Determination of Quaternary Ammonium Compounds (QAC) in Food Products

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Abstract

An analytical method to determine QAC is introduced and validation data related to fresh fruits, vegetables and dairy products are published. Analysing several hundreds of samples, two major representatives of the QACs have been identified:

– Benzalkonium chloride ("BAC") and
– Didecyldimethylammonium chloride ("DDAC").

Results of these two QACs on several food products (different fruits, vegetables and dairy products) are presented and discussed related to different aspects (type of commodity, country of origin).

Introduction/Objectives

An important group of active substances in disinfectant formulations are the "Quaternary Ammonium Compounds", often named "QAC" or "Quats". QACs are positively charged polyatomic ions of the structure NR_4^+. They have antimicrobial activity. Certain quaternary ammonium compounds, especially those containing long alkyl chains, are therefore used as antimicrobials and disinfectants [1]. The increasing use of disinfectant formulations across the food chain is critically discussed as some pathogenic germs may build up resistance phenomena [2]. Considering the a. m. aspects, QACs should not be present in food products with relevant levels. Therefore, it was necessary to develop and establish a robust and reliable analytical method to analyse QACs in food products.

Methods

5 ml of water (saturated with NaCl) are added to 5 g of homogenised sample material while agitating for a short time. After adding 100 ml of extraction mixture (Acetonitrile/water 7:3 with 0.1 % Formic acid) the sample is extracted for 5 min by making use of a horizontal shaker. The extract is centrifuged at 2500 rpm for at least 10 min. 1 ml of the organic part of the liquid is transferred into a 1.5 ml vial and subsequently analysed by LC/MSMS.

HPLC: Agilent 1290 Infinity / MSMS: Agilent 6490, ESI pos. mode
Eluent A: H_2O (0.1 % Formic acid)
Eluent B: Acetonitrile (0.1 % Formic acid)
HPLC-column: Kinetex XB-C18 (100 A; 2.6 µm; 50 x 2.1mm)
Flow: 0.4 mL/min
Gradient: 0 min/0.5 %B → 0.3 min/0.5 %B → 4 min/100 %B → 7 min/100 %B

Compound	Precursor ion 1 [m/z]	Product ion 1 [m/z]	CE (eV)	Precursor ion 2 [m/z]	Product ion 2 [m/z]	CE (eV)
BAC-10	276	91	–26	276	184	–18
BAC-12	304	91	–26	304	212	–18
BAC-14	332	91	–28	332	240	–20
BAC-16	360	91	–28	360	268	–22
BAC-18	388	91	–28	388	296	–22
DDAC-10	326	186	–26	326	85	–26

Table 1: Ion transitions of different BAC compounds

Compound	Paprika (sweet pepper) Extraction efficiency (c = 0.1 mg/kg)	Milk Extraction efficiency (c = 0.1 mg/kg)	Reporting Limit (mg/kg)
BAC-10	87.4 %	112 %	0.01
BAC-12	88.2 %	108 %	0.01
BAC-14	76.0 %	85 %	0.01
BAC-16	79.1 %	72 %	0.01
BAC-18	82.0 %	80 %	0.01
DDAC-10	83.9 %	82 %	0.01

Table 2: Validation parameters of different BAC compounds

The validation parameters showed satisfying performances in terms of recoveries and reporting limits.

Results and Discussion

All in all 319 samples of fruits and vegetables were analysed for QAC. In 29 samples of this pool residues of QAC were detected (9 %). The following tables and figures summarise the significant positive results of QAC findings across Citrus and Exotic Fruit and Vegetable products: *See Table 3 and 4*.

Significant levels have been detected in several samples of citrus fruits as well as in Mangos and Passion Fruit. Vegetables are affected, too. The most dominant molecule is typically BAC-12 with the exception of the Passion Fruit sample, where BAC-14 shows the highest level.

As a conclusion, levels of QAC are present across several different fruits and vegetables at different concentration levels.

In a second step, different types of milk products were checked for QAC. All in all, 332 samples were analysed. The focus was put on dairy products. Different kinds of cheese products, ice cream, butter, curd and curd cheeses, yoghourt, milk, cream and Tsatsiki were analysed for QAC. 258 samples showed positive findings of QAC (78 %). Whereas DDAC residues were detected in a very limited number of samples (1 DDAC result in butter, 4 in milk, 1 in cream and 1 in Tsatsiki) BAC concentrations were positively detected in a significant number of samples (255 samples). The following figures highlight the most relevant results of BAC: *See Figure 1 and 2*.

Commodity Citrus and Exotic Fruits	Origin Country	DDAC (mg/kg)	BAC-10 (mg/kg)	BAC-12 (mg/kg)	BAC-14 (mg/kg)	BAC-16 (mg/kg)	sum BAC (mg/kg)	total QAC (mg/kg)
Clementines	Spain	0.010	0.001	0.002	0.004	0.007	0.014	0.024
Clementines	Spain	0.005	0.001	0.036	0.014	0.005	0.056	0.061
Clementines	Spain	0.004	0.001	0.012	0.006	0.003	0.022	0.026
Clementines	Spain	0.006	0.001	0.064	0.022	0.006	0.093	0.099
Clementines	Spain	0.006	—	0.003	0.003	0.001	0.007	0.013
Clementines	Spain	0.003	—	0.010	0.005	0.001	0.016	0.019
Clementines	Spain	0.002	—	0.010	0.004	0.001	0.015	0.017
Clementines	Spain	0.002	—	0.011	0.003	0.001	0.015	0.017
Clementines	Spain	0.033	—	0.011	0.004	0.001	0.016	0.049
Grapefruit	Spain	0.003	—	0.007	0.004	0.001	0.012	0.015
Grapefruit	Spain	0.004	—	0.005	0.003	0.001	0.009	0.013
Grapefruit	Spain	0.006	—	0.013	0.005	0.001	0.019	0.025
Grapefruit	Spain	0.003	—	0.006	0.003	0.001	0.010	0.013
Limettes	Mexico	0.018	—	0.116	0.039	0.004	0.159	0.177
Mandarines	Spain	0.008	—	0.019	0.008	0.004	0.031	0.039
Mangos	Spain	0.028	0.001	0.036	0.027	0.007	0.071	0.099
Mangos	Brazil	0.003	—	0.011	0.004	0.003	0.018	0.021
Melones	Brazil	0.047	0.001	0.098	0.040	0.008	0.147	0.194
Oranges	Spain	0.006	0.001	0.011	0.008	0.005	0.025	0.031
Oranges	Spain	0.003	—	0.008	0.006	0.001	0.015	0.018
Oranges	Spain	0.006	0.001	0.003	0.003	0.002	0.009	0.015
Oranges	Spain	0.005	0.001	0.015	0.008	0.004	0.028	0.033
Oranges	Spain	0.004	—	0.024	0.009	0.002	0.035	0.039
Passion Fruits	Columbia	0.009	—	0.067	0.628	0.175	0.870	0.879
Lemons	Spain	0.006	0.001	0.010	0.008	0.009	0.028	0.034
Lemons	Spain	0.002	—	0.005	0.002	0.001	0.008	0.010

Table 3: Significant QAC levels in Citrus and Exotic fruits

Commodity Vegetables	Origin Country	DDAC (mg/kg)	BAC-10 (mg/kg)	BAC-12 (mg/kg)	BAC-14 (mg/kg)	BAC-16 (mg/kg)	sum BAC (mg/kg)	total QAC (mg/kg)
Paprika	Spain	0.013	0.001	0.210	0.054	0.009	0.274	0.287
Lollo Bionda	Spain	—	—	0.220	0.120	—	0.340	0.340
Celery	Spain	—	—	0.030	0.018	—	0.048	0.050

Table 4: Significant QAC levels in Vegetables

At this level it becomes already clear that BAC levels in dairy products show significantly higher levels compared to fruits and vegetables. Levels significantly higher than 1 mg/kg BAC were detected (up to 17.9 mg/kg).

The levels found in Tsatsiki samples confirm the higher levels already found in other dairy products (see Figure 2). Concentration levels of BAC at 17 mg/kg were also detected. However, it has to be highlighted that the concentrations found in the 41 Tsatsiki samples vary between 0.01 mg/kg BAC up to 17 mg/kg. As a conclusion, the BAC contaminations deviate significantly across the analysed samples.

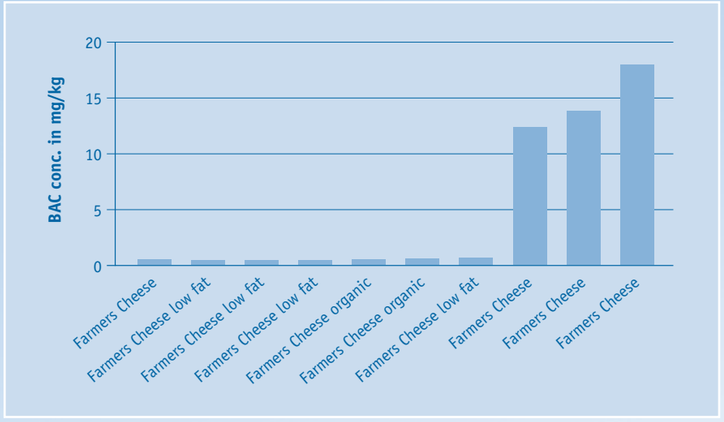

*Figure 1: Benzalkonium chloride (BAC) levels in **Farmer Cheese Products***

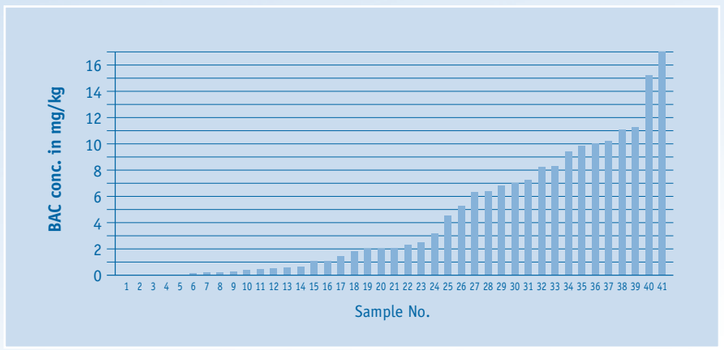

*Figure 2: Benzalkonium chloride (BAC) levels in **Tsatsiki samples***

Finally, the analysis of milk samples (109 in total) presented an average concentration level of 0.20 mg/kg BAC. The lowest level detected was at 0.02 mg/kg and the highest detected a 0.90 mg/kg BAC.

Conclusions

Quaternary Ammonium Compounds (QAC) show antimicrobial activities and thus are used in disinfectant formulations. Therefore, they are also used in the food industry in order to clean processing machines to ensure hygienic standards. However, residues of QAC can cause resistance phenomena for the consumer. The two major representatives of the QAC – namely Benzalkonium chloride ("BAC") and Didecyldimethylammonium chloride ("DDAC") – were monitored in different kinds of food products such as fruits, vegetables and animal products. A method applying the LC-MS/MS technique was developed and set up to provide reliable analytical results.

319 fruit and vegetable samples were analysed for QAC. 29 samples showed residues of QAC (9 %). The highest quantity of total-QAC detected was at 0.879 mg/kg in passion fruits.

Also 332 dairy products were checked for QAC and 78 % of them showed positive results (258 samples). In general, higher concentration levels of QAV were detected in dairy products (up to 17 mg/kg) and a strong presence of BAC (as opposed to DDAC) was monitored. A relationship between the degree of processing the dairy products and the concentration level of QAC was not identified. Whereas (raw) milk samples presented an average level of QAC of 0.20 mg/kg, yoghurts, Tsatsiki samples and Farmer Cheese samples showed levels across a wide concentration range: 0.01 mg/kg up to 17.9 mg/kg. The detected concentration levels also vary significantly within the selected food groups. Thus more research is necessary to investigate the QAC across the food chain.

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