Strategies for reducing acrylamide levels in bread

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Abstract

The formation of acrylamide monomers in cereal products at temperatures higher than 120 °C is a major global health and safety concern in food hygiene first discovered by the University of Stockholm in Sweden. Acrylamide is a carcinogen that causes gene mutation and damages the immune and nervous systems and is therefore a major health concern for humans. No safe limits of consumption that do not cause cancer have yet been determined for acrylamide, as the compound is carcinogenic even at low concentrations, and is 100 times more dangerous to humans than the other known toxins present in the environment. Bread is a main source for the human intake of high levels of acrylamide. In Iran, the rate of the formation of acrylamide and its intake through bread are very concerning. The free asparagine present in wheat becomes the main acrylamide formation agents in acting through the Maillard reaction. The present study was conducted to investigate the measures through which acrylamide levels can be reduced in bread.

Keywords: Acrylamide, bread, cancer, free asparagines

The theoretical basis of the study

The Confederation of the Food and Drink Industries of the EU (CIAA) has proposed a umber of strategies for reducing acrylamide levels in bread, including prolonged fermentation, educed asparagine content in flour, the use of asparaginase and reduced browning time (CIAA, 2009). Flour characteristics vary according to flour extraction rates. Flours with an 82% extraction rate (i.e. with 18% of the bran removed) contain less bran and free asparagine compared to flours extracted at a rate of 93% (i.e. with 7% of the bran removed) **(**Rajabzadeh, 2010). In nutritional terms, flours extracted at 93% have a greater nutritional value; however, they increase acrylamide formation in bread. Reducing the flour extraction rate reduces acrylamide formation in bread (Vahedi, 2012, 2016). L-asparaginase has been proposed as another safe method for reducing acrylamide formation in bread (CIAA, 2009). A study conducted on the use of asparaginase for reducing acrylamide formation in crackers showed up to 70% reduction in acrylamide formation without causing any changes to the product's taste or color (Amrein, 2004).

Introduction

A carcinogenic by-product called acrylamide monomer forms in high-carbohydrate products at temperatures above 120 °C (Tareke, 2002, Lingnert, 2006 & Low, 2006). Acrylamide is considered a major health concern for humans due to causing gene mutation and cancer. No safe limits of consumption that do not cause cancer have yet been determined for acrylamide (Tareke, 2002). Bread is considered a main source for the intake of high levels of acrylamide in humans (Claus, 2002, Swenson, 2003 & Amrein, 2004). Acrylamide is produced through the Maillard reaction of free asparagine with a source of carbonyl (Stadler, Lindsay, 2005 & Edoardo, 2009). The free asparagine in wheat have been identified as the key initiator of acrylamide formation in bread. The extraction rate of flour has a direct effect on the amount of asparagine present in the flour and the formation of acrylamide in the produced bread (Springer, 2003 & Claus, 2006). The absorption of acrylamide in the body leads to the production of Glycidamide, which affects the DNA and causes gene mutation, thereby causing cancer and damage to the nervous system (Lingnert, 2006). In a study examining wafer cookies, acrylamide production was shown to double when the flour used had an ash content of 1.05% instead of 0.55% (Claus, 2002 & Lingnert, 2006). So far, the majority of methods proposed for reducing free asparagine in the agricultural sector and in bakery technologies have been futile and uneconomical and not practical in industrial terms. The use of L-asparaginase in cereal products has not yet become a global industrial practice for reducing free asparagine, except in the case of commercial semi-sweet biscuits. Other studies conducted on this subject have used technological interventions and experimental models. The present study was conducted in Iran and according to the four strategies proposed by the Confederation of Food and Drink Industries of the EU and does not involve any interventions in bakery technologies and has been carried out under normal Sangak bakery conditions and based on different factors, including the wheat cultivar, the flour extraction rate, fermentation, temperature, baking time and L-asparaginase and aims to prevent the formation of acrylamide and to explore the methods through which acrylamide levels can be reduced in bread (CIAA, 2009 & Vahedi, 2012).

Materials and Methods

The present study is based on the design and implementation of three experimental studies, eight article abstracts, and several domestic and foreign papers (1980 - 2012) accessed through the Medline database and also the author's own fields of research and specialization (Vahedi, 2009 - 2016).

Research background in Iran

Holding the first conference on acrylamide and the challenges it presents to the food industry (First Iranian Congress, 2010).

Global research background

In April 2002, the University of Stockholm announced that foods containing carbohydrate and prepared at high temperatures contain substantial amounts of acrylamide. The confirmation of these observations initiated several studies in 30 research centers across the world on the methods that can help reduce acrylamide levels in different food products (Claus, 2002). Measures taken by the FDA included research into methods of reducing acrylamide levels, the analysis of tests performed on food samples, the development of an effective technique for measuring acrylamide levels in food products and posting these techniques on the organization's website, creating practical programs such as holding Q & A sessions about acrylamide, and implementing proposition 65 (the Safe Drinking Water and Toxic Enforcement Act of 1986, holding that, in the state of California, the law requires businesses to warn citizens of the potential risks of chemicals in causing cancer or serious damage). To find a global technique for reducing acrylamide levels in food, the World Health Organization (WHO) formed an association of experts from around the globe. In Canada, the fifth report of the government's challenges was dedicated to chemical management plans. Canada published a report on acrylamide and announced that the intake of acrylamide through food products is at its lowest possible level among the residents of this country (First Iranian Congress, 2010).

Research findings of international centers on the formation and reduction of acrylamide levels in bread

The WHO has estimated the daily intake of acrylamide in developed countries to be between 0.3 and 0.8 µg/kg of body weight (CIAA, 2009). Tareke showed that acrylamide levels are 10-100 times lower in protein-rich foods (meat and seafood) compared to starch-rich foods. In protein-rich foods, acrylamide reacts with the side-chains of amino acids in proteins through thermal processes and thus prevents the formation of large amounts of acrylamide in these foods (Tareke, 2002). Springer announced that the wheat cultivar, organic cultivation, crop yield, the flour extraction rate and the sifting of flour affect the amount of free asparagine and thereby the formation of acrylamide. Rye cultivars contain greater amounts of free asparagine than wheat cultivars (Springer, 2003). Jung argued that the addition of acids and the reduction of pH in corn chips leads to the reduction of acrylamide levels in these products; by reducing the pH from 7 to 6 and from 7 to 4, the formation of acrylamide is reduced by 73% and 99%, respectively (Jung, 2003). Grob stated that the formation of acrylamide requires temperatures higher than 100 °C and low humidities (Fredriksson, 2004). Amrein showed that, in biscuit production, free asparagine and reducing sugars increase with flour extraction rate, and that the addition of 250 mg of free asparagine to each one kilogram of dough leads to a fourfold increase in the formation of acrylamide (Amrein, 2004). Taeymans asserts that wheat cultivar has a dramatic effect on the amount of free asparagine (Taeymans, 2004). Vass suggests that, in cracker production, the use of sucrose instead of invert sugar, the reduction of the temperature from 230 °C to 190 °C and the use of asparaginase reduce the formation of acrylamide by 60%, 60% and 70%, in respective order, and that the addition of amino acids such as glycine, lysine and glutamine has no effect on the formation of acrylamide, but that it improves and intensifies the brown color of the product and leads to the production of greater amounts of melanoidin in the final product 31. (Vass, 2004). Surdyk explains that acrylamide increases linearly with time and temperature, and that no reduction in the formation of acrylamide is observed at high temperatures and that free asparagine inhibits the formation of acrylamide in bread products (Surdyk, 2004). The results of a study conducted by Fredriksson revealed that prolonging yeast fermentation reduces the amount of free asparagine and the formation of acrylamide; however, that given the limited capacity of yeast to metabolize free asparagine, not only does excessive yeast fermentation (3 hours) not reduce the formation of acrylamide, it also weakens the gluten network and reduces the raising of bread. The fermentation of lactic acid by 70% and leaving sourdough to rest for 72 hours reduce the amount of acrylamide; however, if left to rest longer than this time (for example, 96 hours), acrylamide levels increase due to the proteolytic activity of certain microorganisms (Fredriksson, 2004). Amrein showed that the addition of 250 mg of asparagine to one kilogram of dough leads to a four-fold increase in the formation of acrylamide and that the addition of 1000 mg of the enzyme increases the production of acrylamide to more than 8000 µg/kg (Amrein, 2004). Elder states that the addition of divalent cations (magnesium and calcium) to dough at a temperature range of 150

°C to 180 °C reduce acrylamide formation in bread by a maximum of 20% (Elder, 2004). Vass attributed the increase in acrylamide in bread products to the use of ammonium hydrogen carbonate and argued that a acrylamide reduction by one third can be expected in the absence of this compound. However, since ammonium hydrogen carbonate is related to pH and color, the produced bread will not have the desirable appearance and color and will not look attractive to customers. The addition of greater amounts of ammonium hydrogen carbonate will result in a darker color and a higher pH. Replacing ammonium hydrogen carbonate with sodium bicarbonate reduces acrylamide by one third, but the product will have an alkaline taste. The use of tartaric or citric acid eliminates the alkaline taste and reduces the formation of acrylamide (Vass, 2004). In a study on gingerbread, Amrein found that replacing ammonium hydrogen carbonate with sodium bicarbonate reduces the amount of acrylamide by the same amount (Amrein, 2004). Vass reported a 70% reduction in acrylamide by the addition of asparaginase in the production of crackers without changing the products' final taste or color (Vass, 2004). Brathen used glycine amino acid to reduce acrylamide in bread dough and found a reduction from 202 µg to 25 µg in each kilogram of bread (an approximately 90% reduction) and attributed this reduction to the competition between glycine and asparagine (Brathen, 2005). Hendricksen showed that the amount of glucose, fructose and free asparagine determines the amount of acrylamide formed in bread, and that the acrylamide in bread can be reduced through asparaginase, through inactivating the amine group of free asparagine using the asparagine content of proteins and through the acetylation of asparagine into N-acetyl L-asparagine (Hendricksen, 2005). Lindsay showed that trivalent cations react with charged free asparagine groups and prevent their participation in the formation of acrylamide. The addition of 1000 mg/kg of trivalent iron reduces acrylamide formation by 77%; calcium chloride inhibits formation of acrylamide, as well (Lindsay, 2005). Hanley recommended the adjustment of baking time and temperature to reduce the formation of acrylamide (Hanley, 2005). Hebeisen argued that acrylamide levels remain stable for at least three months in bread and cookies and for 12 months in breakfast cereals kept at room temperature (Hebeisen, 2005). Brathen concluded that the formation of acrylamide increases linearly with time and temperature and that no reduction in the formation of acrylamide can be expected at very high temperatures and that high levels of glycine in wheat bread can reduce the formation of acrylamide by up to 80% (Brathen, 2005). Leufven showed that sulfur amino acids and antioxidants are effective in reducing acrylamide formation in crackers (Leufven, 2003). Vattem argued that, due to containing sulfur compounds, the protein content of cereals prevents the formation of acrylamide in these products (Vattem, 2005). Levine showed that the use of ascorbic acid in breakfast cereals reduces their acrylamide levels (Levine, 2005). Amrein demonstrated that the acrylamide formed in the presence of glyoxylate, produced from the thermal decomposition of sugars, is 250 times more than the amount formed in the presence of fructose (Amrein, 2006). Maya proved that the addition of tartaric acid to biscuits reduces the formation of acrylamide by a third (Maya, 2006). Graf showed that using sodium bicarbonate instead of ammonium bicarbonate reduces the formation of acrylamide in semi-sweet biscuits at industrial scale by 70%, and that the use of sucrose instead of invert sugars reduces acrylamide formation even further (Graf, 2006). Claus showed that fermentation reduces acrylamide formation by 50% in wheat bread. Moreover, that the harvest year, bad weather, budding and nitrogen fertilizers have a decisive impact on the bread's asparagine content and the formation of acrylamide in it (Claus, 2006). Fink found that spraying glycine 10% solution on bread dough does not reduce the formation of acrylamide in the bread, but that repeating the spray up to eight times leads to a 16% reduction in the amount of acrylamide formed; however, he found that a mediate substance is then formed in the process, called 3-propionamide. Nevertheless, since glycine should be added at a ratio of 1.5% to 3% of the weight of flour, this strategy was deemed uneconomical (Fink, 2006). Kroll argued that the addition of polyphenolic compounds to wheat bread is more effective in reducing acrylamide, but that this strategy requires further studies (Kroll, 2003). Maya asserted that the use of sucrose in wheat flour biscuits reduces acrylamide formation by 70% (Maya, 2006).

Gokmen showed that the addition of divalent calcium cations at a temperature range of 150 °C to 180 °C reduces the formation of acrylamide by 95% and that the addition of monovalent sodium cations only reduce it by 50% (Gokmen, 2006 - 2007). Studies conducted by Claus on wheat and rye suggest that the climate affects the activity of protease, and that in prolonged fermentations, protease (the exoproteolytic protease group) increases the formation of acrylamide and that bakery yeast prevents the formation of acrylamide by metabolizing the sugars and using up the free asparagine (Claus, 2007). Kolek announced that a 1% salt solution can reduce acrylamide by up to 40%, but that increasing the amount of salt had no further reducing effect (Kolek, 2006). Claus experimented with baking bread at 200 °C for 70 minutes and then at 240 °C for 50 minutes and found that a greater amount of acrylamide formed in the second treatment (124.1 against 92.4 μg/kg); yet, he found that the breads produced were similar in terms of taste, color and aroma. The method of heat transfer was also found to be crucial in the formation of acrylamide; in ovens with air circulation, the faster drying of the bread skin exacerbates the Maillard reaction (Claus, 2002 & Rommens, 2007). Edoardo found that glycine reduces the formation of acrylamide in rye bread but increases the formation of hydroxymethylfurfural, and that asparaginase reduce acrylamide formation by 88% but have no effect on antioxidant activities, color intensification or perceptible properties (Edoardo, 2009).

Research findings of Iranian science institutions on the formation and reduction of acrylamide in bread

In his study, Vahedi showed that the wheat cultivar and a reduced flour extraction rate can reduce the amount of free asparagine significantly. Under laboratory conditions, the effect of L-asparaginase on asparagine hydrolysis was 21.54% with a 250 mg/kg concentration (in bakery industries), 85.36% with a 500 mg/kg concentration and 75.47%-79.89% with a 500 mg/kg concentration again but with two types of dough (Vahedi, 2012). Yeast fermentation reduced free asparagine by 99% after 360 minutes, by 98% after 300 minutes, by 97% after 240 minutes, by 97% after 180 minutes, by 94% after 150 minutes and by 94% after 110 minutes. The effective reduction of free asparagine in the dough made of two types of flour with extraction rates of 93% and 82% reached 90% after 110 minutes of ermentation. Replacing 93% extraction flour with 82% extraction flour increased free asparagine by 33.133%, fructose by 07.347%, glucose by 89.343%, maltose by 43.127% and sucrose by 10.5%. The flour extraction rate from 82% to 93% led to an increase of 133.33% in free asparagine, 347.07% in fructose, 343.89% in glucose, 127.43% in maltose and 5.1% in sucrose (Vahedi, 2012). The individual effects of extraction rate, baking time, baking temperature and L-asparaginase on reduced acrylamide formation in Sangak bread were identified (Vahedi, 2012). The mutual effects of double, triple and quadruple factors on acrylamide formation in Sangak bread were also identified (Vahedi, 2013). The minimum amount of acrylamide formed (a mean amount of 13.07 mg/kg in Sangak bread) was achieved with a baking temperature of 241 °C, a flour extraction rate of 82%, a baking time of 5 minutes and in the presence of L-asparaginase, and the maximum amount of acrylamide formed (a mean of 60.3 mg/kg in Sangak bread) was achieved with a baking temperature of 352 °C, a flour extraction rate of 93%, a baking time of 10 minutes and in the absence of L-asparaginase (Vahedi, 2012). Acrylamide formation increases by 1.572 mg/kg in Sangak bread per each degree of increase in the flour extraction rate at the range of 82% to 93%, by 1.155 per each one minute of increase in baking time at the range of 5 to 10 minutes, and by 0.057 per each degree of increase in temperature at the range of 241 °C to 352 °C. However, per each unit of increase in the enzyme at a range of 0 to 500 mg/kg, acrylamide is reduced by 0.022 in each kilogram of Sangak bread. The mechanisms of the formation of acrylamide act at a high pace and in a very short time in a Sangak baking ovens, and acrylamide is formed at the temperature range of 241 °C to 352 °C and with a baking time of 5 minutes (normal baking) to 10 minutes (browning) in Sangak baking ovens. The mutual effects of different factors were assessed on the formation of acrylamide in Sangak bread, including the mutual effects between each two factors, namely temperature and extraction rate, temperature and the enzyme, temperature and time, extraction rate and the enzyme, extraction rate and time and the enzyme and time, and the mutual effects between each three factors, namely temperature, the enzyme and time, temperature, extraction rate and time, extraction rate, the enzyme and time and finally temperature, extraction rate and the enzyme, and ultimately the mutual effects between all four factors, namely temperature, the enzyme, extraction rate and baking time.

Extraction rate was found to be the most effective factor on the formation of acrylamide and temperature was found to be the least effective, while asparaginase is the most mutually effective factor in the prevention of acrylamide formation in Sangak bread (Vahedi, 2012 - 2013). A study conducted by Motaghi in Kerman showed the concentration of acrylamide in Taftoon, Sangak, Lavash and Barbari breads to be 14, 11, N.D and 9 ng/gram, in respective order (First Iranian Congress, 2010). Mehran showed that the use of acetic and ascorbic acids in the formulation of Sangak bread reduces acrylamide levels by 90%. Malekmohammadi showed that the amount of acrylamide formed in Sangak bread has a parabolic relationship with temperature and a linear one with time. A'rabi showed that, on an industrial scale, the use of sucrose instead of invert sugar and a lower-temperature baking are practical strategies for controlling acrylamide formation in biscuits (Arabi, Mahdavi, & Mohammadi 2011).

Discussion

Iranian researchers found that, under laboratory conditions, the effect of L-asparaginase on pure asparagine hydrolysis was 21.7% when it had a 250 mg/kg concentration (in bakery industries) and 87.81% when it had a 500 mg/kg concentration (a 66.11% difference); however, they found this effect to reach 75.46% - 80.02% when the concentration of L-asparaginase was 500 mg/kg and when there were two different types of dough made from two different types of flour with the different extraction rates of 82% and 93% and in the presence of controls, and no significant differences were observed between them. There were no similar studies that allowed a comparison with the present study (Vahedi, 2012). Overall, the findings of the present study were consistent with the findings of studies conducted by (Zyzak 2003, Amrein & Vass 2004, Edoardo, 2009, Hanne, 2009 & Vahedi, 2012). showing that asparaginase reduces free asparagine. There is a significant difference in the amount of asparagine found in whole - grain flour, bran and endosperm flour prepared from two different types of wheat. The researchers then investigated the effect of the amount of asparagine in whole-grain four, bran and endosperm flour, and the effect of flour extraction rate and L-asparaginase in reducing asparagine in dough (Vahedi, 2012), and obtained results in line with the results of the study conducted by Springer on rye flour (Springer, 2003). except that Springer made no comments on the distribution rates of asparagine in whole-grain flour, bran and endosperm flour, and more importantly, did not investigate the effects of different distribution of asparagine in whole-grain flour bran, and endosperm flour, or the effect of flour extraction rates on the formation of acrylamide in Sangak bread, or the effect of L-asparaginase in reducing free asparagine in dough on an industrial scale. The researchers investigated the separate effect of bakery yeast compared to enzymes in reducing asparagine and found the amount of free asparagine to be 62.33% less in 82% flour compared to in 93% flour (Vahedi, 2012). This finding is in line with the findings of the study conducted by Claus on wafer cookies Amrein, 2004 & Claus, 2006). Changing the extraction rate from 93% to 82% led to a 50% reduction in the ash content of flour (Vahedi, 2012). which is in line with the results obtained by (Hansen, 2004), (Claus, 2006)], and (Springer, 2003). A significant difference was observed in the amount of asparagine yielded in the products made with two different wheat cultivars (Vahedi, 2012).
which is consistent with the results obtained by (Rommens, 2007), (Halford, 2007), (Taeymans, 2004), (Claus, 2006) and (Springer, 2003), but inconsistent with the results obtained by (Lerner, 2006)] who attributed the difference in the amount of asparagine to climatic conditions, increased amounts of nitrogen fertilizer and sulfate deficiency. In doughs prepared from 93% extraction rate flour (in the absence of yeast), the reduction in asparagine was unchanged at minute 0; however, the reduction in asparagine reached 99%, 98%, 97%, 94% after 360, 300, 240, 180 and 150 minutes of yeast fermentation (in the presence of yeast), and reached 94% after 110 minutes of fermentation, while the effective reduction in asparagine (in the presence of yeast) in the two types of dough prepared from two types of flour with extraction rates of 93% and 82% reached 90% after 110 minutes (Vahedi, 2012). but reduced the amount of free asparagine in the dough by 99%, 98%, 97% and 94% after 360, 300, 240, 180 and 150 minutes and by 94% after 110 minutes; however, a 90% reduction was achieved in the amount of free asparagine in the two types of dough prepared from the two different types of flour with the different extraction rates of 93% and 82% after 110 minutes (Vahedi, 2012). These results concur with the results obtained by a study conducted by Fredrickson under laboratory conditions (Fredrickson, 2004), and the

results of studies conducted by (Benedito, 1986), and (Claus, 2002). but not with the results obtained by (Claceys, 2005), who found no reductions in the amount of asparagine up to 5.5 hours after fermentation. The optimum amount of time needed for yeast fermentation to be completed and for a significant reduction to be made in the amount of asparagine in Sangak bread dough was found to be 110 minutes (Vahedi, 2012). These results are consistent with that the results obtained by (Sadd, 2005), (Fredrickson, & Claus, 2004), who showed that fermentation times exceeding 120 minutes cause a destruction and decomposition of the gluten protein network and a loss of admissibility in the dough. Changing the extraction rate of flour from 82% to 93% led to an increase in sucrose by 5.1%, in maltose by 127.43%, in glucose by 343.89% and in fructose by 347.07% (Vahedi, 2012). which are in line with the results obtained by Claus on wafer cookie (Claus, 2006 & Vahedi, 2012). As an inhibitory factor in the formation of acrylamide in cereal products; nevertheless, contrary to the global findings, Iranian researchers found that, although free asparagine was reduced in the dough by L-asparaginase to a degree of near-ineffectiveness, acrylamide was still formed (Vahedi, 2012). There by highlighting the role of reducing sugars in the formation of acrylamide in Sangak bread. Sugars were found to have a decisive role in the formation of acrylamide and the amount of acrylamide found in Sangak bread (contrary to the global findings). The special role of sugars was further highlighted in doughs made from 93% flour, which had 68.71% more glucose compared to doughs made from 82% flour. By increasing the extraction rate of flour from 82% to 93%, the mean amount of acrylamide in bread increases by 50.33% (Vahedi, 2012). which is consistent with the results obtained by (Yaylayan, 2003), but partly inconsistent with the results obtained by (Springer, 2003). who showed that free asparagine is the key element in the formation of acrylamide in bread. They are also consistent with the results obtained by Claus on wafer cookies (Amrein & Claus, 2006), with the other part of the results obtained by (Springer, 2003), with the results obtained by (Claus, 2002). on rye flour and with the results obtained by (Hanson, 2004). The mean amount of acrylamide produced in Sangak bakery ovens became 14.35% by increasing the temperature from 241 °C (the minimum baking temperature) to 352 °C (the maximum baking temperature), and became 14.4% by changing the baking time from 5 to 10 minutes. However, acrylamide was reduced by 22.96% with the addition of 500 mg/kg of L-asparaginase [Vahedi, 2012).These results are consistent with the results obtained by (Claus, 2006), (Edoardo, 2009), and (Hanne, 2009). The differences due to the efficiency of asparaginase depend on the particular baking system used (studies conducted across the world on the use of asparaginase for reducing acrylamide formation in cereal products were conducted under laboratory conditions and involved interventions, except in the study of semi-sweet biscuits; however, the study conducted in Iran was based on an industrial scale and involved no interventions on the Sangak bread technologies in place; it should thus be noted that favorable laboratory conditions are seldom realized in studies that are conducted an industrial scale). The maximum amount of acrylamide formed in Sangak bread was 60.3 mg/kg in a combination of 72 treatments, which resulted from the mutual effects of the four factors of temperature set at 352 °C, baking time at 10 minutes, flour extraction rate at 93% and without the addition of enzymes, while the minimum amount was 13.07 mg/kg with the four factors of temperature set at 241 °C, baking time at 5 minutes, extraction rate at 82% and with the addition of enzymes (Vahedi, 2012). Since Motaghi, Mehran and Arabi did not use the LC/MS/MS system (the most valid device used for measuring acrylamide) (Arabi, 2011), the results obtained from their studies cannot be compared to the results of the present study, which has used this particular system (Vahedi, 2012 - 2013).

Conclusion

Global findings and studies conducted in Iran propose the following strategies for reducing acrylamide formation in bread: Changing wheat species through genetic engineering and cultivating species with a lower asparagine content (given the diversity of climates, cultivating such species cannot be generalized to the entire world, as it may lead to a reduced wheat production and a widespread starvation). Replacing nitrate fertilizers with sulfate ones and changing climatic conditions are not practical solutions. Flour extraction rate has been recognized as the most relevant factor to the formation of acrylamide in bread. Therefore, using flours with low extraction degrees reduces the amount of free asparagine to a large extent and eliminates the health risks of breads containing acrylamide (i.e. their carcinogenic qualities).

However, since such flours have little nutritional value, this strategy both limits the range of flour use and necessitates the enrichment of flour, which is itself neither practical nor economical. Slowing down the Maillard reaction through adjusting reducing sugars, including glucose and fructose, which hasten this reaction. Not only does the use of low extraction rate flour effectively reduce the amount of free aspragines, it also reduces the reducing sugars; however, the elimination of sugars is almost impossible, since they are essential as the raw material required for fermentation reactions by saccharomyces cerevisiae and are also vital for Maillard reactions (responsible for the taste and aroma of bread); yet, the adjustment of sugars is possible. The moderation of glucose using glucose-oxidase, leading to the production of hydrogen peroxide, which should then be neutralized using catalase, thereby making the whole strategy uneconomical. Yeast fermentation reduces the amount of free asparagine and reducing sugars to an effective degree; however, prolonged fermentation times and their subsequent adverse outcomes and the limited metabolism of free asparagine and sugars by different ferments should also be taken into account. Prolonged yeast fermentation processes thus still require further studies. The use of less than 2% salt reduces acrylamide formation in bread, but increases it dramatically with the use of more than 2% salt. Changing formulations and technological stages is associated with the risk of changing the perceptible properties of products and therefore their non-acceptance by the customers. The use of L-asparaginase appears to be an appropriate strategy, and unlike other enzymes, it does not require a later stage of separation (thus making it affordable). It becomes inactive as baking starts, rendering it harmless to humans and not threatening the safety of the bread. Moreover, it eliminates the adverse nutritional risks of using high extraction rate flours (containing greater amounts of bran and asparagine) through bread, and preserves the nutritional value of bran and thus increases variety in flour production and satisfies the expectations of the whole-grain bread market and prevents the spread of cancer across the community. Overall, this enzyme can be effective in reducing acrylamide formation and improving Sangak breads' safety. However, the enzyme is not a food grade one and its action might be slowed down or stopped altogether. The excessive use of this enzyme is not only pointless, it can also cause cancer. Nevertheless, non-competitive reversible inhibitors (such as phytic acid in bran) may inhibit the enzyme's action. Baking time and temperature are among the most influential factors in the formation of acrylamide in bread. Nevertheless, changing them seems impossible, as changing the temperature inhibits the positive aspects of the Maillard reaction (responsible for the taste and aroma of bread) and prolongs the baking time and thus increases the formation of acrylamide. The use of amino acids competing with free asparagine, such as glycine, can reduce the formation of acrylamide, but it also intensifies the color-forming reactions, produces greater amounts of melanoidin and increases the antioxidant capacity. Cysteine amino acid is the strongest known inhibitor of acrylamide formation; however, it is not recommended, since it causes the whitening of the product and creates a bad aroma. Enriching with monovalent cations reduces the formation of acrylamide by 50%, but the accumulation of sodium leads to the accumulation of toxic metals. The use of acrylamidenease reduces the formation of acrylamide in the final product and converts it to acrylic acid, ammoniac and non-reactive products. Sewage bacteria metabolize acrylamide. For example, Rastonia eutropha TDM-3 present in the sewage is able to metabolize more than 1446 mg of acrylamide per liter of sewage. The use of acids and creating an acidic pH can terminate the Maillard reaction; however, this practice entails a risk of producing a sour taste in the product, and more importantly, under acidic conditions, sucrose is hydrolyzed and the formation of acrylamide increases. In general, reducing the formation of acrylamide in bread through agricultural means (which is impossible), asparagine competitors (which is dangerous), L-asparaginase (which is uneconomical and has a limited use), low extraction rate flours (which is uneconomical) and the elimination of sugars is impractical at the present moment. Moreover, the use of additives such as sodium bicarbonate, salt, antioxidants, etc., has not yet been approved.

Suggestions for the Future

Adjusting the amount of initiators (sugars and free asparagine) in flour through yeast fermentation is the only safe way for reducing acrylamide formation in bread, since yeast fermentation can reduce free asparagine just as effectively as can reducing the flour extraction rate and L-aspararginase. However, conducting further studies on the effect of fermentation in reducing acrylamide formation is still required. Measuring the biomarkers of acrylamide in the blood to determine if people are in fact at a risk of cancer due to their consumption of bread. Investigating the effect of the human digestive system's pH on changes in the amount of acrylamide formed in food. Investigating the effect of the immediate cooling of bread after baking in reducing the product's acrylamide formation. A necessary step in reducing acrylamide formation in bread is to teach bakers and the general public to avoid browning their bread or prolonging its baking time.

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