brews were 1.5 and 16.3 µg L^{-1}, after 3 and 15 min of simmering, respectively. However, Mojska and Gielecinska [55] showed higher mean levels of 818±310 µ Kg^{-1} for the ground coffee substitute and 3.21±1.25 µg 160 mL^{-1} (4 g coffee substitute 160 mL) for their respective brew. These higher values because these substitutes contained mainly roasted barley with chicory root, the latter contained high levels of free asparagine (444-2786 mg Kg^{-1}) that result in higher rates of acrylamide formation [18, 56].

Our results indicated that none of the coffee substitutes based on roasted hulled barley varieties exceeded the benchmark level of 500 µg kg^{-1} for the presence of acrylamide in coffee substitutes exclusively from cereals as established by the European Commission [EC, 11]. The European Food Safety Authority [EFSA, 12] estimated a Benchmark Dose Lower Confidence Limit (BMDL10) of 0.170 and 0.430 mg kg^{-1} BW day^{-1} for neoplastic and neurological effects of acrylamide, respectively. In the present study, we have no available data about the consumption pattern and consumer preferences of coffee substitute brews to consider the acrylamide intake, but we could consider the overconsumption. So, we standardized a cup of 70 mL as a standard cup size of coffee in the Arabian and Turkish countries.

Based on our results, adult consumers who are fond of coffee substitutes might drink about five cups per day, corresponding to about 35 g of coffee substitute based on barley variety of Giza133 roasted at 200° C and an acrylamide intake will be of 0.1214 µg kg^{-1} BW day^{-1} (considering 70 kg of BW for an adult), resulting in a margin of exposure (MOEs) of 1400 and 3542 for neoplastic and neurological effects, respectively. These results confirm that the overconsumption of the very light (roasted at 200°C) coffee substitute based on Giza133 could imply an indicator to help risk managers and decision makers to set priorities for further action. Similarly, a health risk for children were found that add 1.5 g of roasted barley to milk daily: acrylamide intake will be 0.027 µg kg^{-1} BW day^{-1}, comparable to MOE values of 6343 and 16,045 for neoplastic and neurological developments, respectively [14].

4. Conclusions

Selection from hulled barley varieties or/ and genotypes with low acrylamide-forming potentials grown under regimen agronomic conditions for high yield potential besides controlling the roasting degree at the factory level could be used significantly to generate coffee surrogates with no excessive acrylamide and with proper physicochemical properties. In this study, the reducing sugars and sucrose levels in the three hulled barley genotypes showed no correlations to the amounts of acrylamide in their coffee surrogates. However, free asparagines accumulated in these genotypes are the main detriment of the final acrylamide levels in their respective coffee surrogates. Final concentrations of acrylamide in the surrogates were significantly influenced by the barley genotype, and roasting degree. The level of acrylamide presents in the medium-roast of Giza133 corresponds only to 77% and 75% of those exist in their respective very-light roasts. Moreover, the overconsumption of the latter exerts an indicator to help risk managers and decision makers to set priorities for further action.

5. Declaration of 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.

6. References

[1] Abdel-Haleem, A. M. H., Agwa, A. M., Mahgoub, S. A., & Shehata, W. M. Characterization of β-glucan gum for food applications as influenced by genotypic variations in three hulless barley varieties. *Journal of Food Science*, **85**(6), 1689-1698 (2020).

[2] FAOSTAT (2018). Statistics- Food and Agriculture Organization of the United Nations Rome, Italy. Retrieved from http://www.fao.org/faostat/en/#data/QC

[3] Baik, B. K., & Ullrich, S. E. Barley for food: Characteristics, improvement, and renewed

interest. Journal of cereal science, 48(2), 233-242 (2008).
[4] Griffey, C., Brooks, W., Kurantz, M., Thomason, W., Taylor, F., Obert, D., ... & Hicks, K. Grain composition of Virginia winter barley and implications for use in feed, food, and biofuels production. Journal of Cereal Science, 51(1), 41-49 (2010).
[5] Majcher, M. A., Klensporf-Pawlik, D., Dziadas, M., & Jeleń, H. H. Identification of aroma active compounds of cereal coffee brew and its roasted ingredients. Journal of agricultural and food chemistry, 61(11), 2648-2654 (2013).
[6] Yahya, H., Linforth, R. S., & Cook, D. J. Flavor generation during commercial barley and malt roasting operations: A time course study. Food chemistry, 145, 378-387 (2014).
[7] Schlörmann, W., Zetzmann, S., Wiege, B., Haase, N. U., Greiling, A., Lorkowski, S., ... & Glei, M. Impact of different roasting conditions on chemical composition, sensory quality and physicochemical
[8] Postles, J., Powers, S. J., Elmore, J. S., Mottram, D. S., & Halford, N. G. Effects of variety and nutrient availability on the acrylamide-forming potential of rye grain. Journal of Cereal Science, 57(3), 463-470 (2013).
[9] Mizukami, Y., Yoshida, M., & Ono, H. Acrylamide elution from roasted barley grains into mugicha and its formation during roasting. Food Additives & Contaminants: Part A, 33(2), 225-235 (2016).
[10] Žilić, S., Dodig, D., Basić, Z., Vančetović, J., Titan, P., Đurić, N., & Tolimir, N. Free asparagine and sugars profile of cereal species: the potential of cereals for acrylamide formation in foods. Food Additives & Contaminants: Part A, 34(5), 705-713(2017).
[11] EU (2017). European Commission.. European Commission Regulation 2017/2158 of 20 November. Establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. Off. J. Eur. Union OJ L304, 304.
[12] EFSA (2015). European Food Safety Authority.. European Food Safety Authority Panel on Contaminants in the Food Chain. Scientific Opinion on acrylamide in food. EFSA Journal, 13(6):4104, 321 pp. doi:10.2903/j.efsa.2015.4104
[13] Availableonline:www.efsa.europa.eu/efsajournal
[14] Mizukami, Y., Yoshida, M., Isagawa, S., Yamazaki, K., & Ono, H. Acrylamide in roasted barley grains: presence, correlation with colour and decrease during storage. Food Additives & Contaminants: Part A, 31(6), 995-1000 (2014).
[15] Bertuzzi, T., Rastelli, S., Mulazzi, A., & Pietri, A. Survey on acrylamide in roasted coffee and barley and in potato crisps sold in Italy by a LC–MS/MS method. Food Additives & Contaminants: Part B, 10(4), 292-299 (2017).
[16] Raffan, S., & Halford, N.G. Acrylamide in food: Progress in and prospects for genetic and agronomic solutions. Annals of Applied Biology, 175(3), 259-281 (2019).
[17] CAC (2009). Codex Alimentarius Comission Code of Practice for the Reduction of Acrylamide in Foods. CAC/RCP, 67-2009.
[18] Capuano, E., & Fogliano, V. Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. LWT-food science and technology, 44(4), 793-810 (2011).
[19] FoodDrinkEurope (2019). Acrylamide toolbox. Brussels, Belgium: FoodDrinkEurope. pp56-58.
[20] Anese, M. Acrylamide in coffee and coffee substitutes. Acrylamide in food: Analysis, content and potential health effects, pp181-195(2016).
[21] Curtis, T. Y., Postles, J., & Halford, N. G. Reducing the potential for processing contaminant formation in cereal products. Journal of Cereal Science, 59(3), 382-392 (2014).
[22] Curtis, T. Y., & Halford, N. G. Reducing the acrylamide-forming potential of wheat. Food and Energy Security, 5(3), 153-164 (2016).
[23] Curtis, T. Y., Powers, S. J., Balagiannis, D., Elmore, J. S., Mottram, D. S., Parry, M. A., ... & Halford, N. G. Free amino acids and sugars in rye grain: implications for acrylamide formation. Journal of Agricultural and Food Chemistry, 58(3), 1959-1969 (2010).
[24] EBC (1998). European Brewery Convention.. 3.2 "Moisture Content of Barely". European Brewery Convention, ed. Analytica-EBC. Fachverlag Hans Carl, Nürnberg, Germany
[25] Soares, C., Cunha, S., & Fernandes, J. Determination of acrylamide in coffee and coffee products by GC-MS using an improved SPE clean-up. Food additives and contaminants Part A, 23(12), 1276-1282 (2006).
[26] CIE (1932). Commission Internationale de l'eclairage. proceedings, Cambridge University, Cambridge.
[27] SCAA (2010). Specialty Coffee Association of America. Handbook series: Roast Color Classification system, www.scaa.org.
[28] Kearsey, M.J., & Pooni, H.S. (1996). The Genetical Analysis of Quantitative Traits. Chapman and Hall, London-UK.
[29] Hartleb, H., Meyer, U., & Lehmann, C. O. Resistance behaviour of common barleys to different isolates of Drechslera teres (Sacc.) Shoem. Archiv für Phytopathologie und Pflanzenschutz, 26 (3), 257-264 (1990).
[30] Zaki, K. I., & Al-Masry, A.I.S. Detection of biochemical genetic markers for net blotch disease resistance and barley grain yield. Egyptian journal of phytopathology, 36, 1-17(2008).
[31] Afiah, S. A. N., & Abdel-Hakim, A. M.. Heterosis, combining ability, correlations and path coefficient analysis in barley (Hordeum vulgare L.) under desert conditions of Egypt. In Proc. 1st Pl. Breed. Conf., Cairo Univ., Special Issue of Egypt. J. Pl. Breed , 3, 53-66 (1999).
[32] Curtis, T. Y., Muttucumaru, N., Shewry, P. R., Parry, M. A., Powers, S. J., Elmore, J. S., ... & Halford, N. G. Effects of genotype and environment

on free amino acid levels in wheat grain: implications for acrylamide formation during processing. Journal of Agricultural and Food Chemistry, 57(3), 1013-1021(2009).

[33] Nagamine, T., Kato, T., Sotome, T., Kaneko, S., & Nogata, Y. Genetic variation in functional constituents of barley grain and their accumulation by malting. Bulletin of the Tochigi Prefectural Agricultural Experiment Station (Japan), (2009).

[34] Weber, E. A., Graeff, S., Koller, W. D., Hermann, W., Merkt, N., & Claupein, W. Impact of nitrogen amount and timing on the potential of acrylamide formation in winter wheat (Triticum aestivum L.). Field Crops Research, 106(1), 44-52(2008).

[35] Curtis, T. Y., Powers, S. J., Wang, R., & Halford, N. G. Effects of variety, year of cultivation and sulphur supply on the accumulation of free asparagine in the grain of commercial wheat varieties. Food chemistry, 239, 304-313 (2018).

[36] Stockmann, F., Weber, E. A., Merkt, N., Schreiter, P., Claupein, W., & Graeff-Hönninger, S. Impact of row distance and seed density on grain yield, quality traits, and free asparagine of organically grown wheat. Agronomy, 9(11), 713 (2019).

[37] Dibaba, K., Tilahun, L., Satheesh, N., & Geremu, M. (2018). Acrylamide occurrence in Keribo: Ethiopian traditional fermented beverage. Food Control, 86, 77-82.

[38] Oosterveld, A., Harmsen, J. S., Voragen, A. G. J., & Schols, H. A. (2003). Extraction and characterization of polysaccharides from green and roasted Coffea arabica beans. Carbohydrate Polymers, 52(3), 285-296.

[39] Vargas-Elias, G.A., Correa, P.C., SOUZA, N.R., Baptestini, F.M., Melo, E.D.C. Kinetics of mass loss of Arabica coffee during roasting process. Journal of the Brazilian Association of Agricultural Engineering (Engenharia Agricola), 36(2), 300-308(2016).

[40] Hoke, K., Houška, M., Průchová, J., Gabrovská, D., Vaculová, K., & Paulíčková, I. Optimisation of puffing naked barley. Journal of food engineering, 80(4), 1016-1022(2007).

[41] Sharma, P., Gujral, H. S., & Rosell, C. M. Effects of roasting on barley β-glucan, thermal, textural and pasting properties. Journal of Cereal Science, 53(1), 25-30(2011).

[42] Nakilcioğlu-Taş, E., & Ötleş, S. Physical characterization of Arabica ground coffee with different roasting degrees. Anais da Academia Brasileira de Ciências, 91(2): e20180191 (2019).

[43] Mridula, D., Goyal, R. K., & Manikantan, M. R. Effect of roasting on texture, color and acceptability of pearl millet (Pennisetum glaucum) for making sattu. Int J Agric Res, 3(1), 61-68 (2008).

[44] Murthy, K. V., Ravi, R., Bhat, K. K., & Raghavarao, K. S. M. S. Studies on roasting of wheat using fluidized bed roaster. Journal of Food Engineering, 89(3), 336-342 (2008).

[45] Fikry, M., Yusof, Y. A., Al-Awaadh, A. M., Rahman, R. A., Chin, N. L., Mousa, E., & Chang, L. S. Effect of the roasting conditions on the physicochemical, quality and sensory attributes of coffee-like powder and brew from defatted palm date seeds. Foods, 8(2), 61(2019).

[46] Hidayat, D.D., Indriati, A., Andriansyah, C.E., Rahayuningtyas, A., Sudaryanto, A. Changes of Some Engineering Properties of Coffee Beans Due to Roasting Process. Asian Journal of Applied Sciences, 8(1):12-21(2020).

[47] Holopainen-Mantila, U. (2015). Composition and structure of barley (Hordeum vulgare L.) grain in relation to end uses. VTT Science.

[48] Clarke, R.J. Appendix 1: International Standards Organization (ISO). Coffee: Recent Developments, 235-237(2001).

[49] Schoeman, L., & Manley, M. Oven and forced convection continuous tumble (FCCT) Roasting: Effect on physicochemical, structural and functional properties of wheat grain. Food and Bioprocess Technology, 12(1), 166-182(2019).

[50] Kim, Y., Lee, Y. C., & Kim, K. O. Optimum roasting and extraction conditions and flavor characteristics of roasted malt extract. Cereal chemistry, 75(3), 282-288(1998).

[51] Bolek, S., & Ozdemir, M. Optimization of roasting conditions of Pistacia terebinthus in a fluidized bed roaster. LWT, 80, 67-75(2017).

[52] Somporn, C., Kamtuo, A., Theerakulpisut, P., & Siriamornpun, S. Effects of roasting degree on radical scavenging activity, phenolics and volatile compounds of Arabica coffee beans (Coffea arabica L. cv. Catimor). International Journal of Food Science & Technology, 46(11), 2287-2296(2011).

[53] Gökmen, V., & Palazoğlu, T. K. Acrylamide formation in foods during thermal processing with a focus on frying. Food and Bioprocess Technology, 1(1), 35-42(2008).

[54] Bagdonaite, K., Derler, K., & Murkovic, M. Determination of acrylamide during roasting of coffee. Journal of agricultural and food chemistry, 56(15), 6081-6086(2008).

[55] Pastoriza, S., Rufián-Henares, J.Á., & Morales, F. J. Reactivity of acrylamide with coffee melanoidins in model systems. LWT-Food science and technology, 45(2), 198-203(2012).

[56] Mojska, H., & Gielecinska, I. Studies of acrylamide level in coffee and coffee substitutes: influence of raw material and manufacturing conditions. Roczniki Państwowego Zakładu Higieny, 64(3), (2013).

[57] Loaëc, G., Niquet-Léridon, C., Henry, N., Jacolot, P., Volpoet, G., Goudemand, E., ... & Tessier, F. J. Effects of variety, agronomic factors, and drying on the amount of free asparagine and crude protein in chicory. Correlation with the acrylamide formation during roasting. *Food Research International*, **63**, 299-305(2014).