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Decreasing Acrylamide Formation in Fried Potato Slices Using Hydrocolloid Coatings and Bene Kernel Oil

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ABSTRACT

This study investigated the effect of CarboxyMethyl Cellulose (CMC), tragacanth, and Saalab hydrocolloids in two concentrations (0.3 and 0.7%) and different frying media (Refined Canola Oil (RCO), RCO+1% Bene Kernel Oil (BKO), and RCO+1 mg L^{-1} UnSaponifiable Matter (USM) of BKO on acrylamide formation in fried potato slices. The hydrocolloid coatings significantly reduced acrylamide formation in potatoes fried in all oils ($P< 0.05$). Increasing the hydrocolloid coating concentration from 0.3 to 0.7% produced no effective inhibition of acrylamide ($P> 0.05$). The 0.7% CMC solution was identified as the most promising inhibitor of acrylamide formation in RCO oil, with a 62.9% reduction in acrylamide content. The addition of BKO or USM to RCO led to noticeable reduction in the acrylamide level in fried potato slices. The findings suggest that a 0.7% CMC solution and RCO+USM are promising inhibitors of acrylamide formation in fried potato product.

Keywords: Antioxidant, CMC, Saalab, Tracaganth, Unsaponifiable matters.

INTRODUCTION

Acrylamide (H_2C=CH-CO-NH_2) is known to be neurotoxic and genotoxic for humans and is classified as a carcinogenic compound by the International Agency for Research on Cancer (IARC, 1994). The presence of considerable levels of acrylamide in heat-treated carbohydrate-rich foods was first highlighted in 2002 jointly by the Swedish National Food Authority and the University of Stockholm. Different countries have researched the presence of acrylamide in food products and many food organizations have affirmed that deep-fried potato chips and slices contain the highest amounts of acrylamide (Medeiros *et al*., 2011).

Independent studies have shown that the main formation pathway of acrylamide in deep fried foods is a non-enzymatic browning reaction between the free amino group of asparagine amino acids and a source of carbonyl, such as reducing sugar. This reaction is responsible for the taste and the aroma of the heated food. Any factor, such as food formulation, pH, a_w, temperature, and duration of frying which influences the Maillard reaction also causes acrylamide formation (Mottram *et al*., 2002).

Research on minimizing acrylamide formation during frying frequently involves pretreatment and/or modification of the process conditions because of the high level of acrylamide precursors naturally found in

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potatoes. Pretreatments such as soaking in water or salt solution (Pedreschi *et al*., 2010), enzyme treatment (Pedreschi *et al*., 2008), blanching (EL-Saied *et al*., 2008), microwave treatment (Erdogdu *et al.,* 2007), addition of natural antioxidants (Ou *et al.,* 2010), and the use of more stable frying oil (Arribas-Lorenzo *et al.,* 2009) have been examined as approaches to control acrylamide level in the final product. Some of these were intended to make conditions favorable for acrylamide formation.

Pistacia atlantica subsp. *mutica* grows in abundance in the Zagros region of Iran. Its fruits, which are locally called Bene, are round to oval, somewhat flat, 0.5–0.7 cm in diameter, and covered with a rather dry hull. The oil from *P. atlantica* fruit is an important new source of edible oil (Sharayei *et al.,* 2011). Iranian bene fruit kernels have an oil content of approximately 55-58%. Small but effective amounts of compounds like polyphenol and tocopherol affect the oxidation stability of edible oil. Sharayei *et al.* (2011) reported that bene kernel oil contains more polyphenolic and tocopherolic compounds than canola oil. The unsaponifiable matter (USM) fraction typically constitutes 0.5–2.5% of the vegetable oils, although some vegetable oils have exceptional amounts (5–6%). The USM fraction of vegetable oils, including hydrocarbons, terpenic alcohols, sterols, tocopherols and phenolic compounds, is of great importance for oil characterization and stability (Sharayei *et al.,* 2011).

Hydrocolloids are hydrophilic polymers that modify the functional properties of food systems, such as thickening, gelling, water retention, and emulsifying properties. Saalab (*Orchis anatolica*), is one of the Mediterranean species, which has wide distribution in Turkey and Iran. It is a safe food ingredient obtained by milling dried tubers of certain wild terrestrial orchids and has been used for many years for its nutritive and demulcent properties (Kaya and Tekin, 2001; Farhoosh and Riazi, 2007). Saalab has been used in medicines, drinks, and ice-creams as a thickening and stabilizing agent.

CarboxyMethyl Cellulose (CMC) is the most important water-soluble cellulose derivative which is formed by reaction of cellulose with sodium hydroxide and chloroacetic acid. Formed sodium carboxymethyl groups (CH_2COONa) promote water solubility. CMC is applied in many food industries and in cosmetics, pharmaceuticals, detergents, etc. (Farhoosh *et al.,* 2008a).

Tragacanth, a dried exudate, is an Iranian native gum obtained from the stems and branches of Asiatic species of *Astragalus* (Balaghi *et al.,* 2011). It is a heterogeneous branched anionic biopolymer with a high molecular weight (Farzi *et al*., 2013) and consists of two main fractions: tragacanthin and bassorin (Farzi *et al.,* 2013). Bassorin has the capacity to swell and form a gel, and tragacanthin is water-soluble part (Balaghi *et al.,* 2011). Gum tragacanth has been used for many years as a stabilizer, thickener, emulsifier and suspending agent in the food, pharmaceutical, cosmetic, textile, and leather industries based on its high viscosity at low concentration and high stability to heat and acidity (Balaghi *et al*., 2011).

Hydrocolloid coatings are used to reduce the oil uptake of fried food products and/or water loss. Zeng *et al.* (2010) observed that acrylamide formation decreased in chemical models in the presence of 2% (w/w) pectin, alginic acid (> 50% reduction), and xanthan gum (20%), while for fried potato strips, they suggested that immersion time was a more important factor than concentration of the immersion solution. They showed inhibition of acrylamide formation by immersing potato strips in a 1% alginic acid solution for 5 hours was two times more effective than immersing in a 5% solution for 1 hour (60% versus 30% reduction).

Despite the wide application of hydrocolloids to improve the quality of food products, their roles in acrylamide formation have not been sufficiently explored. The present study aimed to: (1) evaluate the effective reductions in acrylamide formation

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from hydrocolloids coatings (tragacanth, Saalab, CMC) in fried potato slices, and (2) explore the function of Bene Kernel Oil (BKO) and its USM as frying oil additives to reduce acrylamide reduction.

MATERIALS AND METHODS

Materials

Potatoes were collected from Damghan fields in Semnan province, Iran. The refined free antioxidant canola oil was procured from Segol- Neyshabour Edible Oil Plant. CMC, Tragacanth and Saalab were purchased from Jahan Shimi Corporation (Tehran, Iran). Saturated brome solution, sodium thiosulfate, ethyl acetate and other chemicals were purchased from Merck Corporation.

Extraction of BKO

After drying the bene in the shade, the pericarp was removed and the kernel was grinded to powder. The powder was mixed with normal hexane solvent with a ratio of 1:4 and the extraction of oil took place with mixer for 48 hours in dark at room temperature. The solvent was evaporated under vacuum at 40°C. The extracted oil was capped under nitrogen in dark jars and preserved in the freezer.

Extraction of USM

A 5 g sample of crude bene oil was mixed with 50 mL of normal ethanolic potassium solution and was heated up to 95°C for 1 hour. After cooling down, 100 mL of distilled water was added onto it. Then, it was extracted twice with 100 mL diethyl ether parts. The surface layers were collected and washed with 75 mL of distilled water. After that, 100 mL normal ethanolic potassium solution was added and then washed with 100 mL of distilled water again. The surface layer was separated and mixed with anhydrous sodium sulfate. After filtering, it was dried in oven at 45°C. For more purification, USM were dissolved in the chloroform and after filtering, chloroform was evaporated under vacuum at 45°C (Lozano *et al.,* 1993).

Preparation of Oils and Hydrocolloid Solutions

The Refined Canola Oil (RCO) without additives was used for frying environment. 0.1% of BKO and 100 ppm USM were added to the RCO, separately.

Coating Pretreatments

The potato (the Agria variety) was peeled and cut into slices with manual device up to 6×0.5×0.5±0.2 cm. The potato slices were immersed in hydrocolloids solutions of 0.3 and 0.7% of CMC, tragacanth and Saalab, separately, at room temperature for an hour (Zeng *et al.,* 2010).

Deep Frying Process

The potato slices (20 g, each batch) were fried in a thermostatic home fryer batches at 170±2°C. The batches were fried for 6 minutes with a RCO, blend of RCO and 0.1% BKO and blend of RCO and 100 mg L^{-1} of USM, separately. To ensure oil temperature uniformity, the oil (1.5 L) was heated to the desirable temperature for 2 hours before the frying process. The samples were fried up to 5 minutes and, at the end of frying process, the slices were discharged and the surplus oil was absorbed by an absorbent paper. After cooling the samples, they were wrapped in aluminum foil and kept in the freezer up to the testing time (Tyagi and Vasishtha, 1996). Frying operation was repeated twice.

Water Content Analysis

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A determined amount of potato slices was weighed and kept in an oven of 105°C for 24 hours. After this time, they were discharged from the oven, left to cool down in the desiccator, and weighed again. This operation was repeated until a stable weight was reached. The Water Content (WC) was calculated as the following equation (Tran *et al*., 2006).

$$WC(\%) = \frac{moist\ sample\ weight - dry\ sample\ weight}{moist\ sample\ weight} \times 100 \quad (1)$$

Also, Water Retention (WR) in the fried potato slices that had been treated with a hydrocolloid solution was calculated relative to that in the ones immersed in distillated water as follows:

WR= [(WC of hydrocolloids treatment/WC of distillated water treatment)-1]×100% (2)

Positive and negative water retention indicates, respectively, enhanced water retention and loss as a result of the treatment (Zeng *et al*., 2010).

Acrylamide Measurement

GC-MS analysis was performed using a Varian ion trap Saturn 2200 GC-MS (Agilent Technologies, Santa Clara, California, USA). Separation of the reaction products was done using a Varian DB5 column (DB5 30 m×250 µm×0.25 µm).

A 10 g unknown sample and 100 µL of acrylamide solution (2,000 ng mL^{-1}) were combined in 10 mL water. Standards were also prepared in water using 100 µL internal standards. Samples were mixed for 20 minutes and then centrifuged. The supernatant was filtered using 0.45 µm nylon syringe filters. A brominating reagent (200-300 µL) was added to 3 mL of the filtered sample and standards. They were gently mixed and then allowed to react in an ice bath for 1 hour. One drop of 1N sodium thiosulfate was added to each sample to decompose any remaining bromine. Samples were extracted with 2 mL ethyl acetate and then centrifuged for 10 minutes. The organic layer was transferred to auto-sampler vials for analysis. Brominating acrylamide by this method yields 2,3-bromopropionamide.

Analysis of the more stable 2,3 bromopropionamide created in situ from the thermal decomposition in the injector using ammonia as the reagent gas provided stable adduct ions that were used for PCI SIM (Injector: 180°C; Xfer line: 180°C; Trap: 120°C; Ionization mode: CI, amount of acrylamide at hand) (Robarge *et al*., 2003).

Color Measurement

The images were prepared using a desktop scanner (Canon scan 8400F). The samples were placed on the scanner, which was then covered by a non-reflecting black cloth. The scanner resolution was set to 400 dpi and the images were stored in JPEG format for further analysis. A 5×0.5 cm segment of each image was selected using Photoshop for analysis. Image J software was used to determine the required measurements of each plate. The images were processed to reduce noise. The parameters were in pixels and the program had the capability to convert the measurements to *L, a*, and *b* parameters (Fengxia and Zhanming, 2001). The total color differences were calculated from the *L, a*, and *b* values according to the following equation:

$$\Delta E = [(L-L_{target})^2+(a-a_{target})^2+(b-b_{target})^2]^{0.5} \quad (3)$$

The raw potato slices (before frying) were used as a target for the calculation of *ΔE*.

Statistical Analysis

All experiments and measurements were carried out in triplicate, and the results were analyzed using a completely randomized factorial design by means of SAS software version 9.2. The variance analysis (ANOVA) was used to determine the effect of each treatment on assessment factors. Significant differences between means were determined by Duncan's multiple range

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tests. P-values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Acrylamide Content

Quantitative analysis showed that immersing the potato slices in hydrocolloid solution effectively inhibited the formation of acrylamide content over the control ($P< 0.05$). As shown in Figure 1, variations in the concentration of the immersion solution affected the outcome of the treatment insignificantly ($P> 0.05$). A solution of 0.7% CMC was identified as the most promising inhibitor of acrylamide formation (62.9% reduction). Immersion of potato slices in a 0.3% solution produced 60.6% inhibition of acrylamide formation in fried potato slices.

Increasing the concentration of Saalab solution produced no significant effect on acrylamide content and led to the lowest reduction of acrylamide formation in fried potato slices. In addition, immersion of potato slices in 0.3 and 0.7% solutions produced no significant difference in acrylamide inhibition (94.1 and 91.9 µg kg^{-1}, respectively). Immersion of potato slices in a 0.7% solution of tragacanth produced an insignificant reduction ($P> 0.05$) in acrylamide content compared with immersion of potato slices in a 0.3% solution (57.5% *versus* 54.4% reduction).

Zeng *et al.* (2010) showed that, at 2% (w/w) pectin, alginic acid (> 50% reduction), and xanthan gum (20%) significantly reduced acrylamide formation in chemical models. Their findings revealed that alginic acid and pectin were promising inhibitors of acrylamide formation in fried potato strips. Ahrné *et al*. (2007) reported that acrylamide formation in the outer crust was greater than for the inner crust when baking white bread because of the higher temperature in the outer crust. It was postulated that formation of surface coatings on potato slices might modulate heat transfer from the surrounding oil to the surface of potato and reduce the rate of acrylamide formation. This effect, however, was not observed in this study. Increasing the Saalab concentration more than 2 fold produced a thicker surface layer, but did not significantly decrease acrylamide content. These results suggest that the type of hydrocolloid coating played a predominant role in the inhibition of acrylamide in the final products. The different performance of hydrocolloid coatings is related to their structure. Saalab is known to be a valuable

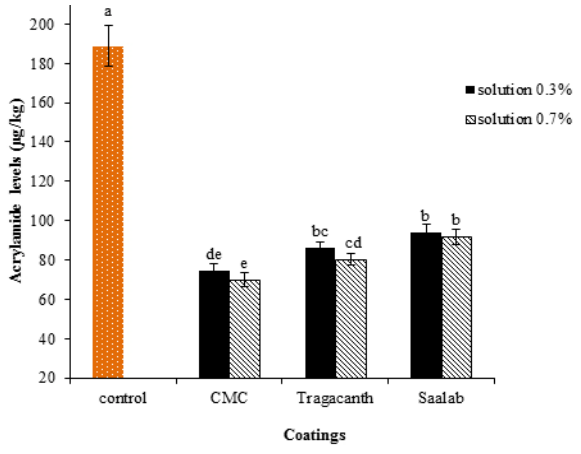

Figure 1. Effects of different concentration of CMC, tragacanth and Saalab solutions on the acrylamide content of potato slices fried in Refined Canola Oil (RCO). Data values are means±SD. Bars with the same letters are not significantly different at $P< 0.05$.

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source for glucomannan (16–55%) which acts as a stabilizer. Saalab also contains starch (2.7%), nitrogenous substance (5%), moisture (12%) and ash (2.4%) (Farhoosh and Riazi, 2007). It is reported that bassorin and tragacanthin of different species have different amount of uronic acid, methoxyl, and neutral sugar contents as well as small amounts of protein (< 4% w/w) (Farzi *et al.,* 2013).

The effect of BKO and USM on acrylamide level is shown in Table 1. As seen, the addition of BKO and USM to RCO led to a significant reduction in acrylamide formation during frying for all coated samples and the control samples (P< 0.05). Results showed that USM was more effective than BKO in the inhibition of acrylamide formation in the control (37.8% *versus* 29.2% reduction in comparison to RCO) and coated samples.

Sharayei *et al.* (2011) indicated that BKO was capable of retarding oxidation at a level as low as 0.1% (1,000 ppm) and its effectiveness at this level was nearly the same as the powerful synthetic antioxidant TBHQ. Kotsiou *et al*. (2011) found that acrylamide content decreased up to 49% when the antioxidant capacity, before heat treatment, reached 13.44 ppm of the BHT equivalents in the model system. It enhanced it up to 20% when the antioxidant capacity, before heat treatment, reached 16.35 ppm of BHT equivalent. This demonstrated that the antioxidant paradox may be related to the structure of the phenolic compound which differs in virgin olive oil from other plant phenols.

It is reported that the unsaponifiable constituents of the BKO were separated into hydrocarbons (12.9±1.1%), carotenes (9.8±0.0%), tocopherols and tocotrienols (65.8±0.6%), sterols (3.0±0.6%), methyl sterols (2.8±0.4%), triterpenic alcohols (3.8±0.1%), and triterpenic dialcohols (2.2±0.6%). These compounds have an antioxidative activity and effectively inhibited lipid oxidation *via* different mechanisms of action (Sharayei *et al.*, 2011). Farhoosh *et al.* (2008b) revealed that total phenolic content of *Pistacia atlantica* subsp. *mutica*, *kurdica*, and *P. vera L. cv. Ohadi* kernel oils were 81.12, 56.51 and 62.84 mg gallic acid kg^{-1}, respectively. This data, however, provided evidence that the existing phenolic or other compounds could improve the inhibition of acrylamide formation by means of scavenging free radicals.

Table 1. Combination effects of frying oils and coatings of CMC, tragacanth and Saalab solutions (0.7%) on the water content, water retention, and the acrylamide content of fried potato slices.[a]

Oil	Pretreatment	Water content (%)	Water retention (%)	Acrylamide (µg/kg)
RCO [b]	Control	20.50± 0.71^{cd}	-0.39	188.82±11.31^{a}
	CMC	43.24± 0.5^{ab}	110.11	70.02±2.83^{d}
	Tragacanth	47.19±0.25^{a}	129.30	80.28±2.91^{cd}
	Saalab	23.50±2.12^{c}	14.19	91.86±4.24c
RCO+BKO [c]	Control	16.05±2.83^{d}	-22.01	133.62±9.89^{b}
	CMC	45.33±1.47^{a}	120.26	20.27±1.41^{e}
	Tragacanth	36.92±1.06^{b}	79.40	22.59±1.43^{e}
	Saalab	20.10±1.41^{cd}	-2.33	30.31±1.70^{e}
RCO+USM [d]	Control	20.50±0.71^{cd}	-0.39	117.50±7.42^{b}
	CMC	43.09±2.55^{ab}	109.38	15.01±0.71^{e}
	Tragacanth	48.95±1.86^{a}	137.85	17.23±1.41^{e}
	Saalab	25.00±2.83^{c}	0.21	20.58±1.56^{e}

[a] Means±SD with the same lowercase letters are not significantly different at $P< 0.05$. [b] Refined Canola Oil; [c] Bene Kernel Oil, [d] UnSaponifiable Matters.

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Table 2. Combination effects of frying oils and coatings of CMC, tragacanth and Saalab solutions (0.7%) on the color indexes of fried potato slices.

Oil	Pretreatment	*a*	*b*	*L*	*ΔE* [c]
RCO [d]	Control	-2.25	43.65	73.37	38.32±4.19 [a]
	CMC	-5.55	46.78	74.19	17.65±1.41 [e]
	Tragacanth	-4.56	46.99	77.87	19.86±1.38 [d]
	Saalab	-5.59	44.40	75.31	29.83±2.18 [b]
RCO+BKO [e]	Control	-2.89	38.34	78.69	26.27±2.57 [c]
	CMC	-3.42	44.87	77.48	13.24±0.90 [g]
	Tragacanth	-3.16	51.46	74.41	17.73±1.05 [de]
	Saalab	-3.06	42.86	72.81	18.55±1.32 [de]
RCO+USM [f]	Control	-2.61	46.69	65.49	20.11±0.85 [d]
	CMC	-3.22	45.42	78.20	13.82±1.02 [fg]
	Tragacanth	-3.10	51.27	76.12	16.21±2.12 [ef]
	Saalab	-6.61	56.06	71.43	16.32±1.81 [ef]

[c] Means±SD with the same lowercase letters are not significantly different at $P< 0.05$. [d] Refined Canola Oil; [e] Bene Kernel Oil, [f] UnSaponifiable Matters.

Water Content

The moisture content of the control and coated samples after final frying in different mediums are shown in Table 1. It can be seen that the control and Saalab coated samples had significantly lower moisture contents in comparison with the CMC and tragacanth coated samples ($P< 0.05$) for all frying media. The greater water retention capacity of CMC and tragacanth hydrocolloids was responsible for this observation. Ahrné *et al*. (2007) reported that acrylamide usually forms in lower a_w. On the other hand, the outer crust of bread formed higher acrylamide because of the lower water content than the inner crust, which is in agreement with the results of this study since the CMC- and tracaganth-coated potato slices had higher water content and less acrylamide.

The water content of the Saalab coated samples was similar to the control, but the acrylamide content was much lower than the control samples for all frying mediums. In addition, neither BKO nor USM treatment significantly changed the water retention of the fried potato slices relative to the RCO ($P> 0.05$). In other words, although it had considerable effect on acrylamide content, frying potato slices in the presence of BKO or USM was not effective in reducing water loss during frying. This data, however, provided evidence that alteration in water content was not a major mechanism in acrylamide formation, which is in agreement with the findings of Zeng *et al.* (2010).

Color

The color of a fried potato is the result of non-enzymatic browning reactions (Maillard reactions). The effect of frying oil and hydrocolloid coatings treatment on the color of fried potato slices is shown in Table 2. All coated samples were slightly less red, more yellow, and lighter (as indicated by smaller negative a^*, larger b^* and higher L^* values, respectively) than the controls. This difference was more significant for potato slices fried in RCO ($P< 0.05$). The control samples had higher ΔE values than the coated ones. It was observed that adding BKO and USM to RCO led to 31.5 and 47.5% reduction of ΔE in the control samples, respectively. Coated potato slices fried in different frying oil mediums behaved similarly. The results suggested that

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the most promising color was for CMC-coated potato slices fried in the presence of USM.

CONCLUSIONS

The findings of the present study indicate that hydrocolloid coatings are very effective in reducing acrylamide formation during potato frying, although the coatings did not succeed in totally inhibiting the formation of this hazardous molecule. The reduction was a consequence of the type of hydrocolloid coating. Moreover, using more stable frying oil led to more inhibition of acrylamide formation during frying. It was observed in the present study that potato slices fried in the presence of USM lowered acrylamide levels. Further work is needed on the percentage of USM to identify the effective components and their mechanisms in combination with hydrocolloid coatings.

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کاهش تشکیل آکریل آمید در برش‌های سیب زمینی سرخ شده با استفاده از پوشش های هیدروکلوئیدی و روغن مغز بنه

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چکیده

این پژوهش به بررسی اثر هیدروکلوئیدهای کربوکسی متیل سلولز (CMC)، تراگاکانت و ثعلب در دو غلظت (۰/۳ درصد و ۰/۷ درصد) و محیط های سرخ کردن متفاوت (روغن کانولا تصفیه شده (RCO)، مخلوط RCO و یک درصد روغن مغز بنه (BKO) و مخلوط RCO و ۱ میلی‌گرم بر لیتر مواد صابونی ناشونده‌ٔ (USM) روغن مغز بنه بر تشکیل آکریل آمید در برش های سیب زمینی سرخ شده می‌پردازد. پوشش‌های هیدروکلوئیدی به طور قابل توجهی میزان تشکیل آکریل آمید را در همه محیط‌های روغنی کاهش دادند (P< ۰/۰۵). افزایش غلظت پوشش‌های هیدروکلوئیدی از ۰/۳ به ۰/۷ درصد اثر بازدارندگی معنی‌داری بر تشکیل آکریل آمید نداشت (P> ۰/۰۵). محلول ۰/۷ درصد CMC با ۶۲/۹ درصد کاهش در محتوای آکریل آمید در محیط

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RCO، بهترین اثر بازدارندگی را در تشکیل آکریل آمید از خود به نمایش گذاشت. افزودن BKO یا USM به RCO منجر به کاهش قابل توجهی در مقدار آکریل آمید در برش‌های سیب زمینی سرخ شده شد. بر اساس یافته‌های این پژوهش بهترین اثر بازدارندگی از تشکیل آکریل آمید در فراورده سیب زمینی پوشش داده شده با محلول ۰/۷ درصد CMC و سرخ شده در محیط روغن شامل مخلوط RCO و USM مشاهده شد.

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