International Food Research Journal 22(3): 936-942 (2015)

Journal homepage: *http://www.ifrj.upm.edu.my*

# Effect of blanching and pectin coating as pre-frying treatments to reduce acrylamide formation in banana chips

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**Article history**

*Received: 3 August 2014*
*Received in revised form: 28 October 2014*
*Accepted: 20 November 2014*

**Keywords**

*Acrylamide*
*Blanching*
*Pectin coating*
*Acrylamide reduction*
*Banana chips*

**Abstract**

This research aims to reduce acrylamide formation in banana chips by blanching and pectin coating as pre-frying treatments. Blanching treatment was conducted at either 90 or 100°C for 1 min, while pectin solution for edible coating was fixed at the concentration of 2% (w/v). The impacts of pre-treatments on acrylamide content, physical and sensory properties of the products were analysed. Banana chips prepared without pre-treatment, served as control, contained 194.4 µg/Kg of acrylamide. Using pectin-based edible coating could reduce 33.0% of acrylamide content in the products, while the reduction values were 20.1 and 53.4% by the pre-treatments of blanching at 90 and 100°C, respectively, compared to that of control. The synergistic effect of blanching and pectin coating was observed and resulted in great acrylamide reduction up to 91.9 and 90.8%, for the samples pre-treated by edible coating following blanching at 90 and 100°C, respectively.



## Introduction

Since there is sufficient evidence of carcinogenicity in experimental animals, acrylamide is classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic to humans (Group 2A) (IARC, 1994). Acrylamide has been found at milligram per kilogram levels, in heat-treated foods such as potato chips and crisps, bread, cookies, and coffee (Skog and Viklund, 2014). This level was much higher than the maximum limit of 0.5 µg/L in drinking water set by World Health Organization (WHO, 2011). Moreover, the mechanism of acrylamide formation in processed food has been revealed by Mottram and Wedzicha (2002) and Stadler *et al*. (2002). Briefly, acrylamide is formed via the Maillard reaction as a result of the reaction between the amino acid asparagine and reducing sugars (glucose and fructose). In addition, the formation of acrylamide is favoured by heating at high temperature and low moisture. It is also recognized primarily as a surface reaction, which may be responsible for the fact that acrylamide in bread is mainly located in the crust, with very low or no amount in the crumb as well as for the high acrylamide content in potato chips, since one piece has essentially two surfaces with very little matter between them (IFST, 2012).

Fried banana chips have been one of the favourite snacks and widely consumed in Southeast Asian countries. It is rich in acrylamide precursor as reported by Daniali *et al.* (2013). Fresh banana contained asparagine in the range of 0.92-2.16 mg/g (fresh wt.), glucose in the range of 0.57-7.61 mg/g (fresh wt.), and fructose in the range of 0.70-4.39 mg/g (fresh wt.). Because of high temperature processing (i.e., deep fat frying) and the notable presence of acrylamide precursors, the acrylamide formation in banana chips may occur at a high level. In this sense, reducing acrylamide content in banana chips is an important issue.

Blanching is a unit operation prior to processing, in which fruits or vegetables are heated for the purpose of inactivating enzymes, modifying texture, preserving colour, flavour and nutritional value as well as removing trapped air. Water blanching is generally performed in hot water at the temperature ranging from 70 to 100°C (Reyes De Corcuera *et al*., 2004). The use of blanching treatment to reduce acrylamide content of fried potato strips has been studied by El-Saied *et al*. (2008). Blanching process with hot water at 70°C for 10 and 20 min as well as at 90°C for 5 and 10 min could reduce acrylamide content of potato strips in the range of 76.8–82.2%.

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Moreover, Pedreschi *et al.* (2011) have reported that the utilization of blanching combined with the application of asparaginase enzyme could reduce acrylamide content in potato chips.

As known, edible film/coating was widely used to preserve and enhance food quality in the late sixties. In fried food processing, edible coating could avoid excessive oil absorption and improve while maintaining the product crispiness (Varela and Viszman, 2011). Coating with hydrocolloids, such as tragacanth, carboxy methyl cellulose (CMC), guar gum and xanthan gum, could significantly reduce oil uptake of various food products such as fried potato chips, vegetables and cereals (Albert and Mittal, 2002; Akdeniz *et al*., 2006; Garmakhany *et al*., 2008). Interestingly, coating with some hydrocolloids could also reduce acrylamide formation in model systems and fried potato product (Zeng *et al*., 2010). In addition, they found that pectin (2%, w/w) might be the most favourable inhibitors for acrylamide formation in the fried potato products.

In contrast to chromatography and electrophoretic methods, enzyme-linked immunosorbent assay (ELISA) requires neither expensive equipment nor time-consuming sample preparation and allows for fast screening of numerous samples (Oracz *et al*., 2011). For this reason, ELISA method was used to evaluate the effects of blanching and pectin coating as pre-treatments to reduce acrylamide formation in banana chips. The presence of synergistic interaction between blanching and pectin coating were studied. Furthermore, the Impacts of pre-treatments on physical and sensory properties of the obtained products were also elaborated.

## Materials and Methods

*Materials*

Pectin and palm oil were purchased from Wako Pure Chemical Industries Ltd. (Japan) and PT. Inti Boga Sejahtera (Indonesia), respectively. Bananas (*Musa paradisiaca* var. *sapientum* Ambon) were bought from local market (Bogor, Indonesia) with maturity index of 1 and 2 indicating the peel colour of green (Yanez *et a*l., 2004).

*Preparation of pectin coating solution*

Pectin coating solution was fixed at the concentration of 2% (w/w) and prepared by dispersing 40 g of pectin powder in 2 L of distillated water while stirring on a magnetic stirrer. The clear solution of pectin was obtained after 6 h of stirring.

*Preparation of banana slices, blanching process, pectin coating application, and frying process*

The bananas were peeled, washed with tap water at room temperature, and then sliced with the thickness of 2 mm. The slices were divided randomly into 6 parts, that is, 1) as control, without pre-treatment applied, 2) pre-treated with pectin coating, 3) pre-treated with blanching at 90$^o$C for 1 min, 4) pre-treated with blanching at 90$^o$C for 1 min followed by pectin coating, 5) pre-treated with blanching at 100$^o$C for 1 min, and 6) pre-treated with blanching at 100$^o$C for 1 min followed by pectin coating, which were labelled as samples C, CP, B90, B90+CP, B100 and B100+CP, respectively.

The frying process was carried out in a thermostatically temperature controlled fryer (Cecilware Corporation; F210262; New York) with a capacity of 4 L of oil. All pieces were fried at 180$^o$C for 9 min in palm oil. The fried banana chips were treated with spinner to remove excessive oil from the surface of the products. Vacuum packer (DZQ-400/2D, Korea) was used to pack all samples for keeping their freshness prior to the analysis.

*Acrylamide analysis*

ELISA method was used to quantify acrylamide content in banana chips by using the Morinaga Acrylamide ELISA kit (Morinaga Institute of Biological Inc., Japan). The principle of this kit is by using an acrylamide derivative (3-CTBA; 3-[(2-carbamoylethyl)thio] benzoic acid) as a hapten since an antibody to acrylamide cannot be raised in animals by immunization simply using acrylamide. For this reason, acrylamide must be derivatized quantitatively to 3-CTBA using a large excess of 3-MBA (3-mercaptobenzoic acid) prior to ELISA. The antibody recognizes 3-CTBA specifically and does not cross-react with intact acrylamide or 3-MBA. Cross-reactivity was found to be only 0.6% and 0.3% for metacrylamide and acrylonitrile, respectively, the highest found among the structural analogs examined. The kit permits a sensitive and specific assay of acrylamide with a detection limit of 0.9 ng/mL (RSD=30%) and a quantification range of 3-200 ng/mL (RSD<20%). The results of ELISA are highly correlated with those of conventional methods (GC/MS/MS and LC/MS/MS) with $R^2$ = 0.99. All chemicals, buffer and solution used for this analysis were already included in the Morinaga Acrylamide ELISA kit. The procedure of the analysis was the same as that described in the Instructions for Morinaga Acrylamide ELISA Kit (Morinaga Institute of Biological Science (MIoBS), 2011). Percent reduction of acrylamide was calculated as:

$$\% \text{ Reduction of acrylamide} = \frac{(AAc - AAs)}{AAc} x100$$

where $AA_c$ = acrylamide content in control and $AA_s$ = acrylamide content in treated samples.

*Colour analysis*

Minolta Chroma Meter CR 400 (USA) was used to determine CIE $L^*$, $a^*$, $b^*$ values of banana chips samples. CIE $L^*$ $a^*$ $b^*$ is an international standard for colour measurement adopted by the Commission Internationale d'Eclairage (CIE) in 1976. L is the lightness component, which ranges from 0 to 100, and parameters a (from green to red) and b (from blue to yellow) are the two chromatic components, which range from -120 to 120 (Papadakis *et al*., 2000). The total colour difference to control (ΔE) was calculated with the equation:

$$\Delta E = \sqrt{(L*-L'*)^2 + (a*-a'*)^2 + (b*-b'*)^2}$$

where $L^{'*}$, $a^{'*}$ and $b^{'*}$ are $L^*$, $a^*$, and $b^*$ values of control (sample without pre-treatment).

Browning index represents the purity of brown colour and is considered as an important parameter associated with browning process (Palou *et al*., 1999). Browning index of the sample was calculated with the equation:

$$BI = \frac{100(x - 0.31)}{0.17}$$

where

$$x = \frac{(a* + 1.75L*)}{(5.645L* + a* - 3.012b*)}$$

*Texture analysis*

Hardness of samples was measured using a Texture Analyser (Model TA-XT2, Stable Micro System, UK) with a 50 N load cell. Samples were punctured with a rounded-end probe (P/0.25 s ball probe) and using the crisp fracture support rig. The crosshead speed was set to 1 mm/s. The hardness value of fried banana chips was defined as the force at maximum compression in the force–deformation curves.

*Sensory analysis*

Seventy untrained panellists were selected to evaluate the quality of the fried banana chips based on colour, flavour, crispness, taste and overall quality. The panellist rated the intensity of a given stimulus by making a mark on a horizontal line with 15 cm in length which corresponds to the amount of the perceived stimulus (Meilgaard *et al*., 1999). The ranges of the values were between 0 (extremely dislike) presented at the left end of the line and 15 (extremely like) presented at the right end of the line. The marks from the line scales were converted to numbers by measuring manually the position of each mark on each scale using a ruler.

*Statistical analysis*

All experiments were measured in triplicate, with batch preparation of banana chips as replication units. SPSS version 17 was used for analysis of variance. Differences in the acrylamide content and properties of banana chips were determined by Duncan's Multiple Range Test using $p < 0.05$ as a level of significance.

## Results and Discussion

*Acrylamide contents*

This present study focused on using blanching and pectin coating as pre-treatments to decrease acrylamide formation in banana chips. The results of acrylamide analysis in banana chips are presented in Figure 1.

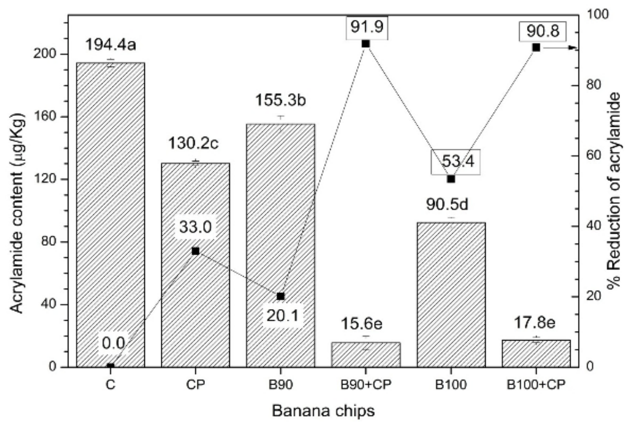


Figure 1. Acrylamide content in banana chips (Y1) and % reduction of acrylamide (Y2) where C = control (without pre-treatment), CP = coated with pectin; B90 = blanched at 90°C for 1 min, B90+CP = combination of B90 & CP; B100 = blanched at 100°C, B100+CP = combination of B100 & CP. Values with different letters above the bar were significantly different at $p < 0.05$.

It is shown that using blanching and pectin coating as pre-treatments could reduce significantly ($p<0.05$) the acrylamide content in banana chips. The highest acrylamide content of 194.4 µg/Kg was observed in control sample (C, without pre-treatment). Pre-treatment with pectin coating (CP) reduced 33.0% of the acrylamide content to 130.2 µg/Kg, while blanching at 90 and 100°C reduced 20.1 and 53.4% to 155.3 and 90.5 µg/Kg, respectively. These results were in good agreement with those reported by Pedreschi *et al.* (2004) and Zeng *et al.* (2010) who used blanching and pectin coating, respectively, to reduce acrylamide content in potato chips. Pedreschi *et al*. (2004) reported that sugars and asparagine might be leached out during blanching process, while Zeng *et al.* (2010) explained coating with pectin could reduce heat penetration from the oil to the banana slices during frying process and might interact with sugar, known as acrylamide precursor, resulting the reduction of acrylamide formation in banana chips.

Figure 1 shows that the acrylamide contents reduced drastically to 15.6 and 17.8µg/Kg in samples CP+B90 and CP+B100, respectively, prepared by pre-treatments combination of blanching and pectin coating. We applied Limpel's formula (Limpel *et al*., 1962) to analyze whether synergistic interaction between coating and blanching as pre-treatments was formed. Limpel's formula was described as $E_e = X + Y - (XY/100)$, in which Ee was the expected effect from the combined pre-treatments, while X and Y were the percentages of acrylamide reduction obtained by using each pre-treatment individually. Synergism was defined to be exhibited when the observed effect ($E_o$) was greater than the expected effect ($E_e$, as calculated by Limpel's formula). From Table 1, $E_o$ values were found greater than Ee values for both combinations of B90+CP and B100+CP, indicating that there was synergistic interaction between blanching and pectin coating treatments in this present study.

Table 1. Synergistic interaction of blanching at 90°C (B90) and 100°C (B100) with pectin coating (CP) pre-treatment in reducing acrylamide content of banana chips by using Limpel's formula

| Pre-treatment | | | % Reduction of acrylamide (single effect) | | % Reduction of acrylamide (combination) | |
|---|---|---|---|---|---|---|
| X | | Y | X | Y | $E_o$ | $E_e$ |
| B90 | + | CP | 20.1 | 33.0 | 91.9 | 46.5 |
| B100 | + | CP | 53.3 | 33.0 | 90.8 | 68.7 |

a)$E_o$ = observed value from experiment.
b)$E_e$ = expected value from Limpel's formula (X + Y –XY/100).
c)Synergism is present if $E_o > E_e$

*Colour properties*

Colour is an important quality factor of most food products influencing consumer acceptability and preference. The results of colour measurements are listed in Table 2 including CIE $L^*$, $a^*$, $b^*$ values and their derivatives, i.e., total colour difference to control (ΔE) and Browning Index (BI). It was found that colour of banana chips was mainly influenced by using blanching as pre-treatment, which could affect significantly ($p<0.0005$) the colour of the final products. The ΔE values were 0, 5.4, 44.9, 31.2, 43.5 and 29.3 for samples C, CP, B90, CP+B90, B100 and CP+B100, respectively. It was indicated that the colour of banana chips pre-treated with only pectin coating was close to that of control, with a minor ΔE value of 5.4, while relatively light colours were observed with larger ΔE values in those with pre-treatments including blanching. The same trend was detected in BI values of banana chips, with the values of 185.9, 176.1, 42.9, 70.2, 46.4 and 75.3 for samples C, CP, B90, CP+B90, B100 and CP+B100, respectively. This observation confirmed the previous statement that sugar and asparagine were leached out during blanching which thus restrained Maillard reaction and acrylamide formation. Nonetheless, immediate coating with pectin after blanching process might reduce the leach of sugar and amino acid from banana strips, resulting in relatively intense colour appearance indicated by lower ΔE values and higher BI values, compared to samples without the following combined treatment of coating.

Table 2. Colour properties of banana chips

| Banana chips | $L^*$ | $a^*$ | $b^*$ | ΔE | BI |
|---|---|---|---|---|---|
| C | 84.6 (1.1)a | -6.4 (0.4)a | 81.2 (0.3)a | 0.0 (0.0)a | 185.9 (5.2)a |
| CP | 85.0 (4.4)a | -9.1 (0.3)c | 80.1 (2.6)a | 5.4 (1.4)a | 176.1 (10.6)a |
| B90 | 92.5 (2.6)c | -7.8 (0.7)b | 37.2 (2.0)c | 44.9 (1.3)c | 42.9 (8.4)c |
| B90+CP | 91.0 (2.6)c | -8.8 (1.5)b | 50.9 (5.8)b | 31.2 (6.4)b | 70.2 (15.6)b |
| B100 | 92.4 (4.2)c | -7.2 (1.1)ab | 38.6 (3.8)c | 43.5 (4.8)c | 46.4 (3.9)c |
| B100+CP | 88.9 (0.8)b | -8.6 (0.8)b | 52.3 (1.5)b | 29.3 (1.3)b | 75.3 (2.3)b |

a) Means of 3 samples of banana chips with standard deviation in parentheses
b) Different letters within a column indicate significantly difference at $p < 0.05$ separated by Duncan's Multiple Range Test (DMRT)

Pedreschi *et al.*, (2006) reported a good linear correlation between the acrylamide concentration and lightness ($L^*$) and redness ($a^*$) values of potato chips with correlation coefficient ($r^2$) of 0.79 and 0.83, respectively. In our case, there was no good linear correlation between colour properties ($L^*$, $a^*$, $b^*$, ΔE and BI) and acrylamide contents in the samples, which might be ascribed to the presence of synergistic effect between blanching and pectin coating.

*Texture properties*

The textural attribute of crispness and crunchiness makes low moisture snack foods, such as potato chips, banana chips, tortilla chips, corn chips, and so forth, popular among consumers. Though it is difficult to define precisely, crispness and crunchiness are two crucial textural characteristics of low moisture foods (Jowitt, 1979). Researchers have shown that crispness and crunchiness are closely related. It has been suggested that the difference might be in the pitch of their respective sounds (Vickers 1984). Objectively, crispness and crunchiness is quantified as the maximum force (hardness) from the force–deformation curves where a lower hardness value corresponds with a higher crispness. Seymour and Hamann (1988) have investigated the relationships between descriptive sensory texture notes (crispness and crunchiness) and mechanical instrumental parameters of five low moisture foods. It was found that crisp products were mechanically weaker than crunchy products; more force was required to fracture a crunchy product.

In the current study, banana chips coated with pectin were most crunchy with the highest hardness, compared to uncoated samples (Figure 2).

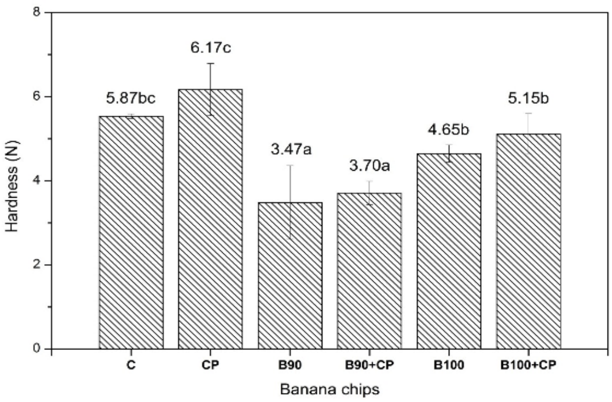


Figure 2. Hardness of banana chips: C = control (without pre-treatment), CP = coated with pectin; B90 = blanched at 90°C for 1 min, B90+CP = combination of B90 & CP; B100 = blanched at 100°C, B100+CP = combination of B100 & CP. Values with different letters above the bars were significantly different at $p < 0.05$.

This observation was in agreement with the finding of Seymour and Hamman (1988) who have reported that the products indicated as crunchy were mechanically stronger than those indicated as crisp. Conversely, banana chips pre-treated with blanching at 90°C showed the lowest hardness, i.e., the highest crispness, compared to other samples. The higher crispness of the blanched samples might be attributed to the softened structure or weakened cell walls in banana strips (Singthong and Thongkaew, 2009). Moreover, it was indicated that coating with pectin was favourable to protect the cellular structure of the banana tissue from damage during deep-fat frying.

Differently from previous studies that concerned about the crispness of banana chips (Singthong and Thongkaew, 2009; Sothornvit, 2011) and expected either higher crispness or lower hardness, this present study showed that crunchiness was a more important attribute to be taken into account since it was well correlated with the sensory analysis results. It was found that samples with higher hardness obtained higher acceptance scores. Hardness of banana chips had a significant positive correlation ($p < 0.05$) with texture acceptance score. This result indicated that generally consumers tended to prefer crunchy banana chips to crispy ones.

Table 3. Sensory properties of banana chips

| Banana chips | Colour | Appearance | Aroma | Taste | Texture | Overall |
|---|---|---|---|---|---|---|
| C | 11.4 (2.3)a | 10.3 (2.7)a | 8.0 (2.5)a | 8.8 (3.0)a | 9.3 (2.5)ab | 9.0 (2.8)a |
| CP | 11.6 (2.1)a | 11.0 (2.5)a | 8.8 (2.6)a | 9.2 (2.8)a | 9.9 (2.1)a | 9.8 (2.2)a |
| B90 | 5.8 (2.8)b | 6.4 (3.2)b | 6.1 (2.9)b | 6.0 (3.0)c | 6.0 (2.2)c | 6.5 (2.6)d |
| B90+CP | 6.0 (2.9)b | 6.6 (3.1)b | 6.9 (3.1)b | 7.6 (2.8)b | 8.9 (2.0)b | 7.8 (2.5)b |
| B100 | 6.0 (2.7)b | 6.4 (3.5)b | 6.3 (2.7)b | 7.0 (2.9)bc | 7.9 (2.7)b | 6.9 (2.6)cd |
| B100+CP | 6.2 (3.0)b | 6.9 (3.3)b | 6.9 (2.9)b | 7.2 (2.9)bc | 8.6 (2.8)b | 7.6 (2.6)bc |

a) Means of 70 consumer panelists; numbers in parentheses refer to standard deviation. Line scale rating test was used from minimum 0 = very dislike extremely until maximum 15 = very like extremely
b) Different letters within a column indicate significantly difference at $p < 0.05$ separated by Duncan's Multiple Range Test (DMRT)

*Sensory properties*

The sensory analysis results of banana chips are presented in Table 3. The sensory properties of banana chips in all attributes were affected significantly ($p<0.005$) by using blanching as either single or combined pre-treatment, while no significant influence was found for those pre-treated with only pectin coating. The application of blanching process lowered the preference scores in colour, appearance, and taste of banana chips, because it reduced Maillard reactant, i.e., reducing sugar and amino acid. It is well known that Maillard reaction is responsible for the brown colour and tasty flavour of baked and fried foods, explaining the lower preference scores of banana chips pre-treated with blanching, compared to control. Moreover, using blanching pre-treatment also reduced the preference scores in texture of banana chips, since it modified the hardness of banana chips as shown in Figure 2.

It was suggested that the application of pectin coating had positive impacts on all attributes of sensory properties, as indicated by the higher preference scores compared to those uncoated ones, i.e., CP > C, B90+P > B90, and B100+P > B100. It explains that pre-treatment with pectin coating could avoid excessive leaching of sugar and amino acids, known as Maillard reactants, while protecting the cellular structure of the banana tissue from damage during deep-fat frying, so increasing preference scores of banana chips in all attributes.

## Conclusion

The synergistic phenomena of acrylamide

reduction in banana chips by blanching and pectin coating has been confirmed by applying Limpel's formula. It was observed that the combination of blanching and pectin coating as pre-treatment had a more pronounced reducing action on acrylamide formation than single pre-treatment. The use of blanching as a single pre-treatment was found not an appropriate method since it induced apparent loss in terms of both physical and sensory properties. Noticeably, applying coating with pectin as single pre-treatment could not only reduce significantly acrylamide formation in banana chips, but also slightly improve the crunchiness and sensory properties of the products, suggesting its prospect as a promising process to manufacture preferable banana chips with low-acrylamide content.

## Acknowledgements

This research was funded by a grant of Indofood Riset Nugraha (IRN) for Year 2011/2012 from Indofood Sukses Makmur Holding Co., Indonesia. The financial support from United Nation University (UNU) and Kirin Holding Company Limited (Japan) to the first author is also acknowledged. We acknowledge Dr. Hanifah N. Lioe (Bogor Agricultural University) for valuable discussions about acrylamide analysis.

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