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Targeted investigations of acrylamide in food: Phase 1

Biscuits

A report prepared for the Food Standards Agency

October 2015

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1st draft:	13th May 2015
Final Version	6th October 2015
Pre-publication amendments	24th February 2016
Sponsor:	Food Standards Agency,
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	London, WC2B 6N
Sponsors Project Title:	Targeted investigations of acrylamide in food: Phase 1 Biscuits
Sponsors Project reference No:	
Distribution:	1.
	2.
	3.
	4.
	5.

1. Summary

A short investigation of acrylamide in UK biscuits was undertaken to determine whether biscuits could be categorised based on available knowledge of recipe and process factors: The overall objective was to attempt to define more detailed sub-groups for biscuits within the context of current EC Indicative Values. Following a review of historical data, a matrix of key process & recipe variables (together with acrylamide) was created from an investigation of approximately 60 biscuits (representing 11 categories) collected from retail outlets in 2014. The key findings from the investigation were as follows:

- Trends in acrylamide
    - Categories of biscuits with highest mean levels of acrylamide were the ‘Cones and wafers’, ‘Breakfast biscuits’ and ‘Ginger biscuits’.
        - These biscuits together with ‘Crackerbread / Crispbread’ also had the greatest range of acrylamide values.
    - Lowest amounts of acrylamide were found in Shortbread, ‘Crispbakes’ and ‘Soda crackers’.
    - Amounts of acrylamide in products followed a trend with respect to raising agents declared on the label: yeast << none or sodium < ammonium.
        - Biscuits declaring yeast had the smallest range of acrylamide values.
        - Within certain categories, there was a trend towards lower acrylamide in biscuits declaring yeast.
- Matrix model
    - Key recipe factors (qualitative data) identified included: added fruit, inverted sucrose, sucrose, ginger, ammonium based raising agents, rye or rye / wheat combinations, yeast raising agent and divalent metal ions (Ca/Mg).
    - Key process factors available (quantitative data) included: Moisture and pH.
        - Measurement of colour as an indicator of baking conditions was not possible due to contributions from (coloured) ingredients.
    - Multivariate regression analysis of all recipe and process factors showed that some of the variability in acrylamide could be accounted for by just three qualitative variables: the presence of ammonium, and / or ginger and / or sugar (sucrose).
        - The remaining variability was most likely due baking conditions (temperature / time) and quantitative differences in key ingredients.
    - It is likely that detailed baking conditions (temperature / time), together with moisture, pH and quantitative recipe data (including asparagine concentrations for raw materials), would be required to predict amounts of acrylamide in biscuits with any certainty.
- EC Indicative Value Categories
    - Seven samples exceeded EC Indicative Values.
        - Four of these were ginger biscuits which had been assigned to the ‘Biscuits & Wafers’ category (Indicative Value 500 µg/kg).
        - Similarities in recipe indicate that Ginger biscuits might be included in the ‘Gingerbread’ category, for which a higher Indicative Value (1000 µg/kg) applies.
            - It may be appropriate to consider extending the definition for the ‘Gingerbread’ category to include Ginger biscuits.
    - This investigation may provide some scientific justification for sub-categories of biscuit based on products declaring yeast in their recipes.

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Terms and abbreviations

AA	acrylamide
ALARA	As Low As Reasonably Achievable
Asn	asparagine
ANOVA	analysis of variance
IV	EC Indicative Value

2. Introduction

2.1 Acrylamide

Absent in raw food, or the raw materials used to make food, acrylamide (AA) is a chemical substance that can form naturally when some foods are subjected to high temperatures during cooking (including home-cooking) and processing. Acrylamide is formed when foods containing the natural amino acid asparagine and sugars are heated at temperatures greater than 120°C. Although AA does not occur in such foods subjected to lower temperatures and relatively short process times e.g. boiled potatoes, it has been found in a wide range of home-cooked and processed foods, including potato crisps, French fries, bread, crispbreads and coffee.

Regular and prolonged exposure over a lifetime to foods containing high levels of AA and furan has the potential to increase the risk of developing cancer. Experts, including the international Joint Food and Agriculture Organisation and the World Health Organisation Expert Committee on Food Additives (JECFA), have concluded that current global levels of dietary exposure to AA indicate a human health concern. In the UK, the Food Standards Agency (FSA) has concluded that exposure to AA should be as low as reasonably achievable (ALARA).

There are no statutory maximum AA levels; however the latest EC Recommendation[1] on investigations into the levels of AA in food specifies 'indicative values' (IV) for AA. These IV are not intended as maximum limits but as a trigger to initiate investigations by enforcement authorities into food business operators' understanding of AA and any action they have taken to mitigate its production. The EC has specified IVs for five sub-categories of biscuits, namely crackers, crispbread, ginger bread and other products similar to these sub-categories (see Table 1).

An AA "toolbox" is available to industry to provide guidance on measures to reduce AA levels in various categories of foods[2]

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[1] Commission Recommendation of 8 November 2013 on investigations into the levels of acrylamide in food (2013/647/EU), oj L 301, 15-17, 12.11.13

[2] Available at: http://www.fooddrinkeurope.eu/publications/category/toolkits/

Table 1. EC Indicative values for acrylamide in biscuits

Foodstuff	Indicative value (μg/kg)	Comment
Biscuits and wafers	500	Product as sold, as defined in Part C.6 of the Annex to Recommendation 2010/307/EU
Crackers with the exception of potato based crackers	500	
Crispbread	450	
Ginger bread	1000	
Products similar to the other products in this category	500	

2.2 Project brief and lines of approach

This study was commissioned to investigate the relationship (correlations) between declared recipe and processing factors and AA formed in retail biscuits. The objective of the study was to determine whether a matrix model could be developed to predict a more appropriate subgroup for a given biscuit type for which a current EC IV might apply. It was anticipated that a more defined sub-categorisation of products would enable a better understanding of the potential impacts of IVs, maximum limits or other enforcement measures. The investigation would review historical data on AA levels from previous FSA Surveys (2007-2014) to inform the subsequent selection and investigation of approximately 60 retail samples collected for this task in 2014.

3. **Review of historical data**

Acrylamide levels in biscuits can vary widely (Leung et al. 2003; Taeymans et al. 2004) and amounts >1000 µg/kg have been reported in some categories (Hamlet et al 2005; 2007) (see Table 2).

Table 2. Summary of acrylamide levels measured in retail biscuits sampled in 2006: mean and range by product type (data from Hamlet et al 2007)

Biscuit category	Acrylamide (µg/kg)			
	n	Mean	Min	Max
Ginger biscuits	10	1111	128	1859
Crispbreads	9	876	152	1546
Crackers	8	643	290	1028
Amaretti	1	348	-	-
Rich Tea	6	334	277	402
Digestive	11	295	159	444
Water biscuit	2	282	258	305
Bourbon	3	245	176	287
Savoury or snack crackers	8	243	16	552
Cream crackers	4	167	115	203
Pretzels	2	68	53	83
Coconut	1	64	-	-
Ginger shortbread	1	61	-	-
Garibaldi biscuits	1	27	-	-
Shortbread	1	13	-	-

Free asparagine (Asn) is believed to be the limiting or dominant precursor of AA formation in cereal products and hence recipes that utilise cereals which are naturally high in Asn, such as rye, oats and wholemeal flours, may form more AA. Conversely, reducing or consuming Asn, e.g. by the addition of either an enzyme or bakers yeast or another microorganism (Fredriksson et al 2004; Hamlet et al 2005; 2007; Sadd & Hamlet 2008) to product recipes, can have a significant impact on AA generated during baking. Divalent metal ions (e.g. Ca / Mg) have also proved to be effective in reducing AA in bakery products possibly due to their ability to form relatively stable, i.e. nonreactive, Asn (and / or other amino acids) / metal ion complexes (Lindsay & Jang 2005).

As Asn is consumed during cooking, e.g. via Maillard reactions (Sadd & Hamlet 2005), it can not be used as a retrospective measure of AA generating potential in finished biscuits.

The thermal conversion of Asn into AA is a multistep series of reactions which are believed to begin with reducing carbohydrates and the subsequent generation of reactive (towards Asn) carbonyl species. Fructose was identified as one of the more reactive sugars at an early stage and the ability of these reducing carbohydrates to initiate or promote reaction steps leading to AA is still a key area of research. Consequently biscuits that utilise high fructose ingredients in their recipes, e.g. inverted sucrose syrups, molasses, etc, may form more AA. Another early discovery was that ammonium (carbonate and hydrogencarbonate) raising agents could act as accelerants for AA formation, possibly due to increased formation of reactive carbonyls from reducing sugar / ammonium reactions (Amrein et al. 2004). Consequently the replacement of these salts with e.g. sodium raising agents to reduce AA during baking was recognised at an early stage (Biedermann and Grob, 2003). Figure 1, which has been taken from investigations in 2006 (Hamlet et al 2007) shows the distribution of AA in retail biscuits as a function of raising agents declared: Within particular categories of biscuit, it can been seen that the lowest levels of AA were found in products declaring yeast, sodium + yeast or sodium as raising agents e.g. crispbreads, cream crackers and pretzels. The designations “ammonium + sodium” or “sodium + ammonium” used in Figure 1 reflect the order of raising agents given on the retail packs. This distinction was included for evaluation only in the event that the order of raising agents given on the pack may relate to the most significant / abundant ingredient.

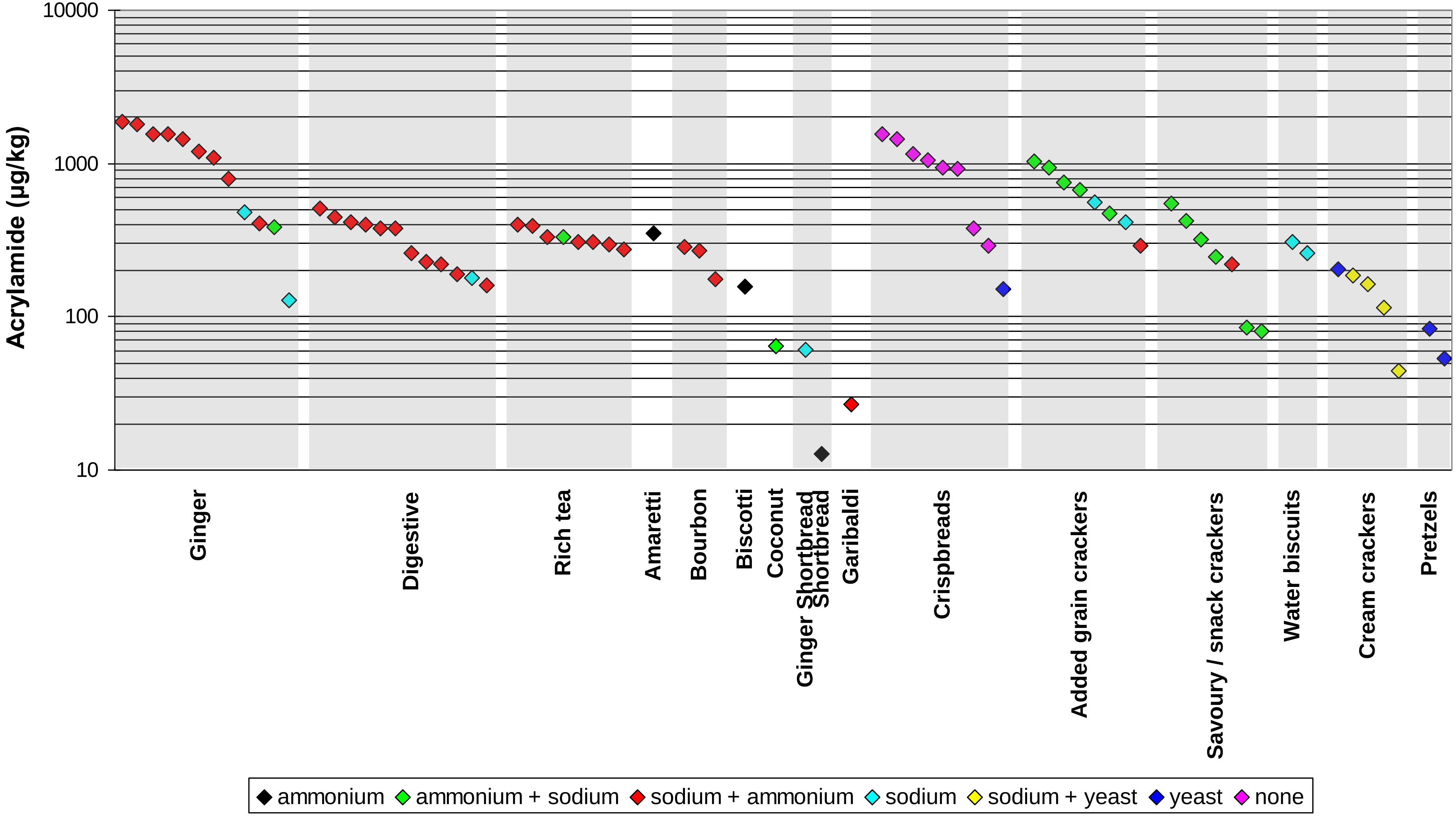

Figure 1. Distribution of acrylamide in biscuit types as a function of raising agents declared (adapted from Hamlet et al 2007)

Within some categories of biscuits (and cake), those containing ginger have previously been found to have higher levels of AA (Hamlet et al 2005; 2007). The combination of high fructose syrups and ammonium raising agents present in ginger biscuits could in part account for higher AA (compared with the continental gingerbread) although there are gaps in the understanding. For example, shortbread biscuits did not contain significant levels of AA. However the equivalent product with added stem ginger (and sodium based raising agent) showed significant formation.

Temperature and time (and moisture and pH) are equally important determinants of AA formation in cereal products (Sadd and Hamlet 2005). Studies have shown that levels of Maillard products (as indicated by colour formation) do not necessarily align with AA; low moisture content was a more important promoter of AA than temperature and therefore a key factor controlling AA levels, and this could account for much of the variation seen between different types of bakery products (Sadd and Hamlet 2005). However, moisture range is relatively narrow in biscuits and hence unlikely to be a significant variable with respect to AA; a low pH is beneficial for reducing AA but its effectiveness in cereal products may be limited by buffering of the dough and flavour constraints (Sadd and Hamlet 2008). Colour changes resulting from the progression of the Maillard reaction during biscuit baking (and hence AA) can provide an indication of AA in baked cereal products. In the case of biscuits, model system studies showed a weak relationship between AA and the tri-stimulus colour space red-to-green (a) value (LAB scale; Hamlet et al 2007).

Predictive models for AA based on measurement of key processing (e.g. temperature / time, colour, moisture, pH) and recipe parameters (e.g. Asn & sugar concentrations in raw materials) have shown good agreement with measured values in cereal (Sadd & Hamlet 2005; Wedzicha et al 2005) and potato products (Parker et al 2012).

3.1 FSA surveys 2007-2014

The UK FSA has collected AA and qualitative ingredient data (declared on label) from a wide range of retail products (mainly cereal and potato based) since 2007, primarily in response to EC Recommendations for pan-European activity on monitoring[3,4] and investigating the effectiveness of reduction methods for AA[5,6]. Within the FSA survey reports, data for biscuits covering the period 2007-2014 was available (Hamlet et al. 2015; 2014; 2011; 2010; 2008) that could be abstracted and assessed (statistically), primarily to inform the subsequent selection and investigation of biscuits for a detailed study (see section 4).

[3] Commission Recommendation of 3 May 2007 on the monitoring of acrylamide levels in food (notified under document number C(2007) 1873) (Text with EEA relevance) (2007/331/EC)

[4] Commission Recommendation of 2 June 2010 on the monitoring of acrylamide levels in food (Text with EEA relevance) (2010/307/EU)

[5] Commission Recommendation of 10.1.2011 on investigations into the levels of acrylamide in food

[6] Commission Recommendation of 8 November 2013 on investigations into the levels of acrylamide in food (Text with EEA relevance) (2013/647/EU)

As raising agents were known to influence AA in biscuits, the data was initially categorised by individual raising agents, i.e. ‘ammonium’, ‘none or sodium’, ‘yeast’ and ‘combinations of all’. The mean AA amounts in each category given in Table 3 shows that the biscuits followed the expected trend, i.e. yeast < none or sodium < ammonium + yeast < ammonium and these data were statistically significant at the 95% confidence level by ANOVA (see Appendix 6.2, Table 8): eight biscuits utilised both ammonium and yeast in their recipe and the mean AA amounts in these products was a little lower than biscuits declaring ammonium suggesting that the former was the dominant variable with respect to AA formation. Furthermore, the range of AA values within each raising agent category was relatively wide indicating that other factors, such as quantitative variation in ingredients and / or processing conditions, were important in determining amounts of AA formed.

Table 3 Summary for acrylamide in the Survey biscuits (2007-2014) categorised by raising agent (declared)

Raising agent declared in biscuit	n	acrylamide (µg/kg)		
		mean	range	sd
yeast	44	244	34-868	1989.0
none or sodium	51	291	27-1538	283.4
ammonium + yeast	8	505	302-868	409.1
ammonium	41	568	27-1573	197.1

To try and account for the relatively wide range of AA values observed for the categories given in Table 3, a total of six key recipe variables (together with AA concentrations) were catalogued, as present or absent, from the 129 biscuit samples covering the period 2007-2014: no quantitative ingredient information or processing details were available. In addition to the key raising agents, i.e. ammonium, none or sodium, and yeast, the variables included: Inverted sucrose; sugar (sucrose), ginger; and rye (=rye and rye / wheat combinations). The historical data was then assessed using the statistical functions of Microsoft Excel (see 6.1.3 for method details) for the survey period as a whole (2007-2014) as well as individual periods (2007-2009, 2010-2013, 2014) to allow for changes to the number / type of biscuits tested during these periods.

Multivariate regression ($p = 0.05$) of the historical data as a whole (2007-2014) showed that approximately half of the variation in AA could be accounted for by three statistically significant variables: the presence of ammonium; and / or the presence of ginger, and / or the absence of rye. Statistically, this was a “strong” finding as no actual quantities for any of the recipe ingredients was available. The correlation was not improved by allowing for the presence of other ingredients such as invert sucrose, sugar or yeast or logarithmic transformation. Yeast was found to be beneficial in reducing AA in the 2007-2009 data but not for the whole data set; ginger was the only statistically significant (AA promoting) factor common to data from all survey periods. The statistical output summaries from all multivariate regressions can be found in section 6.3 of the Appendices.

3.2 Conclusions from historical review

a) Early investigations sponsored by the UK FSA (Sadd & Hamlet 2005; Hamlet et al 2005; 2007; 2008) showed that:

- The highest mean levels of AA were found in the ginger biscuits, crispbreads and crackers. These products, together with digestives and savoury / snack crackers also had the greatest range of AA levels.
- The lowest mean levels of AA were found in the garibaldi and shortbread biscuits.
- Biscuit recipes that utilise cereals which are naturally high in Asn, such as rye, oats and wholemeal flours, may form more AA.
- Recipe ingredients that can promote AA formation include fructose (e.g. inverted sucrose syrups) and ammonium based raising agents.
- Biscuits utilising ginger appear to form more AA.
- Yeast has a protective effect in real bakery processes since lower levels of AA are produced in the final food than would be expected from the chemistry of the main ingredients.
- Calcium supplementation (especially as the chloride salt) appears to be an effective control. Although less effective in biscuits, calcium carbonate supplementation of UK flours plays an important role in AA reduction in breads.
- Low pH is beneficial for reduction of AA formation.
- Temperature and moisture are important determinants of AA in cereal products: Low moisture content is a more important promoter of AA than temperature and may be a key factor for AA variation in biscuits.

b) A statistical review of AA and recipe data from FSA surveys (2007-2014) indicated that much of the variation in AA could be accounted for by three recipe ingredients: the presence of ammonium; and / or the presence of ginger, and / or the absence of rye.

4. 2014 Investigation

The scientific approach for the main investigation followed three main tasks:

1. Identify and construct a matrix of key recipe and process factors for AA formation in biscuits
2. Explore the relationship between the various factors and AA
3. Identify trends in support of a more detailed categorisation within the context of EC IVs.

4.1 Selection and sampling of biscuits

The selection of biscuit types for detailed investigation was based primarily on those surveyed previously in the UK (FSA surveys 2007-2014). The biscuits included in previous FSA surveys represented a wide range of categories for which both qualitative ingredient information and AA concentrations were available. It was expected that this approach would a) extend the pool of data for subsequent analysis / trends and b) provide a calibration or benchmark for new data from the investigation (in comparison with the historical category values). Each category was then expanded to include additional brands so that the data obtained from the investigation would be representative of the market. Additional categories, that were either relatively new to the market (e.g. breakfast biscuits) or had not previously been investigated, were also included for investigation. Details of the 11 biscuit types / categories identified for investigation are given in Table 4: the total number of biscuits for investigation was 58.

Table 4. Details of the 2014 biscuits selected for investigation

Biscuit category	n	Comments
Breakfast biscuits	6	A relatively new category of biscuit containing wholegrain cereals, many contain added fruit
Cones & wafers	6	Wafer sheets baked from a very fluid batter comprising wheat flour and sugar between hot plates; can also be rolled / shaped e.g. cones
Crackers		
Cream	*6*	*Unsweetened fermented (yeast) recipe based on wheat flour, salt and fat. Process involves dough sheet lamination & cutting prior to baking*
Flatbread	*3*	*Typically unleavened wheat and sometimes rye bread-like recipe with added seeds*
Oatcake	*1*	*Traditional Scottish cracker / biscuit, cooked on a griddle / hot plate or in oven*
Soda	*1*	*Typically alkaline pH dough, baked rapidly in very hot ovens*
Wheat germ	*1*	*Chemically leavened and sweetened wheat based dough*
Crackerbread / Crispbread	8	Traditionally prepared from wholemeal rye flour, salt, and water. Modern variants contain wheat flour, spices and grains, and are raised with yeast or sourdough, and milk or sesame seeds can be added. In unleavened crispbread, bubbles are introduced into the dough mechanically. Crispbread is only baked for a few minutes, at temperatures usually between 200 and 250 °C.
Crispbakes	2	Essentially twice baked bread (similar to a rusk recipe) also known as Dutch crispbakes or Beschuit
Digestives	4	Semi-sweet short dough biscuit, based on wheat flour, with relatively high amounts of fat and sugar and a minimal gluten network. Typically utilise sodium based raising agents
Ginger biscuits (gingernut)	6	A sugar rich short dough biscuit based on wheat flour, with a minimal gluten network, utilising inverted sucrose syrups, molasses in the recipe. Flavoured with ginger (stem / powdered) and lemon oil and sodium and / or ammonium based raising agents.
Malted Milk	4	A short dough biscuit, based on wheat, with a minimal gluten network and utilising barley malt extract and whole milk (powder) in the recipe.
Rich Tea	5	Hard-sweet biscuit, based on wheat flour, with a well developed gluten network and relatively low amounts of sugar and fat.
Shortbread	4	Relatively fat-rich short dough biscuit, based on wheat flour, with a minimal gluten network. Baked at a low temperature to avoid browning.
Water biscuit	1	Typically baked from flour and water, without shortening / fats.

4.1.1 Cataloguing of recipe variables

Key recipe information was abstracted from the ‘on-pack’ ingredient declarations of each of the 58 samples to an Excel database as present or absent: the presence or absence of fruit and metal ions (Ca / Mg) was also included in addition to the recipe variables used in the historical review (see section 3); no quantitative recipe information was available.

The distribution of the key recipe variables in each of the biscuit categories is given in Figure 2 and Table 11 of the Appendices (see section 6.5).

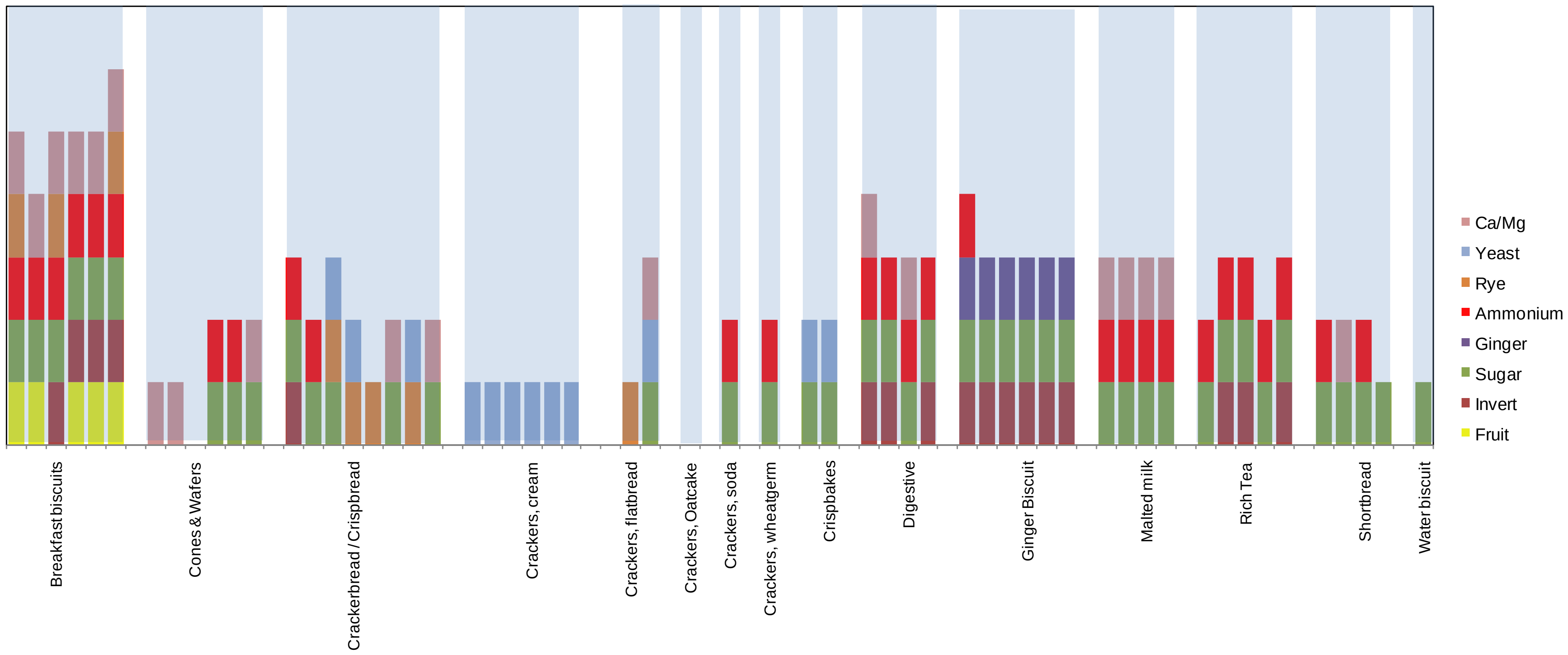

Figure 2 Distribution of key ingredients in the 2014 biscuit categories: base cereal is wheat flour unless stated; rye = rye or rye / wheat combination; ammonium = ammonium or ammonium + sodium based raising agents; invert = inverted sucrose syrup; sugar = sucrose

4.2 Chemical / physical analysis

4.2.1 Acrylamide

Concentrations of AA measured in each of the 58 samples are summarised in Figure 3. Categories of biscuits with highest mean levels of AA were the 'Cones and wafers', 'Breakfast biscuits' and 'Ginger biscuits': the highest amount of AA was measured in a ginger biscuit. Shortbread, 'Crispbakes' and 'Soda crackers' all had mean AA amounts below 20 µg/kg. 'Cones and wafers', 'Breakfast biscuits', 'Ginger biscuits' and 'Crackerbread / Crispbread' showed the greatest variation in AA amounts.

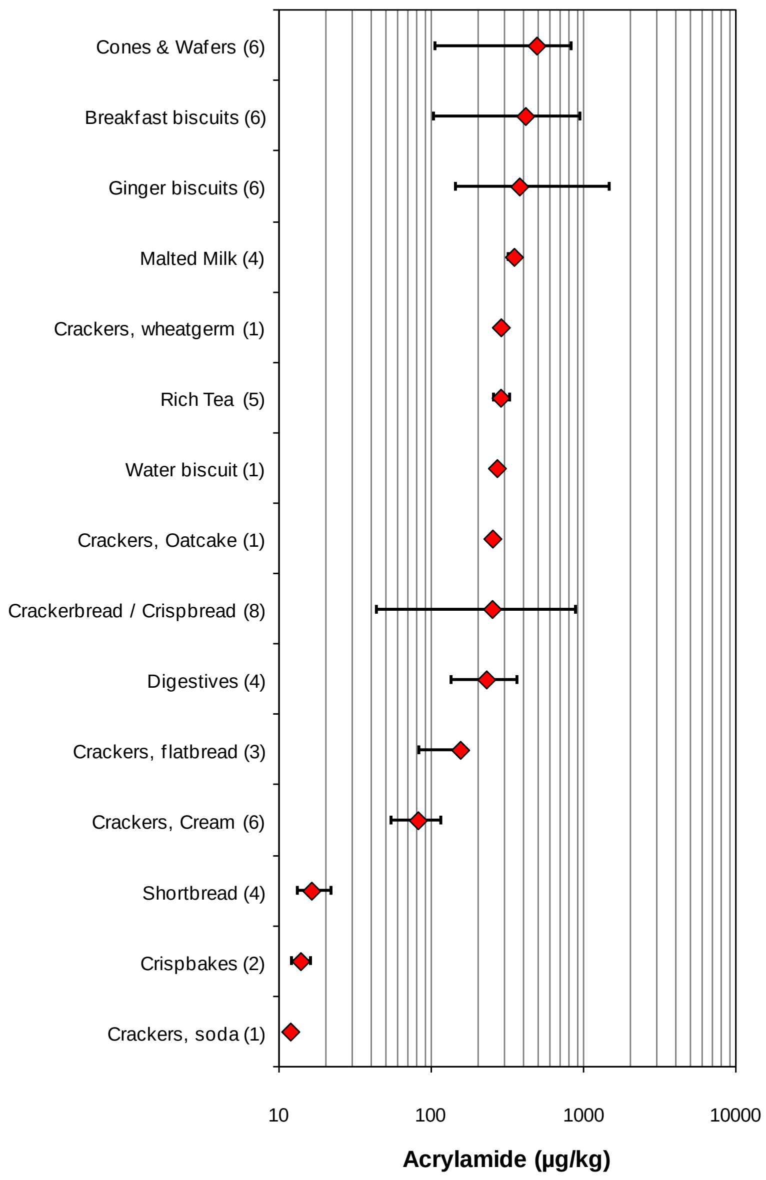

Figure 3. Distribution of AA in the 2014 biscuit categories (logarithmic scale) arranged in order of increasing concentrations: mean, range and number of samples (in parentheses).

When the biscuits were categorised by raising agent, the means for AA also followed the expected trend observed in the ‘historical data’, i.e. yeast < none or sodium < ammonium (see Table 5) and these differences were statistically significant by Anova at the 95% confidence level (see Appendix 6.4, Table 10). Unlike the ‘historical data’ however, the mean and range of AA amounts was much lower for biscuits using ‘yeast’ in their recipes while the ranges for products using ‘none or sodium’ and ‘ammonium’ remained relatively wide.

Table 5 Summary for acrylamide in biscuits categorised by raising agent (declared)

Raising agent declared in biscuit	n	acrylamide (µg/kg)		
		mean	range	sd
yeast	12	94	12-292	76.5
none or sodium	18	238	13-815	238.2
ammonium	28	360	12-1470	307.0

The distribution of AA within the individual categories of biscuit as a function of raising agents (declared) is given in Figure 4. As expected, within some categories, e.g. ‘Crackerbread / Crispbred’ and ‘Ginger biscuits’, lowest amounts of AA were found in biscuits utilising none, sodium or yeast in their recipe. However for other categories, the picture was less clear indicating that other recipe or process factors had influenced amounts of AA formed.

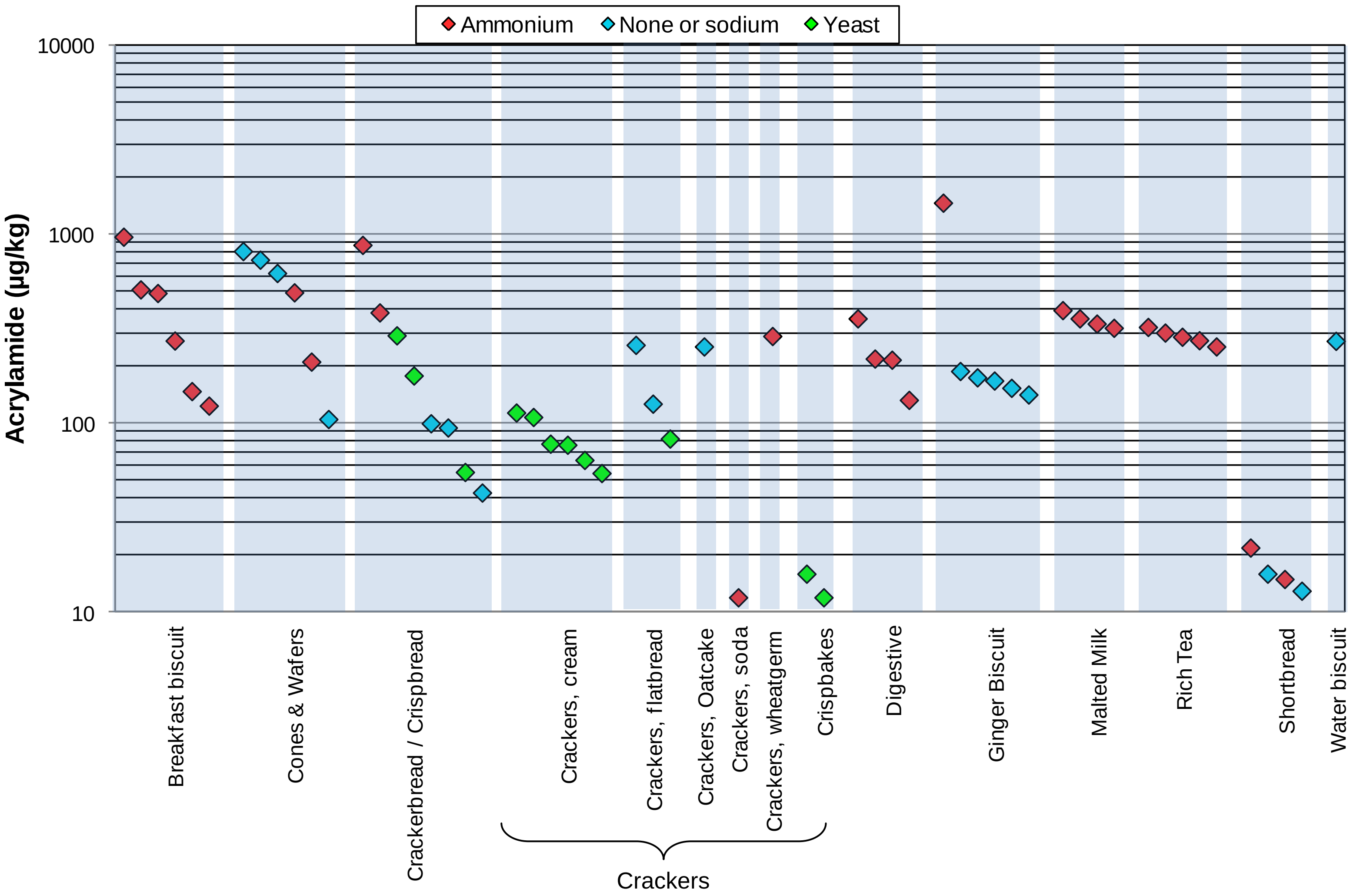

Figure 4. Distribution of acrylamide in each biscuit category as a function of raising agent declared

A summary of AA means and ranges categorised according to current Commission Recommendations for IVs is given in Table 6. The definitions for each biscuit category were rather broad and hence the assignment of some biscuit categories could be subjective. For example, the ginger biscuits were assigned to the 'Biscuits and Wafers' although they might equally have been placed in the 'Gingerbread' category as they appear to share a similar recipe / process: The IV for 'Gingerbread' (1000 µg/kg) is much higher than that for the other categories. Similarly the placement of Breakfast biscuits in the 'Products similar to the other products in this category' was somewhat arbitrary and in this instance based on the inclusion of fruit (which was absent from other 'Biscuits & Wafers'). With the exception of the crackers, all other categories contained samples with amounts of AA in excess of IVs.

Table 6. Distribution of acrylamide in the 2014 samples according to EC IV sub category definitions

Commission sub category	n	Acrylamide (µg/kg)			n>IV
		Mean	range	IV	
Products similar to the other products in this category [a]	8	318	12-971	500	2
Biscuits & wafers[b]	29	315	13-1470	500	4
Crispbread	8	254	43-879	450	1
Crackers with the exception of potato based crackers	13	138	12-290	500	-

[a] Breakfast biscuits & Crispbakes; [b] includes Ginger biscuits

The distribution of AA within the EC IV sub categories of biscuit as a function of raising agents (declared) is given in Figure 5. As expected, within some Categories of biscuit, lowest amounts of AA tended to be found in products utilising none / sodium or yeast in their recipe.

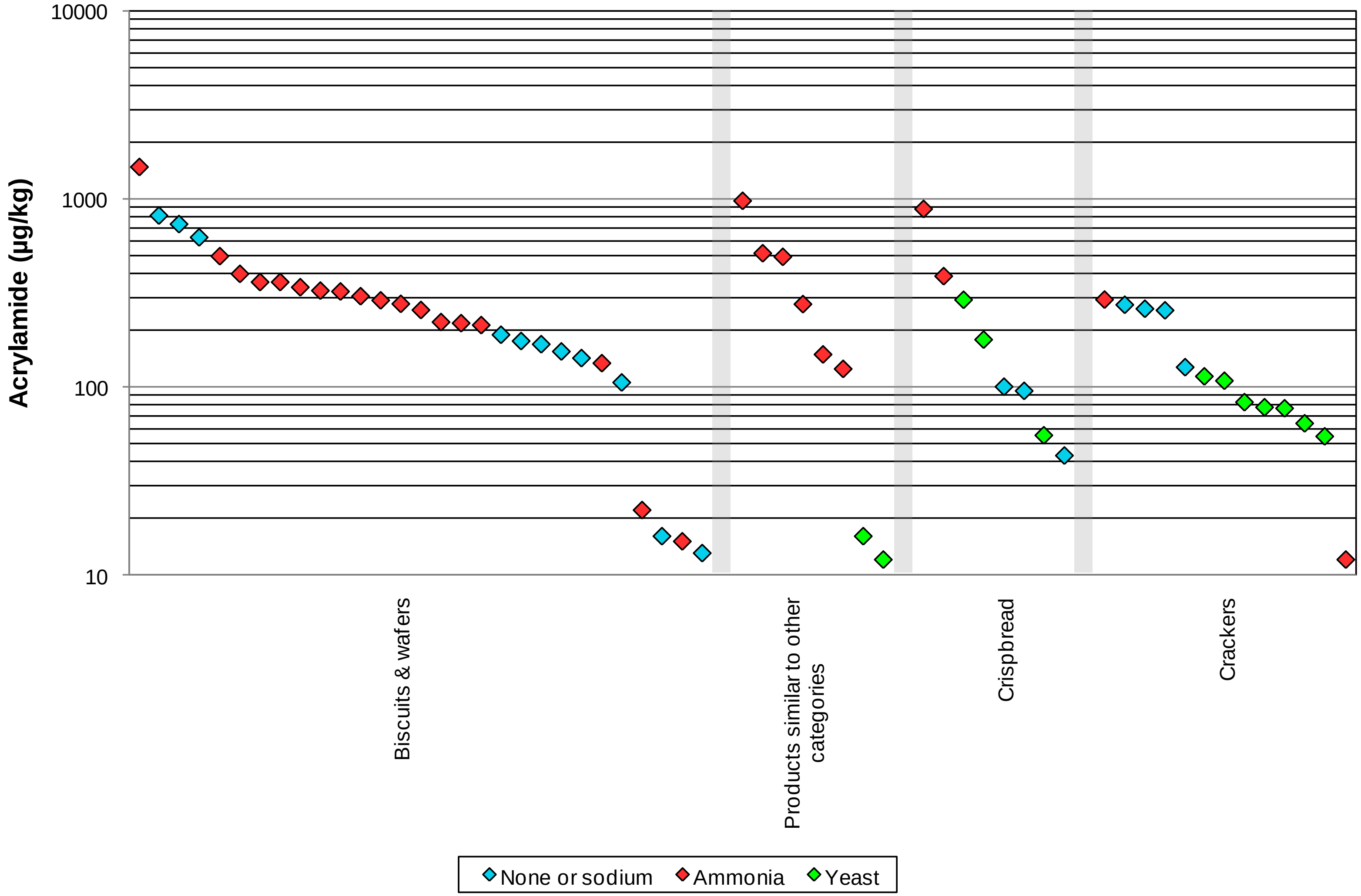

Figure 5. Distribution of acrylamide (logarithmic scale) in each EC sub category as a function of raising agents declared.

4.2.2 Measurement of process variables

Bake temperature / time, and moisture (and to a lesser degree pH) are important process variables for AA formation. At the outset of this project it was proposed to measure biscuit colour (using the lab colour space) as an indication of baking conditions so that this important process variable could be included in the final data analysis. However, inspection of the ingredient declarations indicated that the contribution to overall colour was not solely due to baking as some ingredients were highly coloured (e.g. fruit, cocoa powders, rye). Consequently this task was replaced by the measurement of additional processing parameters known to be important for AA formation, i.e. moisture and pH.

A summary of moisture, pH and the corresponding AA values for each category of biscuit is given in Table 7: Digestives and Rich Tea biscuits had the lowest mean moisture and highest mean pH (both of which can promote AA formation) although these products did not contain the highest amounts of AA; no statistical relationship was apparent between moisture and AA; and pH showed a weak correlation with AA (R square=6.7%, significance F<0.1 ,p=0.0496).

Table 7. Summary of moisture, pH and acrylamide measured in the 2014 biscuit categories.

	n	moisture (g/100g)		pH		acrylamide (µg/kg)	
		mean	range	mean	range	mean	range
Breakfast biscuits	6	4.8	3.1-5.5	6.6	5.9-6.9	420	124-971
Cones & wafers	6	3.8	1.7-7.1	6.8	6.3-7.0	497	105-815
Crackerbread / Crispbread	8	5.4	3.1-6.9	6.4	4.4-7.8	254	43-879
Crackers, Cream	6	4.8	3.4-5.8	6.7	6.3-7.1	83	55-114
Crackers, Flatbread	3	3.4	3.1-3.8	6.0	5.8-6.4	157	83-260
Crackers, Oatcake	1	3.8	-	6.6	-	255	-
Crackers, Soda	1	3.1	-	7.9	-	12	-
Crackers, Wheat germ	1	3.6	-	7.2	-	290	-
Crispbakes	2	5.4	4.7-6.0	5.4	5.3-5.4	14	12-16
Digestives	4	2.5	1.6-3.4	8.7	7.9-9.4	232	133-359
Ginger biscuits	6	3.5	2.8-4.4	7.1	6.9-7.7	383	142-1470
Malted Milk	4	3.4	3.2-3.6	7.8	7.5-8.1	353	320-397
Rich Tea	5	2.6	1.9-3.2	8.1	8.0-8.3	288	255-323
Shortbread	4	4.0	3.1-4.9	7.0	6.5-7.8	17	13-22
Water biscuit	1	2.9	-	6.7	-	273	-

4.3 Data analysis: correlations between acrylamide and recipe and process factors

The matrix of catalogued recipe (see section 4.1.1) and processing variables (see sections 4.2.1, and 4.2.2), together with AA values, was assessed using the statistical functions of Microsoft Excel (see 6.1.3 for method details). Details of the parameters defining each biscuit (10 variables), together with AA can be found in Table 11 of the appendices.

The statistical output summaries from all multivariate regressions can be found in Appendix 6.6, Table 12 A) - C). Multivariate regression (p = 0.05) analysis of all data showed that, as expected, ginger and ammonium were statistically significant variables ($p<0.05$, significance F=0.00189) with respect to AA. Unlike the historical data, rye was not significantly correlated (p=0.566); the fit to the data was not improved using the three significant variables obtained from the 'Historical' (FSA) data (ginger, ammonium and rye); and sugar showed a negative correlation ($p<0.05$) indicating a possible "protective effect" on AA levels. The best correlation was therefore obtained for ginger, ammonium and sugar although the fit to the data was not as strong as that obtained with the 'Historical' (FSA) data (adjusted R square 19.7% compared to 46.3%), indicating that care should be exercised with subsequent interpretation.

This investigation has shown that ginger, ammonium and possibly sugar could account for some of the variation in AA observed within and between categories of biscuit sampled in 2014. The variables could not however predict actual levels in biscuits. It is highly likely that detailed baking conditions (temperature / time), together with moisture, pH and quantitative recipe data (including Asn concentrations for raw materials), would be required to predict amounts of AA in biscuits with any certainty (Sadd & Hamlet 2005; Wedzicha et al 2005; Parker et al 2012). The latter are also likely to account for the majority of the observed variability in AA.

4.4 Conclusions from investigation

- A wide range of ingredients was declared in the biscuits. Ingredients identified as potentially significant for AA formation included: Fruit, Inverted sugar, Sugar, Ginger, Ammonium, Rye, Yeast and Ca/Mg.
- Categories of biscuits with highest mean levels of AA were the 'Cones and wafers', 'Breakfast biscuits' and 'Ginger biscuits': these biscuits together with 'Crackerbread / Crispbread' also showed the greatest variation in AA amounts.
- Lowest amounts of AA were found in Shortbread, 'Crispbakes' and 'Soda crackers'.
- Amounts of AA in products as a function of raising agents followed the trend yeast << none or sodium < ammonium.
- Within categories of biscuit (including categories based on EC IVs) lowest amounts of AA were generally found in biscuits declaring yeast.
- A total of 7/58 biscuits were found to exceed EV IVs.

- Measurement of colour (according to lab colour space) as an indicator of bake temperature / time was not reliable due to colour contributing ingredients in some biscuits.
    - Other measures of the “processing” i.e. moisture and pH were possible but there was no significant relationship with AA
- Multivariate regression analysis of all recipe and process variables (i.e. moisture and pH) indicated that some of the variation in AA could be accounted for by the presence of ammonium, ginger, and sugar in the recipe.
        - The remaining variability was most likely due baking conditions (temperature / time) and quantitative differences in key ingredients.

5. Overall conclusions and recommendations

- Trends in AA
    - Categories of biscuits with highest mean levels of AA were the ‘Cones and wafers’, ‘Breakfast biscuits’ and ‘Ginger biscuits’
        - These biscuits together with ‘Crackerbread / Crispbread’ also had the greatest range of AA values.
    - Lowest amounts of AA were found in Shortbread, ‘Crispbakes’ and ‘Soda crackers’.
    - Amounts of AA in products followed a trend with respect to raising agents declared on the label: yeast << none or sodium < ammonium.
        - Biscuits declaring yeast had the smallest range of AA values.
        - Within certain categories of biscuit, there was a trend towards lower AA in biscuits declaring yeast.
- Matrix model
    - Key recipe factors (qualitative data) identified included: added fruit, inverted sucrose, sucrose, ginger, ammonium based raising agents, rye or rye / wheat combinations, yeast raising agent and divalent metal ions (Ca/Mg).
    - Key process factors available (quantitative data) included: Moisture and pH.
        - Measurement of colour as an indicator of baking conditions was not possible due to contributions from (coloured) ingredients.
    - Multivariate regression analysis of all recipe and process factors showed that some of the variability in AA could be accounted for by just three qualitative variables: the presence of ammonium, and / or ginger and / or sugar (sucrose).
        - The remaining variability was most likely due baking conditions (temperature / time) and quantitative differences in key ingredients.
    - It is likely that detailed baking conditions (temperature / time), together with moisture, pH and quantitative recipe data (including Asn concentrations for raw materials), would be required to predict amounts of AA in biscuits with any certainty.

- EC IV Categories
    - Seven samples exceeded EC IVs
        - Four of these were ginger biscuits which had been assigned to the ‘Biscuits & Wafers’ category (IV 500 µg/kg)
        - Similarities in recipe indicate that Ginger biscuits might be included in the “Gingerbread” category, for which a higher IV (1000 µg/kg) applies.
            - It may be appropriate to consider extending the definition for the ‘Gingerbread’ category to include ‘Ginger biscuits’
    - This investigation may provide some scientific justification for sub-categories of biscuit based on products declaring yeast in their recipes.

6. APPENDICES

6.1 Materials and methods

6.1.1 Biscuit samples

Biscuits (two packs from same batch code) were obtained from retail outlets in Berks and Bucks: the sample details and ingredient declaration were recorded in an Excel database. A single pack was taken for analysis; the second pack was retained for follow-up investigations as required.

6.1.2 Sample Preparation

The contents of one pack of biscuits were blended in a food processor. Homogenised samples were stored in 150 g sample containers at -18°C until required for analysis.

6.1.3 Analysis of acrylamide

Acrylamide was determined as the brominated derivative, 2-bromopropenamide using GC/MS/MS, according to the method of Hamlet, Sadd & Liang (2008).

Method performance (typical): limits of detection and quantification were 0.5 and 3.0 µg/kg respectively; the estimated value for the method uncertainty (single determination) was: ±14% at 49 µg/kg (expanded relative uncertainty with a coverage factor of 2).

6.1.1 Moisture measurements

The moisture was determined gravimetrically in duplicate. Samples (4-6 g) were weighed accurately into pre-dried dishes and heated at 131°C in a laboratory fan oven (Hereaus, Hanau, Germany) for 120 - 130 min. The percentage moisture was calculated from the loss in weight expressed as a mean of duplicate determinations (wwb).

6.1.2 pH measurements

Samples (2-4 g and 45% moisture) were homogenised with x5 weight equivalents of deionised water in a 25 ml glass vial using a 10 mm diameter 10T shaft (Ystral, Sweden). Duplicate pH measurements were made on the stirred slurry (magnetic 'flea') using a Gelplas calibrated generalpurpose electrode, model 309/1050/03 (accuracy ± 0.02 pH unit, BDH/Merck, Lutterworth, UK).

6.1.3 Statistical methods

A database matrix of abstracted recipe ingredient variables (i.e. ammonium, fruit, invert [=inverted sucrose], sugar [= sucrose], rye, yeast), AA concentrations, moisture and pH values (where appropriate) was compiled for each biscuit sample using Microsoft Excel: A value of 1 or 0 was assigned to each cell dependent upon whether a recipe variable was present or absent respectively; quantitative values were entered for AA, moisture and pH. Multivariate regression was carried out using the Data Analysis Tools in Excel at a confidence level of 95%. Correlations were assessed with respect to 'Adjusted R Square', 'Significance F', and 'P-value' (<0.05).

6.2 Anova summaries for FSA survey samples (2007-2014) categorised by raising agents

Table 8. Anova output comparing acrylamide data from FSA Survey biscuits categorised by raising agent

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Ammonium	41	23285	567.9	167336.5		
None or sodium	51	14866	291.5	80311.4		
Yeast	44	10741	244.1	39583.9		
Ammonium + yeast	8	4040	505.0	38839.2		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2762922	3	920974.1	**10.16607**	**0.00000**	**2.66926**
Within Groups	12683010	140	90592.9			
Total	15445932	143				

6.3 Multivariate regression summaries from 'Historical' data (FSA surveys 2007-2014)

Table 9. Excel multivariate regression output summaries (95% confidence level) from FSA survey data

A) 2007-2009 biscuit data (6 variables)

Regression Statistics						
Multiple R	75.7%					
R Square	57.3%					
Adjusted R Square	**50.5%**					
Standard Error	288.5					
Observations	45					
ANOVA						
	df	*SS*	*MS*	*F*	*Significance F*	
Regression	6	4239512	706585	8.5	**7.137E-06**	
Residual	38	3162563	83225			
Total	44	7402076				
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	376.144	136.288	2.760	0.009	100.242	652.045
invert	-161.656	135.326	-1.195	0.240	-435.608	112.297
sugar	-92.908	133.548	-0.696	0.491	-363.262	177.447
ginger	**687.671**	**141.231**	**4.869**	**0.000**	**401.764**	**973.578**
ammonium	166.469	122.145	1.363	0.181	-80.800	413.739
yeast	**-259.829**	**116.859**	**-2.223**	**0.032**	**-496.398**	**-23.261**
rye	**325.267**	**129.222**	**2.517**	**0.016**	**63.671**	**586.862**

B) 2011-2013 biscuit data (6 variables)

Regression Statistics	
Multiple R	75.4%
R Square	56.8%
Adjusted R Square	**51.9%**
Standard Error	209.0
Observations	60

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	6	3043395	507233	11.6	**2.941E-08**
Residual	53	2315581	43690		
Total	59	5358976			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	110.716	68.505	1.616	0.112	-26.688	248.121
invert	122.779	89.212	1.376	0.175	-56.157	301.715
sugar	-36.224	77.065	-0.470	0.640	-190.796	118.348
ginger	**333.987**	**91.386**	**3.655**	**0.001**	**150.691**	**517.283**
ammonium	**339.524**	**71.181**	**4.770**	**0.000**	**196.753**	**482.295**
yeast	38.205	69.237	0.552	0.583	-100.667	177.078
rye	**133.504**	**74.834**	**1.784**	**0.080**	**-16.594**	**283.601**

C) 2014 biscuit data (6 variables)

Regression Statistics	
Multiple R	68.0%
R Square	46.2%
Adjusted R Square	**27.3%**
Standard Error	280.5
Observations	24

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	6	1150622	191770	2.4	**0.06941**
Residual	17	1337916	78701		
Total	23	2488538			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	219.639	161.249	1.362	0.191	-120.566	559.845
invert	140.538	216.003	0.651	0.524	-315.188	596.264
sugar	-158.972	237.990	-0.668	0.513	-661.087	343.143
ginger	**489.522**	**174.551**	**2.804**	**0.012**	**121.252**	**857.792**
ammonium	138.586	172.016	0.806	0.432	-224.335	501.508
yeast	22.495	170.077	0.132	0.896	-336.336	381.326
rye	**-16.904**	**204.143**	**-0.083**	**0.935**	**-447.609**	**413.801**

D) 2007-2014 data (6 variables)

Regression Statistics	
Multiple R	69.2%
R Square	47.9%
Adjusted R Square	**45.3%**
Standard Error	257.3
Observations	129

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	6	7425989	1237665	18.7	**2.473E-15**
Residual	122	8076772	66203		
Total	128	15502761			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	188.723	61.243	3.082	0.003	67.487	309.959
invert	20.408	73.915	0.276	0.783	-125.913	166.730
sugar	-51.244	67.750	-0.756	0.451	-185.362	82.875
ginger	**489.146**	**71.252**	**6.865**	**0.000**	**348.095**	**630.197**
ammonium	**245.209**	**57.862**	**4.238**	**0.000**	**130.665**	**359.753**
yeast	-39.170	59.776	-0.655	0.514	-157.504	79.163
rye	**185.176**	**65.657**	**2.820**	**0.006**	**55.202**	**315.150**

E) 2007-2014 data (6 variables): Regression of Log[acrylamide]

Regression Statistics	
Multiple R	60.0%
R Square	36.0%
Adjusted R Square	**32.9%**
Standard Error	0.3
Observations	129

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	6	8	1	11.4	**3.992E-10**
Residual	122	15	0		
Total	128	23			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	2.102	0.082	25.567	0.000	1.939	2.265
invert	0.101	0.099	1.018	0.311	-0.095	0.297
sugar	0.019	0.091	0.208	0.836	-0.161	0.199
ginger	**0.418**	**0.096**	**4.372**	**0.000**	**0.229**	**0.608**
ammonium	**0.261**	**0.078**	**3.359**	**0.001**	**0.107**	**0.415**
yeast	-0.004	0.080	-0.046	0.963	-0.163	0.155
rye	**0.264**	**0.088**	**2.991**	**0.003**	**0.089**	**0.438**

F) 2007-2014 data, minimum fit (3 variables)

Regression Statistics	
Multiple R	68.9%
R Square	47.5%
Adjusted R Square	**46.3%**
Standard Error	255.1
Observations	129

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	3	7365955	2455318	37.7	**1.986E-17**
Residual	125	8136806	65094		
Total	128	15502761			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	151.935	33.251	4.569	0.000	86.127	217.743
ginger	**501.039**	**60.220**	**8.320**	**0.000**	**381.855**	**620.223**
ammonium	**232.696**	**52.575**	**4.426**	**0.000**	**128.645**	**336.748**
rye	**187.935**	**61.789**	**3.042**	**0.003**	**65.647**	**310.224**

6.4 Anova summary for acrylamide in the 2014 investigation biscuits categorised by raising agents

Table 10. Anova output comparing acrylamide data for biscuits categorised by raising agent

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
None or sodium	18	4289	238.3	56752.16		
Yeast	12	1133	94.4	5859.56		
Ammonium	28	10080	360.0	94266.45		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	614557.7	2	307278.8	**4.72811**	**0.01274**	**3.16499**
Within Groups	3574436	55	64989.7			
Total	4188994	57				

6.5 Compositional and measured variables for individual samples from the 2014 investigation

Table 11. Key parameters defining the individual biscuits used in the 2014 investigation

Sample Number	Biscuit Type	Compositional variables (declared)[a]								Measured variables			EC Indicative values	
		Fruit	Inverted sugar[b]	Sugar[b]	Ginger	Ammonium[c]	Rye	Yeast[c]	Ca/Mg	Moisture (g/100g)	pH	Acrylamide (µg/kg)	Sub Category	Acrylamide (µg/kg)
14C-08902	Malted milk	0	0	1	0	1	0	0	1	3.5	8.1	320	Biscuits & wafers	500
14C-08903	Malted Milk	0	0	1	0	1	0	0	1	3.4	7.8	359	Biscuits & wafers	500
14C-08904	Malted Milk	0	0	1	0	1	0	0	1	3.2	7.8	397	Biscuits & wafers	500
14C-08905	Ginger Biscuit	0	1	1	1	0	0	0	0	2.8	7.0	168	Biscuits & wafers	500
14C-08907	Ginger Biscuit	0	1	1	1	0	0	0	0	3.4	7.1	189	Biscuits & wafers	500
14C-08908	Ginger Biscuit	0	1	1	1	0	0	0	0	3.4	7.2	142	Biscuits & wafers	500
14C-08909	Ginger Biscuit	0	1	1	1	0	0	0	0	3.2	7.0	175	Biscuits & wafers	500
14C-08910	Ginger Biscuit	0	1	1	1	1	0	0	0	4.4	7.7	1470	Biscuits & wafers	500
14C-08912	Shortbread	0	0	1	0	1	0	0	0	3.1	7.8	22	Biscuits & wafers	500
14C-08913	Shortbread	0	0	1	0	1	0	0	0	3.9	6.7	15	Biscuits & wafers	500
14C-08921	Cones & Wafers	0	0	0	0	0	0	0	1	1.8	6.9	815	Biscuits & wafers	500
14C-08922	Cones & Wafers	0	0	1	0	1	0	0	0	2.3	6.3	212	Biscuits & wafers	500
14C-08923	Cones & Wafers	0	0	1	0	1	0	0	0	1.7	6.9	493	Biscuits & wafers	500
14C-08925	Crackerbread / Crispbread	0	0	1	0	0	0	0	1	5.8	6.5	43	Crispbread	450
14C-08926	Crackerbread / Crispbread	0	1	1	0	1	0	0	0	4.2	7.8	879	Crispbread	450
14C-08927	Crackers, Cream	0	0	0	0	0	0	1	0	5.5	6.8	55	Crackers with the exception of potato based crackers	500

Table 11 continued...

Sample Number	Biscuit Type	Compositional variables (declared)[a]								Measured variables			EC Indicative values	
		Fruit	Inverted sugar[b]	Sugar[b]	Ginger	Ammonium[c]	Rye	Yeast[c]	Ca/Mg	Moisture (g/100g)	pH	Acrylamide (μg/kg)	Sub Category	Acrylamide (μg/kg)
14C-08928	Crackers, Cream	0	0	0	0	0	0	1	0	4.3	6.9	77	Crackers with the exception of potato based crackers	500
14C-08929	Crackers, Cream	0	0	0	0	0	0	1	0	3.4	6.3	64	Crackers with the exception of potato based crackers	500
14C-08930	Crackers, Cream	0	0	0	0	0	0	1	0	4.7	7.1	108	Crackers with the exception of potato based crackers	500
14C-08931	Crackerbread / Crispbread	0	0	1	0	0	1	1	0	3.1	6.6	292	Crispbread	450
14C-08933	Crackers, Wheatgerm	0	0	1	0	1	0	0	0	3.6	7.2	290	Crackers with the exception of potato based crackers	500
14C-08934	Water biscuit	0	0	1	0	0	0	0	0	2.9	6.7	273	Crackers with the exception of potato based crackers	500
14C-08936	Crackerbread / Crispbread	0	0	1	0	0	0	0	1	6.4	6.0	95	Crispbread	450
14C-08937	Crackerbread / Crispbread	0	0	0	0	0	1	1	0	6.9	5.8	179	Crispbread	450
14C-08939	Crackerbread / Crispbread	0	0	0	0	0	1	0	0	6.3	6.3	100	Crispbread	450
14C-08941	Crackerbread / Crispbread	0	0	0	0	0	1	1	0	5.8	4.4	55	Crispbread	450
14C-08942	Crackerbread / Crispbread	0	0	1	0	1	0	0	0	4.4	7.5	386	Crispbread	450

Table 11 continued...

Sample Number	Biscuit Type	Compositional variables (declared)[a]								Measured variables			EC Indicative values	
		Fruit	Inverted sugar[b]	Sugar[b]	Ginger	Ammonium[c]	Rye	Yeast[c]	Ca/Mg	Moisture (g/100g)	pH	Acrylamide (µg/kg)	Sub Category	Acrylamide (µg/kg)
14C-08943	Crackers, Flatbread	0	0	0	0	0	0	0	0	3.4	6.4	260	Crackers with the exception of potato based crackers	500
14C-08944	Crackers, Flatbread	0	0	1	0	0	0	1	1	3.8	5.8	83	Crackers with the exception of potato based crackers	500
14C-08949	Crackers, Soda	0	0	1	0	1	0	0	0	3.1	7.9	12	Crackers with the exception of potato based crackers	500
14C-08950	Crispbakes	0	0	1	0	0	0	1	0	4.7	5.3	16	Crackers with the exception of potato based crackers	500
14C-08951	Crispbakes	0	0	1	0	0	0	1	0	6.0	5.4	12	Crackers with the exception of potato based crackers	500
14C-08952	Breakfast biscuit	1	1	1	0	1	0	0	1	5.2	6.9	274	Products similar to the other products in this category	500
14C-08953	Breakfast biscuit	1	0	1	0	1	1	0	1	5.1	6.8	971	Products similar to the other products in this category	500
14C-08954	Crackers, Oatcake	0	0	0	0	0	0	0	0	3.8	6.6	255	Crackers with the exception of potato based crackers	500
14C-08956	Digestive	0	0	1	0	1	0	0	1	2.2	9.3	217	Biscuits & wafers	500
14C-08957	Digestive	0	1	1	0	1	0	0	0	1.6	9.4	220	Biscuits & wafers	500

Table 11 continued...

Sample Number	Biscuit Type	Compositional variables (declared)[a]								Measured variables			EC Indicative values	
		Fruit	Inverted sugar[b]	Sugar[b]	Ginger	Ammonium[c]	Rye	Yeast[c]	Ca/Mg	Moisture (g/100g)	pH	Acrylamide (µg/kg)	Sub Category	Acrylamide (µg/kg)
14C-08959	Digestive	0	1	1	0	1	0	0	0	2.7	7.9	133	Biscuits & wafers	500
14C-08960	Rich Tea	0	1	1	0	1	0	0	0	2.0	8.0	302	Biscuits & wafers	500
14C-08962	Rich Tea	0	1	1	0	1	0	0	0	3.0	8.0	255	Biscuits & wafers	500
14C-08963	Rich Tea	0	0	1	0	1	0	0	0	2.9	8.1	275	Biscuits & wafers	500
14C-08964	Rich Tea	0	1	1	0	1	0	0	0	1.9	8.0	287	Biscuits & wafers	500
14C-09608	Cones & Wafers	0	0	0	0	0	0	0	0	6.0	7.0	624	Biscuits & wafers	500
14C-09609	Cones & Wafers	0	0	0	0	0	0	0	1	7.1	6.8	734	Biscuits & wafers	500
14C-09610	Digestive	0	1	1	0	1	0	0	1	3.4	8.2	359	Biscuits & wafers	500
14C-09611	Shortbread	0	0	1	0	0	0	0	0	4.1	6.5	13	Biscuits & wafers	500
14C-09612	Malted Milk	0	0	1	0	1	0	0	1	3.6	7.5	337	Biscuits & wafers	500
14C-09613	Rich Tea	0	0	1	0	1	0	0	0	3.2	8.3	323	Biscuits & wafers	500
14C-09614	Ginger Biscuit	0	1	1	1	0	0	0	0	3.5	6.9	154	Biscuits & wafers	500
14C-09615	Crackers, Cream	0	0	0	0	0	0	1	0	4.8	6.6	114	Crackers with the exception of potato based crackers	500
14C-09617	Crackers, Flatbread	0	0	0	0	0	1	0	0	3.1	5.9	127	Crackers with the exception of potato based crackers	500
14C-10221	Breakfast biscuit	1	0	1	0	1	0	0	1	5.5	6.6	511	Products similar to the other products in this category	500
14C-10222	Breakfast biscuit	1	1	1	0	1	0	0	1	5.3	6.7	148	Products similar to the other products in this category	500

Table 11 continued...

Sample Number	Biscuit Type	Compositional variables (declared)[a]								Measured variables			EC Indicative values	
		Fruit	Inverted sugar[b]	Sugar[b]	Ginger	Ammonium[c]	Rye	Yeast[c]	Ca/Mg	Moisture (g/100g)	pH	Acrylamide (μg/kg)	Sub Category	Acrylamide (μg/kg)
14C-10223	Breakfast biscuit	0	1	1	0	1	1	0	1	3.1	6.6	489	Products similar to the other products in this category	500
14C-10224	Breakfast biscuit	1	1	1	0	1	1	0	1	4.4	5.9	124	Products similar to the other products in this category	500
14C-10225	Crackers, Cream	0	0	0	0	0	0	1	0	5.8	6.7	78	Crackers with the exception of potato based crackers	500
14C-10226	Shortbread	0	0	1	0	0	0	0	1	4.9	7.0	16	Biscuits & wafers	500
14C-10227	Cones & Wafers	0	0	1	0	0	0	0	1	3.7	6.8	105	Biscuits & wafers	500

[a] 1 = present, 0 = absent; [b] for declared ‘sugar’, sucrose assumed; [c] raising agent

6.6 Multivariate regression summaries from '2014 Investigation' samples

Table 12. Excel multivariate regression output summaries (95% confidence level) from the 2014 biscuit data:

A) All variables

Regression Statistics	
Multiple R	57.6%
R Square	33.1%
Adjusted R Square	**18.9%**
Standard Error	244.1
Observations	58

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	10	1387904	138790	2.3	**0.02536**
Residual	47	2801090	59598		
Total	57	4188994			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	29.748	476.585	0.062	0.950	-929.018	988.514	-929.018	988.514
Fruit	-56.864	160.750	-0.354	0.725	-380.253	266.524	-380.253	266.524
Invert	-54.215	99.584	-0.544	0.589	-254.552	146.121	-254.552	146.121
Sugar	**-263.790**	**107.813**	**-2.447**	**0.018**	**-480.681**	**-46.899**	**-480.681**	**-46.899**
Ginger	**403.038**	**156.618**	**2.573**	**0.013**	**87.964**	**718.113**	**87.964**	**718.113**
Ammonium	**334.039**	**123.719**	**2.700**	**0.010**	**85.148**	**582.931**	**85.148**	**582.931**
Rye	63.518	109.928	0.578	0.566	-157.628	284.665	-157.628	284.665
Yeast	-106.416	104.774	-1.016	0.315	-317.194	104.362	-317.194	104.362
Ca/Mg	124.444	86.646	1.436	0.158	-49.866	298.753	-49.866	298.753
Mosture (g/100g)	25.905	32.068	0.808	0.423	-38.607	90.418	-38.607	90.418
pH	17.239	60.749	0.284	0.778	-104.972	139.450	-104.972	139.450

B) Ginger, ammonium and Rye cf 'Historical' (FSA) data

Regression Statistics	
Multiple R	41.4%
R Square	17.2%
Adjusted R Square	**12.5%**
Standard Error	253.5
Observations	58

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	3	718457.8803	239485.9601	3.726295361	**0.01654**
Residual	54	3470535.905	64269.18343		
Total	57	4188993.786			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	131.046	53.524	2.448	0.018	23.736	238.356	23.736	238.356
Ginger	**216.587**	**113.355**	**1.911**	**0.061**	**-10.676**	**443.850**	**-10.676**	**443.850**
Ammonia	**212.501**	**68.700**	**3.093**	**0.003**	**74.766**	**350.235**	**74.766**	**350.235**
Rye	81.423	98.140	0.830	0.410	-115.335	278.182	-115.335	278.182

C) Minimum fit (3 variables)

Regression Statistics	
Multiple R	48.9%
R Square	24.0%
Adjusted R Square	**19.7%**
Standard Error	242.9
Observations	58

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	3	1003503	334501	5.7	**0.00189**
Residual	54	3185491	58991		
Total	57	4188994			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	242.987	62.711	3.875	0.000	117.258	368.715	117.258	368.715
Sugar	**-228.950**	**96.905**	**-2.363**	**0.022**	**-423.232**	**-34.667**	**-423.232**	**-34.667**
Ginger	**313.216**	**117.159**	**2.673**	**0.010**	**78.325**	**548.106**	**78.325**	**548.106**
Ammonium	**334.783**	**85.178**	**3.930**	**0.000**	**164.010**	**505.555**	**164.010**	**505.555**

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