Challenge
Quick, automated, and reliable determination of total inorganically (TIX) and total organically bound (TOX) chlorine in different types of palm oil

Solution
Water extraction prior to measurements, matrix- and time-optimized combustion elemental analysis with highly sensitive coulometric titration

Intended audience
Quality control labs in palm oil mills and refineries; importers, exporters, and further processors of palm oil; independent oil control labs and authorities

Determination of Organically (TOX) and Inorganically Bound (TIX) Chlorine in Palm Oil by Means of Combustion Elemental Analysis and Coulometric Titration

Introduction

Palm oil and palm kernel oil rank first in world vegetable oil and fat production, accounting for more than 30% of global production volume. Seventy percent of the oil is used for cooking, frying and salad preparation, and as an additive in the food and cosmetics industries. Palm oil is found in many everyday products such as margarine, spreads, chocolates and dressings, but also in detergents and cosmetics. Crude palm oil has a characteristic taste of its own and a high content of colorants such as carotene, therefore it is usually refined. The mild, odorless and almost transparent refined palm oil is a versatile ingredient for many purposes with a long storage life. As with all edible oils, refining palm oil carries the risk of forming potentially harmful substances if the process steps are not closely monitored. This is particularly true for the formation of 2-MCPD and 3-MCPD fatty acid esters, to which palm oil is particularly

susceptible. 2-MCPD and 3-MCPD fatty acid esters have been detected in refined palm oil in various studies, and the mechanism for the formation of these contaminants has been studied in detail. The chlorine-containing compounds are formed when oils and fats in combination with organic and especially inorganic bound chlorine are exposed to high temperatures. This is the case in deodorization, when edible oils are exposed to temperatures up to 270 °C to remove highly volatile, undesirable odors and flavors. 3-Monochloropropane-1,2-diol fatty acid esters (3-MCPD) have gained particular attention in this context because they are suspected of increasing the risk of cancer. Studies in animals have shown that increased intake of this substance can lead to kidney and liver damage and the formation of benign tumors. The World Health Organization (WHO) has set a limit of 2.0 micrograms per kilogram of body weight

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as the maximum daily intake. Similarly, in Regulation (EC) 1881/2006, the European Commission sets limits for the content of 3-MCPD in various foods, including edible oils. Suppliers of edible oils must therefore test their final products for 3-MCPD.

To comply with this, permanent monitoring of these specific compounds in the final products is required. For this, dedicated chromatographic methods are established. They are however often time consuming due to the necessary sample treatment and method optimization, and require skilled lab technicians. But what about monitoring the entire production process, were actions must be taken to prevent the formation of 3-MCPD quickly? Here other techniques, that can detect all Cl compounds which can contribute to the formation of 3-MCPD are more suitable tools. Combustion elemental analysis is becoming increasingly popular for this task. It is a well-established method for total chlorine (TX) determination and described in detail by a multitude of well-approved norms like ASTM D4929-B, ASTM D5808, UOP 779, just to name few. What works fine for mineral oil and

its derived products can easily be adapted to palm oil, which has comparable matrix properties.Besides the total chlorine content, it can be helpful in some cases to have additional information about the nature of Cl contamination. Knowing exactly whether the contamination is of organic or inorganic origin helps to identify possible sources of contamination, allows for better assessment of the risk potential of palm oil during processing, and to take appropriate countermeasures. This application note provides insight into how to use elemental analysis to quantify total organically (TOX) and total inorganically (TIX) bound chlorine. The technique is low cost and combines efficient high-temperature combustion with highly sensitive coulometric detection to provide accurate determination of total chlorine parameters down to the lower ppb range. The total content of both species can be determined directly, and the differentiation into organically (TOX) and inorganically (TIX) bound species is performed after a washing step of the original sample.

Materials and Methods

Samples and reagents

- 2 different edible oil samples
- Kit calibration solutions, c: 0.1 – 10 mg/L Cl, Analytik Jena GmbH+Co. KG, 402-889.071
- Ultra-pure water (prepared by water purification system)

Sample preparation

The standards and the pure palm oils for TX determination have been analyzed directly without sample pretreatment. For the separate determination of the parameters TIX and TOX, the palm oil samples require pretreatment. For this purpose, extraction with warm water, often referred to as „water wash", was performed. This step can be performed manually using a separating funnel or automated using a suitable „shaking" system. General laboratory equipment such as rotary mixers, shakers or centrifuges are often used for this purpose. For the experiments described in this work, the manual method described in Table 1 was used.[4]

Table 1: Sample preparation – process steps of water extraction by separatory funnel

Step	Details
1 – Preparation the sample/water mix	Inhomogeneous (multi-phasic) samples (in closed vessels) are molten at approx. 60 °C in a water bath, while gently shaken. Afterwards one part of the so homogenized sample and one part of 60 °C warm distilled water was put together in a separatory funnel (e.g., 50 mL water/50 mL sample), which was closed afterwards.
2 – Extraction of the TIX	The separatory funnel was shaken for 1 minute vigorously and degassed periodically to prevent gas build-up.
3 – Separation of phases	After approx. 10 – 15 minutes waiting time the phases have separated. The aqueous phase was drained from the funnels bottom carefully, to avoid contamination by the organic phase. If necessary a filtration could be applied. The water extract and the organic phase were stored in suitable vessels until analysis (TIX resp. TOX determination).

Calibration

Prior to sample measurements, the multi EA 5100 was calibrated for chlorine determination using liquid standards based on 2,4,6 trichlorophenol (Cl) in isooctane in a concentration range of 0.1 to 10 mg/L Cl. For the calibration and subsequent verification measurements, the inje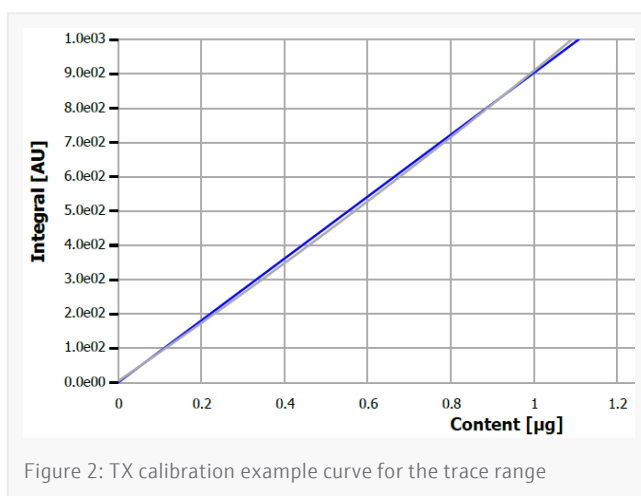ction volume was set to 100 µL. The obtained calibration was verified with certified reference standards. Calibration was performed according to the variable concentration, constant volume type. Calibration parameters were calculated by linear regression. A blank correction was applied to avoid false, underestimated analytical results due to blank effects. Figure 1 and Table 2 show the calibration curve for the trace range and the associated performance data.

Figure 2: TX calibration example curve for the trace range

Table 2: Performance parameters of TX calibration

Parameter	Value
Linearity	Linear
Range	5.5 – 914 AU
Correlation coefficient	1.0000
Limit of detection	19.94 µg/L

Instrumentation

A multi EA 5100 with HiPerSens chlorine detector was used in horizontal operation mode. For automated sample introduction and transfer to the furnace, the system was equipped with an automatic boat drive with flame sensor technology and the MMS-T, a temperature-controlled multi-matrix sampler. The MMS-T can be used in three modes: „cooled" for the automatic dosing of extremely volatile liquids, „heated" for the direct dosing of highly viscous homogeneous materials without dilution, and „standard", without temperature control under ambient conditions like a classic liquid sampler. For samples such as palm oil, the heated mode is recommended at a moderate temperature, e.g. 70 ℃. This allows analysis of both the organic and aqueous phases. For aqueous extracts, this temperature is not critical. For pure palm oil and organic extraction phase, the dilution step is unnecessary. This saves time and provides better results, especially in the ppb range.

For the determination of chlorine, an injection volume of 100 µL was used for samples and standards. Sample digestion was performed by efficient catalyst-free high-temperature combustion in a quartz tube. This process is fully automatically controlled by the flame sensor and adapted to the specific needs of each matrix component, ensuring matrix-independent, optimal results in the shortest possible time. The process is divided into two phases. In the first process phase, the volatile sample components are vaporized in an inert gas stream, followed by combustion of the gaseous products formed in an oxygen-rich atmosphere. In the second phase, the heavier, non-volatile sample components and the pyrolysis products formed are quantitatively oxidized in pure oxygen.

The flame sensor ensures uniform evaporation of exactly the amount of sample that can be completely oxidized by the amount of oxygen present. For this purpose, the combustion flame is monitored in real time. Based on the registered flame values, an optimal digestion program is calculated for each matrix, which includes the right number of waiting points, minimized waiting times and a maximum analysis speed. This allows fully automatic adaptation of the process to the specific requirements of the individual components, thus optimizing the quality of the analysis and its duration. The reaction gases formed are then dried and transferred to the titration cell, where the HX formed is absorbed into the cell electrolyte. The use of a high-performance dryer makes it possible to analyze even large volumes of pure water samples without a decrease in HX transfer, loss of HX or memory effects.

Chlorine is quantified by microcoulometric titration using the „high sensitive" cell. A built-in auto protection system and heated transfer lines guarantee highest operational safety (particle and aerosol trap) and a complete transfer (no condensation loss) of the formed HX gas. This allows a highly sensitive analysis with a detection limit of only 10 ng Cl absolute.

Instrument settings

Method parameters

A standard library method was used for all analyses. The process parameters are summarized in Table 3. The injection volume was set at 100 µL. A temperature-controlled syringe was used for direct injection of pure samples. Both the syringe and the sample (sample tray) were kept at a constant temperature of 70 °C throughout the analysis time.

Table 3: Process parameters multi EA 5100 – horizontal operation

Parameter	Setting
Furnace temperature	1050 °C
2nd combustion	60 s
Ar flow (1st phase)	200 mL/min
O_2 main flow	200 mL/min
O_2 flow (2nd phase)	200 mL/min
Draw up speed	1.0 µL/s
Injection speed	2.0 µL/s
ABD mode	automatic*

* flame sensor controlled automatically optimized combustion

Evaluation parameters

The evaluation parameters for the detection of chlorine by micro-coulometric titration are summarized in Table 4.

Table 4: Detection parameters for Cl ("high sensitive" option)

Parameter	Setting
Max. integration time	1200 s
Cell temperature	22 °C
Titration delay	1
Threshold	300 cts
Threshold value	25 cts
Baseline/drift correction	automatic

Results and Discussion

The results obtained for the washed samples (organic phase), water extracts (aqueous phase), blank and standard solutions, are summarized in Table 5. They are average values of two replicate analysis.

Table 5: Results of TIX, TOX and TX determination for palm oil samples and standard measurements

Sample	TOX ± SD	TIX ± SD	TX ± SD
Palm oil 01 – A	649 ± 7.00 µg/L	493 ± 1.00 µg/L	1.23 ± 0.01 mg/L
Palm oil 01 – B	676 ± 18.1 µg/L	471 ± 9.30 µg/L	
Palm oil 02 – A	5.75 ± 0.10 mg/L	534 ± 4.23 µg/L	6.10 ± 0.1 mg/L
Palm oil 02 – B	5.82 ± 0.03 mg/L	483 ± 8.72 µg/L	
Blank solution			16.1 ± 0.81* µg/L
1.00 mg/L Cl			1.00 ± 0.04 mg/L

*below detection limit

Both phases, the organic and the aqueous, could be analyzed without any problems. Their very different viscosities had no influence on sample dosing and analysis quality. No carryover or memory effects were observed, as confirmed by the low standard deviation of less than 3% for replicate analyses, regardless of the measured concentration. Repeated experiments have demonstrated the high reproducibility. This is comparable to the quality of the analysis of the standard solution. In addition to TIX and TOX, the TX value was also measured to evaluate the quality of the separation step. The measured and calculated TX values are summarized and compared in Table 6.

Table 6: Comparison of calculated and measured TX values of palm oil samples

Sample	TX ± SD	TX$_{calculated}$ ± SD	Delta
Palm oil 01 – A	1.23 ± 0.01 mg/L	1.14 ± 0.02 mg/L	-7.3%
Palm oil 01 – B	1.23 ± 0.01 mg/L	1.15 ± 0.05 mg/L	-6.5%
Palm oil 02 – A	6.10 ± 0.1 mg/L	6.28 ± 0.02 mg/L	3.0%
Palm oil 02 – B	6.10 ± 0.1 mg/L	6.30 ± 0.05 mg/L	3.3%

Taking into account the deviations of the TIX and TOX determinations, the calculated TX values and the directly measured TX values are within the acceptable range. All parameters have been determined fully automated together in a single analysis cycle by combustion method. Besides this, the direct measurement of TIX from the aqueous phase is another method. This allows the direct injection of the sample into the titration cell, saving valuable time, since no combustion and accumulation of the gaseous HX happens. Direct determination of TIX by the so-called cell direct method is also advantageous in a rare case that water soluble halogen compounds are present in the oil sample. The coulometer cell detects only ionic chlorine, this way the organically bound chlorine cannot falsify the results.This methodology can be easily adapted to other edible oils and comparable oil matrices (e.g., UCO, tall oil, or cod liver oil, glycerol).

In the following typical measurement curves for TIX, TOX and TX analysis and standard solutions are shown.
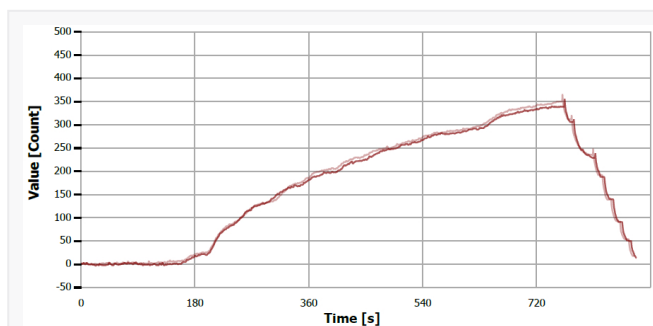

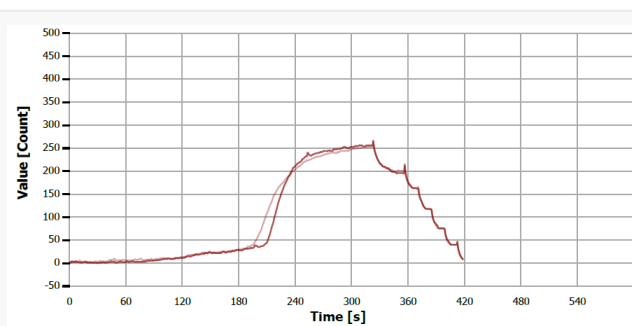Figure 2: TOX analysis curve for sample "palm oil 01-A"

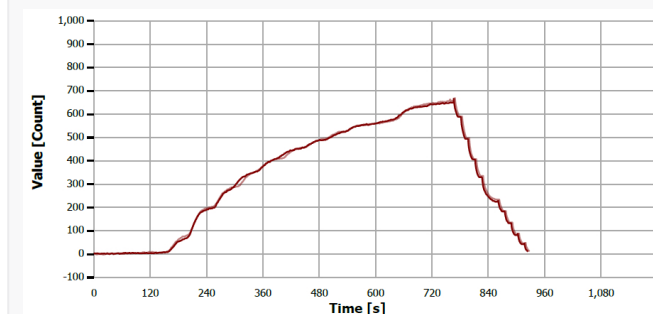Figure 3: TIX analysis curve for sample "palm oil 01-A"

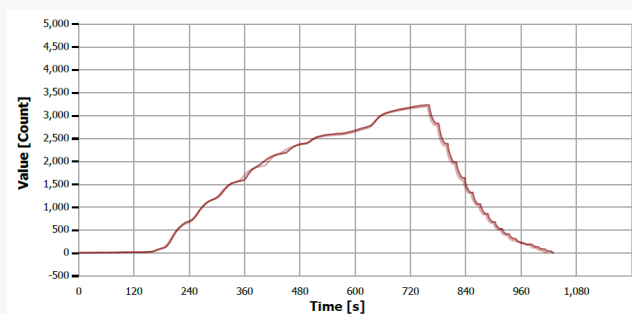Figure 4: TX analysis curve for sample "palm oil 01-A"

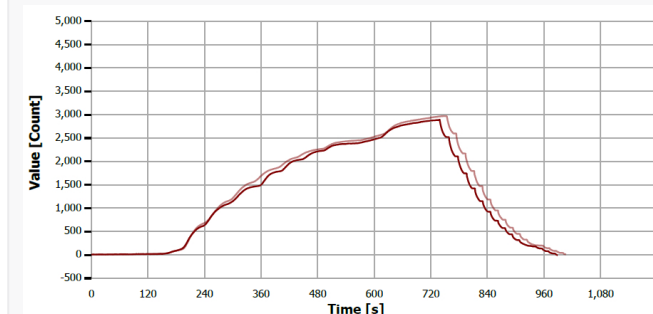Figure 5: TX analysis curve for sample "palm oil 02-A"

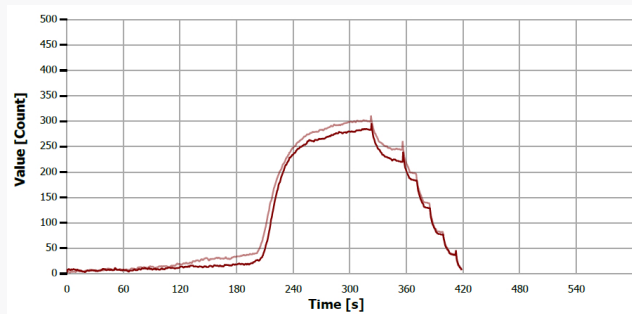Figure 6: TOX analysis curve for sample "palm oil 02-A"

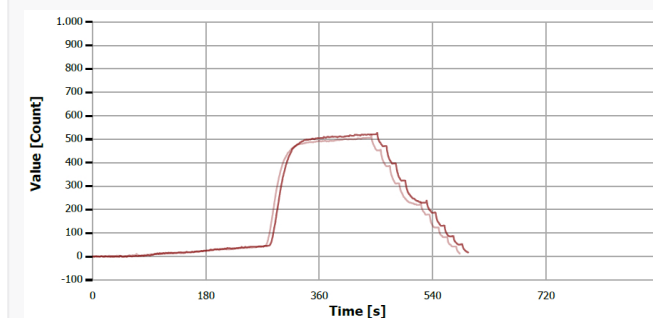Figure 7: TIX analysis curve for sample "palm oil 02-A"

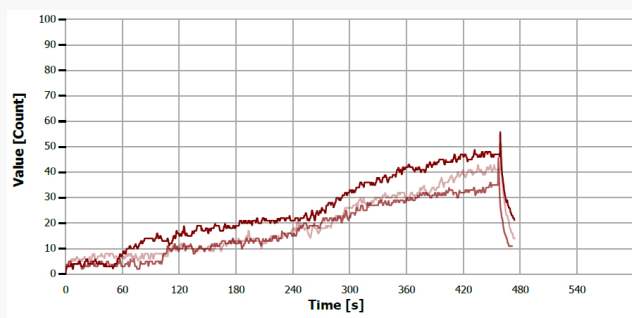Figure 8: TX analysis curve for standard solution "1.00 mg/L Cl"

Figure 9: TX analysis curve for "blank solution"

Summary

Besides the classical determination of total chlorine (TX), combustion-based elemental analysis can also be used to distinguish between organically and inorganically bound chlorine species. For the separate determination of the parameters TIX and TOX, only a single and easy-to-handle sample preparation step is required: by washing with distilled water prior to analysis, the inorganic chlorine compounds dissolved in the palm oil can be extracted quickly and easily. Both the washed organic sample and the resulting water extract can be analyzed with the same analyzer, ensuring the best comparability of measurement results. The multi EA 5100 masters these challenges perfectly thanks to highly sensitive coulometric titration and flame sensor-optimized combustion, so that multiple repeat analyses are not necessary even for contents in the lower trace range. In most cases, a twofold determination already provides reliable analytical results. In addition to the direct determination of the three parameters, there are other interesting aspects for time optimization, such as the use of the difference method to calculate one of the three parameters from the two already measured (e.g., TX - TOX = TIX).

Figure 18: multi EA 5100 with ABD and MMS autosampler

The flame sensor optimized digestion process ensures quantitative combustion of each sample component. Neither soot nor other undesirable pyrolysis products are generated. Supported by the auto protection system, results with excellent reproducibility can be achieved even for smallest chlorine quantities. If required, the application range of the analysis system can be easily extended by accessory modules for the determination of nitrogen, sulfur and/or carbon contents.

Overview of devices and consumables

Table 7: Overview of devices and accessories

Article	Article number	Description
multi EA 5100	450-300.011	multi EA 5100 – combustion elemental analyzer for sulfur, nitrogen, chlorine, and carbon analysis in solids, liquids and gases
Cl module	450-300.023	Extension of multi EA 5100 for chlorine determination with coulometric titration
Extension kit Cl high sensitive	450-300.024	Extension of multi EA 5100 Cl module, for the determination of very low chlorine contents
ABD	450-300.013	Automatic boat drive with flame sensor technology – horizontal operation of multi EA 5100
MMS-T	450-900.453	Temperature controllable autosampler
multiWin software	450-011.803	Operation and data evaluation software for multi EA 5100

References

[1] EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain); SCIENTIFIC OPINION ON THE RISKS FOR HUMAN HEALTH RELATED TO THE PRESENCE OF 3- AND 2-MONOCHLOROPROPANE- DIOL (MCPD), AND THEIR FATTY ACID ESTERS, AND GLYCIDYL FATTY ACID ESTERS IN FOOD., EFSA Journal, 2016, Vol. 14/5, 4426, 159

[2] Matthäus, Bertrand; ORGANIC OR NOT ORGANIC – THAT IS THE QUESTION, Eur. J. Lipid Sci. Technol. 2012, Vol. 114, page: 1333–1334

[3] Federal Institute for Risk Assessment (BfR); Possible health risks due to high concentrations of 3-MCPD and glycidyl fatty acid esters in certain foods, 2020, BfR Opinion No. 020/2020, page 4

[4] Analytik Jena GmbH; Fundamentals - Instrumentation and Techniques of Halogen Analysis, 2022, page 37-39