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Effects of frying conditions and yeast fermentation on the acrylamide content in you-tiao, a traditional Chinese, fried, twisted dough-roll ☆

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ABSTRACT

The effects of frying temperature, frying time, and dough pH on the formation of acrylamide in the processing of you-tiao, a traditional Chinese, fried, twisted dough-roll, were analyzed using response surface methodology. The results obtained showed that the frying time and temperature as well as dough pH had a notable impact on the formation of acrylamide in the products. It was demonstrated that lowering the frying temperature to 175 °C, prolonging the frying time to 86 s, and adjusting the dough pH to 6.0 with citric acid reduced the acrylamide content by 71% in the finished products. An examination of the influence of yeast fermentation on the free asparagine and reducing sugars revealed that the addition of 0.8% yeast fermented for one hour could reduce the amount of acrylamide formed in the fried, twisted dough-roll by 66.7%.

1. Introduction

In early 2002, the National Food Administration of Sweden reported the presence of high amounts of acrylamide in heat-treated grain and potato-based foods. The report by Tareke, Rydberg, Karlsson, Eriksson, and Törnqvist (2002) revealed that carbohydrate-rich foods that are fried may contain very high (150–1000 μg/kg) levels of acrylamide whereas precooked levels were below 5 μg/kg. These findings resulted in worldwide interest because acrylamide is a potent neurotoxin and has been classified as "probably carcinogenic to humans" by the International Agency on Research on Cancer – IARC, (1994). The highest concentrations of acrylamide were found in fried potato-based products such as French fries and potato crisps. Research was initiated to determine both the causative mechanism(s) as well as ways to lower or abolish the formation of acrylamide. Shortly after the Swedish findings were announced, Mottram, Wedzicha, and Dodson (2002), and Stadler et al. (2002) simultaneously reported that reducing sugars and the amino acid asparagine, which are relatively abundant in potatoes and wheat, participate in acrylamide formation through the Maillard reaction. These findings were further substantiated by subsequent publications by Zyzak et al. (2003), Rydberg et al. (2003), and Stadler et al. (2004) who showed the role of temperature and cooking time as well as product composition on levels of acrylamide synthesis.

Recently, approaches to reduce or eliminate the acrylamide content in food have been vigorously sought. For example, one approach is the inclusion of additives in the food processing prior to heating. The enzyme asparaginase has been proven to reduce acrylamide levels by 70–90% without affecting the organoleptic properties (e.g. colour or flavour) of the products (Vass, Amrein, Schönbächler, Escher, & Amadò 2004). Fink, Andersson, Rosén, and Åman (2006) demonstrated that applying glycine to dough prior to fermentation can reduce the acrylamide content effectively. Gökmen and Şenyuva (2007) found that dipping potatoes into calcium chloride solution prior to frying inhibited the formation of acrylamide by up to 95% during frying. Other methods for reducing the acrylamide content have been identified through optimizing the processing conditions, such as cooking temperature and time, dough pH, and water content (Claus, Carle, & Schieber 2008; Gökmen, Palazoglu, & Şenyuva, 2006).

You-tiao is a traditional Chinese, fried, twisted dough-roll. This inexpensive breakfast food is popular with all segments of the population. The processing of you-tiao is unique in that the dough is similar to bread dough that is fermented and fried at high temperature rather than baked. To the best of our knowledge, no report to date has considered the reduction of acrylamide content in you-tiao. The objectives of this study are: (1) to determine the processing

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conditions related to acrylamide content in you-tiao; (2) to observe the effects of added yeast on the dough formula; and (3) to investigate the inhibition mechanism of the yeast.

2. Materials and methods

2.1. Materials

Acrylamide (>99%) was purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA). Methanol (HPLC-grade), *n*-hexane, and glucose (all analytical grade) were supplied by Merck & Co. Inc. (Sinopharm Group Chemical Reagent Co. Ltd., Shanghai, China). Flour, salt, sodium bicarbonate, and aluminum potassium sulfate (alum) were purchased from a local supermarket in Wuxi, Jiangsu, China. Yeast was supplied by Angel Yeast Co. Ltd. (Yichang, Hubei, China).

2.2. Preparation of you-tiao,

The ingredients for you-tiao included 300 g of plain wheat flour, 4.5 g (1.5%) of salt, 5.0 g (1.66%) of sodium bicarbonate, 5.1 g (1.7%) of aluminum potassium sulfate, and 175 mL of deionized water. Flour and yeast (0.1% to 1.2%) were mixed together using a Hobart A120T mixer (Hobart Co., Troy, OH, USA) for 1 min at high speed, then solutions of salt, sodium bicarbonate, and aluminum potassium sulfate in preheated water were added to the flour–yeast mixture followed by addition of the remaining water. Ingredients were mixed for 5 min at medium speed and the dough was rested in a proofing cabinet (38 °C, 85% RH) for 20 to 240 min depending upon the experiment. For dough analysis, 20 g dough was divided with a plastic knife, and frozen (−30 °C) immediately. The remaining dough was used for making you-tiao similar to that described by He, Shen, & Ponte (1987). The dough was rolled out, cut into 25 cm strips and deep-fried at various temperature and time regimes described below. The pH of the dough was adjusted by the addition of citric acid, ranging from 0% to 3.2% (flour weight basis).

2.3. Analysis of free asparagine and reducing sugars in dough

The frozen dough was freeze-dried, crushed, and ground in a centrifugal mill so as to pass through a 0.5-mm screen. Freeze-dried and milled dough (1.0 g) was extracted 25 mL 5% trichloroacetic acid in a volumetric flask. After 24 h with constant shaking, the sample was filtered with filter paper, and 1 mL of the effluent liquid was centrifuged at 5000 rpm for 5 min, and 200 μL of the supernatant was analyzed using an Agilent 1100 Liquid Chromatograph (Agilent, Palo Alto, CA, USA).

Analysis of reducing sugars was modified from the procedure described by Fink et al. (2006). Freeze-dried and milled dough (3.0 g) were extracted with 50 mL of distilled water for 20 min in a temperature-controlled water bath. Then the sample was centrifuged (5 min at 4000 rpm), the supernatant was diluted with water to 100 mL in a volumetric flask, and 0.5 mL of the diluted supernatant was mixed with 1.5 mL of DNS reagent (0.63% (w/v) 3,5-dinitrosalicylic acid in water). The solution was heated for 5 min in a boiling water bath, cooled in running water, and diluted with water to 20 mL in a test tube with stopper. Absorption was measured in a spectrophotometer (model 721E, Shanghai Spectrum Instrument Co. Ltd., China) at 530 nm. A standard curve was made with glucose solutions and molar content of reducing sugars was calculated. All samples were analyzed in triplicate.

2.4. Determination of acrylamide by GC/MS

The pulverized sample (1.5 g) was defatted twice with 20 mL *n*-hexane in a 50-mL centrifuged tube with shaking for 10 min. After the second hexane extraction, residual solvent was evaporated, and the sample was extracted with 10 mL methanol with stirring for 20 min and 1 min shaking in an ultrasonic bath. Following centrifugation at 8000 rpm for 20 min at 4 ºC, the supernatant was removed, placed in a fresh tube, and stored at 4 °C. Sample clean-up was performed by solid-phase extraction using Bond C18 cartridges (Supelco Inc., Bellafonte, PA, USA). The cartridge was preconditioned with methanol (3 mL) and water (3 mL). The sample solution was loaded and the effluent collected and concentrated with nitrogen stream to less than 1 mL then diluted to 1 mL with methanol.

Analysis was carried out using a gas chromatograph/mass spectrometer (GC/MS) (TRACE DSQ, Thermo Scientific, Waltham, MA, USA). An injection volume of 2 μL was made using a splitless injector heated to 150 °C. The GC column was PEG-20M (30 m × 0.25 mm i.d. × 0.25 μm d.f.). The column was held at 60 °C for 1 min, ramped at 12 °C/min to 186 °C, held for 6 min, ramped at 50 °C / min to 230 °C, and held for 5 min. Detection was in selected ion monitoring mode, monitoring ions m/z 71 and m/z 55 were used to quantify acrylamide as described by De Wilde et al. (2005).

2.5. Experimental design

A randomized circumscribed central composite design of 15 experiments was made. The independent variables were frying temperature, frying time, and dough pH (marked with A, B, C); there were three levels of each factor: frying temperature (175, 200, and 225 °C), frying time (48, 67, and 86 s) and dough pH (6.0, 7.2, and 8.4). The response value was acrylamide (marked with Y) (Table 1). Experimental data was analyzed by response surface methodology (RSM) using Design Expert 7.1.2 (Stat-Ease, Inc., Minneapolis, MN, USA).

The traditional you-tiao recipe utilizes chemical leavening, so an experiment was designed to determine the effects of yeast fermentation on acrylamide content. The effect of yeast addition and fermentation time on the free asparagine, reducing sugars, and acrylamide content were investigated. In one set of experiments, yeast concentration was varied from 0.1% to 1.2% at a fermentation time of 120 min, and, in another set, the fermentation time was varied from 20 to 240 min using a yeast concentration of 0.8%.

3. Results and discussion

3.1. Acrylamide detection

Identification of acrylamide was based on the relative retention time, and on two diagnostic ions (precursor (72 > 71.99) and one

Table 1
Factors and levels of randomized central composite

Factor	A	B	C
	Frying temperature (°C)	Frying time (s)	Dough pH
8	200	86	6.0
6	175	67	6.0
15	200	67	7.2
7	175	67	8.4
1	225	67	8.4
14	200	48	6.0
5	175	48	7.2
12	225	67	6.0
13	225	48	7.2
4	225	86	7.2
3	200	86	8.4
9	200	67	7.2
10	200	48	8.4
2	175	86	7.2
11	200	67	7.2

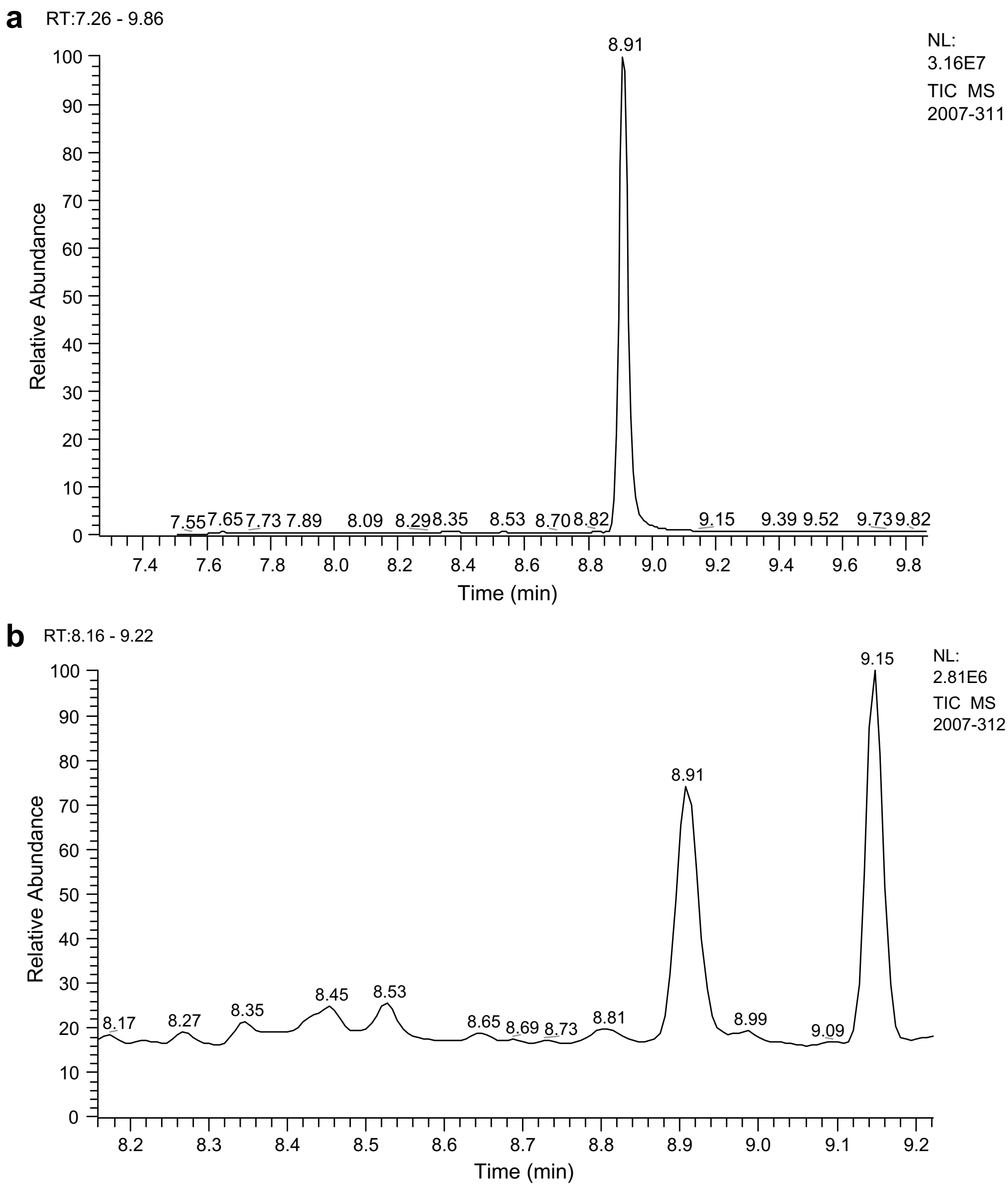

Fig. 1. Chromatogram of: (a) acrylamide standard with selected ion; and (b) typical sample with selected ion.

daughter ion (72 > 55) obtained by collision with Argon. The retention time for acrylamide was 8.91 min in chromatograms of the acrylamide standard and acrylamide peak in the typical sample (Fig. 1). Quantification of acrylamide was made based on its cali-

Table 2
Analysis of variance

Source	Sum of squares	df	Mean square	*F*-value	*p*-Value Prob > *F*	Significance
Model	104932.7	9	11659.19	21.32	0.0018	**
A-temperature	45255.36	1	45255.36	82.76	0.0003	**
B-time	8532.10	1	8532.10	15.60	0.0108	*
C-dough pH	1325.61	1	1325.61	2.42	0.1802	NS
AB	293.95	1	293.95	0.54	0.4964	NS
AC	269.45	1	269.45	0.49	0.5140	NS
BC	264.55	1	264.55	0.48	0.5177	NS
A^2	46079.17	1	46079.17	84.27	0.003	**
B^2	1946.94	1	1946.94	3.56	0.1178	NS
C^2	4166.71	1	4166.71	7.62	0.0398	*
Lack of Fit	1860.52	3	620.17	1.42	0.4386	NS
Residual	734.05	5	546.81			

*Significant at the $p < 0.05$ level.
**Significant at the $p < 0.01$ level.
NS: not significant.

bration curve, y (peak area) = $10^7 \times$ (concentration) − 2×10^6, with a correlation coefficient of 0.99.

3.1.1. Time/temperature experiment

Frying temperature has a significant impact on the acrylamide content of you-tiao, ($P < 0.01$) as does frying time ($P < 0.05$) (Table 2). The amount of acrylamide in the final product increased as frying temperature was raised from 175 °C to 225 °C. The maximum acrylamide content of 340.25 μg/kg was found when dough was fried at approximately 210 °C for 86 s (Fig. 2a). These data agree with those published by several investigators regarding the relationship between cooking temperature and acrylamide formation in carbohydrate-rich foods. The decrease in acrylamide detected above 210 °C is most likely due to acrylamide decomposition. This trend was also reported by Similar trends have been reported by several investigators in multiple systems (Brathen & Knutsen 2005; Mottram et al. 2002; Rydberg et al., 2003; Stadler et al., 2002; Tareke et al., 2002). The authors concluded that the instability of acrylamide at high temperature resulted from evaporation and/or additional reactivity between acrylamide and additional components such free amino acids or protein in the system.

3.1.2. Dough pH

As the dough pH was increased from 6.0 to 8.4, the acrylamide content increased and reached a maximum at around pH 7.2 (Table 1). These results were similar in experiments that simultaneously varied the frying time (Fig. 2b). as well as frying temperature (Fig. 2c). Similar pH-mediated effects have been reported in other systems (Amrein, Schönbächler, Escher, & Amadò 2004; Claus, Mongili, Weisz, Schieber, & Carle 2008). In a low-pH environment the reaction of asparagine and carbonyl compound is blockeded, and the Maillard reaction does not participate in acrylamide formation (Low et al., 2006). From the results of the experimental conditions evaluated above, a lower frying temperature (175 °C), and prolonged frying time of 86 s, and adjusting the dough pH to 6.0 with citric acid reduced the acrylamide content by 71% in the finished product.

3.2. Yeast fermentation

3.2.1. Influence of yeast addition on asparagine and acrylamide content

During fermentation yeast metabolize free amino acids providing a potential control point for asparagine content and subsequent formation of acrylamide (Surdyk, Rosén, Andersson, & Åman 2004). In samples fermented for 120 min, addition of yeast to 0.1% and 0.2% produced a small decrease in both asparagine and acrylamide compared to yeast-free controls (Fig. 3). Considerable lowering of both asparagine and acrylamide were observed as yeast was increased to 0.8%, resulting in a decrease of the final acrylamide content to approximately 35% that of the control. Increasing yeast beyond 0.8% did not result in a reduction in either asparagine or acrylamide beyond that observed at the 0.8% level.

3.2.2. Influence of fermentation time on asparagine, reducing sugars, and acrylamide content

From the results obtained in the experiments described above, 0.8% yeast was used to examine the impact of fermentation time on asparagine and acrylamide contents. As the fermentation time was increased from 0 min to 240 min, the asparagine content was reduced from 15.45 mg/100 g to 7.48 mg/100 g and the acrylamide content decreased from 343 μg/kg to 125 μg/kg (Fig. 4). The results showed that both free asparagine and acrylamide were reduced dramatically when fermented for up to 80 min, after which

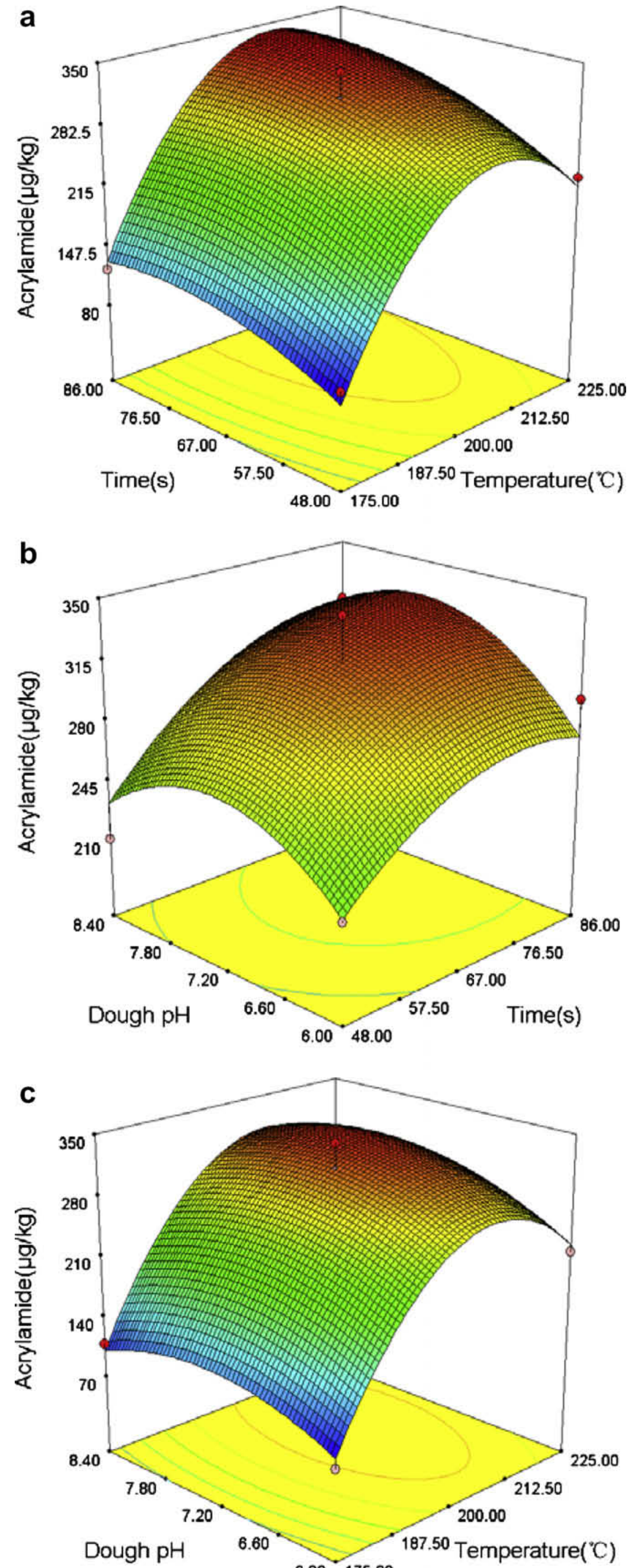

Fig. 2. Response surface curves showing; (a) the effect of frying temperature and frying time on acrylamide content in you-tiao; (b) the effect of dough pH and frying time on acrylamide content in you-tiao; and (c) effect of frying temperature and dough pH on acrylamide content in you-tiao.

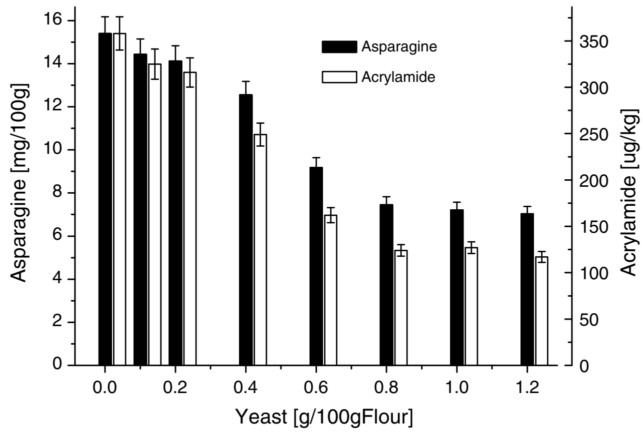

Fig. 3. Impact of yeast addition to dough on asparagine in dough and acrylamide in you-tiao.

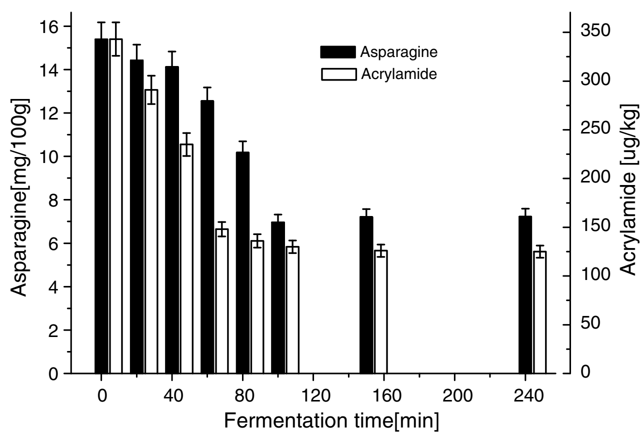

Fig. 4. Influence of fermentation time on asparagine in dough and acrylamide in you-tiao.

no change was observed in asparagine or acrylamide content for the duration of the experiment. This is in general agreement with the results of Fredriksson, Tallving, Rosén, and Åman (2004) who observed decreases of asparagine and acrylamide by 91% and 87%, respectively, in whole-wheat bread. Differences in the yeast strain used, flour composition, and the processing conditions may be sources of variation between the findings. Another possible explanation may involve the concentration of reducing sugars, which in addition to free asparagine, have a profound effect on acrylamide formation through the Maillard reaction (Mottram et al. 2002).

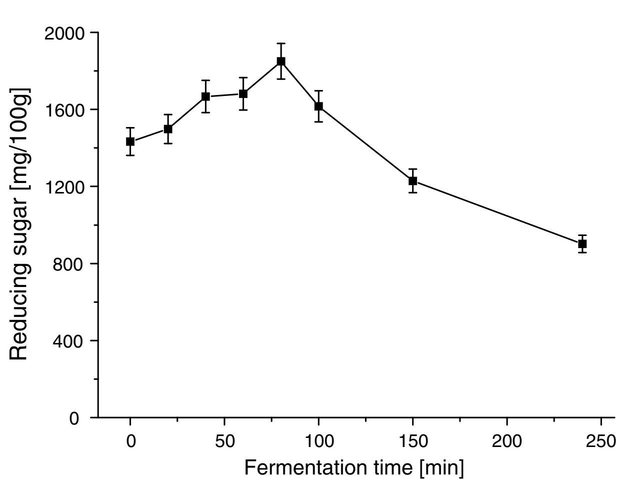

Fig. 5. Influence of fermentation time on reducing sugar in you-tiao dough.

During fermentation reducing sugars achieved a maximum content (1850 mg/100 g) at about 80 min and decreased rapidly as fermentation time increased (Fig. 5). This was likely due to activity of yeast amylase liberating sugar from starch in the initial stage of the fermentation. Although it has been well established that an increase in reducing sugars can lead to production of acrylamide, the simultaneous decrease in asparagine is limiting the formation of acrylamide.

4. Conclusion

This article examined the effects of the processing conditions (frying temperature, frying time, and dough pH) and yeast fermentation on the acrylamide content in the traditional Chinese, fried, twisted dough-roll, you-tiao. The results obtained can be summarized as follows:

1. Frying temperature has a significant impact on the acrylamide content, which reached its maximum at around 200 °C; frying time and dough pH also influenced acrylamide formation with a higher concentration of acrylamide near pH 7. As a result, it has been demonstrated that lowering the frying temperature, prolonging frying time, and lowering the pH of dough by addition of citric acid can reduce the acrylamide content in this fried, twisted dough-roll.
2. Yeast fermentation can reduce the acrylamide content through the reduction of free asparagine.

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