PROCEEDINGS
of Abstracts and Protocols

Training School

Approach to the Assessment of Acrylamide Mitigation Measures in Cereal-based Food Processing

07 – 08 September 2023

National Agricultural and Food Centre
Food Research Institute

Bratislava, Slovakia

Edited by: Zuzana Ciesarova
September 2023

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Title: Proceedings of Abstracts and Protocols of the Training School: Approach to the Assessment of Acrylamide Mitigation Measures in Cereal-based Food Processing.

Editor: Zuzana Ciesarová

Publisher: Národné poľnohospodárske a potravinárske centrum – Výskumný ústav potravinársky

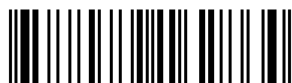
First edition

Bratislava, 2023

Format: e-document online - PDF

Location of digital print: Training Schools – ACRYRED • COST Action CA21149

ISBN: 978-80-89162-86-4



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COST Action CA21149 • Reducing Acrylamide exposure of consumers by a Cereals supply-chain approach targeting Asparagine

Training School

Approach to the assessment of acrylamide mitigation measures in cereal-based food processing

The Training School promotes interdisciplinary training, networking and knowledge exchange to build capacity in monitoring and assessment of acrylamide mitigation measures applied in industrial production.

WG5 Leaders: Zuzana Ciesarová (SK), Vural Gökmen (TR)

7th and 8th of September 2023

NATIONAL AGRICULTURAL AND FOOD CENTRE

FOOD RESEARCH INSTITUTE

Bratislava, Slovakia

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Training School

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NPPC VUP Bratislava, Slovakia

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NATIONAL AGRICULTURAL AND FOOD CENTRE

FOOD RESEARCH INSTITUTE

Number of Trainees: 21 from 11 countries

Number of Trainers: 7 foreign + 7 Slovak

Remote participation: ACRYRED TS 1st day_Teams
ACRYRED TS 2nd day_Teams

1st day

2nd day

Training School – 07 September 2023 ACRYRED TS 1st day_Teams

Time slot	Presentation title	Speaker	Location
09:00 – 09:10	Opening	Zuzana Ciesarová, LO (SK)	VUPOP, Trenčianska 55
09:10 – 09:30	Introduction general: CA 21149 ACRYRED Acrylamide in food and cereal products	Nigel Halford, CA chair (GB)	VUPOP, Trenčianska 55
09:30 – 10:15	Dietary acrylamide exposure and current legislation	Marta Mesias (ES)	VUPOP, Trenčianska 55
10:15 – 10:45	Coffee break		VUPOP, Trenčianska 55
10:45 – 11:30	Acrylamide mitigation options: Toolbox	Vural Gökmen (TR)	VUPOP, Trenčianska 55
11:30 – 12:00	Asparaginase treatment – pros & cons	Zuzana Ciesarová (SK)	VUPOP, Trenčianska 55
12:00 – 13:00	Lunch break		ROZADOL
13:00 – 13:45	Novel techniques in acrylamide and asparagine determination usable in food production	Jana Hajšlová (CZ) Beverly Hradecká (CZ)	VUPOP, Trenčianska 55
13:45 – 14:30	Aspects of acrylamide mitigation measures: impact on quality and sensory profile	Jane K. Parker (GB)	VUPOP, Trenčianska 55
14:30 – 14:45	Coffee break		VUPOP, Trenčianska 55
14:45 – 15:30	Aspects of acrylamide mitigation measures: impact on nutritional profile	Sladjana Zilic (SRB)	VUPOP, Trenčianska 55
17:00 – 18:30	Sightseeing tour Blavacik	On request - prebooked	Start point: VUPOP End point: Dunajský pivovar
19:00 – 21:00	TS dinner	Prebooked	Dunajský pivovar

Training School – 08 September 2023 ACRYRED TS 2nd day_Teams

Time slot	Presentation title	Speaker	Location
09:00 – 09:30	Technological aspects of acrylamide mitigation measures in cereal-based food production (progress and economy)	Jozef Murín (SK)	VUPOP, Trenčianska 55
09:30 – 10:00	Analytical sessions: Sensors for asparagine determination	Tanya Curtis (GB)	VUPOP, Trenčianska 55
10:00 – 10:30	Coffee break		VUPOP, Trenčianska 55
10:30 – 12:00	Tutorials: Sensors for asparagine determination	Tanya Curtis (GB)	VUPOP, Trenčianska 55
12:00 – 13:00	Lunch break		ROZADOL
13:00 – 15:00	Practical session I: LC/MS/MS demo of acrylamide/asparagine determination (3 x 40 min)	Jana Horváthová (SK) Janka Kubincová (SK) Zuzana Dubová (SK)	VUP, Priemyselná 4
13:00 – 15:00	Practical session II: sensory and texture analysis of innovative cereal-based products (3 x 40 min)	Kristína Kukurová (SK) Veronika Vigašová (SK)	VUP, Priemyselná 4
13:00 – 15:00	Practical session III: colour analysis of innovative cereal-based products (3 x 40 min)	Blanka Tobolková (SK)	VUP, Priemyselná 4
15:00 – 15:15	Coffee break		VUPOP, Trenčianska 55
15:15 – 15:30	Debrief, Certificates	Zuzana Ciesarová (SK) Vural Gökmen (TR)	VUPOP, Trenčianska 55
15:30 – 16:00	Closing remarks	Zuzana Ciesarová (SK) Nigel Halford (GB)	VUPOP, Trenčianska 55

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ORAL PRESENTATIONS

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OP-01

Dietary acrylamide exposure and current legislation

Marta Mesías, Francisco J Morales

Chemical modifications in processed foods (CHEMPROFOOD) Research Group, Institute of Food Science, Technology and Nutrition, Spanish National Research Council, Madrid, Spain

mmesias@ictan.csic.es

Since the discovery of acrylamide in processed foods until the publication of the European Regulation, various measures have been implemented to control its presence. These measures include the application of different mitigation strategies, the publication of reference values, and the establishment of controls in food production. The main objective has been to minimize acrylamide levels in foods as much as possible, thereby reducing dietary exposure and the associated health risks. In 2007, EFSA launched a first database to monitor acrylamide content, which has been regularly updated to track changes in levels among different food groups. Cereal-based foods, such as soft bread, breakfast cereals, biscuits, crackers, crisp bread, and similar processed cereal-based baby foods, were covered by this monitoring program. The overall trend did not show a clear reduction in acrylamide levels, except for infant biscuits and cereals during the period 2007–2010 [1]. Based on these findings, in 2011, the European Commission established indicative values for food groups (not constituting safety thresholds), which were revised and updated with stricter criteria in 2013 [2,3]. The main changes introduced were focused on cereal products, since in 2011, only an indicative value was established for soft bread and breakfast cereals, whereas new categories were proposed in 2013 considering the majority cereal. In this way, reduced values were recommended for bread based on wheat (80 µg/kg) and for breakfast cereal based on wheat/rye (300 µg/kg) and maize, oat, spelt, barley, and rice (200 µg/kg). Indicative values were also reduced for crisp bread and processed cereal-based baby foods. In 2015, EFSA published a scientific opinion on acrylamide in food where new values for acrylamide content in foods were indicated, evaluating the acrylamide intake, the acrylamide exposure and the consequent risk related to this exposure [4]. Following the EFSA Opinion, in 2017, the Commission published Regulation 2017/2158/EC, establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food [5], maintaining certain margins with respect to the acrylamide levels reported by EFSA in 2015. Within the Regulation, reference values were revised and again reduced in all the categories for cereal products, except for breakfast cereals based on wheat/rye. The benchmark levels were set with consideration to the acrylamide levels reported by EFSA in 2015 [4] and they will be revised every three years to ensure the lowest technically possible reduction of acrylamide levels in food products. The regulation is targeted at food-business operators, who are required to identify stages in the food chain that are susceptible to acrylamide formation and implement appropriate measures to reduce its presence. In 2019, the latest official publication about acrylamide provided new recommendations on monitoring its presence in certain foods. Among them, bakery products such as donuts, pancakes or churros and other cereal products such as rice or maize crackers are mentioned [6].
The mean acrylamide levels reported by EFSA in 2015 were based on an extensive dataset of more than 40,000 samples from various European countries and food associations. These data were collected to understand the acrylamide content in different food categories commonly consumed in Europe. Based on these findings and considering the dietary habits of the European population, EFSA estimated the mean dietary acrylamide exposures for various age groups to range between 0.4 and

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1.9 μg/kg body weight/day, whose margins of exposure indicate a concern for neoplastic effects based on animal evidence [4]. It is noteworthy that cereal-based products were found to be significant contributors to acrylamide exposure, comprising approximately 20-30% and sometimes even up to 40% of the acrylamide intake for different age groups. Considering these results, it becomes evident that controlling the presence of acrylamide in cereal-based foods is crucial to decrease their contribution to the acrylamide exposure and, consequently, the risk related to this exposure.
To assess the real exposure and risk to acrylamide, it is necessary to know its content in the different food matrices and the consumption habits of the population. Nevertheless, it must be considered that acrylamide, must first be absorbed to exert its biological activity. Toxicokinetic studies in animals showed that the acrylamide monomer is rapidly absorbed due to its low molecular weight, high water solubility and high polarity [7]. The impact of the digestive process on acrylamide's bioaccessibility is not well-studied, although it is a crucial factor for its absorption. Researchers have traditionally assumed that all acrylamide present in food is bioavailable. However, the mechanical action of chewing, pH variations, and the activity of digestive enzymes may affect its chemical integrity and stability. Acrylamide is a bifunctional molecule with an amide group and a vinyl group, making it prone to addition reactions with nucleophilic groups in biomolecules, like SH- or NH_2-. This could explain its absence in food matrices with a high protein content [8]. Given its reactivity, there is growing interest in reassessing its stability during digestion, as this could influence its bioavailability and bioactivity. Hamzalioglu & Gökmen [9] confirmed that the acrylamide content in biscuits and potato-based fried products changes during digestion, with varying reductions after complete digestion, depending on the food type. Sansano et al. [10] also found that the gastric phase of digestion releases acrylamide from the food matrix across different food groups. González-Mulero et al. [11] assessed acrylamide bioaccessibility after gastrointestinal digestion of cereal and potato-based foods, alone or with protein sources, showing that the presence of protein significantly decreased acrylamide bioaccessibility, likely due to the Michael addition of amino acids to acrylamide during digestion. In conclusion, considering acrylamide’s neoplastic properties, understanding its bioaccessibility is crucial; blocking/decreasing its absorption by interaction with other constituents in the food matrix may be vital in mitigating toxic effects. Future revisions of the EU Regulation on acrylamide [5] should consider updated risk/benefit evaluations that account for gastrointestinal digestion's influence.

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OP-02

Acrylamide Mitigation Options: Toolbox

Vural Gökmen

Food Quality and Safety (FoQuS) Research Group, Department of Food Engineering, Hacettepe University, 06800 Beytepe Campus, Ankara, Türkiye

vgokmen@hacettepe.edu.tr

The discovery of acrylamide in carbohydrate rich cooked foods by the Swedish researchers raised worldwide concern among consumers and initiated intense scientific research into possible measures to control the formation of this compound in thermally processed foods. As a probable human carcinogen, acrylamide is formed when food is heated to high temperatures exceeding 100°C and is thus considered a naturally occurring compound. Basically, acrylamide is formed from free asparagine in the presence of carbonyl compounds such as reducing sugars and their degradation products, during heating foods [1,2]. Therefore, a key challenge is to "decouple" the mechanistic route to acrylamide from the general Maillard pathway, retaining the desired attributes of cooked food with beneficial nutritional properties and health effects.
The European Food Safety Authority (EFSA), in its opinion issued in 2015, states "current levels of dietary exposure to acrylamide are not of concern with respect to non-neoplastic effects. However, although the epidemiological associations have not demonstrated acrylamide to be a human carcinogen, the Margins of Exposure (MOE) indicate a concern for neoplastic effects based on animal evidence." [3]. The MOE estimated for acrylamide is below 10,000, indicating a concern from a public health perspective. Therefore, all efforts must be made to reduce dietary exposure of acrylamide. However, in this context it is important to understand that the elimination of acrylamide in foods is virtually impossible, as asparagine is an important amino acid in the nitrogen-storage cycle of plants [4]. The latest EU Regulation 2017/2158 on acrylamide set out benchmark levels which refers to generic performance indicators [5]. According to this regulation, there is no maximum limit set, but the food business operators should at least be able to demonstrate that they follow good manufacturing practices to keep acrylamide lower. This necessitates to take mitigation actions verified through proper sampling and analysis by food business operators to reduce the acrylamide content in their products.
The Acrylamide Toolbox, established in 2005 by the Technical Process Contaminants Expert Group of the European Food & Drink Industries, summarizes the measures designed to achieve reduction of acrylamide in the pertinent food categories, using practical tools and fundamental knowledge attained in mechanistic studies [6]. The latest version of the toolbox was issued in 2019 and represents the most complete knowledge on acrylamide mitigation in the concerned foodstuffs.
Using a selection of tools related to agronomy, recipe, processing, and final preparation, food manufacturers have been able to reduce acrylamide levels in the range of 40–90% in certain selected foods. Where possible manufacturers have made changes to commercial products: for example, ammonium bicarbonate replacement by sodium bicarbonate or use of low fructose invert syrup. Recipe changes including ammonium bicarbonate replacement, reducing sugar modifications, and dilution of high-asparagine ingredients by more highly refined grains or other cereals, accounted for about two-thirds of the commercialized mitigation measures. In the case of the cereals group, most current efforts are focused on mitigation of acrylamide in whole grain and bran products, which are

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naturally high in asparagine; they continue to be monitored and are subject to ongoing development work.
Pretreatment with the commercially available enzyme asparaginase accounts for the very effective measures for certain food categories. This is despite limitations of effective use in some products and affordability for artisanal businesses. Water activity of dough is an important factor for the effectiveness of asparaginase treatment in bakery products. A water activity value exceeding 0.75 is needed in the dough to effectively reduce asparagine, so acrylamide in bakery products [7]. The addition of calcium salts like calcium chloride and calcium lactate is also very effective for lowering acrylamide formation but the change in taste should be assessed in the final product ([8]. Some mitigation methods have been used only for a few specific products or have not even been considered as potential approaches by some manufacturers, because they are in direct conflict with health targets. The replacement of wholemeal flour or bran with high extraction refined flour runs counter to the intake of whole-grain components. Replacing ammonium bicarbonate with sodium salts is in direct conflict with WHO, and EU efforts to reduce hypertension and heart disease through sodium reduction in the diet.
It is obvious that the content of acrylamide increases not only with increasing temperature, but also with the time of heating [9]. So, one may assume that different temperature-time combinations may be a viable alternative to mitigate acrylamide in thermally processed foods. In general, surface of foods reaches high temperatures earlier than interior parts because of simultaneous drying, promoting the Maillard reaction in surface. This is the reason that acrylamide formation is mainly a surface phenomenon. Hence, if the exposure of food surface to high temperatures is limited, excessive accumulation of acrylamide in potato can be prevented. Using temperature programmed frying or partial baking in combination with vacuum and radio frequency post drying are promising technological solutions for the mitigation of acrylamide in potato and cereal products as successfully demonstrated at laboratory scale [10-12].
In conclusion, new tools may be available or modifications to existing ones may be done as innovation in processing technologies advances. Food business operators are thus expected to make every reasonable effort to reduce acrylamide levels in final products and thereby reduce consumer exposure, following the “as low as reasonably achievable” approach.

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OP-03

Asparaginase treatment – pros & cons

Zuzana Ciesarová[1*], Kristína Kukurová[1]

[1]National Agricultural and Food Centre, Food Research Institute in Bratislava, Slovakia

**e-mail: zuzana.ciesarova@nppc.sk*

The use of L-asparaginase enzyme prior to heat treatment of potato and cereal based foods is a progressive way to prevent the excessive formation of acrylamide. Its role is to convert a substantial precursor, L-asparagine, into L-aspartic acid, which does not enter the acrylamide formation pathway. L-asparaginase, originally used as a pharmaceutical agent, after being adapted for commercial production, is ready for use in food processing to effectively reduce the final acrylamide content in many food products. Various applications of L-asparaginase have been described in laboratory or pilot scale in many food processes. It has been successfully used in the preparation of French fries and potato crisps with 62% effectiveness in acrylamide reduction, as well as in cereal processing. It had >90% reduction in acrylamide as demonstrated in bread, biscuit, gingerbread and fried pastry production. Smaller reductions (about 50%) were observed in cereal puffed snacks and roasted malt. No significant adverse effects on the quality of the final products were observed. On the downside, higher food production costs and the need to adjust food processing conditions to ensure optimal enzyme performance must be considered.
Bakery products as staple foods are a commonly used matrix for enrichment with nutritionally valuable compounds, especially of natural plant origin. Among them, the sea buckthorn fruit is the exceptional material used for the production of juices and jams, but the pomace remaining after juice production still has a high content of bioactive substances (carotenoids, tocopherols, flavonoids, tannins, phenolic acids, ascorbic acid, etc.) [1]. Partial substitution of wholemeal flour (wheat, triticale, rye) with dried sea buckthorn pomace in the recipe of the biscuits significantly increased their nutritional value. On the other hand, due to the high content of the free amino acid asparagine in sea buckthorn berries, this ingredient promoted the undesirable formation of acrylamide. This disadvantage was successfully eliminated by introducing asparaginase treatment into the sea buckthorn pomace processing process. The application of the enzyme had to be optimised for the specific acidic conditions of sea buckthorn mash. This innovative process is protected by utility model No. 9572 [2]. The final biscuits enriched with enzymatically treated sea buckthorn pomace powder had a reduced acrylamide content of up to 65% (Figure 1), thus complying with the requirements of EU Regulation No 2017/2158 [3], which sets the benchmark for acrylamide content in biscuits at below 350 µg/kg.

Key facts about asparaginase [5]:
- L-asparaginase has been shown to be an effective tool for reducing acrylamide in food.
- The safety of L-asparaginase as a processing aid is confirmed by the GRAS status granted by the US FDA and by acceptance by certain national authorities.
- In the production of French fries, the use of L-asparaginase as a blanching solution resulted in a 62% reduction in acrylamide.
- In biscuit production, the use of L-asparaginase in dough prior to baking resulted in a 90% reduction in acrylamide.

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- In fried dough pastries, L-asparaginase added to the dough resulted in a >90% reduction in acrylamide.
- In bread production, L-asparaginase was successfully applied to the surface of bread loaves with a 50% effectiveness on acrylamide reduction.
- In toasted bread, the effectiveness of L-asparaginase in reducing acrylamide was between 70% and 88%.
- In breakfast cereals, a 50% reduction in acrylamide was achieved with L-asparaginase when specifically applied to flour.

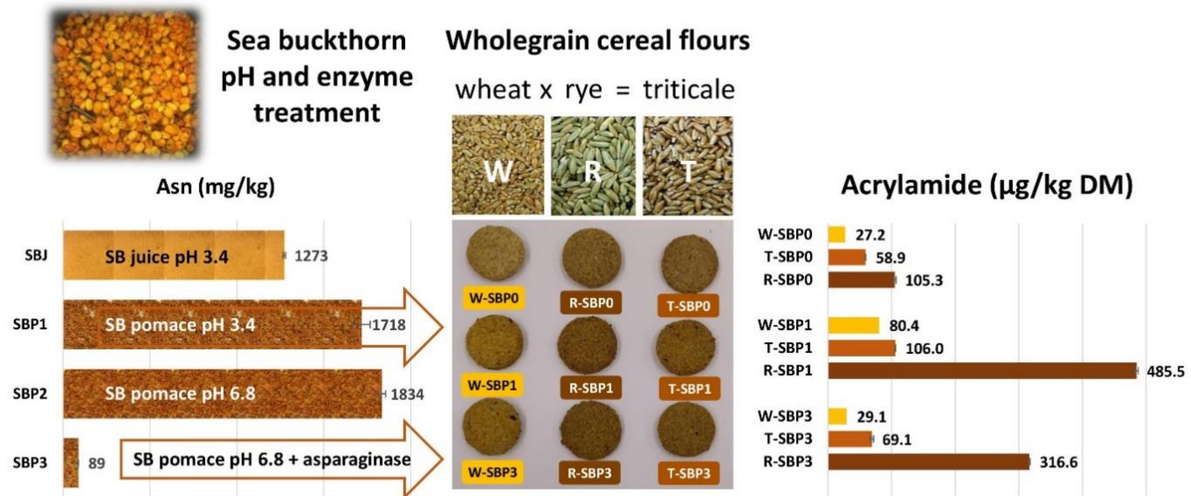

Fig.1. Impact of enzymatically treated sea buckthorn pomace on acrylamide reduction in nutritionally enhanced wholegrain cereal biscuits (adapted from [4])

Acknowledgments: This contribution was supported by the projects APVV-17-0212 “Bioactive compounds of sea-buckthorn and its application in functional foods”. The presentation was given in the frame of the COST Project ACRYRED CA 21149 Reducing Acrylamide Exposure of Consumers by a Cereals Supply-chain Approach Targeting Asparagine.

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OP-04

Aspects of acrylamide mitigation measures: impact on quality and sensory profile

Jane K. Parker

Department of Food and Nutritional Sciences, School of Chemistry, Food and Pharmacy, University of Reading, Reading, UK

**j.k.parker@reading.ac.uk*

Since the realisation in 2002 that acrylamide, a Class 2a probable carcinogen, is present at rather high levels in thermally processed carbohydrate rich foods, there has been a concerted effort to reduce its formation in many foods including biscuits, cereals, bread, potato snacks, French fries, as well as coffee, olives etc. Whilst many mitigation strategies have been successfully tried and tested, many of these mitigation measures do not maintain the quality and sensory properties of the original product, and this leads to consumer rejection. The aim of this lecture is to review the impact of acrylamide mitigation strategies on the development of sensory properties during thermal processing.

Reduction of acrylamide during processing whilst maintaining flavour is somewhat of a challenge since both acrylamide, flavour (and colour) are generated via the same chemical pathways: much of the flavour of baked, roasted, and fried foods is developed via the Maillard reaction which involves the reaction between reducing sugars and amino acids. Acrylamide is also known to be formed in asparagine rich foods via the Maillard reaction [1,2] and also directly via interaction of asparagine and a reducing sugar [3]. The details of the mechanism vary depending on the composition of the food [4] but the obvious solution to reduce time and temperature results in an insipid pale product, as reported for example in French baguettes [5].

Different mitigation approaches have been taken. Addition of inhibitors or competitors can be successful. In some cases, different strategies have been applied where the authors report no change in sensory properties. Antioxidants such as vitamin E [6], lemon peel extract [7] and hydrocolloids such as chitosan and sodium alginate [8] have been shown to successfully reduced acrylamide in cookies with no impact on sensory scores. Whereas addition of fermented wholemeal lupin flour achieved substantial reduction in acrylamide, it also imparted an off-note to the sour dough bread [9]. There are further examples where both sensory and acrylamide have been considered in non-cereal products such as potatoes and coffee and these will be discussed briefly.

However, the way to solve the reduction in sensory qualities is to understand in detail the underlying mechanisms. Early papers [10] on potato cakes demonstrated how glycine could compete with asparagine for reaction dicarbonyl species thus reducing acrylamide, but also reducing pyrazine formation - pyrazines being important for the aroma of baked goods. Later work [11] showed that acrylamide increased when sulfur-deprived wheat flour was cooked, and the volatile profile changed considerably: pyrazines and other important aroma

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compounds such as Strecker aldehydes (malty) were higher in the sulfur-deprived flours but other important aroma compounds such as the sweet sugar degradation products were reduced.
Recent work in model biscuits [12] has shown the mitigation potential of inorganic divalent cations (e.g., Ca^{2+}) and monitored the changes in acrylamide, HMF and aroma compounds. $CaCl_2$ was particularly effective and was associated with a decrease in Strecker aldehydes (malty) and pyrazines (toasty) but an increase in sugar-derived compounds such as furaneol (sweet) 2-acetylfuran (toasty), 2,3-pentanedione (buttery) and HMF (a process contaminant).
In the final section, we will look at how to design low acrylamide systems with better flavour. The example is in potato snacks [13]. By first identifying very precisely the aroma-active compounds, their precursors and their formation pathways, flavour formation in low acrylamide baked potato snacks was manipulated in such a way that a sensory panel observed an increased in baked notes.
In conclusion, a holistic approach to acrylamide mitigation is required such that the sensory profile of the low acrylamide product is at least as good as the original. Equally it is important to assess the influence of mitigation measures on the nutrient and anti-nutrient profile, the formation of other process contaminants and the feasibility and cost of the new product.

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OP-05

Analytical session: novel techniques in acrylamide and asparagine determination usable in food production

Jana Hajšlová, Beverly Hradecká, Aristeidis Tsagkaris

Department of Food Analysis and Nutrition, University of Chemistry and Technology, Prague, Czech Republic

jana.hajslova@vscht.cz, beverly.hradecka@vscht.cz

After discovery in thermally processed foods in 2002, acrylamide has become an issue of food safety concern [1] as it is classified by IARC as a probable human carcinogen (group 2A); moreover, it has neurotoxic effects [2]. Mitigation strategies aimed at protecting consumers' health have become the subject of an intensive investigation. Acrylamide formation occurs during the Maillard reaction at temperatures above 120 °C; reducing sugars and asparagine are the key precursors. Due to safety concerns about the dietary exposure of consumers to acrylamide, the Commission Regulation (EU) 2017/2158 introduced specific obligations for operators who produce and place certain foodstuffs on the market, to establish a programme for their own sampling and analysis of acrylamide content in the foodstuffs and apply specific mitigation measures in order to achieve levels of acrylamide as low as reasonably achievable [3]. Acrylamide Toolbox 2019 presents a useful tool of interventions that may prevent and reduce acrylamide formation in food [4]. In 2019, due to insufficient data on the occurrence of acrylamide in foods, the European Commission established a recommendation to monitor acrylamide in a number of foods, including bakery and cereal products [5].

Widely used instrumental techniques represented either by liquid chromatography (LC) or gas chromatography (GC) coupled with mass spectrometry (MS) are nowadays mainly used for accurate and reproducible acrylamide analysis with sufficiently low limits of quantification (LOQ). For the GC based acrylamide determination, this very polar compound with low molecular weight (Mr = 71.08 g/mol) has to undergo derivatization, typically by bromation or silylation. In this way, analyte stability is enhanced (polymerisation and decomposition are prevented), and LOQ is improved. However, the derivatization step is rather time consuming, and due to high-temperature conditions, acrylamide may originate from precursors contained in the injected sample. Under such conditions, high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) represents currently a ´gold standard´ in acrylamide analysis [6,7]. An official method for acrylamide determination can be found in the European Standard EN 16618:2015 [8].

QuEChERS (quick, easy, cheap, effective, rugged, and safe) procedure is often used for sample preparation. In case of high-fat samples, defatting with a non-polar solvent is performed in the first phase. Acrylamide extraction is achieved by aqueous acetonitrile. After phases separation, induced by the addition of a mixture of salts ($MgSO_4$ and NaCl), the analyte is transferred to the organic phase (acetonitrile). To remove most of the remaining matrix co-extracts that might interfere with the analysis, dispersive solid phase extraction, dSPE ($MgSO_4$ and Al_2O_3) is used. Regarding quantification, this is preferably performed by an isotope-dilution approach employing a labelled internal standard, e.g., acrylamide-D_3, acrylamide-D_5, $^{13}C_1$-acrylamide or $^{13}C_3$-acrylamide for matrix effects compensation [9-11]. Multiple reaction monitoring (MRM) mode is used for selective and sensitive detection of acrylamide, the MS/MS transitions are: *m/z* 72>55 (quantifier), *m/z* 72>54 (qualifier) and for labelled $^{13}C_3$-acrylamide m/z 75>58 (7,11].

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In addition to the above methods, several (semi)quantitative approaches can be considered, e.g., immunoassay methods (ELISA, enzyme-linked immunoassay), capillary electrophoresis, or electrochemical sensing analysis based on biomolecules. Although these methods are in principle simple, fast, and low-cost, their application is limited and needs to be improved, namely, in terms of detection stability [12].

Considering the conceivable mitigation measures, selecting plant material with an inherently low free asparagine (ASN) content is a promising approach. By testing raw plant material, e.g., cereal grains prior to thermal processing, an early warning on risk of excessive acrylamide formation can be obtained. To achieve that objective, the ACRYRED project (COST Action CA21149) has emphasized the need for the availability of a cost-efficient, simple and rapid test enabling ASN screening without the need of laboratory instrumentation such as HPLC. One of the challenging ideas is the development of an asparaginase (ASNase) assay based on the principle that ASNase hydrolyses ASN (the acrylamide precursor) to L-aspartic acid and ammonia. The produced ammonia will be monitored through various chromogenic reagents and correlated with the ASN content of the tested matrix. During the first stage of the experiments, a 96-microtiter plate format will be used and crucial assay parameters, e.g., enzyme concentration, specificity toward other amino acids, ratio between enzyme and test sample, tolerance against organic solvents, will be investigated and optimized. Additionally, the performance of an in-house produced ASNase will be benchmarked toward commercially available ASNase from *Escherichia coli*. The enzyme source plays a pivotal role on assay sensitivity, but it is also an impactful asset on the assay final cost.

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OP-06

Aspects of acrylamide mitigation measures: impact on nutritional profile

Slađana Žilić

Maize Research Institute, Laboratory of Food Technology and Biochemistry, Belgrade, Serbia

szilic@mrizp.rs

In addition to its beneficial effects on food, thermal treatments are accompanied by certain chemical food safety problems. One of them is the formation of thermally induced contaminants. In recent years, acrylamide is among the most studied. Acrylamide is classified as a “probable human carcinogen” by the International Agency for Research on Cancer (IARC). In addition, acrylamide is a neurotoxic and genotoxic compound to animals [1]. It is known today that more than one-third of food products consumed by the U.S. and European populations contain acrylamide. In addition to processed potatoes and coffee, cereal-based food products are groceries with the highest level of acrylamide. According to data, bakery products may contribute up to 50% of the total mean acrylamide intakes. Acrylamide is almost unavoidable compound in cereal-based food products for two reasons. First, precursors of acrylamide (free asparagine and reducing sugars) accumulate in cereal grains under natural field conditions. Second, cereals require thermal transformation into food, that is, as mentioned, a crucial condition for acrylamide formation. Due to their health concern, researchers and authorities of many countries reported that acrylamide formation in food needed to be minimized. The European Commission, as well as other renowned food organizations have published several documents that provide guidance for acrylamide mitigation. In general, the strategies recommended for reducing acrylamide in food can be categorized into several different groups such as: 1. Measures directed at the raw material/pre-harvest and post-harvest (breeding; crop management regimes; storage conditions); 2. Measures directed at primary manufacturing processing and pre-thermal treatment (grain grinding; bioprocesing; nixamalization; selection of raw materials; refolmulation; additives; dough conditions; etc.); 3. Measures directed at thermal treatment (type of thermal treatment; duration of thermal treatment; temperature; type of frying oil; shape and size of food product; etc.). However, when talking about the application of measures to reduce the content of acrylamide, their effect on the nutritional profile of food must also be taken into account. Minimizing acrylamide level and retaining the desired nutritional and functional properties of cooked food at the same time represents a great challenge for food manufacturers and requires the engagement of all subjects in the production chain. Measures to reduce acrylamide can have both negative and positive effects on nutritional profile of food. As one of the first strategies for reducing acrylamide in food, optimization of agronomic regimes aims to reduce the synthesis of free asparagine in the grain. Crop management strategies include, inter alia, avoiding excessive nitrogen application while ensuring adequate sulfur supply. However, there were also a certain effects of nutrient supply on other free amino acids. A clear effect of sulfur supply on wheat grain sulfur and nitrogen concentration existed as well. Sulfur availability does not affect total proteins or their properties like electrophoretic mobility. It, however, does alter the proportions of different protein fractions and their subunits: sulfur-rich (α-, β-, γ-gliadins and B- and C-LMW glutenins), sulfur-poor (ω-gliadins and D-LMW glutenins) and high molecular weight (HMW glutenins) proteins. These changes in the amounts of different protein fractions change their functional properties resulting stronger dough with increased mixing requirements, less extensibility and reduced loaf volume. [2]. As far as corn is concerned, treatment of the plant with sulfur-based fertilizers and herbicides resulted in a maximum

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reduction of free asparagine content by 20%. However, the statistical analysis confirmed that the differences in the content of free asparagine, as well as nutritional and bioactive components suh as total proteins, oils, fibers, sugars, phenolic compounds and carotenoids, were conditioned predominantly by the interaction of environment factors and treatments [3]. Considering that most cereal-based food products are obtained from flour, one of the more effective measures to reduce acrylamide is the separation of flour fractions and the use of the highly refined one which, otherwise, has better technological properties compared to whole-grain flour. However, food products poor in health-promoting bioactive compounds are obtained in this way. Phenolic compounds, carotenoids and tocopherols are concentrated mostly in the aleurone layer, pericarp and germ. About 99% of total phenolic compounds were localized in the bran of wheat grains. Further, compared to the initial average values, debranning reduced the content of ash, total proteins and cellulose by about 60%, 18% and 90%, respectively, in refined wheat flour [4]. Nixtamalization of corn was also reported to have a reducing effect on the formation of acrylamide in tortilla chips. In addition, there was a considerable increase in soluble free and conjugated phenolic compounds contents of corn flour after alkaline cooking (35% to 56%). Contrary, conditions of alkaline cooking had strong effect on the loss of bound phenolic compounds, as well as free tryptophan, total and available niacin, carotenoids (zeaxanthin, lutein and β-carotene) and tocopherols in corn masa [5]. Reduction in acrylamide content by 82% and 73% reported after 0.2% citric acid treatment of fried and baked corn chips, respectively. This organic acid also stabilizes phenolic compounds in cooked food. Blue corn cookies prepared with 0.5% added citric acid had higher contents of total flavonoids and anthocyanins by 60% and 70%, respectively, compared to the control [6]. Addition of asparaginase to dough has some advantages compared to other reduction strategies because it could achieve very high (54% to 96 %) acrylamide reductions depending on the type of the product and it does not affect the sensory quality of the product, as well as does not alter the physico-chemical properties of the product [7]. The last stage in which it is possible to reduce acrylamide is the thermal treatment itself. It should be emphasized that chemical reactions take place at any temperature as long as reactants are able to collide which inevitably leads to changes in the constituents of food to a lesser or greater extent. The changes are primarily related to the thermolabile phenolic compounds, carotenoids, vitamins, amino and fatty acids, denaturation of enzymes, cleavage of large carbohydrate molecules, changes in the structure of storage proteins and starch and their hydrolysis. These changes can reduce the nutritional and functional value of food. On the other hand, they can cause better digestibility and availability of nutrients, as well as release of bioactive compounds and an increase in antioxidant capacity, formation of resistant starch and increase in viscosity and fermentability of fibers due to their hydrolysis, etc. When optimizing the temperature and cooking time care should be taken. Among others, microorganisms, antinutritional compounds, hydrolytic and oxidative enzymes can survive at insufficiently high temperatures and cause serious health problems or food deterioration. It could be concluded that effective mitigation strategies should be applied according to the product. All food product processing and formulations should be well evaluated individually from all aspects such as compatibility with commercial applications in the food industry, cost-effectiveness and protecting nutritional value, microbiological and chemical safety.

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OP-07

Technological aspects of acrylamide mitigation measures in cereal-based food production (progress and economy)

Jozef Murín[1*], Zuzana Ciesarová[2], Kristína Kukurová[2]

[1]CELPO Ltd., Očová, Slovakia
[2]National Agricultural and Food Centre, Food Research Institute in Bratislava, Slovakia

**e-mail: obchod@celpo.sk*

To improve the nutritional quality of cereal products and meet consumer expectations, manufacturers are expanding their portfolios to include innovative non-wheat cereal products such as rye, oats, spelt, buckwheat, lentils, etc. On the other hand, in addition to their nutritional benefits, they can be a source of undesirable heat-induced contaminants such as the probable carcinogen acrylamide.

Heat-treated cereal-based products have high levels of acrylamide, depending on the presence of precursors in the raw material, heat and processing conditions. Based on our observations of acrylamide in puffed snacks, bio-rye and bio-spelt snacks were recognised as the highest in acrylamide, well above the benchmark level (300 µg/kg) set by Commission Regulation No 2017/2158.

One of the most effective ways to reduce acrylamide in food is the use of asparaginase in the production of rye puffed snacks with prior flour extrusion. Asparaginase was applied during the moistening of the rye flour prior to extrusion, which resulted in a significantly reduced level of created acrylamide in finished products - in average about 60% comparing with untreated products. The sensory properties are also influenced and should be futher investigated in conjunction with the expectation of better or worse sales. A surprise for practice was the improved processability of the treated raw material in the process of manufacturing finished products - the products showed less waste in the puffing process, the corpuses were smoother with more regular edges which also affects better packaging process.

For practice is very important Economic calculation, where the input data are the costs of investment in new technology, premises, staff and the cost of asparaginase. The return on investment depends on the marketability of the product - so the treatment of acrylamide formation sensitive raw material makes sense only for highly desirable and nutritionally rich products or popular baby food products where the legislation is stricter.

The entire process of using asparaginase in this technology was registered with the Industrial Property Office of the Slovak Republic under utility model number 9269.

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OP-08

"ASNInsta Test" – Instant test for asparagine in food

Tanya Curtis*, Emma Honan
Curtis Analytics limited, Sandwich, The United Kingdom

**tanya.curtis@curtisanalytics.co.uk*

Asparagine (ASN) is currently tested using GC-MS, HPLC or LC-MS lab methods provided by service testing companies like PAS Ltd. Prices are £50-60/test and rely on posting samples to the lab and a costly time lag before test results are obtained. This issue with testing for acrylamide is that industry is testing once the damage has already been done to their products. Because of the strong correlation between ASN and acrylamide in wheat and coffee, testing ASN levels allows for a strategy of accepting low ASN ingredients before cooking into final products. ASN is also tested using GS-MS.

ASNInsta Test, an innovative at-the-gate, rapid and easy-to-use test for asparagine in flour. *The development of an* "ASNInsta®Test": significantly adds to the global effort to reduce acrylamide formation in wheat products. The acrylamide issue is a difficult problem facing the food industry globally, and this project will consolidate all previous knowledge on the testing for asparagine and generate substantial new fundamental knowledge, which will translate into strategies that will further reduce asparagine accumulation in the wheat gain. Additionally, the "ASNInsta®Test" could also be used for potato as a follow-on project where the acrylamide problem is significantly more complex.

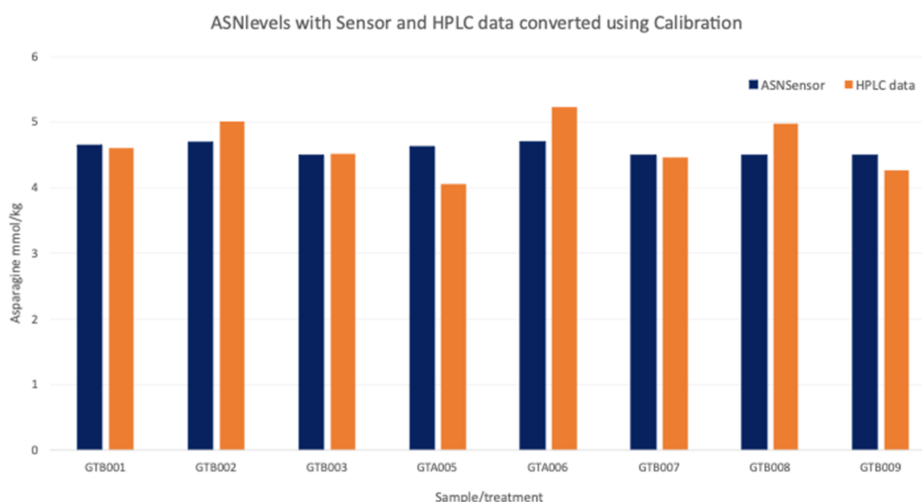

Table 1. Caption [Calibri size 10]

Sample	GTB001	GTB002	GTB003	GTA005	GTA006	GTB007	GTB008	GTB009
ASN Sensor	4.64	4.69	4.50	4.62	4.71	4.50	4.50	4.50
HPLC data	4.603	5.01	4.52	4.06	5.23	4.46	4.972	4.26

References: be formatted according to the examples presented below [Calibri Size 10]
[1] Patent applicaiton pending, Tanya Curtis (2023).

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PRACTICAL SESSIONS

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PS-09

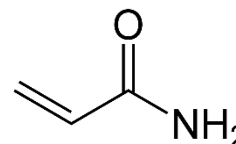
Acrylamide (ACR) analysis using HPLC-MS/MS in cereal products

Zuzana Dubová[1*], Kristína Kukurová[1]

[1]National Agricultural and Food Centre, Food Research Institute, Department of Chemistry and Food Analysis, Priemyselná 4, 824 75 Bratislava, Slovakia

**zuzana.dubova@nppc.sk*

Instrument:
Agilent HPLC 1200 Series coupled to 6460 Triple Quad LC-MS/MS detector



Standard:
Acrylamide (≥ 99 %, Sigma-Aldrich, Steinheim, Germany)

Internal standard (ISTD):
2,3,3-D3-Acrylamide (≥ 98 %, Cambridge Isotope Laboratories, Androver, USA)

Chemicals:
Glacial acetic acid p.a. (Sigma-Aldrich, Steinheim, Germany), methanol gradient grade (Sigma-Aldrich, Steinheim, Germany), potassium hexacyanoferrate trihydrate for Carrez I (Merck, Darmstadt, Germany), zinc sulphate heptahydrate for Carrez II (Merck, Darmstadt, Germany)

Extract solution:
1000 µL of acetic acid/1000 mL of deionized water (0.1%, v/v)

Chromatographic conditions:
Column: Atlantis dC18, 3.0 x 100 mm, 3 µm (Waters, Milford, MA, USA)

Mobile phase: 500 mL deionized water, 5 mL methanol, 1 mL acetic acid (isocratic elution)

Flow: 0.4 ml/min

Column temperature: 25°C

Injection volume: 10 µL

Detection: *Ion source parameters:* ESI (+)
drying gas (N_2) flow 8 L/min at temperature 350 °C, nebulizer 50 psi,
sheath gas flow 11 L/min at temperature 250 °C, capillary voltage 2.5 kV

MRM acquisition: fragmentor 50 eV, collision energy 10 *(18)* eV

ACR 72 →55 (quantifier); *72 →27 (qualifier)*
d_3-ACR 75 →58 (quantifier); *75 →30 (qualifier)*

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Quantification of ACR:
The quantification of acrylamide was calculated from a calibration curve of the standard compound in the range from 2 to 400 ng/mL (Retention time: 2.6 min, Total analysis time: 11 min)

ACR calibration curve:
to 10 mL volumetric flask add:

+ 2, 5, 10, 20, 50, 100, 200, 400, 800 µL ACR solution (stock solution 0.5 mg/mL → dilute 100x = 0.005 mg/mL)
+ 50 µL ISTD solution (stock solution 0.2 mg/mL → dilute 10x = 0.02 mg/mL)
+ fill in with solution of 0.1% acetic acid

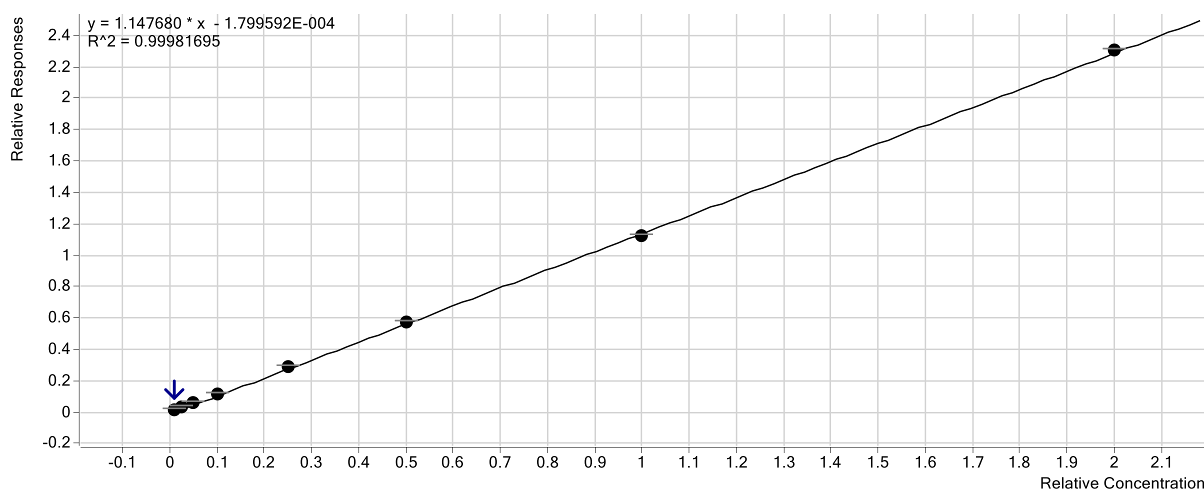

Fig. 1 Calibration curve for calculation of acrylamide content in cereal samples

Calculation in sample:
The content of ACR in µg/kg was calculated using Agilent Mass Hunter Quantitative Software (version B.04.00).

Limit of quantification LOQ: 15 µg/kg

Limit of detection LOD: 5 µg/kg

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Sample preparation and extraction of ACR:

1. **Grind** dry cereal sample into powder using laboratory knife mill & homogenizator at standardized conditions e.g. 5 000 rpm for 20 s using Grindomix GM 200 (Retsch, Germany).
2. **Weigh 2 g** (< 0.0040 g) of finely ground sample into 30 mL plastic centrifuge tube with a cap (e.g. Sarstedt, Germany).
3. Add **100 µL of internal standard** (0.02 mg/mL) and **18 mL of extract solution** (0.1% acetic acid) and shake mixture properly by hand and in vortex for 1 min.
4. Add **1 mL of Carrez I** and shake properly and next **1 mL of Carrez II** and shake mixture vigorously (Fig. 2).
5. Place tubes into ultrasonic bath for 5 min.
6. Centrifuge tubes for 10 min at 10 000 rpm and temperature of - 5°C for effective separation of the fat layer on the top of mixture.

Fig. 2 Acrylamide water extraction by 0.1 % of acetic acid and clarification by Carrez solutions

Fig. 3 Separation of 5 ml of clear supernatant (after centrifugation)

7. Separate **5 mL of clear water supernatant** into a clean plastic tube (Fig. 3).
8. Eliminate interferences and suppressors of MSD signal (e.g. inorganic salts) by **pre-extraction of water extract into ethyl acetate** (effective procedure comprises from **3 times addition of 5 mL of ethyl acetate** and intensive shaking by hand for 1 min of each ethyl acetate addition).
9. Collect all three 5 mL upper layers of ethyl acetate into evaporation flask (Fig. 4) and evaporate to dryness by rotatory vacuum evaporator at 35 °C.
10. Dissolve dry residue in 1 ml of 0.1% acetic acid and filter through a nylon syringe filter (0.13 mm diameter of disk, 0.45 µm size of pores) before LC-MS/MS analysis.

Fig. 4 Pre-extraction into ethyl acetate (3 times by 5 mL of ethyl acetate; collection of the upper layers into evaporation flask)

Fig. 5 Evaportion of collected ethyl acetate layers by rotary vacuum evaporator

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Chromatograms of ACR in cereal sample:

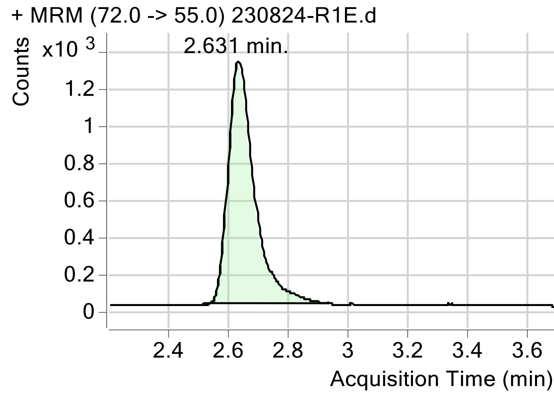

Fig. 6 Chromatogram of Acrylamide quantifier

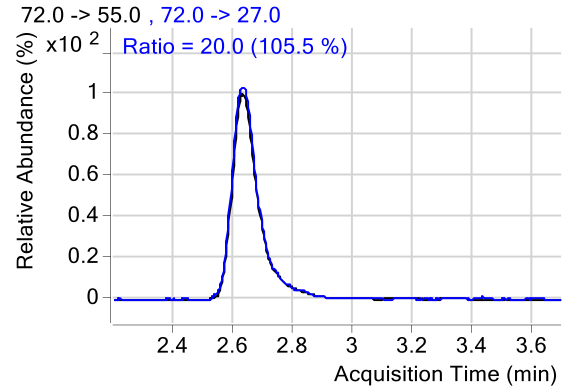

Fig. 7 Chromatogram of Acrylamide *qualifier*

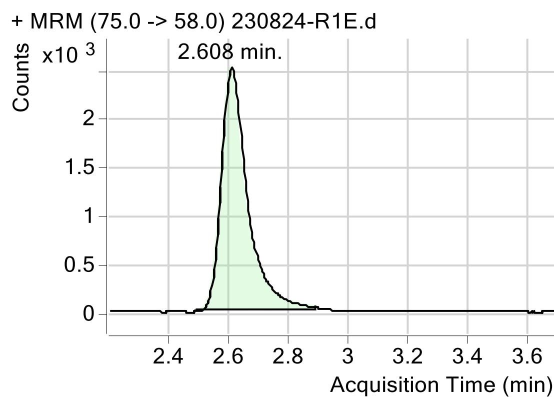

Fig. 8 Chromatogram of d3-Acrylamide (ISTD) quantifier

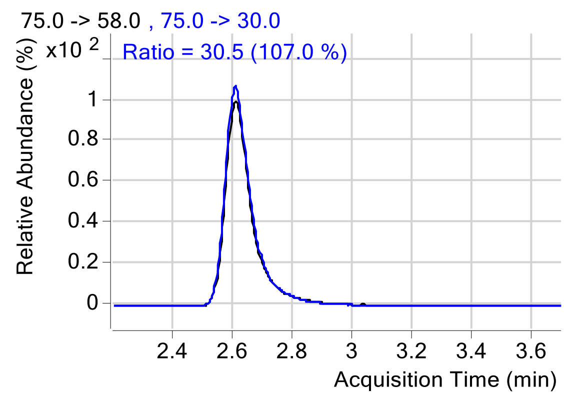

Fig. 9 Chromatogram of d3-Acrylamide (ISTD) *qualifier*

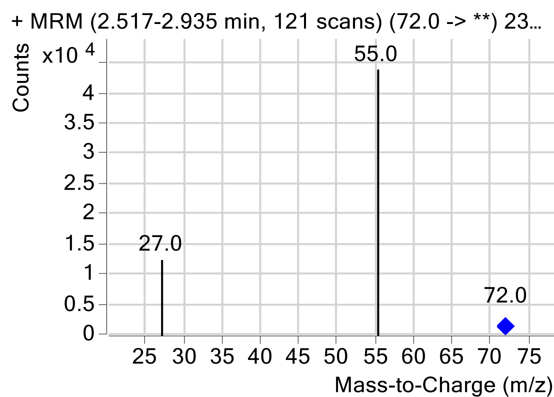

Fig. 10 MRM of Acrylamide

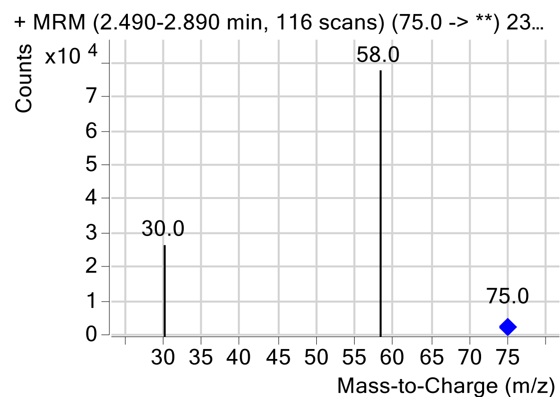

Fig. 11 MRM of d3-Acrylamide (ISTD)

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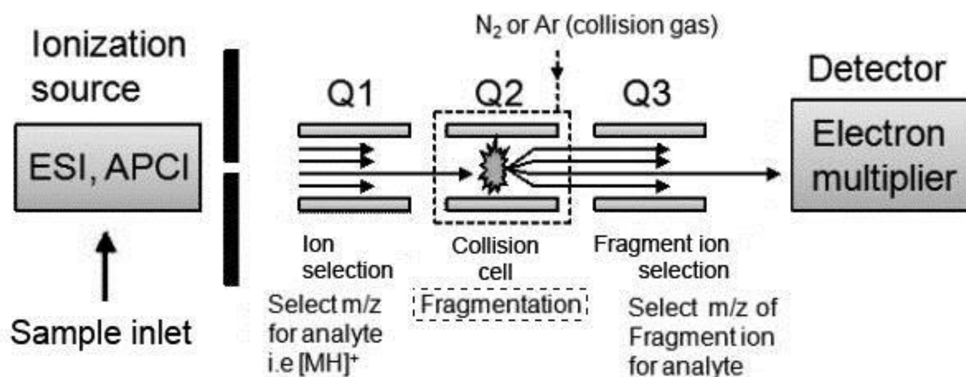
Triple quadrupole scheme



References:

[1] Ciesarová, Z.; Kukurová, K.; Jelemenská, V.; Horváthová, J.; Kubincová, J.; Belović, M.; Torbica, A. Asparaginase Treatment of Sea Buckthorn Berries as an Effective Tool for Acrylamide Reduction in Nutritionally Enriched Wholegrain Wheat, Rye and Triticale Biscuits. *Foods* **2023**, *12*, x. https://doi.org/10.3390/xxxxx (in press).

[2] Ciesarová, Z.; Kukurová, K.; Torbica, A.; Belović, M.; Horváthová, J.; Daško, Ľ.; Jelemenská, V. Acrylamide and 5-hydroxymethylfurfural in thermally treated non-wheat flours and respective breads. *Food Chem.* **2021**, *365*, 130491.

[3] Kukurová, K.; Ciesarová, Z.; Mogol, B.A.; Açar, Ö.Ç.; Gökmen, V. Raising agents strongly influence acrylamide and HMF formation in cookies and conditions for asparaginase activity in dough. *Eur. Food Res. Technol.* **2013**, *237*, 1–8.

[4] Ciesarová, Z.; Kukurová, K.; Bednáriková, A.; Morales, F.J. Effect of heat treatment and dough formulation on the formation of Maillard reaction products in fine bakery products—Benefits and weak points. *J. Food Nutr. Res.* **2009**, *48*, 20–30.

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PS-10

Aminoacids analysis using HPLC-MS/MS in cereal products

Jana Horváthová[1*], Janka Kubincová[1]

[1]National Agricultural and Food Centre, Food Research Institute, Department of Chemistry and Food Analysis, Priemyselná 4, 824 75 Bratislava, Slovakia

jana.horvathova@nppc.sk

Instrument: Agilent HPLC 1200 Series coupled to 6460 Triple Quad LC-MS/MS detector

Chromatographic conditions:
Column: Purospher STAR RP-8ec column (150 × 4.6 mm, 2.7 µm), Agilent, USA

Mobile phase: 100 mL acetonitrile, 500 mL aqueous solution of 0,05 mM perfluorooctanoic acid (PFOA), 1 mL glacial acetic acid (isocratic elution)

Flow: 0,5 mL/min

Column temperature: 25°C

Injection volume: 5 µL

Ion source: ESI (+): drying gas temperature: 320 °C; drying gas flow: 8 L/min (N_2); sheath gas flow 11 L/min; nebulizer pressure: 50 psi; capillary voltage: 3 kV

Detection: MS/MS with MRM

Amino acid	**RT (min)**	**Precursor ion [M+H]+**	**Production ions**		**Fragmentor (eV)**	**Collision energy (eV)**		**R^2**	**LOD (ng/mL)**	**LOQ (ng/mL)**
			quantifier	***qualifier***		**quantifier**	***qualifier***			
Ala	4.53	90	44	*90*	50	6	*2*	0.9990	10	30
Arg	13.64	175	70	*175*	100	25	*5*	0.9992	10	30
Asn	4.3	133	74	*87*	50	12	*5*	0.9998	8	25
Asp	3.74	134	74	*88*	50	12	*5*	0.9996	10	30
Cys	3.92	122	76	*59*	60	10	*25*	0.9991	10	30
Gln	4.9	147	84	*130*	50	15	*5*	0.9919	10	30
Glu	4.21	148	84	*102*	50	15	*10*	0.9994	6	20
Gly	4.47	76	76	*30*	50	2	*6*	0.9997	11	33
His	11.67	156	110	*156*	100	15	*2*	0.9996	10	29
Hyp	3.63	132	86	*132*	50	15	*2*	0.9998	13	39
Ile	7.62	132	86	*132*	80	5	*2*	0.9997	8	24
Leu	7.11	132	86	*132*	80	5	*2*	0.9997	11	33
Lys	13.28	147	84	*130*	80	15	*5*	0.9991	7	23
Met	5.45	150	56	*133*	80	15	*5*	0.9972	8	24

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Orn	12.35	133	116	*70*	80	5	*20*	0.9997	9	28
Phe	8.21	166	120	*103*	80	10	*30*	0.9997	10	30
Pro	3.95	116	70	*116*	50	10	*0*	0.9978	9	29
Ser	4.1	106	60	*106*	50	5	*2*	0.9987	10	30
Thr	4.18	120	56	*74*	50	15	*8*	0.9978	8	26
Trp	15.17	205	146	*188*	100	15	*5*	0.9998	11	34
Tyr	8.14	182	182	*136*	100	2	*10*	0.9979	10	30
Val	5.32	118	72	*118*	80	10	*2*	0.9994	7	21

Quantification of aminoacids:

The quantification of acrylamide was calculated from a calibration curve of the standard compound in the range from 20 to 4000 ng/mL using method of internal standard.

Aminoacids calibration curve:

to 10 ml volumetric flask add:

+ 2, 5, 10, 20, 50, 100, 150, 200, 400 µL solution of aminoacids (0,1 mg/mL)
+ 50 µl of ISTD solution – d3-Glu (0,1 mg/mL)
+ fill in with solution of acidified water (0,1% glacial acetic acid)

Sample preparation:

1. 2.000 g of finely ground or homogenized dry sample is weighed into a capped centrifuge tube and mixed with 20 mL of acetic acid extraction solution (0,2 mM).

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2. The mixture is vortexed for 1 min and then shaked for 30 min using shaker (150 rpm, ambient temperature) and centrifugated at 10000 x g for 10 min at −5 °C.

3. 1 mL of clear supernatant is transferred into a glass tube containing 50 µL of an internal standard solution of d3-Glu (0.1 mg/mL) and 9 mL of acetic acid (0.2 mM) and mixed.

4. After filtration through a 0.45 µm pore size nylon syringe filter were samples injected and analyzed by HPLC-MS/MS.

Calculation:

The content of Asn (X) in ng/g of the sample is calculated:

$$X\ [ng/g] = \frac{Area_{sample} * m_{ISTD} * f_R}{Area_{ISTD} * m_{sample} * b}$$

$Area_{sample}$ – area (response) of Asn in sample

$Area_{ISTD}$ – area (response) of ISTD in sample

m_{sample} – weight of sample [g]

m_{ISTD} – addition of ISTD to the sample [ng]

f_R – dilution factor

b – slope of the calibration curve **y = b*x**

y – Area Asn / Area ISTD

x – Conc. Asn / Conc. ISTD

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Chromatogram results:

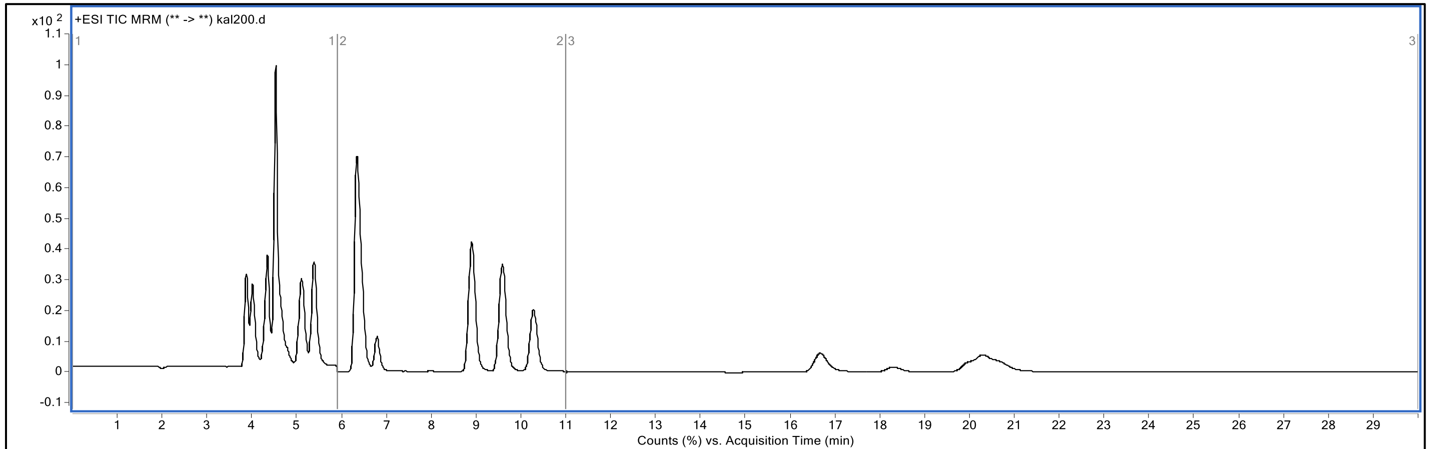

Fig. 1 Chromatogram of 22 aminoacids in cereal sample

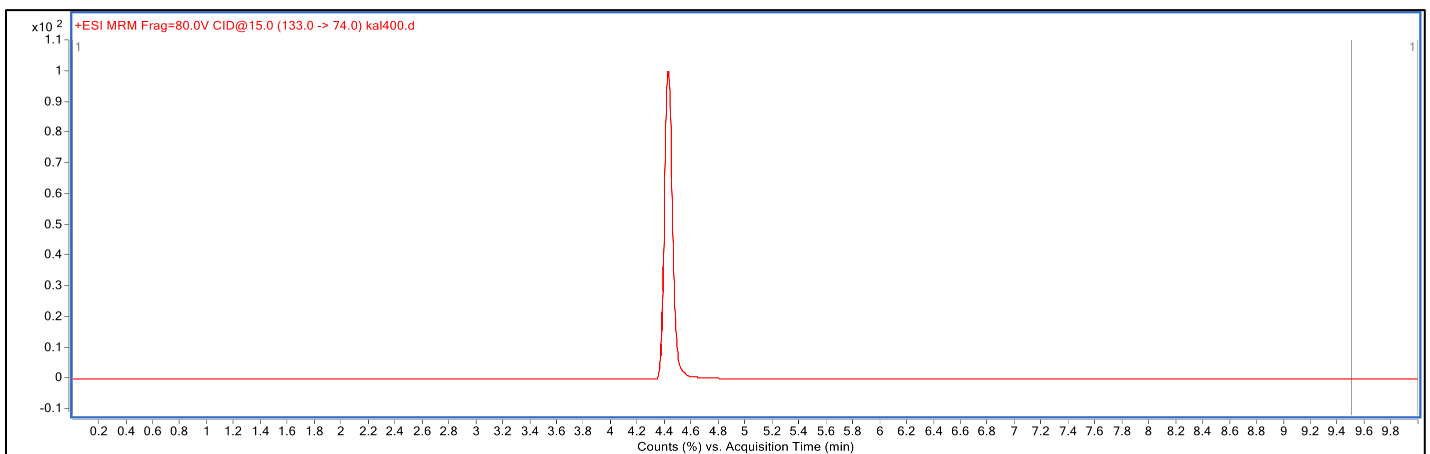

Fig. 2 Chromatogram of Asparagine in cereal sample (quantifier 133,0 → 74)

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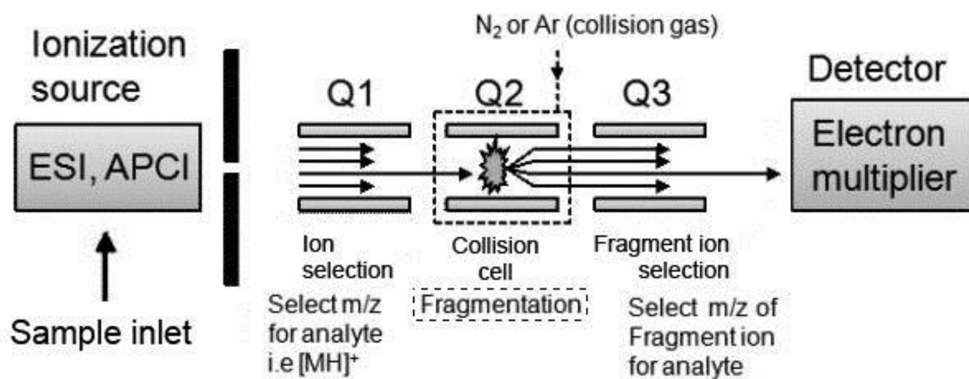
Triple quadrupole scheme



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PS-11

PROFILE ANALYSIS OF PUFFED SNACKS

Veronika Vigašová, Zuzana Ciesarová, Kristína Kukurová
National Agricultural and Food Centre
Food Research Institute in Bratislava, Slovakia

veronika.vigasova@nppc.sk, *zuzana.ciesarova@nppc.sk*, *kristina.kukurova@nppc.sk*

After log into Compusense, we see options of running tests and we can choose which test we are going to complete.

N P
P C

Welcome,
Points Balance: **0**

CEST

Sign Out

Home | Schedule | Account

New Test

Resume Test

Expiry Date: 09/24/2023 4:49 PM

New Test

Start

Expiry Date: 09/29/2023 7:10 PM

Profile analysis of puffed snacks

Welcome!

Click the *next* button to begin

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Profile analysis collect quantitative data on our products. Panelists evaluate properties of samples of puffed snacks, which are:

- color intensity,
- odor intensity,
- texture (hardness, fragility, cohesion),
- taste,
- presence of off-flavour,
- overall acceptability of product.

Panelists evaluate properties of samples on a scale from 1 to 9 (1-the least in atribute, 9-the best in atribute).
In this test we have four samples to evaluate:

- BIO RYE no enzyme (sample 1),
- BIO RYE with enzyme (sample 2),
- BIO SPELT no enzyme (sample 3),
- BIO SPELT with enzyme (sample 4).

On a scale from 1 to 9, evaluate the color intensity of the sample.
Note: 1 - the lightest, 9 - the darkest

Sample: 1

Color intensity

Low								High
1	2	3	4	5	6	7	8	9

On a scale from 1 to 9, evaluate fragility of the sample.
Note: 1 - the least fragile, 9 - the most fragile

Sample: 2

Fragility of the sample

Low								High
1	2	3	4	5	6	7	8	9

On a scale from 1 to 9, evaluate taste of the sample.
Note: 1 - the least pleasant taste, 9 - the most pleasant taste

Sample: 3

Taste

Low								High
1	2	3	4	5	6	7	8	9

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On a scale from 1 to 9, evaluate overall acceptability of the sample.
Note: 1 - the worst, 9 - the best

Sample: 4

Overall acceptability

Low								High
1	2	3	4	5	6	7	8	9

Thanks for completing this test!

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ANALYSIS OF DIFFERENCES IN PUFFED SNACKS

Veronika Vigašová, Zuzana Ciesarová, Kristína Kukurová
National Agricultural and Food Centre
Food Research Institute in Bratislava, Slovakia

veronika.vigasova@nppc.sk, zuzana.ciesarova@nppc.sk, kristina.kukurova@nppc.sk

Analysis of differences is a test which we use to ask panelists which sample in a pair of samples has higher intensity in specific attributes (for example, which of the two samples are sweeter).

Panelists compare two samples and select one sample which has higher intensity in specific attributes, which are:

- color intensity,
- texture (hardness, fragility, swelling).

In this test we have four samples to evaluate:

- BIO RYE no enzyme (sample 1),
- BIO RYE with enzyme (sample 2),
- BIO SPELT no enzyme (sample 3),
- BIO SPELT with enzyme (sample 4).

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In front of you are two samples.
Taste both samples and select the sample that is **darker**.

Color intensity

In front of you are two samples.
Taste both samples and select the sample that is **darker**.

1	4

In front of you are two samples.
Taste both samples and select the sample which texture is harder.

Hardness

In front of you are two samples.
Taste both samples and select the sample which texture is harder.

1	4

In front of you are two samples.
Taste both samples and select the sample that is more fragile.

Fragility

In front of you are two samples.
Taste both samples and select the sample that is more fragile.

1	4

In front of you are two samples.
Taste both samples and select the sample that is more swelling.

Swelling

In front of you are two samples.
Taste both samples and select the sample that is more swelling.

1	4

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Thanks for completing this test!

THANK YOU FOR YOUR PARTICIPATION!

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PS-12

Protocol for textural analysis of puffed breads from pea and buckwheat by Three Point Bend

Kristína Kukurová

National Agricultural and Food Centre, Food Research Institute in Bratislava, Slovakia

kristina.kukurova@nppc.sk

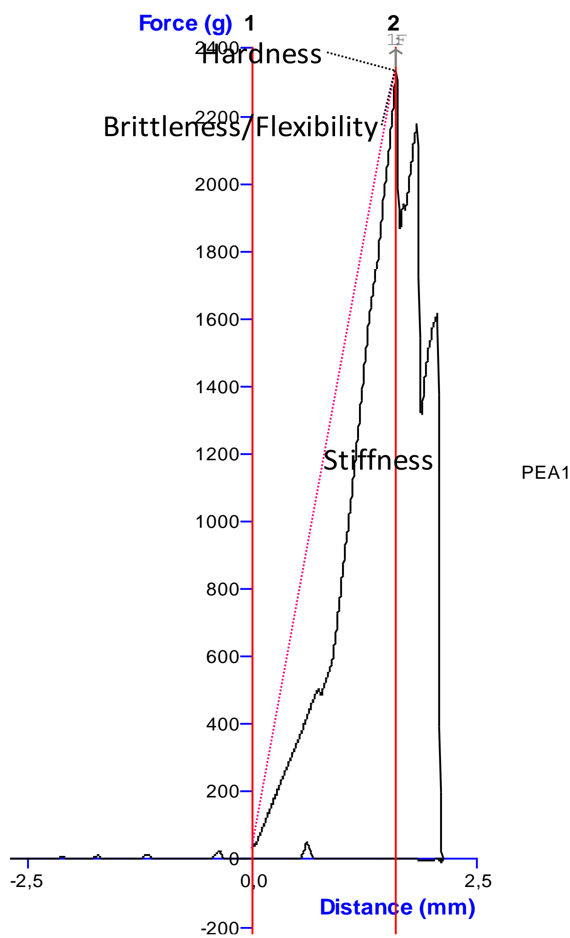

Figure 1 Typical plot of 3 Point Bend for puffed bread.

HDP/3PB Rig (Stable Micro Systems Ltd.)

Diameter of the sample: 8 cm
Thickness of the sample: 8.5 mm (variable*)
Distance of gap: 30 mm
Mode: Force in compression
Pre-test speed: 1.0 mm/s
Test speed: 3.0 mm/s
Post-test speed: 10.0 mm/s
Distance: 5 mm
Trigger force: Auto – 50 g
Tare mode: Auto

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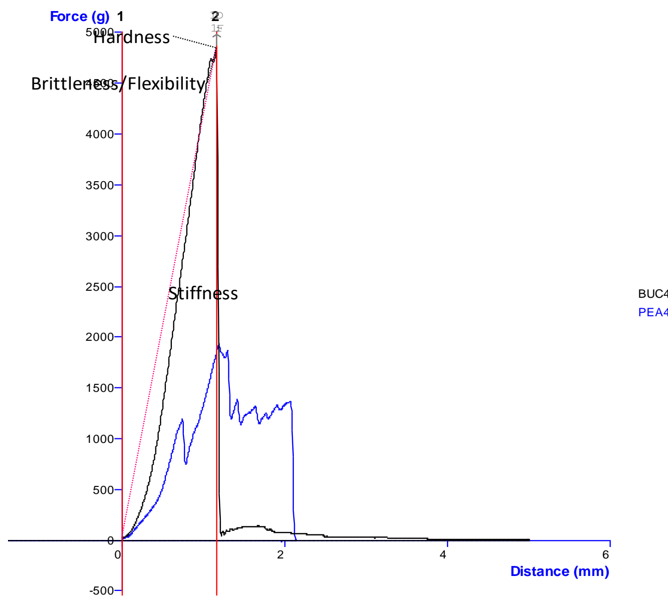

Figure 2 Comparison of typical plots for puffed breads from pea and buckwheat.

Table 1 Results of 3 Point Bend for puffed breads from pea.

Test ID	Hardness Force 1 (g)	Brittleness/ Flexibility Distance 1 (mm)	Toughness Gradient F-D 1:2	Thickness* (mm)
PEA1	2334	1.61	1431	8.11
PEA2	3505	0.96	3627	8.99
PEA3	2773	1.29	2134	7.78
PEA4	1944	1.19	1610	8.00
PEA5	2779	0.98	2798	8.24
PEA6	3166	1.89	1658	9.00
PEA7	4124	1.25	3289	7.39
PEA8	2539	0.72	3496	8.40
PEA9	3104	1.46	2105	7.60
PEA10	3045	1.83	1646	8.02
End Batch				
Average:	**2931**	**1.32**	**2379**	**8.20**
S.D.	581	0.36	805	0.53
C.V.	20	28	34	7

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Table 2 Results of 3 Point Bend for puffed breads from buckwheat.

Test ID	Hardness Force 1 (g)	Brittleness/ Flexibility Distance 1 (mm)	Toughness Gradient F-D 1:2	Thickness* (mm)
BUC1	4280	0.96	4413	8.63
BUC2	4517	0.96	4659	8.84
BUC3	3499	1.08	3199	8.83
BUC4	4856	1.17	4134	8.17
BUC5	4563	0.87	5219	8.61
BUC6	4278	1.20	3540	8.35
BUC7	5268	1.44	3644	8.21
BUC8	4340	1.01	4259	8.54
BUC9	4023	1.47	2709	8.57
BUC10	4323	1.56	2746	8.95
End Batch				
Average:	**4395**	**1.17**	**3852**	**8.60**
S.D.	446	0.23	786	0.26
C.V.	10	20	20	3

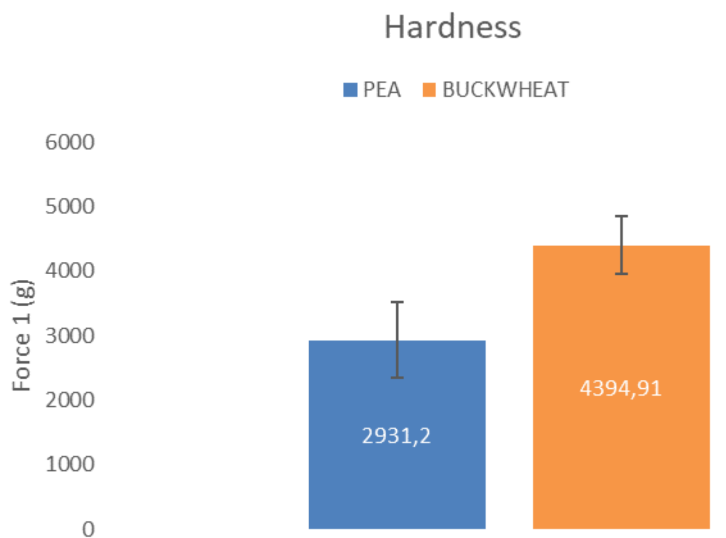

Figure 3 Comparison of hardness of puffed breads from pea and buckwheat

Table 3 Anova: Single Factor for determination of hardness of puffed breads

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	10	29311.95	2931.195	375691.9
Column 2	10	43949.06	4394.906	221510.9

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ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	10712249	1	10712249	35.87475	1.15E-05	4.413873
Within Groups	5374825	18	298601.4			
Total	16087075	19				

Results are statistically different (p-value < 0.05).

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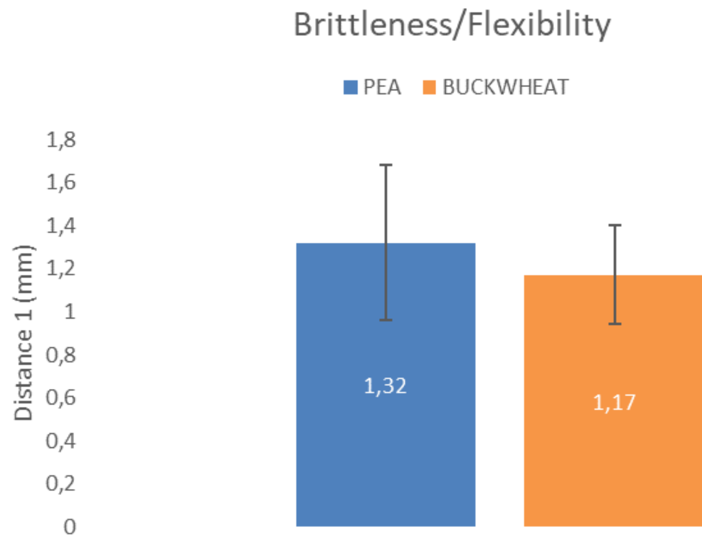

Figure 4 Comparison of brittleness/flexibility of puffed breads from pea and buckwheat

Table 4 Anova: Single Factor for determination of brittleness/flexibility of puffed breads

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	10	13.18	1.318	0.146284
Column 2	10	11.72	1.172	0.05864

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.10658	1	0.10658	1.040188	0.321291	4.413873
Within Groups	1.84432	18	0.102462			
Total	1.9509	19				

Results are not statistically different (p-value > 0.05).

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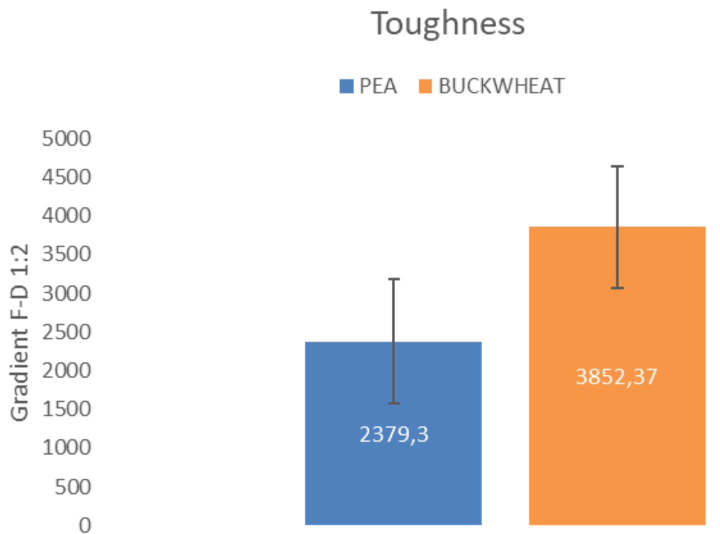

Figure 5 Comparison of toughness of puffed breads from pea and buckwheat

Table 5 Anova: Single Factor for determination of toughness of puffed breads

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	10	23793.01	2379.301	721112.4
Column 2	10	38523.65	3852.365	685984.5

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	10849588	1	10849588	15.42124	0.000988	4.413873
Within Groups	12663872	18	703548.5			
Total	23513460	19				

Results are statistically different (p-value < 0.05).

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Conclusion:

Puffed bread from buckwheat were characterized by higher value of hardness and toughness in band and snap test comparison to puffed bread from pea. Brittleness and flexibility were comparable for both types of samples. Different textural properties could be associated with the different thickness of samples (that was measured by calliper and it is presented in the last column* in the tables) and other mechanical properties affected by capacity for air retention and homogeneity of the extruded materials that was dependent to raw material used. Puffed breads from pea were characterized by bigger variability in thickness and homogeneity in comparison to buckwheat. In addition to better homogeneity of buckwheat puffed breads this products were distinguished by more delicate impression from more effective retaining of air during extrusion.

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REF: BIS4/3PB
Revised: Oct 08

Application Study for

TA.XTplus TA.HDplus TA.XTExpress

Product: **BISCUITS/COOKIES**

Objective: **Measurement of the hardness and resistance of biscuits/cookies to bend or snap**

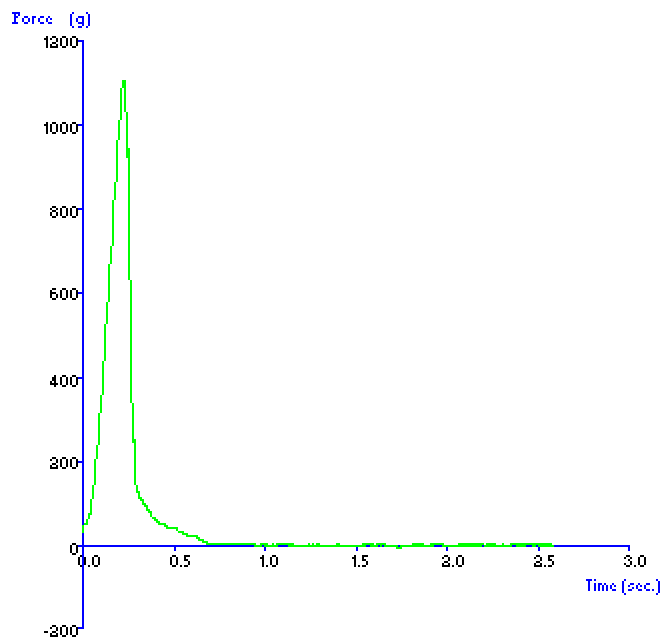
TA Settings:	*Mode:*	Measure Force in Compression
	Option:	Return To Start
	Pre-Test Speed:	1.0 mm/s
	Test Speed:	3.0 mm/s
	Post-Test Speed:	10.0 mm/s
	Distance:	5mm
	Trigger Force:	Auto - 50g
	Tare Mode:	Auto
	Data Acquisition Rate:	500pps
	LOAD PROJECT	

Accessory: 3-Point Bending Rig (HDP/3PB) using 5kg load cell
Heavy Duty Platform (HDP/90)

Test Set-Up:

The two adjustable supports of the rig base plate are placed a suitable distance apart so as to support the sample e.g. 40mm. For comparison purposes this gap distance should be noted and kept constant. The base plate is then secured onto the Heavy Duty Platform. The Heavy Duty Platform is manoeuvred and locked in a position that enables the upper blade to be equidistant from the two lower supports. The sample is removed from its place of storage and is placed centrally over the supports just prior to testing.

Typical plot:



The above curve was produced from a plain dough biscuit, tested at 20C.

Observations:
Once the trigger force is attained the force is seen to increase until such time as the biscuit/cookie fractures and falls into two pieces. This is observed as the maximum force and can be referred to as the 'hardness' of the sample. The distance at the point of break is the resistance of the sample to bend and so relates to the 'fracturability' of the sample i.e. a sample that breaks at a very short distance has a high fracturability.

Data Analysis:
Once tests have been performed, values of particular interest for sample analysis can be automatically obtained by a MACRO, e.g.

Clear Graph Results	
Redraw	
Search Forwards	
Go to Min. Time	
Go to Abs. +ve Value	Force
Mark Value	Force
Mark Value	Distance

This macro is a general example for the analysis of a curve such as the one above. Any changes made to the test parameters or significant differences to the shape of the curve profile may require optimisation of this macro.
The macro may also include analytical features which are not present in all versions of Stable Micro Systems software. The above macro is supported by:

Sample Results:
Test results obtained from 10 plain dough biscuit samples give, the following typical mean maximum force value, the mean distance compressed before breaking value:

Mean Max. Force *'Hardness'* (+/- S.D.)	Mean Distance at Break *'Fracturability'*

(g)	(+/- S.D.) (mm)
1150.3 +/- 130.4	0.68 +/- 0.7

Notes:

The Noise emitted during this test can be measured and analysed using an Acoustic Envelope Detector.

In some varieties of biscuits/cookies the uppermost surface may be quite variable due to inclusions such as chocolate chips. The trigger force may need to be increased slightly to avoid early triggering.

When comparing biscuits/cookies consideration should be taken that the diameters of the samples and the distance between the supports are identical. A sample of larger diameter (and hence larger contact area) will require more force to fracture. Similarly a larger force would be required to fracture the samples if the lower support blades were moved closer together.

If the sample has a pattern/writing on the surface it should always be orientated in the same direction for each test.

Inclusions may also interfere with the fracturability and indeed may serve to strengthen the structure of the biscuit/cookie if in the line of the blade. Also, the structure of the cookie i.e. the presence of large air pockets may cause large fluctuations in the force. It is for these reasons that the variation of test results may appear to be quite high.

Storage, packaging and handling of the sample before testing are considered variable conditions under which the biscuits are tested. These conditions should be identified and kept constant for comparison purposes.

When attempting to optimise test settings it is suggested that the first tests are performed on the hardest samples to anticipate the maximum testing range required and ensure that the force capacity allows testing of all future samples.

N.B. This application study has been designed for a specific sample(s) and it therefore must be noted that any deviation from this sample in terms of sample size, shape, formulation etc. may cause large deviations or indeed may require a different testing method.

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Stable Micro Systems Ltd, Vienna Court, Lammas Road, Godalming, Surrey, UK. GU7 1YL
Tel: +44 1483 427345. Fax: +44 1483 427600
http://www.stablemicrosystems.com
Support: app.support@stablemicrosystems.com

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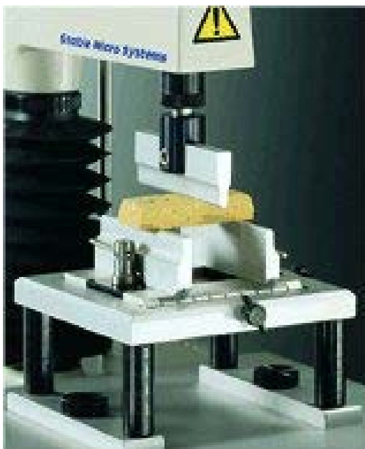
HDP/3PB Three Point Bending Rig

Breaking strength/stress of rigid samples can be determined by performing a three-point bend test. This fixture supports the sample across a span of known distance. A force is applied to the centre of the sample (which is also central to the supports) and the breaking stress is determined. Such a test may, for example, be used to assess the effect of different ingredients used, or to examine typical finished product problems such as moisture uptake.
The breaking strength (force per unit width) or breaking stress (force per unit area) of the sample is taken as the maximum strength or stress value of the curve. Other textural characteristics that may be of interest are the distance to break and the gradient of the slope during application of force. The distance to break gives an indication of the brittleness of the sample as this shows how far a sample can be deformed before fracture. The gradient of the slope indicates sample toughness; the higher the gradient, the tougher the sample.

This three point bend rig, which provides a variable support length up to 70 mm and sample width up to 80mm, is located on the heavy duty platform. Typical applications include measuring the break strength of bread sticks, biscuits and chocolate bars.



Manufacturing Material: Aluminium & Delrin
Maximum Operating Temperature: 100C
Maximum Applied Load (Compression): 100kg*
Maximum Applied Load (Tension): N/A

View Video Clip

Load Typical Project for this Fixture

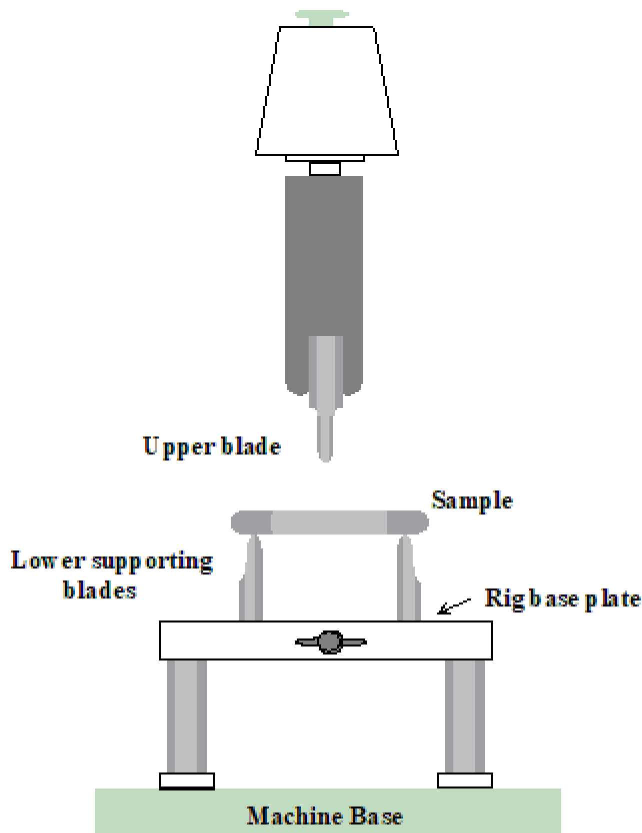
*Applies to the mode of loading as described in the set-up instructions.

LIMITATIONS:
Supports 5mm wide. Recommended minimum test distance between supports 10mm. Support length 90mm

TEST SET-UP:

Typical Set-up Instructions:

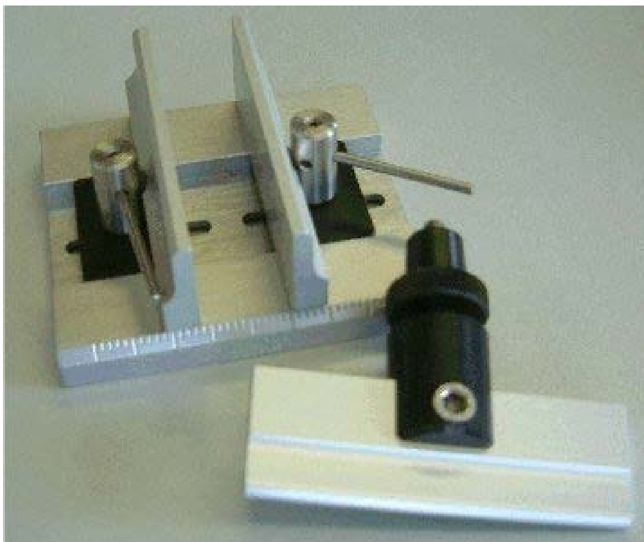
Screw the flexure attachment into the load cell carrier. Locate the base into the heavy duty platform and loosely attach this to the instrument bed using



its fixing screws. Secure the base position onto the heavy duty platform by tightening the two plate securing screws. Position the two adjustable supports a suitable distance apart so as to support the sample and lock at this position. Manoeuvre the heavy duty platform and secure tightly in a position that enables the upper blade to be equidistant from the two lower supports.

Note: For comparison purposes the support gap should be noted and kept constant.

CONTENTS:



1 - Base with two adjustable supports
1 - Lower Adjustable Support

Reference: **Stable Micro Systems Application Studies**

- Measurement of the hardness and resistance of biscuits/cookies to bend or snap
- Measurement of the hardness and fracturability of pretzel sticks
- Comparison of breaking stress/strength of 3 types of dry lasagne using a three-point bend test
- ISO 5628 - Determination of bending stiffness of paper and board

Reference: **Google Scholar Search**

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Colour of puffed breads

Blanka Tobolková*[1]

[1]National Agricultural and Food Centre, Food Research Institute, Department of Chemistry and Food Analysis, Priemyselná 4, 824 75 Bratislava, Slovakia

**blanka.tobolkova@nppc.sk*

Colour is an important attribute to the food industry. Traditionally, the colour is evaluated visually. However, this method is subjective as colour perception differs among individuals. On the other hand, spectral analysis plays dominant role in quantifying colour. In general, the colour of an object can be described by several colour system, e. g. by Hunter Lab, Commission Internationale de l'Eclairage's CIE L*a*b*, CIE XYZ or CIE L*u*v*, which differ in the symmetry of the colour space [1].
Colour of cereal products is usually measured by reflectance spectroscopy using the CIE L*a*b* colour space. This system classifies colour in three dimensions: L* - lightness (brightness), a*- redness/greenness (+a*/-a*), and b* - yellowness/blueness (+b*/-b*). Chroma (C*) and hue angle (H*) represent quantitative and qualitative parameters of colour derived from a* and b* values [1-3].

Fig.1. Homogenized puffed breads prepared from various cereals and pulses (rye, buckwheat, red lentil, green and yellow pea, spelt, corn and quinoa)

Reflectance spectra of homogenized puffed breads (Fig. 1) were recorded using a UV-3600 spectrophotometer (Shimadzu) with Large Integrating Sphere Assembly LISR 3100 (Shimadzu) using a 1 cm quartz cuvette QS in spectral range 200 nm - 800 nm with 2 nm intervals and slit width 12 nm.
Colour values and other spectral characteristics in CIE L*a*b* were calculated from reflectance spectra using Panorama 3.1.16 advanced ColorLite (Labcognition). The standard Illuminant D65 (representing average daylight) and 10° standard observer (angle of perception of a human observer) and reflectance spectral data from 380 nm - 780 nm interval were used in these calculations. Besides basic L*, a*, b*, C* and H* values, other spectral characteristics such as yellowness index (YI, quantifying product degradation by light, processing, or chemical exposure), whiteness (WI, indicating the extent of discoloration during processing) [1], tinting index (TI, describing the amount of greenish or reddish

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tint in the almost white product), Z% brightness (measuring brightness of white materials that tend to get yellowish with age or degradation) [4], were also evaluated.

Table 1. Comparison of selected colour and spectral characteristics of puffed breads

Calibri	L*	a*	b*	C*	H*	YI	WI	TI	Z% Brightness	BI
Buckwheat	73.91	4.93	14.81	15.61	71.58	41.64	69.60	-17.44	31.33	27.05
Corn	80.09	4.25	24.76	25.12	80.27	54.90	67.94	-19.54	31.79	40.38
Lentil	76.33	5.70	25.38	26.01	77.34	58.84	64.83	-23.97	27.23	45.38
Pea	74.61	4.66	24.57	25.01	79.26	57.43	64.36	-21.64	25.93	43.99
Rye	73.04	3.91	17.18	17.62	77.18	45.19	67.79	-16.55	28.80	30.46
Spelt	71.67	3.96	15.29	15.79	75.49	42.36	67.56	-15.96	28.50	27.81

L* - lightness, a* - redness, b* - yellowness, C* - chroma, H* - hue angle, YI – yellowness index, WI – whiteness index, BI – Browning index

The colour of the final bread is influenced by the colour of the ingredients and the manufacturing process. As can be seen from the results (Table 1), significant differences ($p < 0.05$) in the colour of the puffed breads were confirmed. Corn breads were characterised by the highest values of lightness (L*), while the highest a* and b* values were found in red lentil breads. Lentil breads also had the highest yellowness index (YI), tinting index (TI) and browning index (BI), which measures the intensity of brown colour in processed foods.

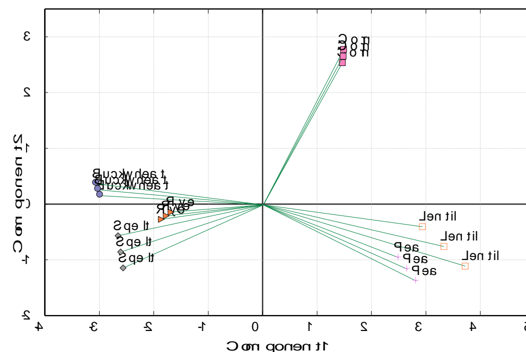

Fig.2. Discrimination of puffed breads by principal component analysis (selected colour and spectral characteristics were used as variables)

Many strong correlations were found between the monitored colour/spectral descriptors of puffed breads, as most of them are mathematically derived from the basic colour tristimulus values. When principal component analysis was applied to the data set of colour characteristics, a clear differentiation of breads was achieved (Fig. 2). The parameters b*, YI, BI, L* and Z% brightness were the most important for discrimination.

Acknowledgments: This work was supported by the COST Action 21149 ACRYRED.

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PROCEDURE FOR COLOR EVALUATION OF PUFFED BREADS

Blanka Tobolková[1*]

Blanka.tobolkova@nppc.sk

Homogenized puffed breads prepared from various cereals and pulses (rye, buckwheat, red lentil, green and yellow pea, spelt, corn+quinoa). Homogenization was performed using the knife mill Grindomix GM200 (RETSCH) – 5 000 rpm, 20 s.

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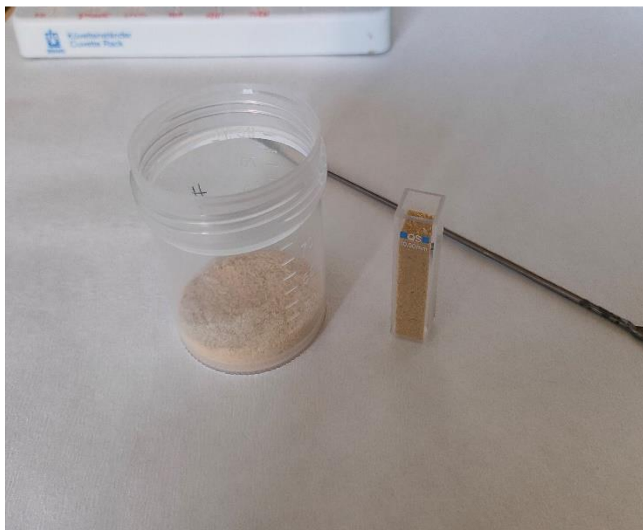

Samples are transferred to the quartz cell (Suprasil 100-QS, Hellma, optical length 10 mm).

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Spectra measurements by UV-VIS-NIR UV3600 Shimadzu

Wavelength range (nm): 200-800 nm
Scan speed: Fast
Sampling interval (nm): 1
Measuring mode: Reflectance
Slit width (nm): 12
Detector unit: External

Open UVProbe program - Spectrum mode and set measurement parameters (wavelength range in nm, scan speed, sampling interval).

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Set Instrument parameters (measuring mode – reflectance, absorbance, transmittance; slit width in nm, and detector unit (for reflectance measurement External(2Detectors)!!!).

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Connect instrument and perform baseline correction using standard white plate ($BaSO_4$).

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For reflectance measurements, place the sample in the holder of the special integrating sphere attachments.

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Perform the measuring.

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The measured spectrum must be saved as a UVPC spectrum file (*.spc).

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Color evaluation using Panorama 3.1 advanced ColorLite

Open Panorama 3.1. advanced ColorLite.

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Open the measured spectra.

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Select Colorimetric Analysis and set parameters for color evaluation – Standard Observer (Degrees 2 and **Degrees 10**) and Illuminant (Illuminant A, C, D55**, D65**, D75, F2, CWF,…) and range of wavelengths (380 – 780 nm = VIS region).

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Colorimetric Analysis Result for: Buckwheat_a (Circle)
Observer: Degrees10
Illuminant: IlluminantD65
Calculate Complementary Values: False
Wavelength Range: 380-780 [nm]

Name	X	Y	Z
Chromaticity Values	0,3544	0,3599	0,2857
Tristimulus Values	45,6383	46,3425	36,7817
Perfect Reflecting Diffuser	94,8100	100,0000	107,3400
Reflecting Diffuser	0,3127	0,3290	0,3583

Name	L*	a(u)*	b(v)*	Chromaticity	Hue Angle
L*a*b* Color	73,7673	4,9300	14,8166	15,6153	71,5958
L*u*v* Color	73,7673	15,9555	19,6721	25,3293	50,9555
L*a*b* Hunter	68,0753	4,5354	11,8400	12,6790	69,0402

Name	Value
Excitation Purity	0,2027
Complementary Purity	0,2712
Yellowness Index	41,7074
Whiteness	-39,5892
Tinting Index	-17,4746
Whiteness-Blue-Reflectance	31,1541
Dominant Wavelength	576,5449
X-Dominant Wavelength	0,5186
Y-Dominant Wavelength	0,4814
Complementary Wavelength	0,0000
Normalization Factor	0,0430

Final report of colorimetric analysis visualizes samples analyzed in different color systems and contains 36 parameters that could be used for color evaluation

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Colorimetric Analysis Result for: Buckwheat_a (Circle)
Observer: Degrees10
Illuminant: IlluminantD65
Calculate Complementary Values: False
Wavelength Range: 380-780 [nm]

Name	X	Y	Z
Chromaticity Values	0,3544	0,3599	0,2857
Tristimulus Values	45,6383	46,3425	36,7817
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L*u*v* Color	73,7673	15,9555	19,6721	25,3293	50,9555
L*a*b* Hunter	68,0753	4,5354	11,8400	12,6790	69,0402

Name	Value
Excitation Purity	0,2027
Complementary Purity	0,2712
Yellowness Index	41,7074
Whiteness	-39,5892
Tinting Index	-17,4746
Whiteness-Blue-Reflectance	31,1541
Dominant Wavelength	576,5449
X-Dominant Wavelength	0,5186
Y-Dominant Wavelength	0,4814
Complementary Wavelength	0,0000
Normalization Factor	0,0430

Colorimetric Analysis Result for: Corn_A (Diamond)
Observer: Degrees10
Illuminant: IlluminantD65
Calculate Complementary Values: False
Wavelength Range: 380-780 [nm]

Name	X	Y	Z
Chromaticity Values	0,3705	0,3793	0,2502
Tristimulus Values	55,0729	56,3753	37,1901
Perfect Reflecting Diffuser	94,8100	100,0000	107,3400
Reflecting Diffuser	0,3127	0,3290	0,3583

Name	L*	a(u)*	b(v)*	Chromaticity	Hue Angle
L*a*b* Color	79,8269	4,1404	24,7482	25,0922	80,5023
L*u*v* Color	79,8269	20,5198	32,8980	38,7729	58,0465
L*a*b* Hunter	75,0835	3,9248	19,3153	19,7100	78,5140

Name	Value
Excitation Purity	0,3017
Complementary Purity	0,3943
Yellowness Index	54,8888
Whiteness	-75,3506
Tinting Index	-19,3537
Whiteness-Blue-Reflectance	31,5001
Dominant Wavelength	574,1410
X-Dominant Wavelength	0,5043
Y-Dominant Wavelength	0,4957
Complementary Wavelength	0,0000
Normalization Factor	0,0430

Comparison of colorimetric reports of homogenized buckwheat and corn puffed breads.

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Whiteness index $WI_{JUDD} = 100 - [(100 - L^*)^2 + (a^*)^2 + (b^*)^2]^{1/2}$

Browning index $BI = 100 \times \frac{X - 0.31}{0.172}, \quad X = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*}$

Table 1. Comparison of color and spectral characteristics of puffed breads.

	*L**	*a(u)**	*b(v)**	*Chromaticity*	*Hue angle*	*Excitation Purity*	*Complementary Purity*	*Yellowness Index*	*Whiteness*	*Tinting Index*	*Whiteness-Blue-Reflectance[a]*	*Dominant Wavelength*	*Browning index*
Buckwheat	73.91	4.93	14.81	15.61	71.58	0.20	0.27	41.64	69.60	-17.44	31.33	576.55	27.05
Corn	80.09	4.25	24.76	25.12	80.27	0.30	0.39	54.90	67.94	-19.54	31.79	574.21	40.38
Lentil	76.33	5.70	25.38	26.01	77.34	0.32	0.41	58.84	64.83	-23.97	27.23	575.22	45.38
Pea	74.61	4.66	24.57	25.01	79.26	0.32	0.41	57.43	64.36	-21.64	25.93	574.58	43.99
Rye	73.04	3.91	17.18	17.62	77.18	0.23	0.31	45.19	67.79	-16.55	28.80	574.93	30.46
Spelt	71.67	3.96	15.29	15.79	75.49	0.21	0.28	42.36	67.56	-15.96	28.50	575.37	27.81

[a]Whiteness-blue-reflectance also called Z% Brightness

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Color and color evaluation

Blanka Tobolková*

**blanka.tobolkova@nppc.sk*

1 Color

When a person views an object, light from a light source that is reflected from the object (or passes through the object) enters the eye and is collected by several types of photoreceptor cells in the retina. The proportion of light collected by these cells is sent to the brain and sensed as color. In practice, the simple proportion of light collected undergoes various processing as it passes along the nerves before being recognized by the person as color. Color measurements are a method of expressing the colors sensed by humans as values. Color measurements are related to illumination, spectral characteristics of the object, and the spectral sensitivity characteristics of the human eye. As the spectral distribution of the illumination and the spectral sensitivity characteristics (color-matching function) of the eye are defined in the JIS standards, a color value can be calculated if the spectral reflection of the object is known. (If the light passes through the object, the spectral transmittance can be used for the calculation. However, the spectral reflectance is used in the explanations below.) To explain in more detail, in the JIS standard, the spectral distribution of the illumination and colormatching function are calculated using multiple conditions. We are familiar with a change in color when the illumination is changed. Therefore, a different coefficient is set for each illumination spectral distribution. In addition, the color also changes according to the viewfield (viewing angle), due to the relationship with the sensitivity distribution characteristics of the retina. Consequently, the JIS standard sets different colormatching functions according to the viewfield. Color measurements require a wavelength range from 380 nm to 780 nm, which is equivalent to the wavelengths that can be sensed by the human eye. Color measurements can be made by calculations based on spectral reflectance measurements by a UV-VIS spectrophotometer across this wavelength range. Color measurement software is available for simple color measurements.

2 Color Measurement

To perform color measurements with a UV-VIS spectrophotometer, first measure the spectral reflectance of the object. Calculations based on the spectral distribution of the illumination, the spectral reflectance obtained for the object, and the color-matching function express the color as a numeric value. Illumination spectral distributions and colormatching function values are stored in the color measurement software to obtain color measurement values when the spectral reflectance spectrum is measured. The XYZ tristimulus values are the basis of color measurement. JIS Z 8722 "Methods of color measurement -- Reflecting and transmitting objects" calculates the XYZ tristimulus values using the expressions below.

$$\left.\begin{aligned} X &= K\sum_{380}^{780} S(\lambda)\bar{x}(\lambda)R(\lambda)\Delta\lambda \\ Y &= K\sum_{380}^{780} S(\lambda)\bar{y}(\lambda)R(\lambda)\Delta\lambda \\ Z &= K\sum_{380}^{780} S(\lambda)\bar{z}(\lambda)R(\lambda)\Delta\lambda \\ K &= \frac{100}{\sum_{380}^{780} S(\lambda)\bar{y}(\lambda)\Delta\lambda} \end{aligned}\right\} \quad \cdots\cdots\cdots\cdots(1)$$

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Where,
S(λ): illumination spectral distribution value at wavelength λ
x(λ), y(λ),z(λ): color-matching function values in the XYZ color system
R(λ): sample spectral reflectance
Δλ: wavelength interval for calculation

In addition to the XYZ tristimulus values, several other color specification systems for expressing colors are known. The color measurement software can perform calculations in the following color specification systems: XYZ tristimulus values, xy color coordinates, Hunter Lab color scale, L*a*b* color system, L*u*v* color system, and U*V*W* color system. Values for color specification systems other than the XYZ tristimulus value system are calculated from the XYZ tristimulus values.

3 Setting Color Measurement Conditions

Several conditions are set for the color measurement calculations. These conditions are the illumination (light source) and viewfield (viewing angle/standard observer). Settings for the illumination are required, as the color varies according to the illumination on the sample. Illumination settings include A, B, C, and D65. In the JIS standards, these are called standard illuminant and supplementary standard illuminant. The spectral distribution is different for each illumination. For example, standard illuminant A is used to calculate object colors under illumination by an incandescent light bulb. Standard illuminant D65 is used to calculate object colors in daylight including the UV light region.

The viewfield (viewing angle) must also be set, as the color appears different when a sample is observed close-up or from a distance. For a viewfield up to 4 degrees, a 2° mean viewing angle is used for the calculations (color viewed from a distance); for a viewfield over 4 degrees, a 10° mean viewing angle is used for the calculations (color viewed close-up).

4 Color models

Two popular color models are used in this application. The color values are calculated from the reflectance spectra by different equations, that are not described in detail here.

One color model is the tristimulus model, which describes color as combination of three (abstract) color stimuli X, Y and Z. This model is based on the three kinds of color receptors in human eyes and how the mixture of stimuli is interpreted as color by our brain.

The CIELab model is based on a three-dimensional color space and was developed to represent color in a manner that is consistent with human perception of color. The axes of the three-dimensional coordinate system are based on the three stimuli bright – dark, red (+a*) – green (-a*) and blue (-b*) – yellow (+b*).

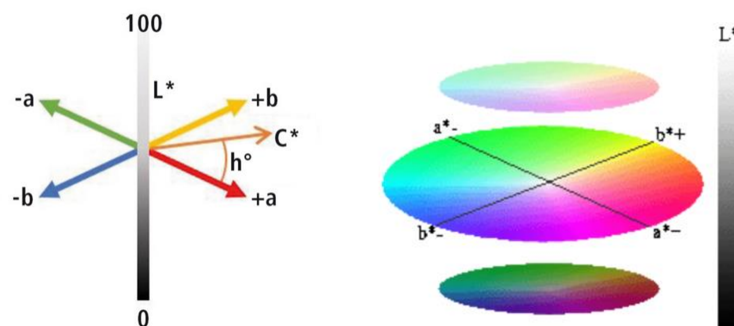

Fig. 1. CIELab color scheme illustrated as vectors

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Any point in the CIELab color space can be described by its lightness index L* and either in cartesian color coordinates a* and b* or in polar coordinates hue angle h° and chroma C*. Hue is the color as in the color circle and chroma/chromaticity is the intensity of that color.

Another colour/spectral parameters include:
Excitation purity - the excitation purity of any color possessing a dominant wavelength is an exactly defined ratio of the distances in the chromaticity diagram indicating how far the given color is displaced towards the spectrum color from the achromatic color.

Yellowness index - quantifying product degradation by light, processing, or chemical exposure

Whiteness index - indicating the extent of discoloration during processing

Tinting index - describing the amount of greenish or reddish tint in the almost white product

Whiteness-blue-reflectance (Z% brightness) - measuring brightness of white materials that tend to get yellowish with age or degradation

Dominant wavelength - defining principal wavelength of the colour

Just as our perception of colors depends on the lighting conditions, the color values in each model differ with the chosen reference light source and observation angle (see Section 3 Setting Color Measurements Conditions). This choice depends on the purpose of the color measurement, e.g. color fastness of products that are presented to customers under defined lighting conditions.

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Názov: Proceedings of Abstracts and Protocols of the Training School: Approach to the Assessment of Acrylamide Mitigation Measures in Cereal-based Food Processing

Editor: Zuzana Ciesarová

Vydavateľ: Národné poľnohospodárske a potravinárske centrum – Výskumný ústav potravinársky, Bratislava

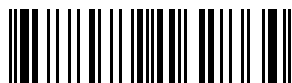
Vydanie: prvé

Rok vydania: 2023

Forma vydania: e-dokument online - PDF

Neprešlo redakčnou úpravou

ISBN: 978-80-89162-86-4



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