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FACTORS AFFECTING THE ANALYSIS OF FURAN IN HEATED FOODS

C. Crews*, S. Hasnip, D. R. Roberts and L. Castle

Central Science Laboratory, Sand Hutton, York YO41 1LZ (UK)

* Author for correspondence

tel: ++44-1904-462000

email: c.crews@csl.gov.uk

KEYWORDS

furans, heated foods, heat-induced toxicant, analysis, determination

ABSTRACT

Various experimental factors that might affect the measurement of furan by automated headspace gas chromatography/mass spectrometry have been investigated. It was established that furan was not lost during sample heating through leakage or decomposition and that deuterium labelled furan used as an internal standard was stable with respect to incubation in the presence of food and stable in model food systems at raised temperature. Saturation of the aqueous phase of the sample with ammonium sulphate improved the partitioning of furan from samples into the headspace. There was possibly a very small decrease in the peak areas of both d₀-furan and d₄-furan when heated at acid pH. For food samples heated at normal headspace incubation temperature (50 °C), the level of furan measured was highest at the most acidic conditions used (pH 2.4) and did not differ significantly between pH 5 and 10. Under strong heating, production of furan decreased markedly at very high pH. Quantification based on standard additions or on external calibration gave comparable results for foods containing furan at relatively low, moderate and high levels.

INTRODUCTION

Background

Furan is a possible human carcinogen found in a number of heated foods, particularly those heat-processed in cans and jars, and in strongly heated foods such as roasted coffees. On account of its high volatility furan is best measured by headspace sampling techniques including direct headspace sampling [Hoenicke et al., 2004] or solid-phase microextraction [Goldmann et al., 2005; Bianchi et al., 2006]. In both of these approaches the food sample is weighed into a sealed vial which is heated (incubated) to effect a favourable partition of furan between the sample and the headspace gas. Quantification is usually achieved by means of the method of standard addition or by using external calibration standards, both using deuterated furan as an internal standard.

The first publication of a method suitable for the quantification of furan at the low levels found in foods was issued by the US FDA [FDA, 2004a], and was used to collect data from a number of foods likely to contain furan [FDA, 2004b]. Since the issue of this method a number of refinements have been made, most notably a reduction in the incubation temperature from 80° to 50°C or lower, because it has been established that furan can be formed in some foods heated at temperatures above 50°C [Hasnip et al., 2006; Senyuva and Gokmen, 2005].

The studies presented here were part of a wider investigation into the factors affecting the formation of furan in foods and were carried out as part of the EU 'Heatox' project on heat-generated food toxicants [Hellenas et al., 2005]. Our aim was to further characterize and improve the methodology for testing foods and model foods systems for furan.

To this effect, studies were made into several areas of the methodology. These were:

1. The potential for losses of furan through leakage through the headspace vial seals.
2. The potential for loss or decomposition of furan or deuterated furan on heated incubation for headspace sampling.

3. The potential for loss of internal standard through deuterium exchange with water or by reaction with food.
4. The effect of pH on the stability of furan and deuterated furan during the headspace incubation.
5. The addition of salt (ammonium sulphate) to increase headspace partitioning and thus analytical sensitivity.
6. A comparison of the use of external calibration with the standard additions method.

Materials and methods

Reagents and samples

Furan (99+%), d₄-furan (>99 atom % D) and ammonium sulphate were purchased from Sigma-Aldrich (Poole, Dorset). Sodium hydroxide, hydrochloric acid, sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate (Analar grades) were purchased from Fisher Scientific UK Ltd. (Loughborough, Leicestershire). Vegetable samples — fresh green (French) beans and a dried bean-based vegetable soup mixture containing dried peas, lentils, beans, onion, pearl barley, and pasta, were obtained from a local retailer.

Apparatus

Furan was measured using gas chromatography-mass spectrometry (GC-MS) using a ThermoFinnigan Voyager benchtop system fitted with a HS2000 headspace autosampler. The temperature of the headspace incubation oven was 50°C and the syringe needle was held at 100°C. Samples were incubated for 30 minutes with shaking. The injection volume was 1 ml and the injection speed was 30 ml/minute. Split injections were made at a ratio of 2:1 on to a HP-Plot Q column of 15 m x 0.32 mm i.d. with a 20 µm phase thickness. Helium carrier gas was used at a constant flow of 1.7 mL/min. The GC oven was held at 50°C for 1 min, then heated at 10°C min⁻¹ to 225°C and held for 12.5 minutes.

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3 The mass spectrometer was operated in electron ionization mode. Furan was detected by
4 selected-ion monitoring of the molecular ion at m/z 68 and confirmed by monitoring the
5 fragment ion, $[C_3H_3]^+$ at m/z 39. The internal standard was detected by monitoring the
6 molecular ion at m/z 72.
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13 Each analytical batch included a reagent blank and a tomato sauce in-house reference
14 material which had been characterised by repeated analysis as containing furan at
15 $2 \mu\text{g}/\text{kg} \pm 1.8 \mu\text{g}/\text{kg}$.
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19 20 21 **Experimental approaches**

22 *Losses of furan through leakage or decomposition*

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24 There were concerns that furan might leak from the vial seals during, for example, high-
25 temperature experiments of model systems, or that the deuterium labelling of the
26 internal standard would be lost in similar circumstances, or through reaction with food
27 matrices. Possible loss of furan was tested for by measuring the stability of furan and
28 deuterated furan heated in sealed vials with and without a food matrix. Aqueous
29 solutions (1 ml) containing $2.5 \mu\text{g}$ of unlabelled furan and deuterium labelled furan
30 were prepared in 20 ml headspace vials which were sealed with PTFE faced silicone
31 septa. One set was heated at 100°C for 1 hour and the other was left at room
32 temperature for the same time. The samples were analysed by GC-MS and the
33 responses measured for the molecular ion of d_4 -furan (m/z 72), and the ions
34 corresponding to successive substitution of deuterium atoms by hydrogen, i.e. m/z 71,
35 70, 69 and also for undeuterated furan (m/z 68).
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50 Potential reaction of the internal standard with food was studied by heating 0.5 g of a
51 dried bean-based vegetable mixture in water (8 ml) with $2.5 \mu\text{g}$ d_4 -furan internal
52 standard which was added before or after the heating period. This was intended to
53 reveal any deuterium exchange with hydrogen from water or food components.
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60 *Stability with respect to pH*

Stability in different pH regimes was studied by heating d_4 -furan at 100°C for 1 hour in

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3 water with the pH adjusted to acid (pH 4), neutral (pH 7) or basic (pH 11) conditions
4 using 0.2M phosphate buffer adjusted with sodium hydroxide and hydrochloric acid. In
5 these experiments the peak area response of the d₄-furan was recorded.
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10 *Effect of pH on furan quantification*

11 The effect of pH on furan determination was also tested on two sample matrices, runner
12 beans and a dried bean-based vegetable mixture. The runner beans were purchased fresh
13 and homogenised in a domestic food blender. The dry bean-based vegetable mixture
14 was milled to a homogenous fine powder. Portions of about 5g fresh bean and about
15 0.5g dry bean-based vegetable mix were mixed with 8 ml of pH buffer solution in 20 ml
16 headspace vials. Each matrix was prepared in duplicate at pH 2.4, 4.7, 7.1, 10.1 and
17 11.6.
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25 The samples were warmed in the headspace autosampler incubation oven under the
26 normal operating conditions of 50°C for 30 minutes. To amplify any effect of pH
27 further samples were heated at 80°C or 120°C for 60 minutes. After cooling internal
28 standard was added and furan was measured using a single point standard addition.
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35 *Addition of ammonium sulphate*

36 Addition of inorganic salts is known to improve the partition of volatile organic
37 compounds from aqueous solution into the headspace and ammonium sulphate is often
38 used for this purpose. Two samples of tomato sauce, which contained about 15 µg/kg
39 furan, were mixed in a headspace vial with 2 ml water and the aqueous phase saturated
40 by the addition of 5 g ammonium sulphate. These samples were compared with two
41 others without ammonium sulphate saturation. The samples were analysed under the
42 standard conditions with incubation at 50°C and the peak area response of the furan was
43 recorded. Sets of calibration standards containing 0.025, 0.050, 0.125, 0.25, 0.5, and
44 1.0 µg furan per vial were prepared in 2 ml water with and without ammonium sulphate
45 addition. These were analysed as above, recording the peak area of the d₀ and d₄-furan.
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59 *Comparison of quantification methods*

60 Two of the different methods used to quantify furan were compared on the same

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3 samples. These were the standard additions procedure of the FDA method [FDA,
4 2004a] and external calibration used in this laboratory and others [Hoenicke et al., 2004;
5 Hasnip et al., 2006]. The procedures were applied to samples having low (tomato
6 sauce), medium (tomato soup) and high (coffee powder) levels of furan. Each of the
7 samples was analysed on five separate days with furan quantified by standard additions
8 at five levels (5, 10, 25, 50, 100 $\mu\text{g}/\text{kg}$) and by means of an external calibration graph.
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15 16 17 **RESULTS**

18 19 20 21 *Potential for loss of furan through leakage or decomposition*

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23 The effects of heating unlabelled and deuterium labelled furan in headspace vials at
24 100°C for 1 hour are shown in Table 1. The repeatability of the determination was
25 good. The peak area for the unlabelled furan molecular ion (m/z 68) had a range of 598
26 million to 640 million units in the unheated sample, and 550 to 661 million units in the
27 heated sample. Similar data were obtained for the d_4 -labelled furan. These sets of results
28 could not be differentiated and thus it was clear that there were no physical losses from
29 the headspace vial or decomposition of the furan or internal standard caused by heating.
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39 Table 1 also shows the percentage of the response corresponding to the molecular ion of
40 furan with full deuteration (m/z 72) and with successive substitutions of deuterium by
41 hydrogen. The relative abundance of the molecular ion of the d_4 -furan remained at 95%
42 throughout, demonstrating that there was no isotopic exchange with hydrogen atoms in
43 the matrix.
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50 The range and average response for d_4 -furan in five samples of vegetable matrix when
51 the internal standard was added before or after heating for one hour at 100°C were very
52 similar. When the internal standard was added before heating the range was 71400 to
53 96800 peak area units, mean 85600 peak area units, and when it was added after heating
54 the range was 77000 to 88800, mean 82900 peak area units.
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3 The peak areas were also similar in the unheated sample (82500 to 100800, mean 93200
4 peak area units). This showed that there was no significant isotopic exchange of
5 deuterium with the food sample, or other losses of d₄-furan.
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10 11 *Stability with respect to pH*

12 Because deuterium loss by deuterium-proton exchange could be catalysed by acid or
13 base, the influence of pH was investigated. When d₀-furan and d₄-furan standards were
14 heated together at 100°C for 1 hour in water at pH 4, 7 and 11 there was a possible
15 small decrease (4%) in the d₀-furan and d₄-furan absolute peak areas in the acidic
16 medium (pH 4), but no difference between the samples heated at neutral and alkaline
17 pH. The peak area ratios remained constant across the samples. The slight decrease in
18 the peak areas at low pH, but constant peak area ratio of d₀/d₄-furan, is interpreted as
19 being due to a partial protonation of the ether oxygen atom of furan, which would
20 reduce the concentration of free (neutral, uncharged) furan in solution and thus lead to a
21 reduction in the partial pressure of furan in the headspace gas.
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33 *Effect of pH on furan quantification*

34 Table 2 shows the furan levels measured with warming at the incubation temperature of
35 50°C for 30 minutes as part of the normal analytical procedure. For both green beans
36 and the dried vegetable mixture the level of furan measured was highest at the most acid
37 pH (2.4). Furan levels did not differ significantly between pH 4.7 and 10.1 for the dried
38 vegetable mixture, but at pH 11.6 very little furan was detected in the green beans.
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47 When the samples were heated at 80°C, furan levels produced in the beans were little
48 changed between pH 2.4 and 10.1 (18 to 29, average 22 µg/kg), but decreased to less
49 than 2 µg/kg at the high pH of 11.6. Furan levels in the dried bean based vegetable
50 mixture appeared to decrease in a less linear fashion with increasing pH, from 50 to 7
51 µg/kg. These effects were more evident when the beans were heated at 120°C for 60
52 minutes. Figure 1 shows a linear decrease in the furan with rising pH measured in
53 duplicate samples of heated green beans. Figure 2 shows that for a bean based vegetable
54 mixture heated under the same conditions there is a sharp decrease in the level of furan
55 formed between pH 2 and 4 from about 500 to 200 µg/kg followed by a steadier decline
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3 to about 20 µg/kg at the highest pH. The fact that the response profiles for different pH
4 values are so different, suggests that different precursors and/or different formation
5 mechanisms operate in the two food types when heated. There appears to be three or
6 more important routes of furan formation in heated foods, depending on the food
7 composition and therefore the precursors available in that type of food [Locas and
8 Yaylayan, 2004; Mark *et al.*, 2006; Crews and Castle, 2006]. With this diversity of
9 formation routes in mind, the temperature used for headspace incubation has to be
10 considered carefully. As a pragmatic measure, it can be assumed that in the absence of
11 any specific information to the contrary, that an incubation temperature of 50°C or
12 lower is acceptable for all food types.
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24 *Addition of ammonium sulphate*

25 The results of the determination of furan in tomato sauce made with or without
26 saturation of the aqueous phase with ammonium sulphate are shown in Table 3. The
27 addition of the salt caused a 2.5 fold increase in peak area for both d₀-furan and d₄-furan
28 and the level of furan measured was the same within experimental error. Calibration
29 lines for standards prepared with and without ammonium sulphate addition were both
30 linear. Thus, as expected, the addition of ammonium sulphate improved the partitioning
31 of furan from samples into the headspace. Such salting-out might be of some use should
32 an increase in analytical sensitivity be required but for most food testing purposes the
33 sensitivity is sufficient without this enhancement.
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45 *Comparison of quantification methods*

46 The results of the experiments comparing the quantification method are summarised in
47 Table 4. The coffee powder sample contained 59 µg/kg furan (range 46-72 µg/kg) when
48 measured by external calibration and 62 µg/kg furan (range 56-67 µg/kg) by standard
49 additions. The tomato soup sample contained 24 µg/kg furan (range 20-29 µg/kg) by
50 external calibration and 28 µg/kg furan (range 22-33 µg/kg) by standard additions. The
51 tomato sauce sample contained 3 µg/kg furan (range 2-3 µg/kg) by external calibration
52 and 2 µg/kg furan (range 2-3 µg/kg) by standard additions.
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3 These results are essentially identical and this provides further evidence that both
4 methods of quantification appear to be equally suited. It means that, as anticipated, the
5 deuterated furan behaves well as an internal standard and the relative partitioning of
6 d_0/d_4 furan is not affected significantly by the nature of the food matrix. The external
7 calibration procedure is especially useful where a large number of food samples are
8 analysed as it reduces the need for duplication of sample preparation.
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16 CONCLUSIONS

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18 Studies into the methodology of furan determination have been applied to identify and
19 improve those methods currently available. This work has shown that there is no major
20 difference in furan levels measured in standards over a range of pH values, or any loss
21 of furan by leakage from automated headspace sampler vials, or any loss of deuterium
22 from the labelled internal standard.
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30 The labelled internal standard was shown to retain its deuterium when heated with both
31 water and a vegetable material, confirming that analytical data were not affected by the
32 normal analytical conditions or experimental heating of food samples or model systems.
33 Extreme acidic conditions increased the level of furan measured in food samples during
34 normal incubation compared with neutral conditions, whereas very basic conditions
35 reduced the amount furan measured. In food samples heated experimentally, the pH had
36 a pronounced effect on the level of furan measured, indicating that control of the pH is
37 required in quantitative experimental work.
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48 Methods of quantification based on standard additions or external calibration have been
49 reported as applied to surveys of furan in foods. The methods were compared to
50 establish that results obtained by both procedures were equivalent. For foods containing
51 furan at relatively low, moderate and high levels the two approaches were shown to be
52 equally effective. Saturation of the samples with ammonium sulphate enhances the
53 sensitivity of the analytical method but, in practice, is not normally required because
54 direct headspace methods have sufficient sensitivity anyway.
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Table 1 Effect of heating on furan and deuterated furan in solution

sample	Peak Area (million peak area units) at given m/z						Percent abundance at given m/z					
	68	69	70	71	72	Total area	68	69	70	71	72	
d0-furan unheated	min	598	28.0	2.6	0.8	0.7	630	95%	4.4%	0.4%	0.1%	0.1%
	max	640	30.0	2.7	0.8	0.8	675	95%	4.5%	0.4%	0.1%	0.1%
	n=5 average	621	29.1	2.7	0.8	0.8	654	95%	4.4%	0.4%	0.1%	0.1%
d0-furan at 100°C	min	550	25.9	2.3	0.7	0.7	580	95%	4.5%	0.4%	0.1%	0.1%
	max	661	31.1	2.8	0.9	0.8	697	95%	4.5%	0.4%	0.1%	0.1%
	n=5 average	622	29.2	2.7	0.8	0.8	656	95%	4.5%	0.4%	0.1%	0.1%
d ₄ -furan unheated	min	1.6	0.2	0.8	5.7	171	179	0.9%	0.1%	0.5%	3.2%	95%
	max	1.7	0.2	0.9	6.2	185	194	0.9%	0.1%	0.5%	3.2%	95%
	n=5 average	1.7	0.2	0.9	6.0	178	187	0.9%	0.1%	0.5%	3.2%	95%
d ₄ -furan at 100°C	min	1.7	0.2	0.8	5.6	167	175	0.9%	0.1%	0.5%	3.2%	95%
	max	1.9	0.2	0.9	6.0	179	187	1.0%	0.1%	0.5%	3.2%	95%
	n=5 average	1.8	0.2	0.8	5.8	173	182	1.0%	0.1%	0.5%	3.2%	95%

Table 2 Effect of pH on furan formation in runner beans during incubation (heated 30min at 50°C

pH	furan $\mu\text{g}/\text{kg}$	
	green beans	vegetable mixture
2.4	5	17
4.7	2	7
7.1	3	6
10.1	3	8
11.6	> 2	6

Table 3 Effect of added ammonium sulphate on furan peak area (incubated 30 minutes at 50°C)

	furan peak area	IS peak area	ratio	furan µg/kg
Tomato sauce	2,716	25,077	0.11	14
Tomato sauce	3,032	28,657	0.11	14
Tomato sauce saturated with NH ₄ SO ₄	7,106	73,998	0.10	12
Tomato sauce saturated with NH ₄ SO ₄	6,176	66,222	0.09	12

Table 4 Furan levels (range and average, n=5) in coffee, tomato soup and tomato sauce, quantified by external calibration and standard additions methods

Sample	Furan $\mu\text{g}/\text{kg}$	
	external calibration	standard additions
Ground coffee	46 to 72 (59)	56 to 67 (62)
Tomato soup	20 to 29 (24)	22 to 33 (28)
Tomato sauce	2 to 3 (3)	2 to 3 (2)

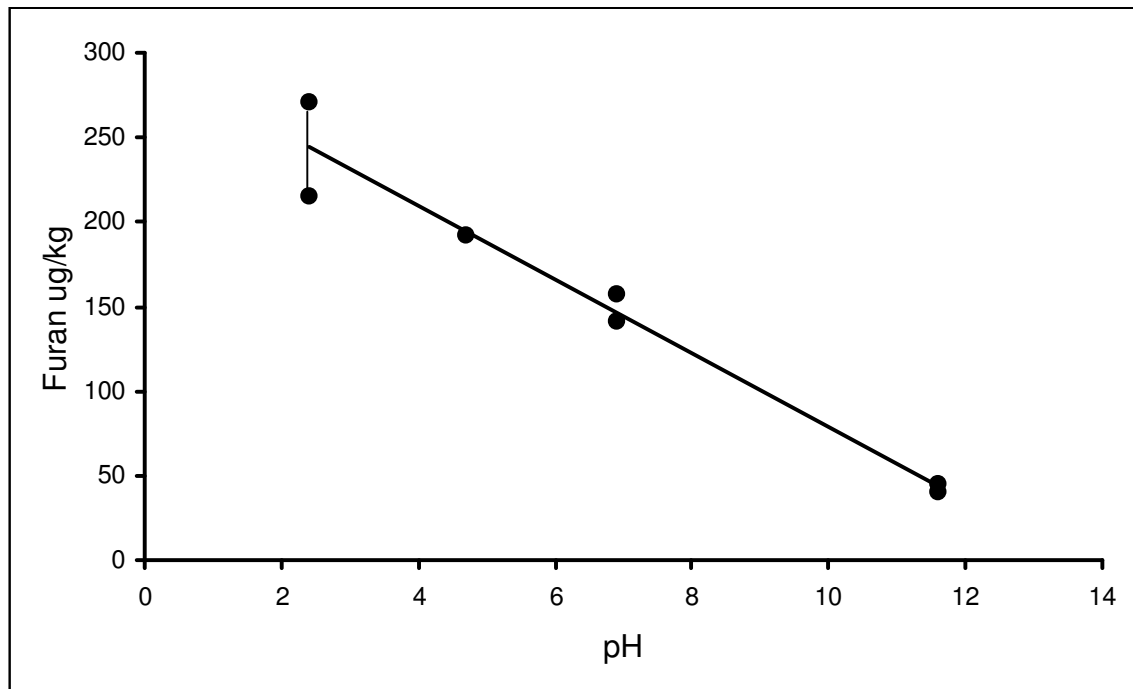


Figure 1 The effect of pH on furan formation in green beans heated at 120°C for 60 minutes (n=2).

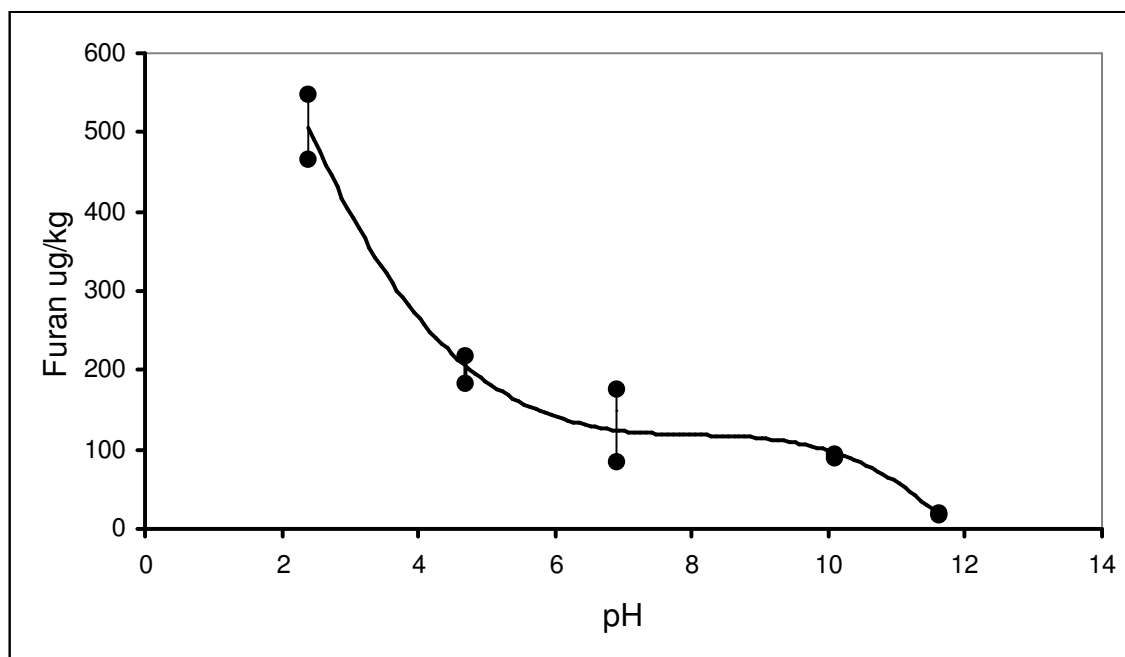


Figure 2 The effect of pH on furan formation in a dried bean based mixture heated at 120°C for 60 minutes (n=2).