



Original research

Reduction of acrylamide by orange waste extract phenolic compounds in potato chips

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ABSTRACT

Acrylamide can lead to carcinogenic, genotoxic, and neurotoxic risks. Adding plant extracts containing phenolic compounds significantly affected acrylamide formation. In this research the effect of orange waste extracts on the reduction of acrylamide, color attribute and sensory properties in fried potato chips were investigated. The average amount of total phenolics in the methanolic extract of orange waste was 39.43 mg GAE/g of dry material. Reduction of acrylamide content in fried potato chips with different doses of phenolic extracts was significantly ($p < 0.05$). The results showed that the addition of orange waste extract significantly reduced the acrylamide content to 44.8%. Furthermore, significantly difference between control and treatments in color was observed ($p > 0.05$). Sensory evaluation results showed that doses of phenolic extracts in 0.05 g had the highest acceptance. The effect of orange extract on the reduction of acrylamide formation was positive. Thus, we can conclude that pre-treating potatoes with fruit processing waste extract before frying produces beneficial effects such as a reduction in acrylamide content.

Keywords: Acrylamide; Potato chips; Phenolic compounds; Gas chromatography-mass spectrometer

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1. Introduction

Today, cancer and carcinogens are one of the important issues in the scientific community, and a number of cancerous or cancer-causing substances are discovered every day (Kris-Etherton et al., 2002). The International Agency for Research on Cancer (IARC) has identified acrylamide as "possibly carcinogenic to humans." Acrylamide (prop-2-enamide) has the molecular formula C_3H_5NO , which is formed by the Millard reaction between reducing sugars (glucose and asparagine), which is responsible for the formation of acrylamide (IARC, 1994). Some studies reported that acrylamide was formed at high temperatures during the heating of starchy foods (especially potatoes and cereals (Pedreschi et al., 2004).

Asparagine amino acid plays a key role in the production of acrylamide. The highest amounts of asparagine are in potatoes, wheat and legumes. Of course, it should be noted that high levels of asparagine in the raw material alone do not represent high levels of acrylamide, but along with other conditions (reducing sugars and heat), it is advisable to carry out an acrylamide formation reaction,

which is commonly used in food products such as Chips are all these conditions (Stadler et al., 2002). Except for asparagine, other amino acids also produce a small amount of acrylamide, including the amino acids of alanine, arginine, aspartic acid, cystine, glutamine, methionine, threonine and valine (Rydberg et al., 2003). Many studies on the formation of acrylamide in potato chips have shown that the main factors of acrylamide production are temperature and frying time. When the frying temperature is very high (180-190 °C), the level of acrylamide increases symbolically at the end of frying (Tareke et al., 2002). Parameters such as potato varieties, frying oil and frying conditions such as frying time and temperature, pH, adding additives such as salt, amino acids and plants minimize acrylamide in potato chips (Zyzak et al., 2003). Recent studies have been conducted to determine the amount of acrylamide. A new platform for measuring acrylamide has been developed, as well as the design of biosensors that can directly determine the amount of acrylamide in food samples (Asnaashari et al., 2018, 2019).

There are several ways to reduce acrylamide levels, such as adding amino acids such as cysteine, glycine, alanine, lysine,

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Glutamic acid, which reduce the formation of acrylamide in heated potatoes. However, no practical application for the industry is possible due to the unpleasant smell and flavor in potatoes (Rydberg et al., 2003). Genetic enhancement or stopping the activity of genes that encode free-acting asparagine biosynthesis controlling enzymes in potatoes may help reduce the amount of asparagine.

The phenolic compounds are widely distributed in plants. In many reported studies, phenolic compounds with antioxidant and anti-radical properties can play an important role in preserving food products and to maintain human health (Fathiazad et al., 2010). Various studies have shown that the antioxidant properties of plants and fruits depend on the phenolic compounds in them (Jayaprakasha et al., 2008). Phenolic compounds and antioxidant activity in citrus fruit processing pomace was investigated (Espinosa-Pardo et al., 2017). Peppermint, cumin, and fennel extracts produced the highest reduction on acrylamide formation (Zhu et al., 2009). Adding antioxidants also reduces acrylamide levels. The use of Rosemary extract and the confirmation of sunflower oil in potato also reduced the amount of acrylamide during deep frying (Kim et al., 2005). Soaking and washing the potato with room temperature water for at least 15 minutes before frying reduced the acrylamide content to 63% (Jackson et al., 2005). Some methods, although they reduce the amount of acrylamide, but practical application in the industry due to unpleasant smell and flavor in potatoes is not possible (Rydberg et al., 2003).

Orange after apple is the second most commonly used fruit in the world (Adubofuor et al., 2010). Orange has phenolic compounds. The essential oil of orange peppermint contains antimicrobial limonin and di-cyclic aldehyde and also contains linalool and di-terpentol oil and a large number of flavonoids with anticancer effects (Mirheidar, 1993). The use of oranges in the industry, for example, the production of orange juice, only made up about half the weight of the orange juice and produced a great deal of waste of the skin, pulp, seeds, and all the fruits that did not have the quality required for processing (Calabrò et al., 2016). In order to improve the management of these wastes, there are serious processes to recover them, for example, through the production of Organic Fertilizer, essential oils, pectin, biodegradable oil, essential oils and antioxidant compounds (Martín et al., 2010).

This study investigates the inhibitory effect of phenolic compounds extracted from orange waste on acrylamide formation in potato chips.

2. Material and Methods

2.1. Plant materials and chemicals

Distilled water, Folin-Ciocalteu, sodium carbonate, gallic acid, methanol with a purity of 100% and all other materials used were purchased from the Merck Co., Germany. Orange and potatoes were purchased from Tehran's local Fruit Market.

2.2. Orange waste phenolic compounds

Orange waste phenolic compounds were extracted by maceration method (Xu et al., 2015). Orange juice was completely taken with the juicer after washing oranges, and orange pulps dried inside the cabin drying at 40 ± 2 °C for 24 h and then with the chopper (Gosonic GSJ-5000) was converted to powder. The dried

sample was stored at 4 °C. For extraction of phenolic compounds, 100 g of dried sample were solved in 1000 ml of methanol and stored at room temperature for 24 hours. Extract was then filtered. The solvent was then evaporated by a rotary evaporator (Heidolph Co.) at low pressure and at 45 °C. The sample was then dried with a freeze dryer (GAMMA-1-16-LSC) at a low pressure and -50 °C for 24 h and turned into powder. The freeze dried extract was stored in a refrigerator.

2.3. Production of potato chips

Potatoes were washed, peeled and sliced by hand slicer into sheets of 1.5 mm thickness. Potato slices were rinsed and soaked in distilled water for 1 min to eliminate surface starch. Blanching was done by immersing 15 potato slices in 10 L of water in 83 °C temperature for 2.5 min. After draining with stainless-steel colander and drying in room temperature, 15 pieces of control samples (immersed in distilled water) and treated sample with orange waste extracts solutions (50, 75 and 100% w/v) were fried at 180 °C (limited commercial frying temperature) for 10 min in a home fryer (Molineux, Spain), then cooled to room temperature (3 repetitions). These conditions allowed the fried chips to reach final moisture contents of ~1.5 g water/100 g (wet basis). After frying they were cooled to room temperature and stored in -20 °C for further analysis.

2.4. Determination of total phenolic content

The total phenolic content of orange pomace extract was determined by a modified Folin-Ciocalteu reagent method (Slinkard & Singleton, 1997). A mixture of 135 µL distilled water, 750 µL 1/10 dilution Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) and 600 µL 7.5% (w/v) Na₂CO₃ was added to 50 µL of sample extract in a 1.5 mL micro-tube. After vortexing for 10 s, the mixture was incubated at 45 °C in a water bath for 15 min. Samples were allowed to cool at room temperature before measuring the absorbance at 765 nm by a spectrophotometer (U-1100, Hitachi Ltd. Japan). Gallic acid standard was prepared from a freshly made stock solution of 1 mg/ml gallic acid (Merck, Germany). The results were expressed in mg gallic acid/g.

2.5. Quantification of acrylamide

Acrylamide was measured according to Tareke et al. (2002) and Lehotay et al. (2006). First, 5 g of the sample solved in 5 ml of hexane, then equilibrated with distilled water and acetonitrile, and mixed completely. Then adding 5 g of sodium sulfate and sodium chloride, after centrifugation for 10 min, at 4500 rpm, the acetonitrile layer was completely isolated. Then, the acetonitrile layer was collected, for bromination, potassium bromide, hydrobromic acid and saturated bromine water were used. The solution was placed in a refrigerator at 4 °C for one night. Then, extra bromine was discolored by adding 0.7 M sodium thiosulfate, and after adding the sodium sulfate, the solution obtained with 65 ml of ethyl acetate and extracted in two steps. The resulting organic phase was then evaporated under vacuum with a sufficient amount of sodium sulfate, then concentrated under nitrogen gas to a volume of 250 µL. The sample was injected until freezing was performed.

2.6. Quantitative analysis by GC

Samples were analyzed on the chromatographic system including a gas chromatograph from Agilent Technologies (Palo Alto, CA, USA) coupled with Agilent's 6890N microelectron capture detector. One microliter of sample was injected on-column with a 7683 automatic liquid sampler and injector system (Agilent) onto a 19091N-113 HP-INNOWax capillary column (polyethylene glycol, 30-m length, 0.32-mm i.d., 25 μm film thickness, J & W Scientific, Agilent, CA, USA). Separations were performed using nitrogen as the carrier gas, applying the following temperature program: 110 $^{\circ}\text{C}$ (hold time 1 min), then at 10 $^{\circ}\text{C min}^{-1}$ to 140 $^{\circ}\text{C}$ (hold time 15 min), and at 30 $^{\circ}\text{C min}^{-1}$ to the final temperature of 240 $^{\circ}\text{C}$ (hold time 7 min). The GC-ECD sample injector interface temperature and detector interface temperature were both held at 250 $^{\circ}\text{C}$ (Zhang et al., 2006). The experiments were repeated three times and their mean values reported.

2.7. Color

The color of potato chips was measured by both Hunterlab (model TES-135, Taiwan). The color indices were expressed as L^* , as Lightness, b^* , as yellowness and a^* , redness. The parameter of total color difference (ΔE) was calculated by Eq. 1 (Pedreschi et al., 2007).

$$\Delta E = ((L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2)^{\frac{1}{2}} \quad (1)$$

2.8. Sensory evaluation

The sensory properties of the fried potato chips (appearance, texture, color, taste, general acceptance) were made through a 5-point preference test (Hoseinabadi et al., 2012). Ten panelists (Iranian Research Organization for Science and Technology staff and students) were used for this test. After examining the samples by the panelists, the numbers went from 1 to 5, respectively, in terms of expressing the levels: 1 = very bad, 2 = fairly bad, 3 = moderate, 4 = fairly good and 5 = very good, were assigned to the samples.

2.9. Statistical analysis

Results were expressed as the mean and standard deviation of three independent replicates. All the data were statistically analyzed using one-way analysis of variance (ANOVA) through Duncan post hoc at $p < 0.05$. All of the statistical analyses were performed using SAS software version 9.3 (SAS Institute Inc.).

3. Results

The average amount of total phenolic compounds content was 39.43 mg GAE/g of dry material.

The potato chips moisture content of various treatments is shown in Fig. 1. There is significant difference between the mean of the control group and the mean of other treatments at 95% level in the Duncan test. It was observed that doses of phenolic extracts in 0.5 and 5 g, samples moisture content was increased and doses of phenolic extracts in 0.05 g, samples moisture content was decreased compared to the control.

The color difference is shown in Fig. 2. The lowest color in dose of phenolic extract at 0.5 g was observed. Compared to the control sample in dose of phenolic extracts at 5 g increase of L^* , a^* and b^* , 0.5 g increase of L^* and b^* and decrease of a^* , and 0.05 g increase of a^* and b^* and decrease of L^* was observed. In the Duncan test, there is significant difference between the treatment group and the control was observed ($p < 0.05$).

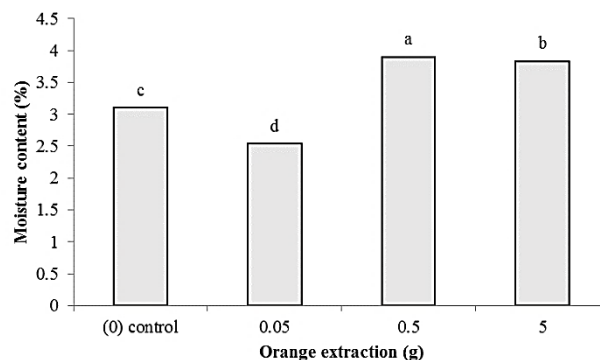


Fig. 1. Moisture content of fried potato chips of the control and pre-treated immersed in different concentration of orange waste extract.

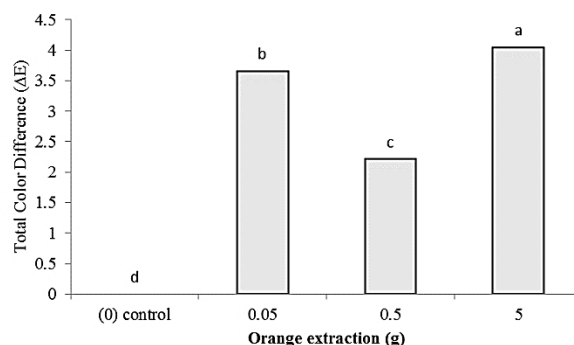


Fig. 2. Color difference of fried potato chips of the control and pre-treated immersed in different concentration of orange waste extract.

The sensory evaluation of each treatment is shown in Fig. 3. There is significant difference between the treatment group and the control sample ($p < 0.05$). The highest acceptance was observed in potato chips treated with phenolic extracts at 0.05 g. Sensory evaluation of immersed sample had (appearance, texture, color, taste, general acceptance) better than control.

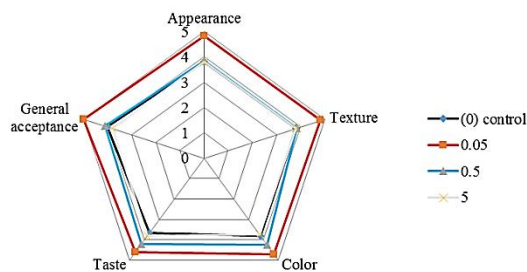


Fig. 3. Sensory evaluation of fried potato chips of the control and pre-treated immersed in different concentration of orange waste extract.

Fig. 4 shows the changes in acrylamide levels in treated samples at different concentrations of orange waste phenolic extracts. The measured acrylamide chromatograph at different treatment of orange waste phenolic extract is shown in Fig. 5. According to results, the minimum (doses of phenolic extracts at 5 g) and maximum (control sample) acrylamide levels was 880 and 1479 $\mu\text{g}/\text{kg}$, respectively. There significant difference between the treatment group and the control sample was observed ($p < 0.05$). The results showed that with increasing of doses of phenolic extracts, acrylamide content were significantly decreased ($p < 0.05$).

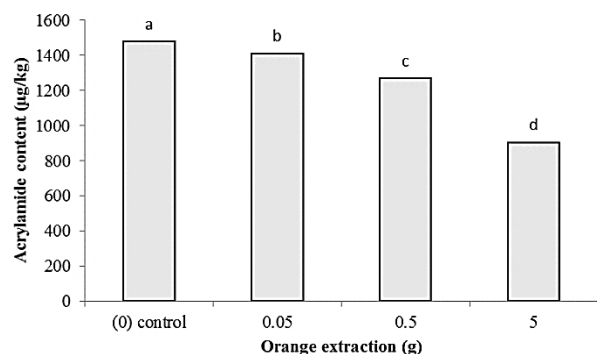


Fig. 4. Acrylamide content ($\mu\text{g}/\text{kg}$) in control and pre-treated fried potato chips after immersion in the orange waste extract at different concentrations.

4. Discussion

Phenolic compounds are important natural components in the crude aqueous extracts of plants and their types and contents differ widely among various dietary plants (Kita et al., 2004). Plant material like fruit processing wastes contain high levels of phenolic

compounds, which have antioxidant, anti-inflammatory, antiviral and anticancer activities. Importantly, most of this phytonutrient is found in the orange peel and inner white pulp (Bouskela et al., 1997).

In a study, extraction of essential oil, polyphenols and pectin from orange peel was investigated using microwave and ultrasound technology. The amount of polyphenols in orange peels waste was 50.02 mg GA/100 g dry material (Boukroufa et al., 2015). The highest concentrations of flavonoids in citrus fruit occur in peel that oranges peel represents roughly 30% of the fruit mass. Narirutin and hesperidin are the major polyphenols in sweet orange peels and naringin and neohesperidin in bitter orange peels (Sawalha et al., 2009). Phenolic compounds in citrus pulp, seed and peel was determined about 52 mg of GAE /g (Sir Elkhatim et al., 2018). It was reported that increase in temperature caused a significant decrease in total phenolic content (Shavandi et al., 2020b).

Moisture reduction should be limited due to the economic importance related to the weight loss. Furthermore, higher heat transfer rate, greater heat penetration into the samples and formation of wet layers effect on the rate of water loss during thermal processing (Shavandi et al., 2020a).

In the potato processing industry, color is an important quality criterion. Color change is the result of Maillard reaction at the surface. Color is strictly related to consumer perception (Marquez & Añon, 1986). In a study, it was reported that the presence of phenolic compounds had no direct effect on the browning process during the frying of potato samples (Napolitano et al., 2008).

It was reported that, natural extracts to have great potential as effective inhibitors of the formation of genotoxic substances in thermally processed foods (Zhang et al., 2007). Polyphenols might also scavenge reactive carbonyls formed in the Maillard reaction (might lead to reduced levels of acrylamide) (Stadler et al., 2004). In another study, quinone-amine interaction was proposed to reduction of acrylamide formation (Zhang et al., 2008).

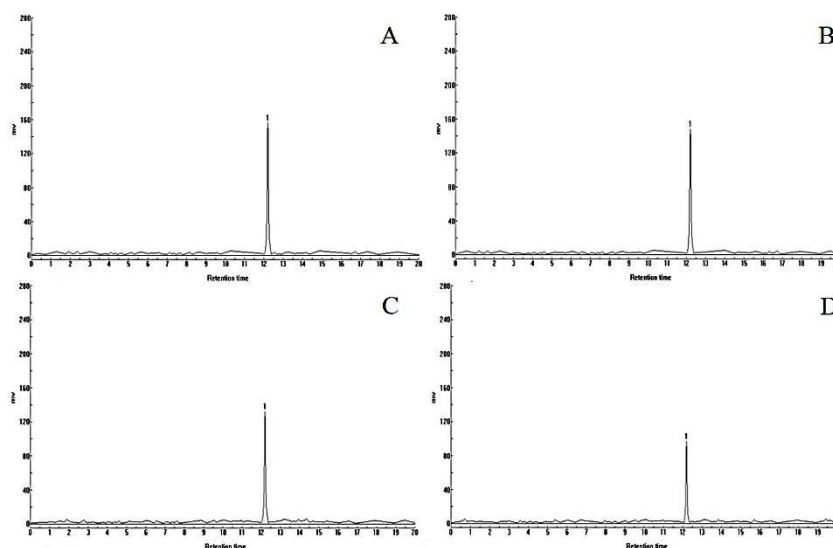


Fig. 5. Acrylamide chromatograph ($\mu\text{g}/\text{kg}$), A (Control), B (Potato chips treated with 0/05 g extract), C (Potato chips treated with 0/5 g extract) and D (Potato chips treated with 5 g extract).

The bitter phenols (aglycons of secoiridoids) are a major problem to the taste of the potato crisps (Sacchi et al., 2002; Brenes et al., 2002). The effects of mild heats on the formation of acrylamide along with adding of citrus flavonoid, naringenin, on the formation of acrylamide were investigated. It was reported that, by increasing the amount of naringenin, the formation of acrylamide to 20–50% significantly decreased. This effect via scavenged asparagine-derived intermediates (also amide source of the reaction) at positions 6 and 8 of the A-ring through naringenin, diverted them from the pathways leading to acrylamide formation (Cheng et al., 2009).

In several study, effect of various antioxidants, phytochemicals and antioxidative extracts on acrylamide formation was investigated. In the reports identified, this effect might led to decrease, no effect or increase (Jin et al., 2013, Kahkeshani et al., 2015).

Rosemary with a known antioxidant properties, had been proposed as one effective inhibitor of acrylamide. It was reported that rosemary aqueous extract (1%) decreases the acrylamide formation in bread when added to dough prior to baking. (Hedegaard et al., 2008).

In a similar research, the potential of natural coatings to reduction of acrylamide formation in potato chips during the frying process were evaluated. The effect of Arabic gum and soluble soybean polysaccharide as coating hydrocolloids on acrylamide formation and physico-chemical attributes of potato chips were investigated. It was reported that coating agents decreased acrylamide formation, and maintain quality (Torabi et al., 2017). In a study, 35 kinds of crude aqueous extracts of plants and 11 phenolic acids to reduction of acrylamide was investigated. Finding that 34 out of 35 plants extract exerted reduction effect while 9 phenolic acids except for ferulic acid and hesperetin inhibited acrylamide formation (Zhu et al., 2009). On the other hand, apple, blueberry, mangosteen, longan and dragon fruit extracts was investigated for their activities against acrylamide formation in chemical model. The result showed that apple extract cause of potent decrease, extracts of blueberry, mangosteen and longan not significant impact and dragon fruit extracts enhanced acrylamide formation. It can be concluded that polyphenolic compounds in apple extract reduce the amount of acrylamide formation (Cheng et al., 2010). In another study reported that grape seed extract had no effect on acrylamide formation in bakery products (Açar & Gökmen, 2009). polyphenols and flavonoids in plant extracts by trapping of carbonyl compounds, react with lipid radicals to form stabilized phenoxyl radicals, supplying carbonyl groups for acrylamide formation, Inhibiting the acrylamide elimination and speeding the conversion from 3-APA to acrylamide could be probable mechanisms on acrylamide formation (Liu et al., 2015). Due to its antioxidant properties, phenolic compounds have a promising role as food additives due to their strong antioxidant power and other biological activities beneficial to health (Xu et al., 2015). The association between the antioxidant activity of phenolic compounds and the inhibition of their acrylamide was also investigated by polyphenols in muscadine grapes. Foods that contain antioxidants have been shown to be effective in inhibiting acrylamide formation. However, studies on the mechanism of action of potential inhibitors are ongoing and should be continued. It is assumed that polyphenols may reduce the formation of acrylamide due to their strong antioxidant properties (Xu et al., 2011).

5. Conclusion

This study revealed that orange pomace as fruit processing waste has significant inhibitory effects on acrylamide formation in pre-treated potato slices (chips) which immersed in the extracts. This pretreatment has no significant negative effect on color and sensory properties of fried chips from the panelist's point of view.

Many other studies on the formation of acrylamide in potato chips have shown that the main factors of acrylamide production are temperature and frying time. Adding plant extracts and phenolic compounds has significantly affected acrylamide formation. Antioxidative activity is one of the probable mechanisms, by which phenolic-containing sources such as Orange processing wastes are able. It was contain high levels of phenolic compounds.

Approaches such as pretreatment the potato chips with fruit extracts such as fruit processing waste could minimize the level of acrylamide in fried potato products. Due to the ability of these materials to be used in industries, their use is to reduce the amount of acrylamide in potato chips and other products and types of foods rich in carbohydrates such as bread and biscuits.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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