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Original scientific paper

THE EFFECT OF ADDING DIFFERENT ADDITIVES ON ACRYLAMIDE CONTENT AND ANTIOXIDANT ACTIVITY OF INNOVATIVE FUNCTIONAL CEREAL PRODUCTS

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Abstract

Acrylamide (AA) is a chemical pollutant that naturally forms in starchy food products during high-temperature cooking, including frying, baking, and industrial processing, at +120°C and low humidity. The main chemical process that causes this is the Maillard reaction, the same reaction that "browns" food and affects its taste. AA is formed from sugars and amino acids (mainly one called asparagine ASN) that are naturally present in many foods. AA is found in crisps, chips, bread, biscuits and coffee. (Nematollahi et al., 2020). The health impact of acrylamide has been the subject of concern and research for several years. Research on laboratory animals has shown that exposure to high levels of acrylamide can cause a variety of adverse effects, including damage to the nervous system and an increased risk of some types of cancer. It is important to note that these studies often involve the administration of acrylamide at significantly higher doses than are typically found in food (Başaran et al., 2023). Various supplements such as fennel, nigella, pomegranate, wild berries, cumin, black cumin, bamboo leaves and many other supplements have an effect on reducing acrylamide levels, which comes from their antioxidant activity (Al-Ansi et al., 2019; Ashkezari & Salehifar, 2019; Borczak et al., 2022; Abdel-Shafi Abdel-Samie et al., 2011; Li et al., 2012). Besides impacting the acrylamide level, these supplements have antioxidant activity, antimicrobial activity, immune support, and other health benefits that make products with added ingredients like these functional products.

Keywords: acrylamide, additives, antioxidant activity, functional product.

1. Introduction

Acrylamide is formed in heated food by the condensation of the amino group of an amino acid (asparagine) with the carbonyl group of a sugar. Asparagine needs a carbonyl compound to convert to acrylamide. The carbonyl can come from several sources. Due to the diversity of antioxidants in structure and properties and the complexity of reactions, different antioxidants are involved in different reactions during the Maillard reaction process, thereby causing different effects on the formation of acrylamide (Jin et al., 2013).
Antioxidants can inhibit acrylamide formation in high-temperature processed foods in three ways. The first is to destroy the formed acrylamide from their oxidized products. The second is the formation of quinones or carbonyl compounds such as vitamin C which then react with the main precursor of acrylamide and asparagine. The inhibition effect depends on how easily they are oxidized and the rate of oxidation and their oxidized products react with asparagine. The third is the inhibition of the production of carbonyl compounds produced by frying oil (Ou et al., 2010).
Acrylamide inhibition studies include those by Cheng et al. (2015) who found that several antioxidant flavones and isoflavones (apigenin, luteolin, naringenin, tricin, daicein, daicin, genistein, genistin) inhibited acrylamide formation by up to 52.1% in a model system, possibly by reacting with key Maillard intermediates such as which is 3-aminopropionamide. The rate

of inhibition was correlated with the change in the antioxidant capacity of trolox equivalent of the flavonoids measured by the DPPH assay. Depending on the concentration, natural antioxidants can either favor or prevent acrylamide formation in model systems.

Natural extracts of plant origin could come up with alternatives to synthetic preservatives, especially antioxidants, which also provide bioactive properties and some other benefits to the final products (Caleja et al., 2017). Several studies have reported that natural extracts of aromatic plants, spices, and fruit extracts are used as antioxidants in meat, dairy and bakery products (Gandhi et al., 2001; Caleja et al., 2017; Reddy et al., 2005). However, increasing interest has been given to studies of additives from natural sources as potential antioxidants. In recent years, several antioxidants of plant origin, mainly from medicinal and aromatic plants, have been valuable in delaying the process of lipid peroxidation in food products (Bajaj et al., 2006).

Polyphenols are recognized as secondary metabolites in fruits and vegetables, which have some functionalities including antioxidant, antitumor and anti-inflammatory activities. They are also able to modulate the immune system and protect the cardiovascular system (Munin & Edwards-Lévy, 2011). Several studies have shown that plant extracts containing polyphenols can reduce acrylamide content and increase antioxidant activity in many food systems (Fernández et al., 2003; Zhang et al., 2007; Marková et al., 2012).

2. Materials and Methods

Fennel (*Foeniculum vulgare L.*) belongs to the *Apiaceae* family, with a long history of herbal applications and is widely cultivated in Egypt and India and is characterized by its edible leaves and strongly flavored seeds (Kulisic et al., 2004). The antioxidant activity of FS was analyzed by various antioxidant methods, including ABTS and DPPH. These antioxidant properties were contrasted with several standard synthetic antioxidants such as α-tocopherol, BHA (Butylated Hydroxyanisole), and BHT (Butylated Hydroxytoluene). FS ethanol and aqueous extracts showed strong antioxidant activity (Roby et al., 2013).

Black cumin seeds (*Nigella sativa L.*) commonly known as Nigella or black seed (BS), belonging to the family Ranunculaceae, is a well-known herb planted in Pakistan, India, and Iran (Oktay et al., 2003). BS is enriched with nutritional benefits (Saxena et al., 2017). BS oil contains flavonoids, phenolic, and some other related compounds, which increase its antioxidant properties (Oktay et al., 2003; Dubey et al., 2016).

Pomegranate flower extract (PFE) contains polyphenols (gallic and ellagic acids) and triterpenoids that have potential antioxidant capabilities (Sreekumar et al., 2014). In addition, an inhibitory effect of water-soluble vitamins on acrylamide formation from fried potatoes has been reported; Water-soluble vitamins such as B2, B5, and B12 can reduce acrylamide synthesis by 20%, due to their strong antioxidant properties and structural stability. It is precisely these vitamins that are more effective in reducing the formation of acrylamide during food processing (Zeng et al., 2009).

The nutrients of wild plants are comparable, sometimes even better than those of cultivated varieties (Sidor & Gramza-Michałowska, 2019; Borowska & Brzóska, 2016; Samec & Piljac-Zegarac, 2011; Zhang et al., 2001; Baltacioglu et al., 2011; Hukkanen et al., 2006; Polumackanycz et al., 2020; Lipowski et al., 2009; Kruczek et al., 2012; Sidor & Gramza-Michałowska, 2015; Młynarczyk et al., 2018; Cristea et al., 2021; Nazhand et al., 2020; Singh et al., 2021; Liu et al., 2022; Tereshchuk et al., 2020). Wild fruits that are cultivated are berry crops (bushes) classified in different families: Rosaceae (Aronia melanocarpa, Crataegus L., Sorbus aucuparia L., Rosa canina L.), Elaeagnaceae (Hippophae rhamnoides L.), and Adoxaceae (*Sambucus nigra L.*) (Sidor & Gramza-Michałowska, 2019; Borowska & Brzóska, 2016; Samec & Piljac-Zegarac, 2011; Zhang et al., 2001; Baltacioglu et al., 2011; Hukkanen et al., 2006; Polumackanycz et al., 2020; Lipowski et al., 2009; Kruczek et al., 2012; Sidor & Gramza-Michałowska, 2015; Młynarczyk et al., 2018).

Black cumin (*Nigella sativa L.*) is unique in its nutritional profile, and black cumin fixed and essential oils have phytochemical-rich fractions (Tauseef et al., 2009).

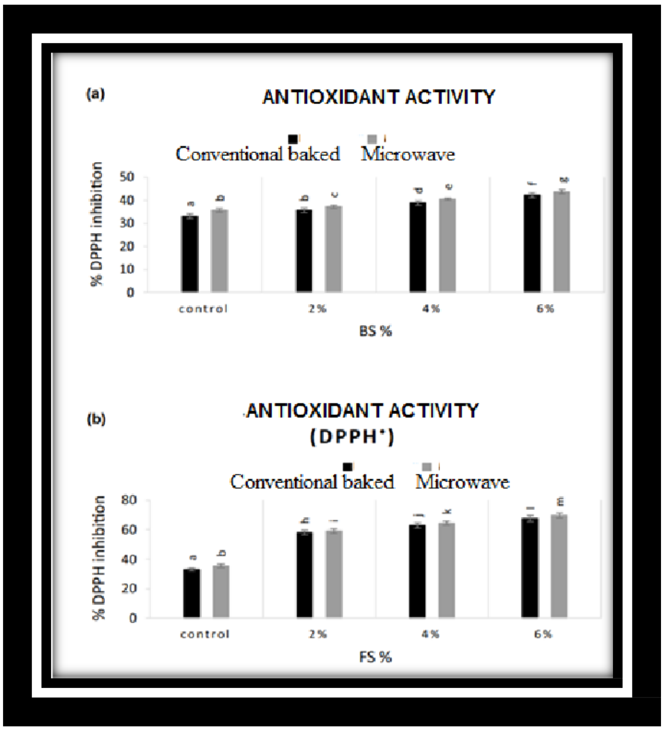
Black cumin seeds possess high antioxidant activity (Mariod et al., 2009; Salih et al., 2009). Black cumin seeds are used to produce cakes with high TPC content, high DPPH without radical scavenging abilities and a strong effect on reducing β-carotene oxidation. The dominant phenolic compounds in black cumin cakes are hydroxybenzoic and β-coumaric acids (Mariod et al., 2009).

In a study by Abdel-Shafi Abdel-Samie et al. (2011), the antioxidant activity and acrylamide content of biscuits with low-gluten flour were decreased with the addition of cumin and black cumin in different amounts of 5%, 10%, 15% and lentil flour at a concentration of 0-15 g/ 100 g of flour.

3. Results and Discussion

Co Al-Ansi et al. (2019) made biscuits with added fennel and cumin seeds which were ground and added in different amounts, the biscuits were made in two batches:

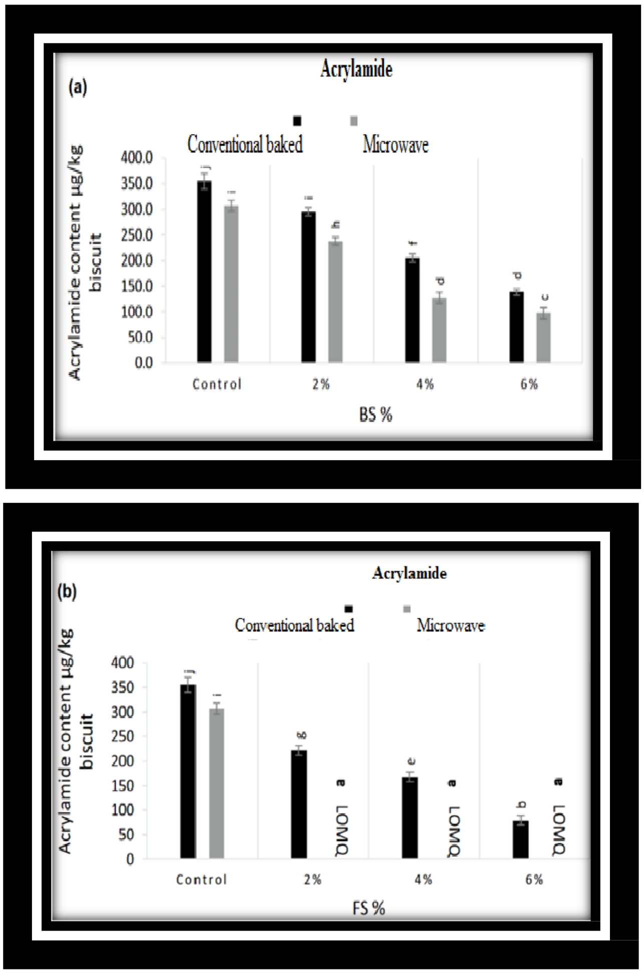
- ✓ The first batch was baked in conventional conditions at 190 °C for 10 min,
- ✓ The second batch was in the microwave at 700 W for 90 seconds.



Graph 1. Antioxidant activity of biscuits with fennel and cumin seeds (BS% - cumin, FS% - fennel)

Antioxidant analyzes of FS in DPPH showed an IC50 value of 746.9 mcg/mL (Mallick et al., 2016). Conventional baked biscuits showed the lowest antioxidant activity and capacity and increased with increasing amounts of BS or FS. However, the presence of natural additives provided some additional benefits. FS biscuits had higher antioxidant capacity and activity compared to BS biscuits. Similar results were observed by Caleja et al. (2017) in their comparative study between natural and synthetic antioxidants. They reported that the addition of FS extract (as a natural antioxidant) provided similar antioxidant activity in biscuits compared to synthetic ones.

Liu (2007) reported that whole grains are rich in antioxidant phytochemicals. The antioxidant activity of FS extracts in ethanol and water shows strong antioxidant activity (Oktay et al., 2003). Higher antioxidant activity in a microwave oven, for two samples of BS and FS biscuits was due to the ability of the microwave oven, as stated by Mahdi et al. (2019) who observed that the microwave technique has great significance in improving the release and extraction of bioactive compounds from plant sources.



Graph 2. Level of acrylamides in biscuits with fennel and cumin (BS% - cumin, FS% - fennel)

Acrylamide content ranged from LOMQ to 355.2 µg/kg (Al-Ansi et al., 2019). According to the authors, the levels of acrylamide content were lower than what was indicated by the European Commission (500 mg/kg). The highest levels of acrylamide were observed in the control conventionally baked biscuits. The addition of BS reduced acrylamide formation to 17%, 31%, and 53% in conventionally baked biscuits, while reductions of 23%, 58%, and 68% were in microwave-baked biscuits to 2%, 4%, and 6% at BS (chart. 2a). The authors also determined that the addition of FS significantly reduced acrylamide formation up to LOMQ in microwave-baked biscuits and up to 78% in conventionally baked biscuits (graph. 2b).

3.1 Effect of Pomegranate Flower Extract (PFE) and Vitamin B3 on Acrylamide Level and Antioxidant Activity in Donuts: Table 1 presents the level of acrylamide in donuts with added pomegranate flower extract and Vitamin B3, which were dried at room temperature and then ground into powder form (Ashkezari & Salehifar, 2019).

Table 1. Acrylamide content in donuts with pomegranate flower extract and Vitamin B3

Resources	Acrylamide content μg/kg
Model (p)	<0,001
A	<0,001
B	0,007
AB	0,5817
A^2	0,0042
B^2	0,3083
R^2	0,9601
adapted R^2	0,9316

The authors (Ashkezari & Salehifar, 2019) used the F model with a value of 33.68 and p value of less than 0.0001 which indicated that the model was significant. In addition, the p values of the linear coefficients (A for PFE and B for vitamin B3) and the squared coefficient of determination (A2 for PFE) were less than 0.05. The coefficient of determination (R2) and the adjusted coefficient of determination (adjusted R2) were 0.96 and 0.93, which indicated that the model was suitable for predicting the experimental data of acrylamide content in doughnuts.
The authors further determined that the addition of PFE and vitamin B3 caused a decrease in the acrylamide content of donuts, mainly due to the antioxidant activities of the compounds, scavenging of carbonyls and limitation (scavenging) of the degradation of sugars during the Maillard reaction (Urbančič et al., 2014). Ciesarová et al. (2008) reported a similar result and showed that the addition of plant extracts derived from pimento, black pepper, marjoram and oregano to potatoes reduced the acrylamide content by up to 75%. Additionally, Budryn et al. (2013) reported that high concentrations of green tea and green coffee extracts reduced acrylamide formation in fried doughnuts.

3.2 Effect of wild fruits on acrylamide level and antioxidant activity in biscuits: Borczak et al. (2022), analyzed the level of acrylamides and antioxidant activity in wheat flour biscuits added (5% percent) six different wild fruits, namely: chokeberry, rowanberry, hawthorns (hawthorn), wild rose, elder and sea buckthorn berries (Sea buckthorn berries), which are presented in table 2.

Table 2. Antioxidant activity and acrylamide content in biscuits with added wild fruits

	Chokeberry	Wild Rose (Rosa canina L.)	Bossel (Sambucus nigra L.)	Hawthorn (Crataegus L.)	Rowanberry (Sorbus aucuparia L.)	Sea buckthorn berries (Hippophae rhamnoides L.)	Control
Antioxidant activity							
ABTS $\mu mol \cdot g^{-1}$ во с.м	15.22 ± 0.05 ^{a}	5.38 ± 0.04 ^{d}	9.42 ± 0.01 ^{b}	7.58 ± 0.01 ^{c}	7.61 ± 0.05 ^{c}	4.99 ± 0.03 ^{e}	1.11 ± 0.00 ^{f}

FRAP μmol·g^{-1} во с.м	17.47 ± 0.05 ^{b}	26.12 ± 0.83 ^{a}	11.37 ± 0.17 ^{e}	12.30 ± 0.17 ^{d}	13.66 ± 0.16 ^{c}	9.83 ± 0.27 ^{f}	2.46 ± 0.08 ^{g}
Acrylamide content							
Acrylamide μg·1000 g^{-1} во с.м	81.98 ± 0.95 ^{a}	173.90 ± 0.54 ^{d}	120.26 ± 1.09 ^{b}	524.96 ± 1.98 ^{f}	370.63 ± 1.76 ^{e}	136.06 ± 0.65 ^{c}	1290.77 ± 1.23 ^{g}

Regarding the acrylamide content, the authors determined that all additives showed a strong inhibition efficiency of the formation of acrylamide in the biscuit samples. The acrylamide content in the control biscuit sample was 361.2 μg/kg. The single addition of lentils to the biscuit formula reduced the amount of acrylamide formed to 346.8, 330.4 μg/kg and 288.3 μg/kg when lentils were added at 5% (T2), 10% (T3) and 15 % (T4). The addition of 15% lentils to the biscuit formula inhibited 20.2% of acrylamide formation in the biscuit control samples.

Cumin also reduced acrylamide formation to 285.2, 199.1 μg/kg and 117.0 μg/kg when cumin was added at 5% (T5), 10% (T9) and 15% (T13). The maximum addition of cumin (15% in T13) inhibited 67.6% of the acrylamide formed in the control biscuit samples. The combination of 15% lentils with 15% cumin (T16) reduced the acrylamide level to 66.7 μg/kg, (81.5% inhibition of acrylamide formation) compared to the biscuit control samples.

Black cumin achieved the highest acrylamide inhibition among the additives. The addition of 5%, 10% and 15% black cumin to the biscuit formula reduced the acrylamide content of the biscuits to only 140.6, 104.7 μg/kg and 87.2 μg/kg. The addition of 15% black cumin to the biscuit formula reduced acrylamide formation by 75.9% compared to control biscuit samples. The combination of 15% buckwheat with 15% black cumin (T28) reduced acrylamide by 89.5% compared to control biscuit samples, resulting in the lowest acrylamide content of 38.0 μg/kg.

4. Conclusions

The use of antioxidant compounds was found to be effective in reducing acrylamide formation due to three effects: (1) carbonyl trapping, (2) reduction of sugar degradation through Maillard reaction processes, and (3) radical scavenging activities. The relationship between acrylamide reduction and amounts of FS (fennel) or BS (cumin) was positive, indicating that antioxidant levels increased with increasing amounts of BS or FS.

From the results of the study (Ashkezari & Salehifar, 2019) it can be concluded that the addition of pomegranate flower extract and vitamin B3 can have a positive effect on the nutritional value and reduction of acrylamide in doughnuts.

Due to its polyphenolic compounds, pomegranate flower extract increases the antioxidant properties of the doughnut. The high antioxidant activity and structural stability of vitamin B3 lead to a significant reduction of the acrylamide content in the final product. In general, pomegranate flower extract and vitamin B3 can be used as food antioxidants and an alternative to synthetic preservatives to reduce the acrylamide content of high-temperature-treated food products.

The addition of wild fruits can be an innovative approach in the production of functional biscuits with improved antioxidant properties characterized by reduced levels of acrylamide while maintaining organoleptic properties acceptable and desirable to potential consumers.

Increasing the level of addition of lentil flour, cumin, and black cumin reduced the acrylamide content of prepared biscuits, despite increased acrylamide precursors in formulated flours. This may be due to the high antioxidant activity of the flours formulated with the addition of lentil flour, cumin, and black cumin.

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