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## Factors Influencing the Acrylamide Content of Fried Potato Products

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### Abstract

**BACKGROUND:** Acrylamide (CAS No. 79-06-1) is known to be a carcinogenic compound, and is classified as a Group 2A compound by the International Agency for Research on Cancer (IARC, 1994). Acrylamide can be generated during the browning process via the non-enzymatic *Maillard* reaction of carbohydrates such as reducing sugars and of amino acids such as asparagine, both of which occur at a temperature above 120°C. Potato tubers contain reducing sugars, and thus, this will affect the safety of processed potato products such as potato chips and French fries. In order to reduce the level of acrylamide in potato processed products, it is therefore necessary to understand factors that affect the reducing sugar content of potatoes, such as environmental factors and potato storage conditions, as well as understanding factors affecting acrylamide formation during potato processing itself.

**METHODS AND RESULTS:** Potatoes were cultivated in eight regions of Korea; For each of these different environments, soil physico-chemical characteristics such

as pH, electrical conductivity, total nitrogen, available phosphate, and exchangeable cation content were measured and correlations with potato reducing sugar content and potato chip acrylamide levels were examined. The reducing sugar content in potato during storage for three months was determined and acrylamide level in potato chip was analyzed after processing. The storage temperature levels were 4°C, 8°C, or 10°C, respectively. The acrylamide content of chips prepared from potatoes stored at 10°C or 20°C for one month was analyzed and the different frying times were 2, 3, 5, and 7 min.

**CONCLUSION:** This study showed that monitoring and controlling the phosphate content within a potato field should be sufficient to avoid producing brown or black potato chips. For potatoes stored at low temperatures, a reconditioning period (20°C for 20 days) is required in order to reduce the levels of reducing sugars in the potato and subsequently reduce the acrylamide and improve chip coloration and appearance.

**Key words:** Acrylamide, cv. Goun, Potato, Processing

### Introduction

Acrylamide (CAS No. 79-06-1) is known to be a

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carcinogenic compound and has been classified as a Group 2A compound by the International Agency for Research on Cancer (IARC, 1994). In April 2002, the Swedish National Food Administration and the University of Stockholm in Sweden demonstrated the presence of relevant amounts of acrylamide in several carbohydrate-rich foods that had been cooked at temperatures above 120°C (Swedish National Food Administration, 2002). The *de novo* generation of acrylamide in cooked food most likely occurs via a known non-enzymatic reaction, referred to as a *Maillard* reaction, but this has not been completely proven (Mottram *et al.*, 2002; Stadler *et al.*, 2002; Claus *et al.*, 2007).

The dispute over whether presence of acrylamide in cooked food is harmful has continued since the original report in 2002. Friedman (2003) reported that in animal toxicity studies, acrylamide has adverse effects, such as carcinogenicity and neurotoxicity, with a reported No Observable Adverse Effect Level (NOAEL) of 0.5 mg/kg/day. This level of acrylamide exposure cannot be achieved by eating processed potato products, and thus, it has been concluded that these products are not harmful to humans (Friedman, 2003). Mucci *et al.* (2003) reported that dietary exposure to acrylamide did not affect the risk of developing bowel, kidney, or bladder cancer. In contrast, Wilson *et al.* (2006), through a combination of *in vitro* and *in vivo* studies, provided evidence for a carcinogenic effect of acrylamide. Therefore, the question of whether a known carcinogenic compound, acrylamide, generated during food processing represents a potential carcinogenic risk to humans still needs to be resolved.

An important factor in the formation of acrylamide in cooked potato products is the content of reducing sugar in the potato tuber. Potato chip manufacturers in certain areas of Korea have expressed dissatisfaction with the color of chips prepared from freshly harvested potatoes with them having a brown coloration instead of the more preferred yellow or white color. The potato-tuber reducing sugar content is determined by the type of cultivar, the cultivation conditions, and the storage conditions. Therefore, in any particular case, it is necessary to analyze the relationship between the potato-field soil environment and tuber reducing sugar content, and to understand how the reducing sugar content is changed by different storage conditions.

Changes in temperature during storage may affect several different physiological processes in the potato tuber; especially important is the balance between starch content and reducing sugar content. The transformation of starch into reducing sugars such as glucose and fructose occurs in potato tubers stored at low temperatures (Isherwood, 1973). This phenomenon has been studied extensively over the years (Burton, 1965; Ewing, 1974; Sowokinos, 1990; Hertog *et al.*, 1997; Edwards *et al.*, 2002; Zommick *et al.*, 2014). The accumulation of reducing sugars at low temperature is referred to as "low temperature sweetening (LTS)" (Edwards *et al.*, 2002; Zommick *et al.*, 2014) and was noted to occur after prolonged storage of potatoes at 10°C (Burton, 1965) or below 8°C (Agle and Woodbury, 1968). However, Burton (1965) also noted that sugar accumulation differed in different potato varieties stored under the same conditions. Hertog *et al.* (1997) have developed a dynamic mathematical model based on physiological processes that models the accumulation of reducing sugars by potato tubers as a function of both time and temperature. They also observed that increases in the reducing sugar content varied both with cultivar type and with the season. Sowokinos (1990) have also described that when potato tubers are stored at low temperatures, the potatoes accumulate sugars including reducing sugars such as glucose, fructose, and sucrose, although in their study storage at temperatures from 8 to 12°C inhibited sugar accumulation. Ewing (1974) reported that generally the reducing sugar content in the potato tuber does not change during the first three days of storage at low temperatures (4–6°C). However, at later times, the reducing sugar content increased dramatically compared to the content in tubers stored at higher temperatures. Edwards *et al.*, (2002) examined the reducing sugar content in yellow-fleshed potatoes such as Yukon Gold, Red Gold, Saginaw Gold, Augsberg Gold, and A08223 stored at temperatures of 3.3°C, 8.3°C, and 10°C. Lower concentrations of sucrose, glucose, and fructose were observed in all cultivars stored at 10°C.

Potato tubers that have undergone low temperature sweetening after long-term storage can be reconditioned by then increasing the storage temperature up to 15–20°C (Pritchard and Adam, 1994). This reconditioning (i.e. increasing the storage temperature to 15–20°C for up to 28 days before processing) decreases the content of reducing sugars in the potato and thereby reduces the browning phenomenon that occurs during

frying. Similarly, Edwards *et al.*, (2002) have also demonstrated that the sugar content of potatoes decreases upon reconditioning.

The aims of this study were several-fold. First, we set out to explore the relationship between different soil conditions and the levels of reducing sugars in potatoes grown in these soils. Second, we sought to understand the effect of these different soil conditions on the acrylamide content in processed potato chips. Finally, we aimed to understand the effect of both potato storage conditions and processing conditions on potato chip appearance as well as acrylamide content.

## Materials and Methods

### Cultivation and collection of potato samples

Potatoes used in this study were cultivar cv. 'Atlantic' and cv. 'Goun', which were bred by the National Institute of Highland Agriculture, Rural Development Administration (RDA) in Korea. They were cultivated at Gangneung city (30 m above sea level) in the Gangwon province of Korea. The planting area was 80×25 cm. All cultivars were harvested 100 days after planting. For the part of the study examining the effect of different growing environments, only cv. 'Atlantic' was used. Potatoes were cultivated in three different areas of Gangwon at different altitudes namely, Gangneung city (30 m above sea level), Jinbumyeon (600 m above sea level) and Daegwallyeong (800 m above sea level). The other Korean potato fields were Habdeokmyeon in Dangjin city, Sinpyeongmyeon in Dangjin city, and Boseonggun in Jeoneup city. Potato chip preparation for analysis of acrylamide and measurement of potato reducing sugar content occurred 1-2 days after harvest.

### Analysis of soil physico-chemical characteristics

Analysis of soil physico-chemical characteristics was performed using soil and plant analytical methods developed by the National Institute of Agricultural Sciences (NIAS). Soil pH was measured by preparing a mixture of soil:deionized water (ratio 1:5) and measuring with a pH meter (Orion 3 Star, Thermo Electron Corp., USA). Soil electrical conductivity (EC) was determined in a mixture of soil:deionized water (ratio 1:10) using an EC meter (Orion 3 Star, Thermo Electron Cop., USA). Total nitrogen content (T-N) was measured using a Vario Max CN element analyzer

(Elementar Analysensysteme GmbH, Germany). Organic matter was analyzed by the Walkley-Black method (Walkley and Black, 1934). Available phosphate was analyzed by a method based on 'Analyses of soil and plant' by NIAS. After extraction of exchangeable cations with 1N ammonium acetate (pH7.0), the extracted cations were analyzed using ICP (Inductively Coupled Plasma, Optima 2100DV of PerkinElmer, U.S.A.).

### Storage condition of potato tubers

The storage temperature levels were 4°C, 8°C, or 10°C maintained for 100 days after potato harvest.

### Processing of potato chips

Three to five tubers from each treatment were sliced to give 1-mm thick slices and then washed in distilled water for 5 min. After removal of water from the slices, they were fried at 180°C for 3 min in soybean oil. For the analysis of acrylamide content, potato chips were crushed into a fine powder.

### Extraction of acrylamide from the fried potato chips

Potato chip acrylamide content was determined by the method of FDA (2003), Zyzak *et al.* (2003), and Zhao *et al.*, (2013) with some modifications. Potato chips (1 g) were homogenized in 5 mL of a 2M sodium chloride solution containing an internal standard (13C-acrylamide). The homogenate (5 mL) was centrifuged at 7,000 rpm for 10 min. The supernatant (1.5 mL) was then passed through an HLB reverse phase extraction cartridge (Waters Oasis HLB, 6 mL per 200 mg). For clean-up, the eluent was then passed through an MCX cation exchange extraction cartridge (Waters Oasis MCX, 3 mL per 60 mg) pre-equilibrated with 2 mL of MeOH, and the cartridge was then rinsed with 0.5 mL MeOH. The eluent was evaporated and reconstituted in 0.4 mL water. The sample was then filtered through a polyvinylidene fluoride (PVDF) membrane (0.45 µm, Millipore, Bedford, MA, USA) prior to LC/MS analysis.

### Analysis of acrylamide by LC/MS

Samples of processed potato chips were analyzed with a Waters 2695 LC (Milford, USA) interfaced with a Micromass ZQ mass spectrometer (Milford, USA) operated in positive electrospray ionization mode. The following parameters were used; Mobile phase: 0.1%

**Table 1. Physico-chemical characteristics of soil in the main Korean potato fields**

Area	pH (1:5)	EC (1:10) (dS/m)	O.M. (g/kg)	Av. P <sub>2</sub> O <sub>5</sub> (mg/kg)	T-N %	CEC (cmol <sub>c</sub> /kg)
Gangneung <sup>a)</sup>	6.0	0.3	48	639	0.2	8.8
Jinbu <sup>b)</sup>	5.7	0.2	24	819	0.1	7.8
Daegwallyeong <sup>c)</sup>	5.7	0.5	18	390	0.30	13.1
Habdeok <sup>d)</sup>	5.8	1.15	16	906	0.10	11.4
Sinpyeong <sup>e)</sup>	6.5	4.07	20	926	0.18	14.7
Jeongeup <sup>f)</sup>	7.3	2.44	33	849	0.19	9.4
Boseong <sup>g)</sup>	5.3	2.35	36	1178	0.23	17.3

pH : - log [H<sup>+</sup>], EC : Electrical Conductivity, O.M. : Organic Matter, Av. P<sub>2</sub>O<sub>5</sub> : Available Phosphate, T-N : Total Nitrogen, CEC : Cation Exchange Capacity

<sup>a)</sup> Gangneung city in Gangwondo, <sup>b)</sup> Jinbumyeon Pyeonchanggun Gangwondo, <sup>c)</sup> Daegwallyeongmyeon Ganwondo,

<sup>d)</sup> Habdeokmyeon Dangjin city Chungcheongnamdo, <sup>e)</sup> Sinpyeongmyeon Dangjin Dangjin city Chungcheongnamdo,

<sup>f)</sup> Jeongeup city Jeolabukdo, <sup>g)</sup> Boseonggun Jeolanamdo

formic acid, Column: Atlantis dc18 (2.1 mm×150 mm, 5 mm) (Waters; Milford, MA), Flow rate: 0.2 mL/min, LC/MS interface: direct (no split), Injection volume: 10 mL, Mode: SIM (selected ion monitoring, m/z 72), Dwell time: 0.5 s, Source temp: 150°C, Desolvation temperature: 350°C: Desolvation Gas: 500 L per hr.

#### Reducing Sugar analysis

Reducing sugar content was determined as described by Ohara-Takada *et al.*, (2005) with some modifications. Raw potato tubers (10 g) were homogenized in 20 mL of 85% (v/v) ethanol, and the sugars in the homogenate were extracted at 80°C for 1 h. After centrifugation at 10,000 × g for 20 min, the supernatant was dried under a vacuum, dissolved in distilled water and passed through a membrane filter (0.45 mm, Millipore, Bedford, MA, USA). The solution of mobile phase was 0.2M NaOH. The concentrations of reducing sugars (glucose and fructose) in the filtrate were determined using a Sugar Analyzer (SU-300, DKK-TOA, Japan).

#### Statistical Analysis

Each experiment in this study was repeated three times. Regression analysis was used to quantify the relationship between reducing sugar content and phosphate content. All mean comparisons were carried out using analysis of variance (ANOVA) with mean separation by DMRT at *P*<0.05 with the SAS software (Version 9.2 for Windows, Statistical Analysis Systems Institute Inc., NC, USA).

## Results and Discussion

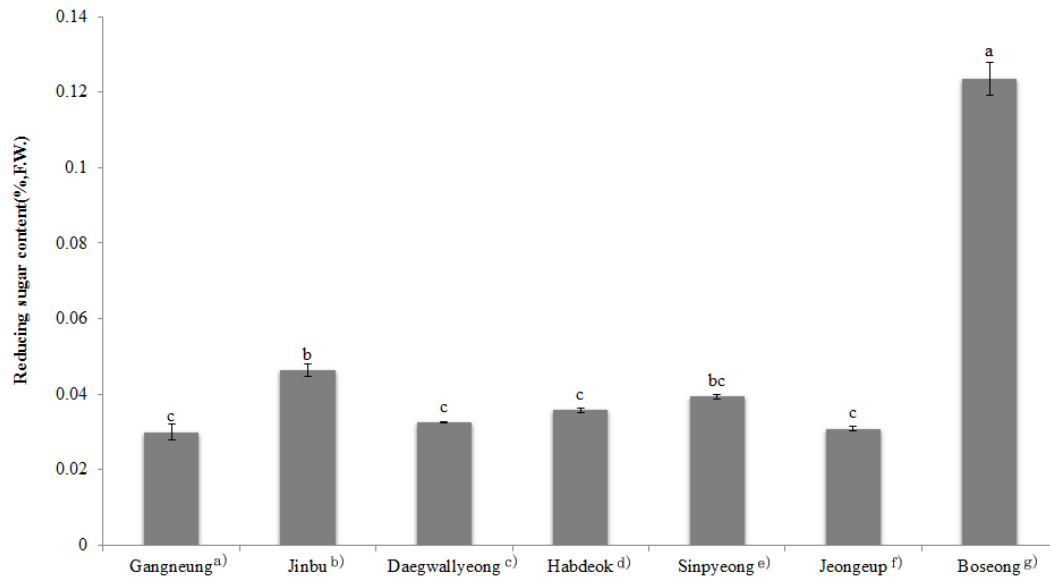
### Correlation between soil characteristics and reducing sugar in potato tuber

The physico-chemical characteristics of soil in the main potato fields in Korea used in this study are shown in Table 1. It was noted that the potato fields' phosphate contents were higher than recommended (Table 1).

In particular, the field in the Boseong area contained twenty times more phosphate and three times the EC than an experimental field belonging to the National Institute of Highland Agriculture.

The reducing sugar content of potatoes collected from these potato fields is shown in Fig. 1. The limit of acceptability in reducing sugar content with respect to color of processed potato products is 0.25% (Jeong, 1995). The reducing sugar content in potatoes collected from the Boseong area approached 0.25%, which suggested that these may produce processed potato products of inferior color with increased acrylamide content.

It is also thought that potato chip browning and blackening is related to the physico-chemical characteristics of the soil. Increased reducing sugar content could be affected by high soil phosphate levels (Vinci *et al.*, 2012). In our study, as shown in Fig. 2, a correlation analysis revealed that soil phosphate content was significantly positively correlated (coefficient of 0.88) with potato reducing sugar content. In particular, in the Boseong area of Jeonranamdo, the



**Fig. 1. Reducing sugar content in potato tubers from different potato fields in Korea.**

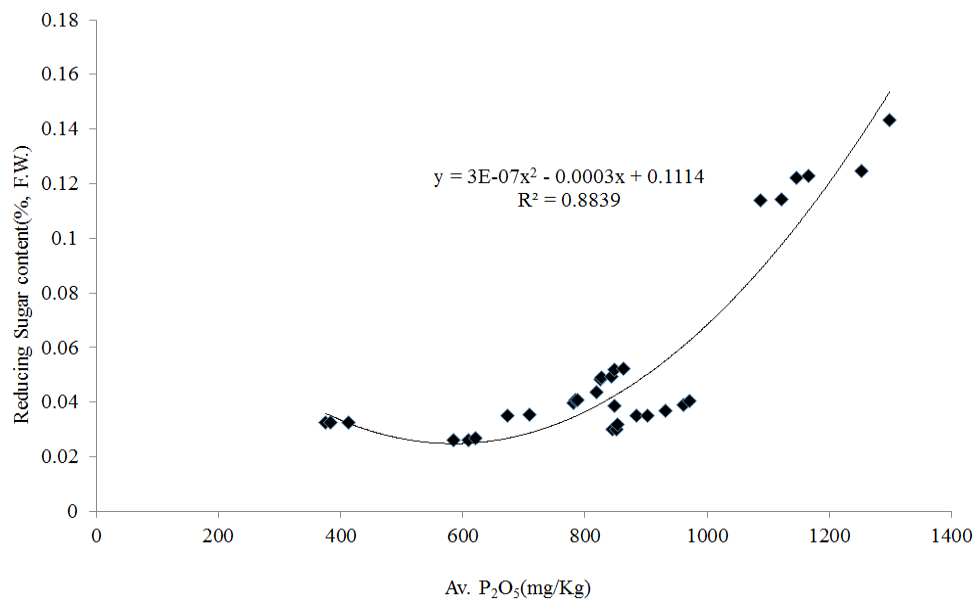
\* Means in a column of the same source by different letters on top of bar are significantly different at 0.05 significance level by Duncan's multiple range test.

† The bars represents standard error (n=3)

<sup>a)</sup> Gangneung city in Gangwondo, <sup>b)</sup> Jinbumyeon Pyeonchanggun Gangwondo, <sup>c)</sup> Daegwallyeongmyeon Ganwondo,

<sup>d)</sup> Habdeokmyeon Dangjin city Chungcheongnamdo, <sup>e)</sup> Sinpyeongmyeon Dangjin Dangjin city Chungcheongnamdo,

<sup>f)</sup> Jeongeup city Jeolabukdo, <sup>g)</sup> Boseonggun Jeolanamdo

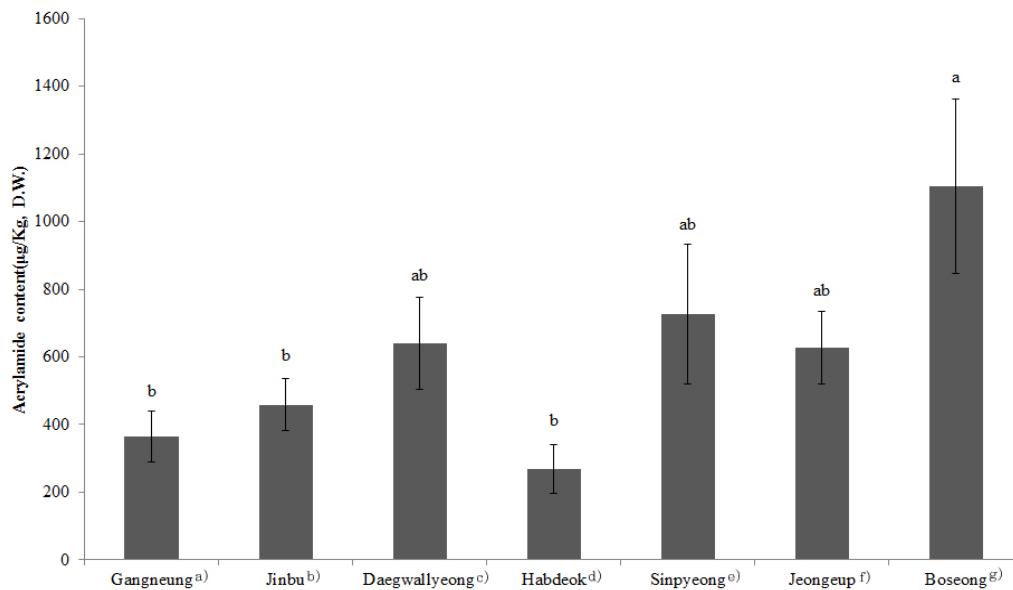


**Fig. 2. Relationship between potato reducing sugar content and soil phosphate content across different Korean potato fields.**

\* Av. P<sub>2</sub>O<sub>5</sub> : Available Phosphate

soil phosphate content was 1178 mg/kg and the potato reducing sugar content in potato was 0.12% (F. W.), which was the highest observed in any area of Korea studied. Potato chips prepared from Boseong area potatoes also had the highest acrylamide content

presumably due to their high potato reducing sugar content. Therefore, in order to reduce the acrylamide content in processed potato products farming methods must be used to keep the potato reducing sugar content low. Our data suggest that one way to



**Fig. 3. Acrylamide content in potato chips prepared from potatoes grown in different Korean potato fields.**

\* Means in a column of the same source by different letters on top of bar are significantly different at 0.05 significance level by Duncan's multiple range test

† The bars represents standard error (n=3)

<sup>a)</sup> Gangneung city in Gangwondo, <sup>b)</sup> Jinbunmyeon Pyeonchanggun Gangwondo, <sup>c)</sup> Daegwallyeongmyeon Ganwondo, <sup>d)</sup> Habdeokmyeon Dangjin city Chungcheongnamdo, <sup>e)</sup> Sinpyeongmyeon Dangjin Dangjin city Chungcheongnamdo, <sup>f)</sup> Jeongeup city Jeolabukdo, <sup>g)</sup> Boseonggun Jeolanamdo

achieve this is prevent excessive accumulation of phosphate in the potato field by removing any accumulated phosphate salts.

#### Effect of potato storage conditions on potato chip acrylamide content

We measured the acrylamide content of potato chips prepared from two different varieties of potatoes, which had been stored for three months at different temperatures (Table 2). In both potato varieties, the acrylamide levels increased from pre-storage levels and the magnitude of the increase was greater as the storage temperature decreased, being greatest at 4°C (Table 2). In both potato types, over the storage temperature range 4-10°C, the acrylamide levels were all in excess of 1000 µg/kg.

In parallel, we also measured the reducing sugar content in the two different potato varieties after storage at these different temperatures. The potato reducing sugar content mirrored the potato chip acrylamide content (Fig. 4, and compare with Table 2) being greatest at 4°C (Fig. 4).

Following reconditioning at 20°C for 20 days, the acrylamide content of all potato chips decreased significantly (Table 2), and similarly, the potato

**Table 2. Acrylamide content in cv. Atlantic and cv. Goun depending on storage condition**

Source		Acrylamide (µg/kg)
Before storage		
cv. Atlantic		635±10 <sup>b1)</sup>
cv. Goun		675±17 <sup>a</sup>
Storage temp.(C) for 3 month		
Atlantic	4°C	10540±60 <sup>b</sup>
Goun	4°C	14424±182 <sup>a</sup>
Atlantic	8°C	6449±205 <sup>a</sup>
Goun	8°C	3693±70 <sup>b</sup>
Atlantic	10°C	2743±38 <sup>a</sup>
Goun	10°C	1234±57 <sup>b</sup>
After reconditioning		
Atlantic	4°C	1057±45 <sup>a</sup>
Goun	4°C	849±100 <sup>b</sup>
Atlantic	8°C	900±220 <sup>ns</sup>
Goun	8°C	648±168 <sup>ns</sup>
Atlantic	10°C	804±163 <sup>ns</sup>
Goun	10°C	742±111 <sup>ns</sup>

<sup>1)</sup> Means in a column of the same source by different letters are significantly different at 0.05 significance level by Duncan's multiple range test

\* ns: non-significant

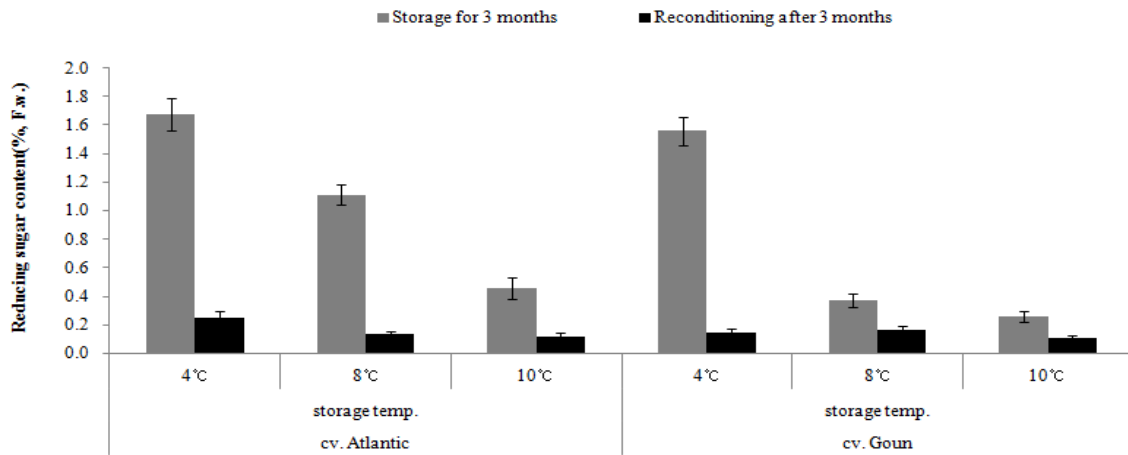


Fig. 4. Change of reducing sugar content by reconditioning in potato cultivars.  
 \* The bar represents standard error (n=3)

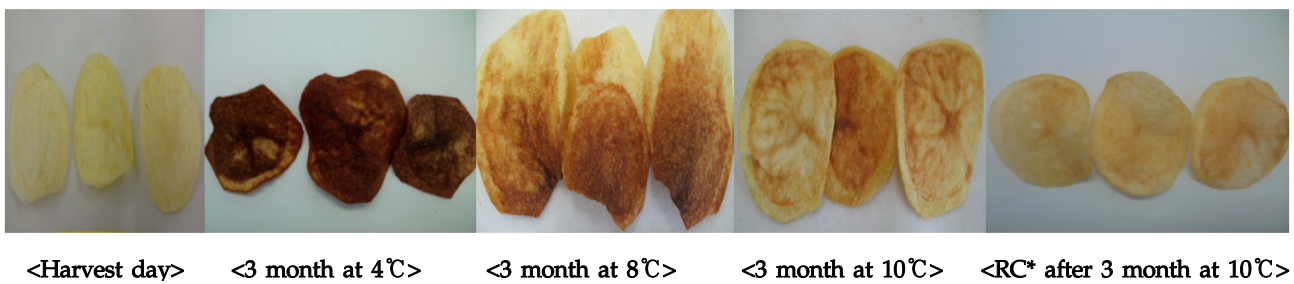


Fig. 5. Changes in chip color in cv. Goun after different potato storage conditions.  
 \*RC : Reconditioning

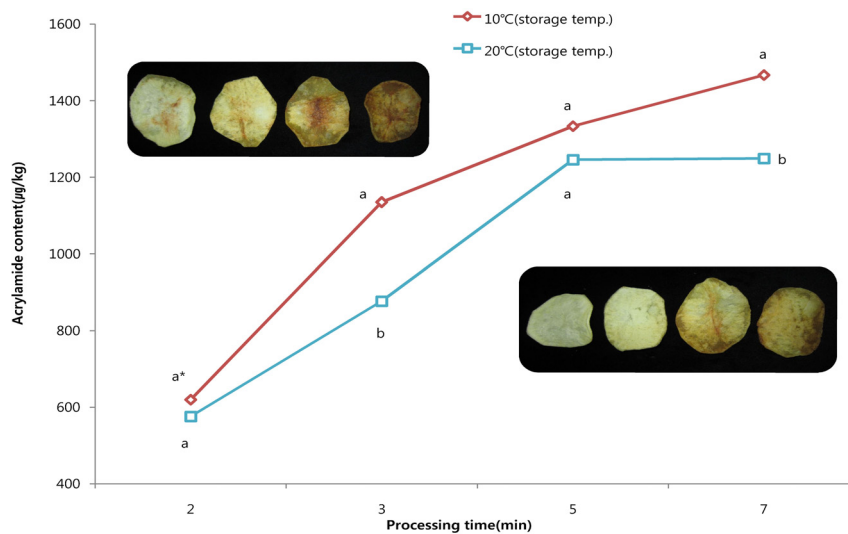


Fig. 6. Effect of potato storage at higher temperatures and processing time on potato chip appearance and acrylamide content.

\* Mean separation within columns by Duncan’s multiple range test at 5% level

† Processing temperature : 180°C, storage period of processed potato: 1 month

‡ The order of potato chip images from left to right are 2, 3, 5, and 7 min frying time respectively

reducing sugar content decreased significantly (Fig. 4). The cv. Goun potato chip color became dark brown

or black when the potato was processed directly following storage at low temperatures. (Fig. 5). The

darkening of the potato chip correlated with storage temperature, with the darkest chips being seen with potatoes stored at 4°C. Reconditioning of cv. Goun improved potato chip color and lightness, and in all cases, the acrylamide content fell below 1000 µg/kg. (Table 2 and Fig. 5). The effect of reconditioning on cv. Goun at 4°C was much better than that of cv. Atlantic since the acrylamide content of cv. Goun could be reduced more than cv. Atlantic (Table 2). Therefore, we conclude that, following cold storage, reconditioning (20°C for 20 days) is required to produce a low acrylamide content in the processed potato product and that the storage temperature does not need to be as low as 4°C but can be as high as 10°C.

#### Changes in potato chip acrylamide content under different processing conditions

We examined the effect of cv. Atlantic potato storage temperature on potato chip acrylamide content and appearance over a range of different processing (frying) times. Fig. 6 shows the acrylamide content of chips prepared from potatoes stored at 10°C or 20°C for one month; the different frying times were 2, 3, 5, and 7 min. Potatoes stored at 20°C and fried for 3 min had an acrylamide content lower than the regulatory standard required by the Ministry of Food and Drug Safety (MFDS), which is 1000 mg/kg. Both the quality of chip color and the low levels of acrylamide in potatoes stored at 20°C for 1 month were so good that a reconditioning process is not be needed. However, this was not the case for potatoes stored at 10°C, these chip were darker in color and had an acrylamide content greater than the regulatory standard of 1000 µg/kg meaning that reconditioning would need to be applied.

In all cases, frying times in excess of 3 min resulted in dark colored chips, and therefore, under these conditions, the chip had lost their product value. Taeymans *et al.* (2004) reported changes in acrylamide content in French fries at different frying temperatures and frying times. The typical conditions were a frying temperature of 180-190°C for 2-4 min. The results presented by Taeymans *et al.* (2004) showed that acrylamide generation followed a non-linear rate of formation depending on the increased temperature. It has also been reported that there are differences in the level of acrylamide in processed food depending on blanching conditions and processing temperature and duration; there was also

a relationship between processed food color and acrylamide content (Pedreschi *et al.*, 2006). At high temperatures over 140°C, which is a temperature well above the typical frying range, the negative effect of overcooking becomes more noticeable. At lower frying temperatures and longer frying times, the acrylamide content in French fries was substantially reduced. However, other issues occur at lower frying temperatures, such as greater fat uptake and poorer texture (Taeymans *et al.*, 2004). Therefore, during processing of potatoes, long processing times and higher temperature should be avoided to produce a good quality processed product.

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