Safety of chia seeds (*Salvia hispanica* L.) subject to thermal processing in relation to the formation of process contaminants as a novel food for extended uses

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**Abstract**

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on the safety of chia seeds in foods subject to thermal processing which may result in the formation of process contaminants. The safety assessment of this novel food (NF) is based on previous assessments of chia seeds by the EFSA NDA Panel, information received from a public call for data by EFSA and information retrieved from an extensive literature search performed by EFSA. In 2019, during the overall safety assessment of chia seeds, the NDA panel retrieved one reference which, among others, investigated the formation of process contaminants, i.e. acrylamide, hydroxymethylfurfural and furfural, in wheat flour-based biscuits with added chia seeds flour. Based on this study, the Panel considers that there is a potential for substantial acrylamide formation in biscuits with 10–20% added chia seeds flour with low residual moisture contents (≤ 2%). The Panel is not aware of further scientific evidence corroborating these findings. The extensive new literature searches performed by EFSA did not show any relevant articles regarding either asparagine content or formation of process contaminants in chia seeds and products thereof. Information received from the call for data were either limited or inconclusive. The available evidence does not provide a basis to conclude whether or not the addition of chia seeds to foods undergoing heat treatment (at temperatures above 120°C) results in increased formation of acrylamide as compared to these foods without chia seeds. Reported concentrations of hydroxymethylfurfural and furfural in heat-treated chia seeds do not pose a safety concern. No information on other process contaminants in chia seeds was found.

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**Keywords:** Novel foods, chia seeds (*Salvia hispanica* L.), process contaminants, heat-treatment, risk assessment

**Requestor:** European Commission

**Question number:** EFSA-Q-2019-00254

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Acknowledgements: The Panel wishes to thank the following EFSA statutory staff members (Declaration of interest available upon request) for the support provided to this scientific output: Valentina Bocca, Davide Gibin and Irene Munoz Guajardo.

Suggested citation: EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck D, Castenmiller J, de Henauw S, Hirsch-Ernst KI, Kearney J, Maciuk A, Mangelsdorf I, McArdle HJ, Naska A, Pelaez C, Pentieva K, Siani A, Thies F, Tsabouri S, Vinceti M, Cubadda F, Engel K-H, Frenzel T, Heinonen M, Marchelli R, Neuhausser-Berthold M, Poulsen M, Schlatter JR, van Loveren H, Gelbmann W, Matijević L, Romero P and Knutsen HK, 2020. Scientific Opinion on the safety of chia seeds (Salvia hispanica L.) subject to thermal processing in relation to the formation of process contaminants as a novel food for extended uses. EFSA Journal 2020;18(9):6243, 12 pp. https://doi.org/10.2903/j.efsa.2020.6243

ISSN: 1831-4732

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The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.
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1. Introduction

1.1. Background and Terms of Reference

On 14 March 2019 the NDA Panel adopted the opinion “Safety of chia seeds (Salvia hispanica L.) as a novel food for extended uses pursuant to Regulation (EU) 2015/2283” (EFSA NDA Panel, 2019) addressing only those novel food applications and extensions of use which do not include thermal processing and/or cooking procedures at temperatures which may result in the formation of levels of concern of the process contaminants. Thus, the overall safety assessment of chia seeds in accordance with Article 29 (1) of Regulation (EC) No 178/2002, was only partially addressed.

In order to complete the assessment on the safety of chia seeds, EFSA proposed to the Commission to issue a separate opinion which would address possible concerns relating to the thermal stability of chia seeds in certain foods, and in particular those foods whose production includes thermal processing at temperatures which may result in the formation of levels of concern of the process contaminants, i.e. acrylamide and furans.

Taking into account the scope of the above-mentioned adopted EFSA opinion on chia seeds and the initial request by the Commission relating to the overall safety assessment of chia seeds, the Commission agreed with EFSA’s proposal.

2. Data and methodologies

2.1. Data

The data considered in this assessment are (i) previous assessments of chia seeds by the EFSA NDA Panel (EFSA NDA Panel 2005, 2009, 2019), (ii) information received from a public call for data by EFSA and (iii) information retrieved by EFSA from extensive literature searches.

In addition, the draft opinion underwent a public consultation from 23 June 2020 to 4 August 2020. The comment received and how it was taken into account when finalising the scientific opinion was published in an EFSA Technical Report (EFSA, 2020).

2.2. Methodologies

Two focused literature searches were performed to retrieve relevant studies on the content of asparagine and process contaminants (acrylamide, furan and alkylfurans) in chia seeds and products thereof.

The sources of information searched were Food Science and Technology Abstracts (FSTA) database via Web of Science platform, and Google Scholar using Publish or Perish software.

No limits on time or language of publication were applied. The search strategies used to interrogate the sources of information are reported in Appendix A.

After reviewing the results retrieved by this search, a second search with a broader scope was performed to identify studies reporting contamination of chia seeds as well as thermal processes applied to chia seeds. The sources of information used to retrieve this information are reported in Table 1.
The complete search strings as run in the databases are available in Appendix A.

3. Assessment

3.1. Introduction

In 2005, the NDA Panel concluded that the safety of chia could not be established from the available information and additional data were required (EFSA NDA Panel, 2005). The first authorisation for placing on the European Union market of chia seeds as a novel food to be used in bread products was given by European Commission (EC) Decision 2009/827/EC6 following the NDA Panel opinion (EFSA NDA Panel, 2009) on safety of chia seeds (Salvia hispanica L.) as a novel food. Subsequent authorisations following applications on extension of use of chia seeds received under Article 4 of Regulation (EC) No 258/977 were based on risk assessments carried out by the Member States national authorities with no objections having been raised by the other Member States. This led to further authorisations of chia seeds in the following food categories: bread products, baked products, breakfast cereals, fruit, nut and seed mixes, fruit juice and fruit/vegetable blend beverages, pre-packaged chia seed as such, fruit spreads, yoghurt, sterilised ready-to-eat meals based on cereal grains, pseudocereals and/or pulses.

The requests for extensions of use which were submitted but not assessed prior to 1 January 2018 were forwarded by the European Commission to EFSA in accordance with Article 35(1) of Regulation (EU) 2015/22838.

On 16 July 2018,9 the European Commission asked EFSA to carry out an overall safety assessment for chia seeds in order to update the EFSA NDA Panel opinion from 2009 to ensure that the currently authorised uses, and the requested extensions of use, comply with the requirements of Article 7 of Regulation (EU) 2015/2283.

When carrying out the risk assessment, EFSA retrieved one reference which, among others, investigated the formation of process contaminants, i.e. acrylamide (AA), hydroxymethylfurfural (HMF) and furfural, in wheat flour-based biscuits with added chia seeds flour (Mesias et al., 2016).

In general, those process contaminants can be formed when certain foods are processed at temperatures above 120°C and at low moisture, especially in foods containing asparagine (Asn) and reducing sugars. Neurotoxicity, adverse effects on male reproduction and developmental toxicity were identified as possible critical endpoints for AA’s non-neoplastic toxicity from experimental animal studies. Furthermore, AA (via its metabolite glyciamide) is considered to be genotoxic and carcinogenic, with margins of exposure (MOEs) indicating a concern for human health (EFSA CONTAM Panel, 2015). HMF showed renal toxicity based on the available animal studies (EFSA CEF Panel, 2011). In 2004, EFSA established an acceptable daily intake (ADI) for furfural and the furfural component of furfural diethylacetal of 0.5 mg/kg body weight (bw) based on an NOAEL (no-observed-

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6 Commission Decision 2009/827/EC of 13 October 2009 authorising the placing on the market of Chia seed (Salvia hispanica) as novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council. OJ L 294, 11.11.2009, p. 14.
7 Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43, 14.2.1997, p. 1-6.
8 Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. OJ L 327, 11.12.2015, p. 1–22.
9 http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2018-00684
adverse-effect level) for hepatotoxicity in a 90-day study in rats of 54 mg/kg bw per day to which a safety factor of 100 was applied (EFSA AFC Panel, 2004).

In the study by Mesias et al. (2016) on biscuits, chia seeds flour was added as a partial substitution of wheat flour in gradually increasing amounts of 0% (control), 5% (sample A), 10% (sample B), 15% (sample C) and 20% (sample D) of the total flour weight. For each sample and the control, two batches of dough and 12 biscuits per batch were prepared. The biscuits were baked at 190°C for 20 min.

In the control samples, the mean AA level was 151 μg/kg. In sample A, the AA level was significantly higher (approx. 200 μg/kg) but still in range of concentrations commonly seen in biscuits, and below the benchmark level set by Regulation 2017/2158 (i.e. 350 μg/kg for foodstuff ‘Biscuits and wafers’). In samples B, C and D, the AA levels were much higher than in the control and sample A, at around 1,200 μg/kg. Similar results were obtained for HMF content, which significantly increased from approx. 25 mg/kg in control to approx. 70 mg/kg in samples B, C and D. The content of furfural, however, showed a linear increase reaching 5.6 mg/kg in sample D, in comparison to approx. 1.3 mg/kg in control.

The formation of furan and alkylfurans, other known process contaminants in heat-treated foods (EFSA CONTAM Panel, 2017), was not reported in the study on biscuits with added chia seeds flour (Mesias et al., 2016). Furan can be formed in foods during thermal processing from a variety of precursors including carbohydrates, ascorbic acid, amino acids, unsaturated fatty acids and carotenoids. Furan is hepatotoxic in rats and mice, primarily leading to cholangiofibrosis in rats and hepatocellular adenomas/carcinomas in mice. The calculated MOE for intake of furan and methylfurans from the diet indicated a health concern (EFSA CONTAM Panel, 2017).

Considering the results from the study by Mesias et al. (2016) and the lack of data on the formation of other process contaminants (furan and alkylfurans), the NDA Panel was not able to finalise the overall safety assessment of chia seeds, i.e. to evaluate the safety of uses of heat-treated chia seeds at temperatures above 120°C during processing or cooking procedures (EFSA NDA Panel, 2019).

Thus, in order to complete the safety assessment of chia seeds, the European Commission submitted an additional request to EFSA for a scientific opinion on the safety of chia seeds in foods subject to thermal processing which may result in the formation of process contaminants.

3.2. Outcome of the literature search

No relevant articles regarding either asparagine or process contaminants in chia seeds were retrieved.

3.3. Data received by EFSA after call for data

EFSA launched a public call for data and received information from six stakeholders which included analytical data of the content of AA and Asn in different foods with added chia seeds.

In total, 75 analytical results on AA and 2 analytical results on Asn were submitted. Those results were obtained from commercially available foods (n = 24) and from foods prepared in the laboratory (n = 51) subjected to thermal processing and containing up to 10% of chia seeds in the final product. Some data sets also included controls, i.e. samples without added chia seeds (n = 14) or samples without available data on exact percentage of added chia seeds (n = 1). According to the EFSA FoodEx2 food hierarchy (EFSA, 2011), the highest number of samples were reported for ‘Bread products’ (n = 62) and ‘Cereal bar with fruits’ (n = 6). Other represented food categories included ‘Biscuits’ (n = 4), ‘Corn chips’ (n = 2) and ‘Pasta’ (n = 1). Total of 21 results (28%) were ‘left-censored’ (analytical data below the limit of detection (LOD)/limit of quantification (LOQ)). AA levels were analysed by liquid chromatography (LC) with mass spectrometry (MS) (n = 69) or by gas chromatography (GC) (n = 6).

The highest concentration of AA (524 μg/kg) was found in a bread product with 5% chia seeds prepared in a laboratory. In this particular data set from one submitter, the mean concentration of AA in the wheat-based breads with 5% of chia seeds from the same batch (n = 10) was 219 μg/kg (range: 88–524 μg/kg). Interestingly, breads with added 2% (n = 10) and 10% (n = 10) of chia seeds from the same batch, contained mean concentrations of AA of 263 μg/kg (range: 212–320 μg/kg) and

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10 Commission Regulation of 20 November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food (2017/2158). OJ L, 304, 21.11.2017, p. 24-44.
11 http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2019-00254
257 µg/kg (range: 95-470 µg/kg), respectively, thus not following a trend in regards to increasing proportions of chia seeds in the final product. Control samples without added chia seeds contained a mean AA concentration of 171 µg/kg (range: 137–213 µg/kg). All concentrations reported by this data set exceeded the benchmark levels set by the Regulation (EU) 2017/2158 for wheat-based bread (50 µg/kg). Two results from another submitter reported Asn concentrations (not known whether as free or total Asn) in bread of 23.13 mg/100 g and 11.58 mg/100 g, respectively. Those two samples did not contain detectable amounts of AA (LOD < 6.33 µg/kg). In another data set on laboratory-prepared foods, containing up to 8% defatted chia flour, no difference in AA concentrations compared to the control without added chia seeds was observed and all AA concentrations were below the benchmark level set-out for wheat-based bread (50 µg/kg). Furthermore, in another data set on laboratory-prepared breads without and with added whole chia seeds up to 10%, no quantifiable levels of AA were reported. However, the analytical method used, had a high LOD/LOQ of 100 µg/kg. Occurrence data reported for commercial products were all in line with the applicable benchmark levels.

4. Discussion

This assessment concerns the safety of heat-treated chia seeds (at temperatures above 120°C) during processing or cooking procedures and which may result in the formation of levels of concern of the process contaminants.

The reported AA concentrations from the study of Mesias et al. (2016) in biscuit samples with 10% of chia seeds flour or more, exceeded the benchmark level set by Regulation 2017/2158 (i.e. 350 µg/kg for foodstuff 'Biscuits and wafers') and were significantly higher compared to the control without added chia seeds flour. These biscuits exhibited a low residual moisture content (< 2%) which generally promotes AA formation. In the data sets received in the call for data, occurrence data for AA in products with and without added chia seeds in combination with moisture content typical for biscuits were not reported. In its opinion on AA in food, EFSA reported occurrence data for AA in the same food category ('Biscuits and wafers'). Based on a total number of 682 samples, mean and 95th percentile occurrence levels of AA were 201 and 810 µg/kg, respectively (EFSA CONTAM Panel, 2015). Based on the study by Mesias et al. (2016), the Panel considers that there is a potential for substantial AA formation in biscuits with 10–20% added chia seeds flour, with low residual moisture contents (< 2%). The Panel is not aware of further scientific evidence corroborating the findings reported by Mesias et al. (2016).

Data sets from the call for data did not show a correlation between higher AA concentrations and increasing proportions of added chia seeds. This may be due to the high variability in the composition of different batches of chia seeds (EFSA NDA Panel, 2009), depending on the origin/geographical location and other agricultural parameters related to the cultivation of chia seeds.

The data set submitted to EFSA with wheat-based breads baked under controlled conditions with 0%, 2%, 5% and 10% chia seeds, showed higher mean AA concentrations (based on 10 results for each percentage tested) in formulations with added chia seeds vs. control. This indicates that the addition of chia seeds in baked products may cause an increase in the formation of AA. However, there was no trend of increasing concentrations of AA with increasing content of chia seeds in the formulations. Furthermore, formulations with 5% and 10% added chia seeds exhibited high variations in AA concentrations in comparison to control and formulation with 2% added chia seeds. Thus, the data are inconclusive. In addition, the concentrations of AA in all samples (both control and chia-containing) were higher than the AA benchmark level for wheat-based bread (50 µg/kg) set by Regulation 2017/2158. This is unexpected in view of the consistent, light brown colour in the provided pictures of the baked products, but according to the submitter may be due to disproportional sampling, i.e. non-representative ratio of crust and crumb parts.

Mesias et al. (2016) suggested that the higher formation of process contaminants in the formulations with added chia seeds flour could be related to the concentrations of respective precursors in chia seeds. While chia seeds flour showed a lower content of reducing sugars (1.6 g/100 g in comparison to 5.6 g/100 g in wheat flour), the content of free Asn (42.8 ± 1.2 mg/100 g) was higher than in wheat flour (23.4 ± 1.1 mg/100 g). The NDA Panel agrees that this is a possible explanation but might not be a general concern related to chia seeds, as the Asn level in the chia flour reported by Mesias et al. (2016) seems not to be particularly high in comparison to wheat in general. Corol et al. (2016) reported concentrations of free Asn in a variety of bread wheat cultivars to be up to 156 mg/100 g of dry matter.
Furthermore, Mesias et al. (2016) reported high concentrations of glyoxal (GO) and methylglyoxal (MGO) in biscuits with added chia seeds flour as well in chia seeds flour as such. Those are process contaminants which can also lead to the formation of AA (EFSA CONTAM Panel, 2015).

HMF can be present in many foodstuffs as a process contaminant formed during thermal processing. EFSA reported occurrence levels up to 19.1 mg/kg in wheaten bread (EFSA CEF Panel, 2011). HMF concentrations in commercial samples of rye bread were reported to show high variability depending on the manufacturing process and reached concentrations up to 147 mg/kg (BFR, 2011). Delgado-Andrade et al. (2009) reported concentrations of HMF in commercial biscuits from the Spanish market up to 182.5 mg/kg. The HMF concentrations reported by Mesias et al. (2016) for biscuits with added chia flour (approx. 70 mg/kg) were lower compared to those in commercial samples and do not pose a safety concern.

Another process contaminant, furfural, can occur in wholegrain bread (up to 26 mg/kg) (EFSA AFC Panel, 2004). The furfural concentrations reported by Mesias et al. (2016) for biscuits with added chia seeds flour (up to 5.6 mg/kg) were much lower compared to occurrence data reported for wholegrain bread and do not pose a safety concern.

Although theoretically furan and alkylfurans can be formed in certain foodstuffs which are subject to heat treatment, it is not known whether adding chia seeds to such foods will substantially increase the occurrence levels of these process contaminants.

Further analytical data on concentrations of process contaminants (e.g. AA, furan and alkylfurans) in heat-treated foods with chia seeds are required. In addition, information on the concentrations of compounds in chia seeds that may act as precursors of process contaminants would help to determine whether there is a specific risk when chia seeds are subject to heat treatment (above 120°C).

5. Conclusions

The limited available evidence does not provide a basis to conclude whether or not the addition of chia seeds to foods undergoing heat treatment (at temperatures above 120°C) results in increased formation of AA as compared to these foods without chia seeds.

Reported concentrations of HMF and furfural in heat-treated chia seeds do not pose a safety concern. No information was identified on other process contaminants.

Documentation provided to EFSA

1) Response for call for data relevant to the safety assessment of heat-treated chia seeds (Salvia hispanica L.) in the framework of Regulation 2283/2015 (analytical data on AA levels in certain foodstuffs), submitted on 3 March 2020 by Federal Public Service (FPS) of Public Health, Food Chain Safety and Environment - General Directorate Animal, Plant and Food (Belgium).

2) Response for call for data relevant to the safety assessment of heat-treated chia seeds (Salvia hispanica L.) in the framework of Regulation 2283/2015 (analytical data on AA levels in certain foodstuffs), submitted on 23 March 2020 by Veterinary Public Health Institute of Sicily (Italy).

3) Response for call for data relevant to the safety assessment of heat-treated chia seeds (Salvia hispanica L.) in the framework of Regulation 2283/2015 (analytical data on AA levels in certain foodstuffs), submitted on 23 March 2020 by Ministry of Agriculture and Rural Development of the Slovak Republic.

4) Response for call for data relevant to the safety assessment of heat-treated chia seeds (Salvia hispanica L.) in the framework of Regulation 2283/2015 (analytical data on AA levels in certain foodstuffs), submitted on 24 and 25 March 2020 by Functional Products Arica S.A. (Chile).

5) Response for call for data relevant to the safety assessment of heat-treated chia seeds (Salvia hispanica L.) in the framework of Regulation 2283/2015 (analytical data on AA and Asn levels in certain foodstuffs), submitted on 8 April 2020 by Unit 133 - Data Analysis and Reporting Federal Office of Consumer Protection and Food Safety (BVL) (Germany).

6) Response for call for data relevant to the safety assessment of heat-treated chia seeds (Salvia hispanica L.) in the framework of Regulation 2283/2015 (analytical data on AA levels in certain foodstuffs), submitted on 17 May 2020 by The Chia Company (Australia).
7) Response for public consultation on the draft Scientific Opinion on the Safety of chia seeds (Salvia hispanica L.) subject to thermal processing in relation to the formation of process contaminants as a novel food for extended uses (analytical data on AA level in rye bread sample), submitted on 13 July 2020 by the Danish Veterinary and Food Administration (Denmark).

8) Data under 1–6 are available in a file annexed to the scientific opinion under ‘Supporting information’. For data under 7, please see Section 2.1.

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Abbreviations

AA Acrylamide
ADI Acceptable daily intake
AFC Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
Asn Asparagine
BfR Bundesinstitut für Risikobewertung
| Abbreviation | Description |
|--------------|-------------|
| bw           | Body weight |
| CEF          | Food Contact Materials, Enzymes, Flavourings and Processing Aids |
| CONTAM       | Contaminants in the Food Chain |
| EC           | European Commission |
| EFSA         | European Food Safety Authority |
| EU           | European Union |
| FSTA         | Food Science and Technology Abstracts |
| GC           | Gas chromatography |
| GO           | Glyoxal |
| HMF          | Hydroxymethylfurfural |
| LC           | Liquid chromatography |
| LOD          | Limit of detection |
| LOQ          | Limit of quantification |
| MGO          | Methylglyoxal |
| MOE          | Margin of exposure |
| MS           | Mass spectrometry |
| NDA          | Nutrition, Novel Foods and Food Allergens |
| NOAEL        | No observed adverse effect level |
| NF           | Novel food |
Appendix A – Literature searches

Chia and asparagine

FSTA (Web of Science platform)
Date of the search: 3 of June 2019

| Search string                                                                 | Results |
|-----------------------------------------------------------------------------|---------|
| TITLE: ((chia AND (seed* OR flour)) OR "salvia hispanica" OR "s hispanica") AND TOPIC: (composition OR asparagine) | 83      |
| Indexes=FSTA Timespan=All years                                             |         |

Updated search (6 April 2020) identified 9 additional references.

Google Scholar (via Publish or Perish)

Several searches were performed, and results were combined, and duplicate removed using EndNote software.

| Search string                                                                 | Results |
|-----------------------------------------------------------------------------|---------|
| Title: Chia                                                                  | 20      |
| All the words: Asparagine                                                   |         |
| Title: Salvia hispanica                                                     | 4       |
| All the words: Asparagine                                                   |         |
| All of the words: asparagine “chia seed”                                    | 62      |
| All of the words: asparagine “chia flour”                                   | 15      |
| All of the words: asparagina “semilla de chia”                              | 85      |
| All of the words: asparagina “semillas de chia”                             | 94      |
| After de-duplication among searches and with search in FSTA:                | 140     |

Updated search (6 April 2020) identified 71 additional references.

Chia and process contaminants

FSTA (Web of Science platform)
Date of the search: 3 of June 2019

| Search string                                                                 | Results |
|-----------------------------------------------------------------------------|---------|
| TS=((chia AND (seed* OR flour)) OR "salvia hispanica" OR "s hispanica") AND TS=((process NEAR/3 contaminant*) OR acrylamide OR furan OR alkylfuran OR “alkyl furan”) Indexes=FSTA Timespan=All years | 1       |

Updated search (7 April 2020) identified 1 additional reference.

Google Scholar (via Publish or Perish)

Several searches were performed, and results were combined, and duplicate removed using EndNote software.

| Search string                                                                 | Results |
|-----------------------------------------------------------------------------|---------|
| Title: Chia                                                                  | 39      |
| Any of the words: acrylamide furan alkylfuran “process contaminant” “process contaminants” |         |
| Title: Salvia hispanica                                                     | 23      |
| Any the words: acrylamide furan alkylfuran “process contaminant” “process contaminants” |         |
| After de-duplication among searches and with search in FSTA:                | 55      |

Updated search (7 April 2020) identified 11 additional references.
Chia and thermal processes

FSTA Database

Date of the search: 24/6/2019

| Set | Query                                                                 | Results |
|-----|-----------------------------------------------------------------------|---------|
| # 1 | TS= (chia OR "salvia hispanica" OR "s hispanica") AND TS= (contamina* OR cook* OR (therm* NEAR/3 process*) OR bake* OR "baking" OR roast* OR grill* OR heat* OR toast*) Indexes--FSTA Timespan--All years | 114     |

Updated search (6 April 2020) identified 4 additional references.

Web of Science, Core collection

Date of the search: 24/6/2019

| Set | Query                                                                 | Results |
|-----|-----------------------------------------------------------------------|---------|
| # 1 | TS= (chia OR "salvia hispanica" OR "s hispanica") AND TS= (contamina* OR cook* OR (therm* NEAR/3 process*) OR bake* OR "baking" OR roast* OR grill* OR heat* OR toast*) Indexes--SCI-EXPANDED, CPCI-S, BKCI-S, ESCI, CCR-EXPANDED, IC Timespan--All years | 141     |

Updated search (6 April 2020) identified 30 additional references.