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SCREENING OF POTATO GENOTYPES BASED ON GLUCOSE AND ASPARAGINES CONTENT TO MINIMIZE ACRYLAMIDE FORMATION IN POTATO CHIPS AND FRENCH FRIES

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ABSTRACT

Seven potato genotypes that are available in Bangladesh, were grown at the field laboratory under the Crop Botany Department, Bangladesh Agricultural University in 2014. Reducing sugars and free asparagine were determined at freshly harvested potato tubers and those after storing at 8 ^{0}C for 8 months. There was no significant variation of asparagine content in all genotypes of freshly harvested tubers. But a significant difference was found in reducing sugar content. The lowest was in the samples of the genotypes Cardinal and Rumanapakri, and the highest in Hagrai. The variety Diamant appeared to contain the lowest amount of reducing sugars after 8 months storage. The results showed that freshly harvested Cardinal, Rumanapakri and Diamant after storage produced less amount of acrylamide after frying as potato chips or French fries. It may be concluded that screening potato genotypes primarily on their reducing sugar contents could be useful tool to minimize acrylamide formation in potato chips and French fries. Further investigation is needed to find out the factors affecting reducing sugar and asparagine content in potato tubers.

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INTRODUCTION

Heating of glucose with asparagine yields acrylamide according to the Maillard reaction (Mottram et al. 2002) and the rate is strongly increasing with temperature increasing from 120°C to 170°C (Stadler et al. 2002). Acrylamide (plant's origin) is a neurotoxin to human (Tareke et al. 2002) and known as a carcinogen in experimental studies (Mucci and Wilson, 2008), and it is classified as a "probable human carcinogen" by the International Agency for Research on Cancer (IARC, 2002). In 2002, the Swedish National Food Administration detected high concentrations of acrylamide in common heated starch rich foods such as French fries (www.slv.se.). It has evoked great concern for the health effects of acrylamide to the public. Soon after, the World Health Organization published that the average daily intake of acrylamide for the general population was about 1 *µ*g/kg of body weight (bw) (FAO/WHO, 2005). The main precursors for this undesirable substance are reducing sugars and the amino acid asparagine (Stadler et al. 2004, Mottram et al. 2002, Weisshaar et al. 2002). Heating of reducing sugars (glucose and fructose) with asparagine yields acrylamide. Therefore, the accumulation of asparagines and reducing sugars in the harvested organs of plants had implications for food safety. Interestingly, asparagine accumulation can be caused by stresses like salinity, drought, mineral deficiencies, toxic metals and pathogen attack. Crop production by escaping the above mentioned challenges is practically impossible. In addition, asparagine may also be formed following the detoxification of cyanide.

Potato tuber (*Solanum tuberosum* L.) is rich in asparagine content (33-59% of the total free amino acids) (Eppendorfer and Bille, 1996). So, potato is very susceptible to acrylamide formation during heating at temperature above 120 °C for the preparation of several potato products such as potato chips, potato crisps, French fries, roasted and baked potatoes. However, reducing sugar (glucose), another important precursor of acrylamide is limiting in potato tuber at harvest. Interestingly, storage of potatoes at temperature below 8-10°C induces a strong increase in sugar contents. The phenomenon is commonly known as "low-temperature sweetening" (Coffin et al. 1987). Prolonged storage at low temperature markedly increases in glucose, fructose and sucrose. As a consequence, the potential of acrylamide formation at 120°C rose by a factor of 28 (Biedermann et al. 2002, Chuda et al. 2003).

Acrylamide is ubiquitous in the human diet, and more than one-third of the calories we take in each day come from foods with detectable levels of acrylamide (Petersen and Tran, 2005). The levels of acrylamide can exceed 1000 parts per billion in potato products (Lea and Azevedo, 2007). For comparison, the tolerance level for water set by WHO is 1 ppb. Estimated dietary acrylamide intake in populations has been calculated by national food administrations for several countries. For adults, estimated average intakes range from approximately 0.3 to 0.6 *µ*g/kg of body weight (bw)/day. Children and adolescents tend to eat more acrylamide on a per body weight basis. This may be due to a combination of children's higher caloric intake relative to body weight as well as their choice for higher consumption of certain acrylamide-rich foods, such as French fries and potato crisps (Dybing et al. 2005).

To date, modified processing methods has been the main approach used in the mitigation of the acrylamide problem. Decreasing the content of precursors may reduce the amount of acrylamide in the products. Keeping this point in mind, selection of genotypes should be done that contain low levels of asparagine to minimize acrylamide in the processed foods. Therefore, attention is turning to improving the raw material by decreasing the levels of sugars and/or free asparagine and thereby the risk of acrylamide formation. The aim of this study was to identify potato genotypes available in Bangladesh that have a lower potential for acrylamide formation during frying. Being a public health issue (especially child health concern), which is highly related to plant origin and glucose/asparagine ratio in the raw material, the present study is designed to minimize acrylamide problem by selecting potato genotypes. Potato genotypes with low reducing sugar as well as low asparagine content may be a more useful target for crop improvement nutritionally in future. To the best of my knowledge, it will be the first scientific investigation in Bangladesh.

METHODOLOGY

Experimentation

An experiment was carried out at the field laboratory, Department of Crop Botany, Bangladesh Agricultural University, Mymensingh (24° 75' N, 90°50' E and the elevation of the area is approximately 19 m

from the average sea level) during November 2014–March 2015. The plant materials are seven potato genotypes, namely Cardinal, Diamant, Rumanapakri, Fata pakri, Gutypakri, Tel pakri and Hagrai. The experiment was laid out in the Randomized Complete Block Design (RCBD) with three replications. The total number of plots was 7×3. The size of the unit plot was 6m × 1m. A spacing of 0.5 m was provided between the plots and 1.0 m spacing was provided among three blocks. The seed tubers were planted at a depth of 5 cm in the experimental plots on 15 November, 2014. A spacing of 60 cm × 30 cm was used and tubers were planted in rows. The soil along the rows of seed tubers were ridged up immediately after planting. All intercultural practices were done when necessary.

Sampling and data recoding

Data were recorded on different morphological, yield components and yield from 5 randomly selected sample plants. Data were recorded on different morphological, yield components and yield from 5 randomly selected sample plants. With the help of digital weight machine 100 g of potato flesh was weighed, dried in an electric oven at 80^0c for 72 hours until the weight become constant. It was then cooled and weighed. Percent moisture content was calculated according to the following formula:-

(%) Moisture = $\frac{IW - FW}{IW} \times 100$

Where,

IW= Initial weight of flesh, FW= Final weight of oven dried flesh

Then percentage of dry matter content of the flesh was calculated from the data obtained during moisture estimation using the following formula.

% Dry matter content = 100 -% moisture content

Estimation of water soluble carbohydrates (WSCs) in potato tuber

The WSCs in tubers were extracted and measured using anthrone method (Yemm and Willis, 1954) as described in Hossain et al. (2009, 2010 and 2011). The fresh tubers were chopped and oven dried and then milled to rough powder. The tuber powder was weighed and extracted once with 80% ethanol at 60°C for 30 minutes followed by 2 successive 15 minutes extractions with distilled water at 80° C. The extracts were combined and evaporated to dryness at 65°C. The dried carbohydrates were resolved in 5 mL distilled water. A fraction of the extract solution (about 1 mL) was taken in a Micro-centrifuge tube (1.5 mL) and charcoal powder was added to it. After mixing the powder and extract solution with a vortex (touch mixture), the solution was centrifuged at 5000 rpm for 5 min to make a clear solution. The clear solution was diluted 200 times with distilled water. Diluted solution (0.06 mL) was mixed with ice-cold anthrone reagent (3 mL). The mixture was heated for 10 min in a boiling-water bath and subsequently cooled with ice water. The absorbance of the reacted solution for standard and samples was measured with a spectrophotometer at 620 nm. The content of WSCs in the sample was calculated as mg WSCs per gram of tuber dry mass using regression equation.

Determination of reducing sugar

Reducing sugar content of potato tuber was determined by dinitrosalicylic acid method as described by Miller (1972).

Preparation of tuber extract

The fresh tubers were chopped and oven dried and then milled to rough powder. The tuber powder was weighed to 1g and dissolved with 70% ethanol and was kept for overnight and then filtered to collect the extract solution. The extracts were then evaporated to remove ethanol in a sand bath. After evaporating ethanol the volume of the extract was made up to 10 ml.

Reagent required

Dinitrosalicylic acid (DNS) reagent (1 g of DNS, 200 mg of crystalline phenol and 50 mg of sodium sulphite were placed simultaneously in a beaker and mixed with 100 ml of 1 % NaOH by stirring). 40% solution of Rochelle salt (It was prepared by dissolving 40 g of sodium potassium tartarate with 100 ml of distilled water in 100 ml volumetric flask).

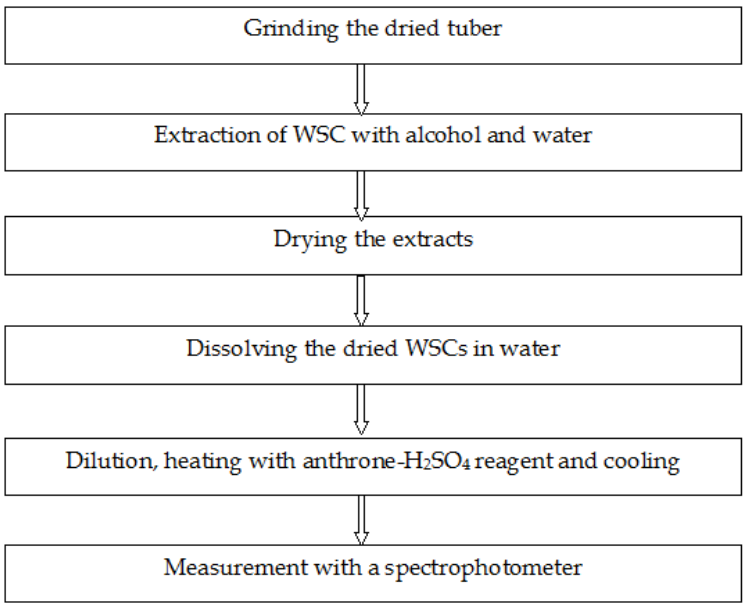

Figure 1. Protocol for measuring tuber WSCs by anthrone method

Estimation of reducing sugar of the potato tuber

Three ml of the extract was pipetted into a test tube and 3 ml of DNS reagent was added to the solution and mixed well. The test tube was heated for 5 minutes in a boiling water bath. After the color had developed, 1 ml of 40% Rochelle salt was added when the contents of the tube was still warm. The test tubes were then cooled under a running tap water. A reagent blank was prepared by taking 3 ml of distilled water and 3 ml of DNS reagent in a tube and treated similarly. The absorbance of the solution was measured at 575 nm in a colorimeter. The amount of reducing sugar was calculated from the standard curve of glucose.

Free Amino Acid Content

Ninhydrin assay (Friedman, 2004) was adapted for free amino acids determination including asparagines. Briefly, the lyophilized (freeze-dried) (approximately 100 mg) sample was deproteinized by stirring in 50 mL of a 0.3 *M* sulfosalicylic acid solution for 5 min. This was followed by centrifugation at 8200 × *g* for 5 min. An aliquot of the supernatant (250 µL) was used for free amino acids determination based on a color reaction using a buffered ninhydrin solution and a continuous measurement of the absorbance at 570 and 440 nm (De Wilde et al. 2005).

Statistical analysis

The data obtained for yield contributing characters and yield were statistically analyzed to find out the significance of the differences among the treatments, The mean value of all the characters for seven genotypes were calculated and the analysis of variance was performed by 'F' (variance ratio) test. The significance of difference among treatment means was evaluated by least significant difference (LSD) test at 5% and 1% levels of probability (Gomez and Gomez, 1984).

RESULTS

Morphological characteristics of stem

The variation of stem characteristics of the genotypes under study is shown in Table 1. Stem characters like stem colour, number, hairiness of the genotypes under study varied considerably. The stem color of the genotypes Cardinal and Diamant were green, whereas that of Fata pakri was green to light pinkish. On the other hand, the stem color of Gutipakri was light green, but the nodal region is pinkish. The stem color of the remaining genotypes was green to medium pinkish except Hagrai. Hagrai had pinkish stem. The main stems

of the genotypes Cardinal, Gutipakri, Rumanapakri and Diamant were few in number, while hagrai and Tel pakri were many in number. On the other hand, the number of main stem of the variety Fata pakri was least. The stems of genotypes Cardinal and Gutipakri were slightly hairy, whereas Diamant was moderately hairy. The remaining genotypes had very hairy stem (Figure 1).

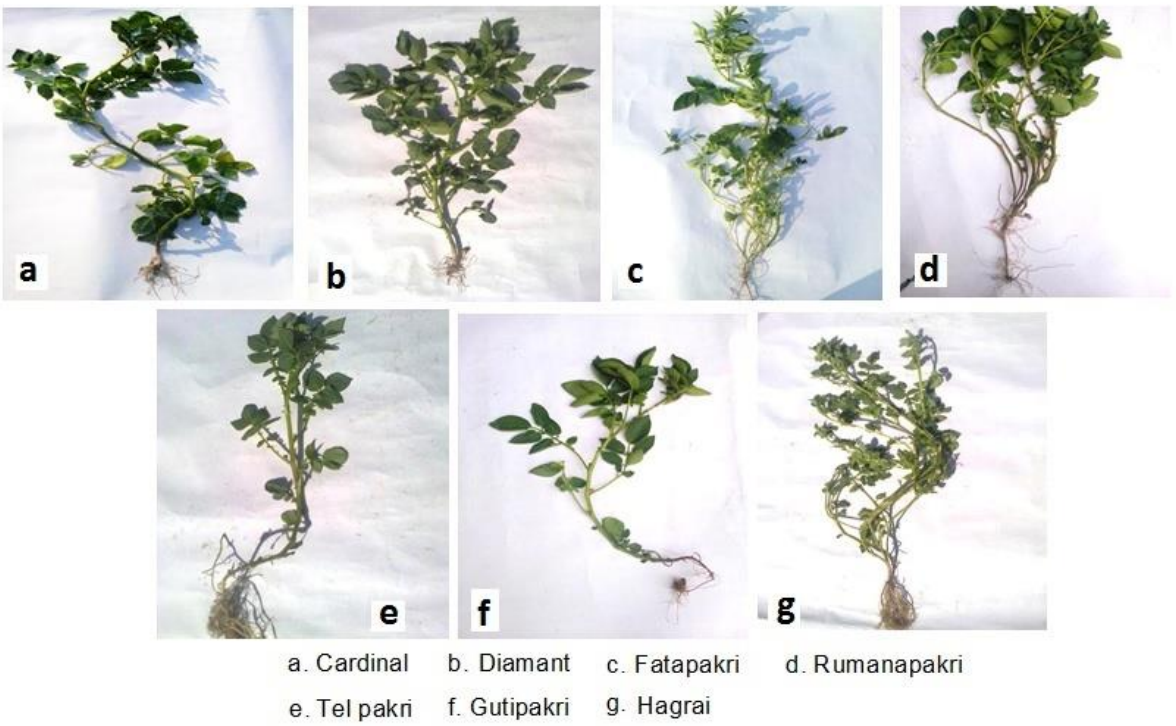

Figure 1. Photographic view of leaves and stem characteristics of seven potato genotypes

Table 1. Stem characteristics of seven potato genotypes

Potato Genotypes	Stem		
	Color	**Number**	**hairiness**
Cardinal	Green	Few	Slightly hairy
Diamant	Green	Few	Moderately hairy
Rumanapakri	Green to medium pinkish	Many	Very hairy
Fata pakri	Green to medium pinkish	Least	Very hairy
Gutipakri	Light green, pinkish at the nodal zone	Few	Slightly hairy
Tel pakri	Green to medium pinkish	Many	Very hairy
Hagrai	Pinkish	Few	Very hairy

Least = <5, Few = 5-9, Many = >9

Tuber characteristics

Table 2 is shown the tuber characteristics of seven potato genotypes under study. Remarkable variation was observed in different characters of tubers. The tubers of the genotypes Cardinal and Diamant were large in size whereas those of the remaining genotypes were medium in size. The tuber of the genotypes Fata pakri was round, while they were oval in Tel pakri. The tubers of the genotypes Hagrai were round irregular, while those of the Gutipakri and Rumanapakri were oval round. The tubers of the variety Cardinal were elongated. The skin of the tubers of Cardinal and Gutipakri were pinkish with creamy patches where eyes were more

pinkish but the base of the tubers of the Gutipakri show a characteristic concentration of creamy patches. In the case of Hagrai the skin of tubers were pinkish white. The skin of the tubers Diamant were creamy white with the exception of eyes of distal end of Diamant which was pink in color. In case of Rumanapakri and Fata pakri the skin of the tubers were red (Figure 2). But the eyes of Rumanapakri were deep red while those of Fata pakri were pink with white eyebrows. Skin of tuber of Tel pakri was redish with yellow patches.

The skin of the tubers of the genotypes Tel pakri and Diamant were smooth, whereas those of Cardinal and Fata pakri were rough. On the other hand, smooth and shiny skin was observed in the genotypes Gutipakri and Hagrai and medium smooth skin in Rumanapakri. The average number of eyes on the tuber of the genotypes Rumanapakri and Diamant were moderate, while those of the remaining genotypes were low. Eyes were not uniformly distributed in the genotypes Cardinal, Fata pakri and Diamant. On the other hand, eyes were more or less uniformly distributed in Hagrai and Rumanapakri.

Table 2. Tuber characteristics of 7 potato genotypes

Potato genotypes	Tuber		
	Size	**Shape**	**Skin color**
Cardinal	Large	Elongated	Pinkish with creamy patch. Eyes are more pinkish.
Diamant	Large	Round irregular	Creamy white. Eyes of distal end are pink color.
Rumanapakri	Medium	Oval round	Red. Eyes are more red than other position.
Fata pakri	Medium	Round	Red. Eyes are pink and eyebrows are white.
Gutipakri	Medium	Oval round	Pinkish with creamy patch. Pink color cone, at the eye and creamy patches conc. at the base.
Tel pakri	Medium	Oval	Red. Yellow patchs are present at the surrounding of eyes.
Hagrai	Medium	Round irregular	Pinkish white

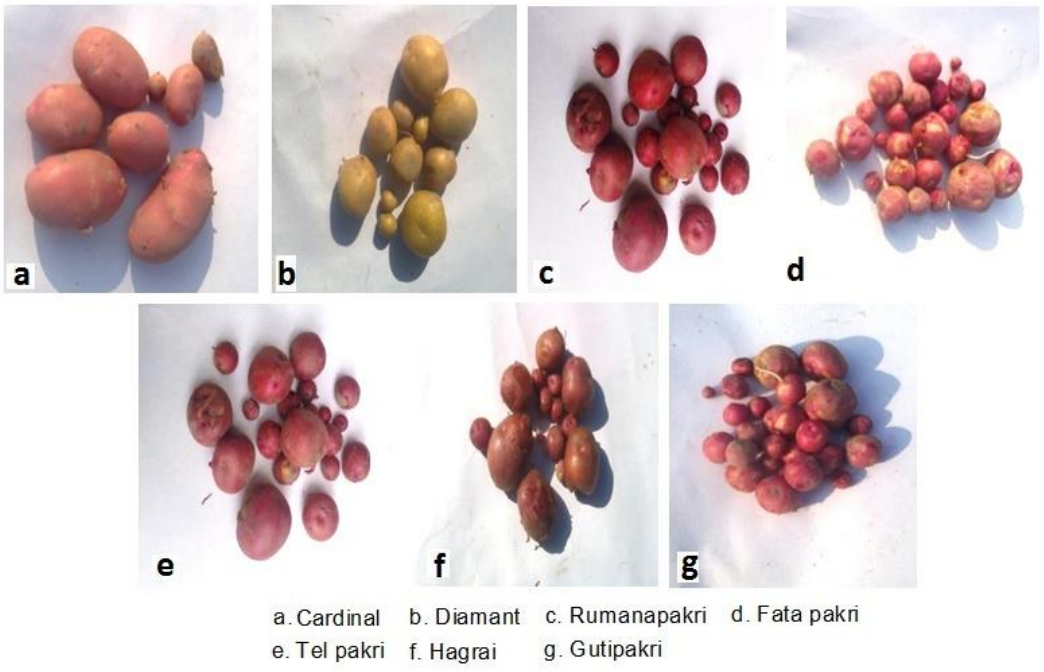

Figure 2. Photographic view of tuber of seven potato genotypes

Yield and yield components

Significant variation was observed among the experimental potato genotypes in the yield of tuber per plot and other yield components (Data not shown). Cardinal produced the highest yield of tubers (19.0 Kg plot^{-1}). The lowest yield of tubers was produced by Hagrai (8.3 Kg plot^{-1}). When the yield per plot was converted into yield per hectare, Cardinal was the highest yielded (26.49 tons ha^{-1}) followed by Diamant (24.36 t ha^{-1}), Gutipakri (19.76 t ha^{-1}), Tel pakri (15.8 t ha^{-1}), and Fata pakri (18.51 t ha^{-1}). The lowest tuber yield (10.23 t ha^{-1}) was obtained from the variety Hagrai (Figure 3).

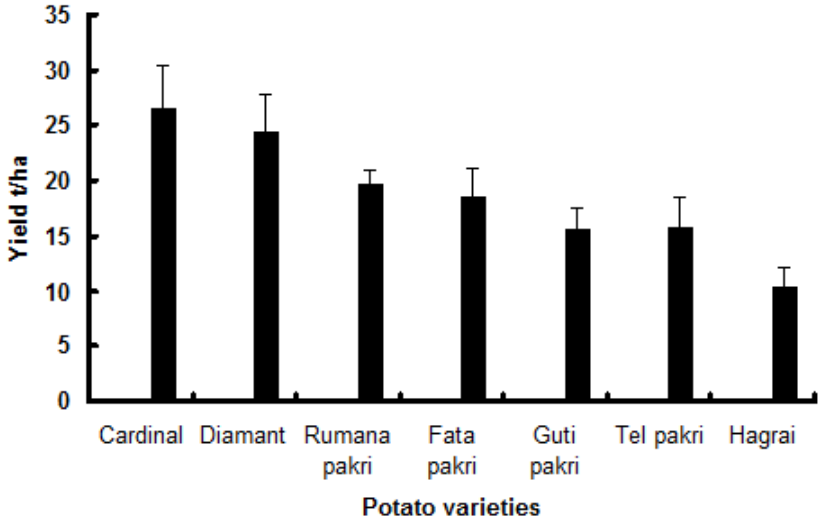

Figure 3. Yield of different potato genotyps available and cultivated in Bangladesh

The DM content, crude protein and total free amino acid content were determined for almost all of the potato samples (Table 3). Dry matter content was significantly varied among the 7 genotypes. Per cent moisture content was significantly greater in Cardinal and Diamant than the others. The concentrations of the assumed precursors of acrylamide (reducing sugars and free asparagine) were listed in Table 4 together with total soluble sugar. Reducing sugar content ranged from 0.094 to 0.482 mg g^{-1}, with the lowest values found in the samples of the cultivars Rumanapakri and Cardinal, and the highest in Hagrai. The highest sugar contents in the samples of the cultivar Tel pakri and the lowest in Diamant and gutipakri. Free asparagine content was statistically insignificant in the freshly harvested tubers of seven genotypes. Free asparagine was found at concentrations between 0.96 and 1.51 mg g^{-1}, and therefore was generally more abundant than reducing sugars after harvest.

Table 3. Dry matter, crude protein and total free amino acid content of freshly harvested potato tubers of 7 genotypes

Genoypes	Dry matter (%)	Crude protein (% of DM)	Total free amino acid (% of DM)
Cardinal	22.51 bc	9.48 c	3.42 a
Diamant	22.17 c	9.59 c	3.09 b
Rumanapakri	24.13 b	10.44 b	3.11 b
Fata pakri	24.49 b	10.54 b	3.23 b
Gutipakri	23.04 bc	10.27 b	2.89 c
Tel pakri	23.77 b	9.85 c	3.01 b
Hagrai	25.51 a	11.20 a	2.98 c
$LSD_{0.01}$	1.679	0.93	0.41

Table 4. Total soluble sugar, reducing sugar and asparagine content of freshly harvested potato tubers of 7 genotypes

Genotypes	Total soluble sugar (mg g^{-1} DM)	Reducing sugar (mg g^{-1} DM)	Asparagine content* (mg g^{-1} DM)
Cardinal	40.13 b	0.100 e	1.29
Diamant	20.57 e	0.141 d	1.48
Rumanapakri	40.74 b	0.094 e	0.96
Fata pakri	25.25 d	0.153 c	1.25
Gutipakri	22.14 e	0.154 c	1.51
Tel pakri	52.85 a	0.341 b	1.02
Hagrai	36.13 c	0.482 a	1.46
$LSD_{0.01}$	18.830	0.118	

In column, dissimilar letter differ significantly, and * indicates non-significant.

Table 5. Total soluble sugar, reducing sugar and asparagine content of potato tubers of 7 genotypes that stored at 8^0 C over 8 months

Genotypes	Total soluble sugar (mg g^{-1} DM)	Reducing sugar (mg g^{-1} DM)	Asparagine content* (mg g^{-1} DM)
Cardinal	55.36 b	0.462 c	1.35
Diamant	38.04 c	0.201d	1.50
Rumanapakri	68.60 a	0.763 ab	0.96
Fata pakri	31.44 d	0.525 bc	0.79
Gutipakri	42.32 c	0.653 b	0.97
Tel pakri	58.55 b	0.853 a	1.00
Hagrai	67.23 a	0.952 a	0.95
$LSD_{0.01}$	13.24	0.426	

In column, dissimilar letter differ significantly, and * indicates non-significant.

The main acrylamide precursors (reducing sugars and free asparagine) were determined after 8 months storing at at 8^0C and data were shown in Table 5. During storage time, asparagine concentrations of all genotypes did not change significantly whereas the reducing sugar concentrations of all genotypes of the tubers increased significantly. The variety Hagrai showed the highest value (0.95 mg g^{-1}) and was significantly different from all other genotypes. The variety Diamant appeared to contain the lowest amount of reducing sugars (0.201 mg g^{-1}). A significant difference of starch breakdown was observed in the studied genotypes, which was reflected by total soluble sugar content. Total soluble sugar content was significantly highest in the variety Rumanapakri, which was the lowest in Fata pakri.

DISCUSSION

Potato tubers contain substantial amounts of the acrylamide precursors free asparagines and reducing sugars (Becalski et al. 2004), which may explain the high concentrations of acrylamide in certain potato products. Potato tuber is rich in asparagine content (33-59% of the total free amino acids) (Eppendorfer and Bille, 1996). In the present study, asparagines content was greater than reducing sugar content in freshly harvested tubers of all genotypes (Table 4). Since asparagines content was identical, the susceptibility to acrylamide formation depends on the content of reducing sugar. Cardinal, Diamant and Rumanapakri could be

used for potato chips and French fries just after harvest (Table 4). Amrein et al. (2003) reported that the asparagine content did not correlate with acrylamide formation in french fries while the high correlation between the potentials os acrylamide and the reducing sugars.

Both the total soluble sugar and reducing sugar content increased during longer storage periods because of the degradation of starch in all genotypes under the present investigation (Table 5). This increase can be described as “senescent sweetening” (Burto 1989). Low temperature storage also influenced the content of asparagines in potato tubers. Asparagine oxidation occurred due to oxidative stress (H_2O_2) during storage (Tareke et al. 2009). Actually, low-temperature storage is very detrimental for acrylamide formation (De Wilde et al. 2005). In the present study, Cardinal and Diamant could be used for frying as potato chips and French fry after storage.

From these results, selection of the appropriate variety seems of extreme importance to control acrylamide formation during frying. Because acrylamide formation is strongly correlated with the amount of reducing sugars present in the raw material, it could be useful to screen potato genotypes primarily on their reducing sugar contents to select the genotypes suitable for frying. Moreover, fresh potatoes sold in retail should be labeled clearly that they are suitable for frying, because potatoes used for other applications are often stored at low temperatures to suppress sprouting. The main precursors for this undesirable substance are reducing sugars and the amino acid asparagine (Stadler et al. 2004, Mottram et al. 2002, Weisshaar et al. 2002). However, despite the high content of free asparagine in potatoes (Olsson et al. 2004), the limiting factor for the creation of acrylamide in potato products is the content of reducing sugars (Amrein et al. 2003, Becalski et al. 2004). It is well-known that the storage temperature influences the amount of reducing sugars in potato tubers, but this does not appear to be the case for asparagine (Olsson et al. 2004). It was also reflected in the present study (Table 4 and Table 5).

CONCLUSIONS

Reducing sugar content was significantly varied among the genotypes whilst asparagines content remained unchanged. The results revealed that (a) the content of reducing sugars is more important factor than the content of asparagine for acrylamide formation. So, reducing sugar content should be determined immediately before home and commercial processing of the potatoes; (b) to reduce cold-storage- induced sugar levels, potatoes should be reconditioned after storage.

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