APIS

Conference Abstract/Poster/Slides

High concentrations of Spermidine in Drone Milk (Apilarnil)

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ABSTRACT

Spermidine is a biogenic amine. Spermidine induces autophagy and may extend life span, reduce dementia and other common diseases. Therefore, it gained popularity as a food additive derived e.g. from wheat germs. Drone milk or Apilarnil (ApiDrohn®) is a high value hive product extracted from the male larvae and conserved by lyophilisation. Apilarnil is used in traditional apitherapy for versatile applications.

In this project the presence of Spermidine in Apilarnil (fresh/different-larva-stages/dry-techniques/vehicle-solutions) was identified and quantified. A workflow was set up comprising an extraction procedure from the freeze-dried Apilarnil (supplied in high purity within Api-Zentrum Ruhr) and a liquid chromatography coupled with a high-resolution time-of-flight mass spectrometer (LC-ESI-QTOF).

Apilarnil in different stages of larva developmental age contains Spermidine in higher concentrations as in wheat germs. Furthermore, Apilarnil contains other bioactive amines and polyamines besides Spermidine. This is the first time that spermidine and several other biogenic amines have been definitely proven to be present in a bee product.

The spermidine content is another column for explanation the various health applications for Apilarnil in apitherapy and is most directly related to its health benefits, although further research has to be done. One of the next tasks will be to understand the synergy between the presence of different bioactive substances like Spermidine and typical hive products e.g. flavonoids.

Keywords: Apilarnil, drone milk, Spermidine, biogenic amines, ApiDrohn®

INTRODUCTION

Drone milk or Apilarnil (ApiDrohn®) is a high value bee hive product extracted from the male larvae and conserved by lyophilisation. Apilarnil is used in traditional apitherapy for versatile applications, often around fertility of men and women. Besides this amine it contains besides other bioactive molecules hormones: Testosterone, Progesterone, Estradiol and Prolactin [1]. This explains partly the regulative function on e.g. women´s menopause symptoms or men's spermatogenesis. The application of Apilarnil shows also good results with animals [2]. In this case the quality of sperm and the number of offspring for stud rams were studied. Mixtures with honey and other bee products like propolis and royal jelly had been traditionally used to strengthen very weak and ill patients. The recipes vary greatly although it seems that Apilarnil is a crucial component for the efficacy.

The research on biogenic amine like Spermidine has recently shown a number of interesting health effects. In the blood of 90 to 100-year old people high concentrations of Spermidine were found [3]. Showing that the surviving fraction of elderly people has a significant difference in metabolism. Spermidine induces autophagy, immune modulation, cardioprotection, neuroprotection, tumour suppression and may extend life span, reduce dementia and other common diseases. Therefore, it gained popularity as a food additive derived e.g. from wheat germs.

As other germ cells (as sperm or wheat germs) contain a lot of Spermidine it was obvious that Apilarnil could also contain Spermidine.

This research project focussed on the detection and quantification of Spermidine and evident biogenic amines at different lava stages.

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MATERIALS AND METHODS

Apilarnil (drone milk) in several stages of age was supplied by Api-Zentrum Ruhr.

High purity pipeline workflow was set up comprising an extraction/re-extraction procedure from the freeze-dried Apilarnil and a liquid chromatography coupled with a high-resolution matrix-assisted laser desorption ionisation time-of-flight mass spectroscopy (LC-ESI-QTOF(HR MALDI-TOF MS).

A targeted LC-ESI-QTOF method in positive mode was developed to analyse the extracts from drone samples. The mass spectra were analysed using Agilent MassHunter Qualitative and Quantitative Analysis Software and Excel.

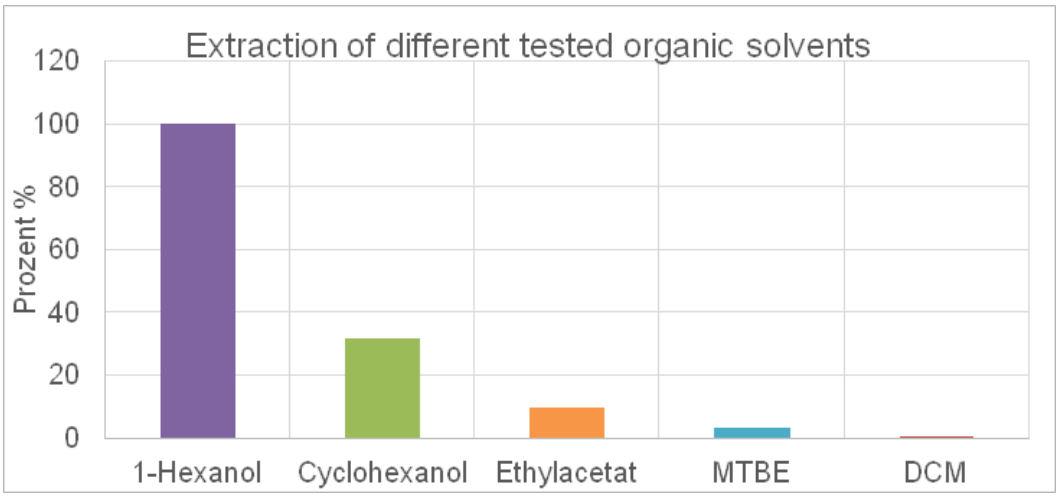

Figure1: Extraction efficiency of tested organic solvents

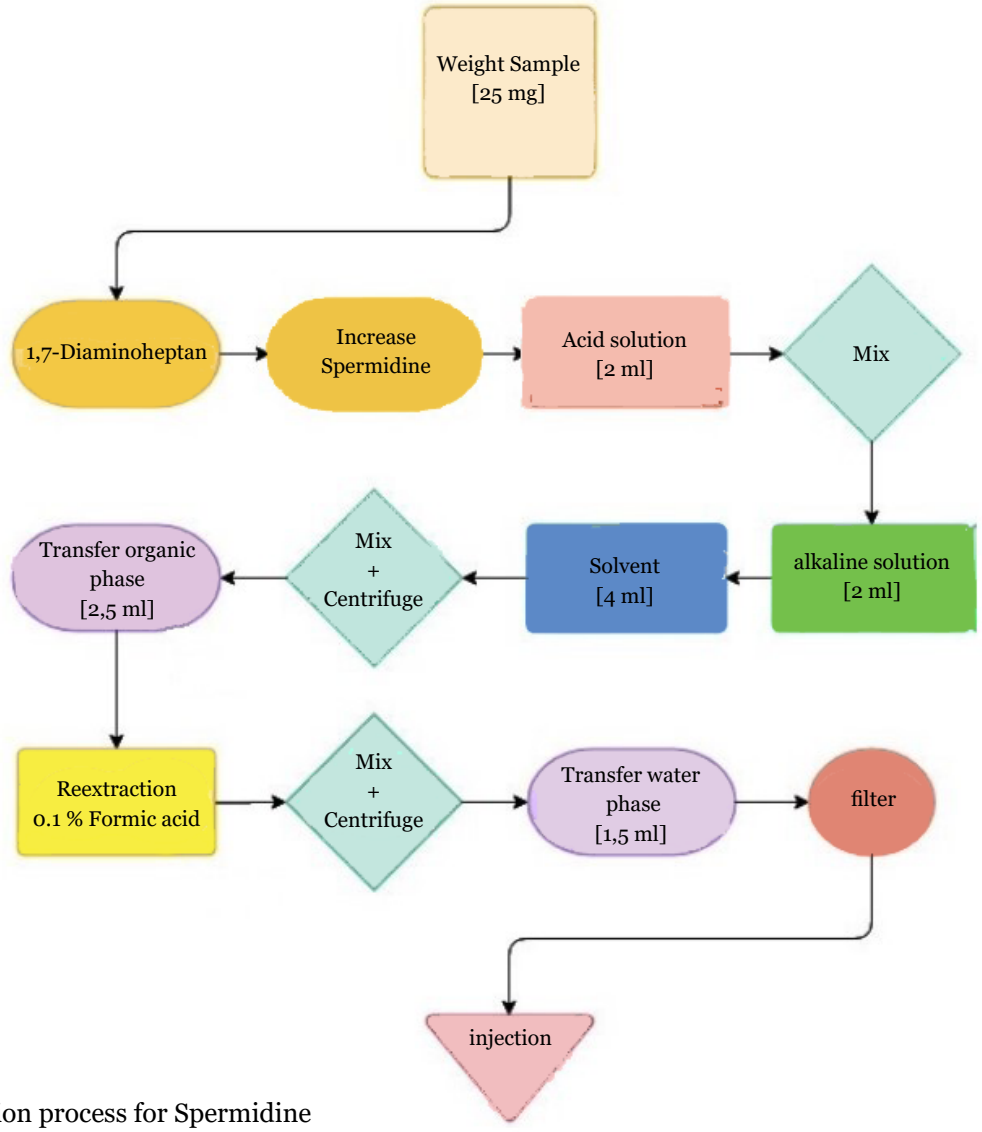

Figure 2: Extraction/re-extraction process for Spermidine

It was found that neutral water extracted less spermidine from the Apilarnil sample than acidified water. In addition to formic acid, hydrochloric acid and sulphuric acid were also tested, but showed no significant improvement in extraction yield. Good results were obtained using 1 % formic acid. In future studies, other acids and different concentrations may be tested to optimize the method.

To further optimize the sample preparation, an organic solvent for extracting the amines from the aqueous phase had to be determined. 1-hexanol, cyclohexanol, ethyl acetate, MTBE (Methyl tert-butyl ether) and DCM (Dichloromethane) were tested. The best extraction yields were achieved with 1-hexanol (see figure 1).

Principle process: Spermidine is obtained by extraction/re-extraction separating from interfering matrix (see figure 2). Optimization separated matrix components successfully and reduced Ion suppression and enhancement effects.

In a subsequent optimization of the automation process, filling speeds of the syringe, penetration depths of the needle, washing units, agitator speeds and times were tested individually for each step of the sample preparation and further optimized in several rounds

RESULTS

To determine the best time to harvest the drones, freeze-dried drone samples from different stages of development and from different locations were supplied by the Api-Zentrum Ruhr.

The stages were defined as day brackets of development of the male drone larva (see table 1).

stage	days of development	description of the development
mix	0-24	Mix as it is produced in Api-Zentrum Ruhr: main fraction is between 7-14 days
uncapped	0-9	
only larva	10-14	This fraction contains only larvae in a capped stage
lava+ pupa	12-17	This fraction contains drones in larva and partly pupped stage (“brood board”)
pupa	15-20	All larvae are already with a chitin shell.
black	21-24	Dark fraction, coloured by the black eyes of the puppa drones

Table 1: Age brackets of the samples analyzed

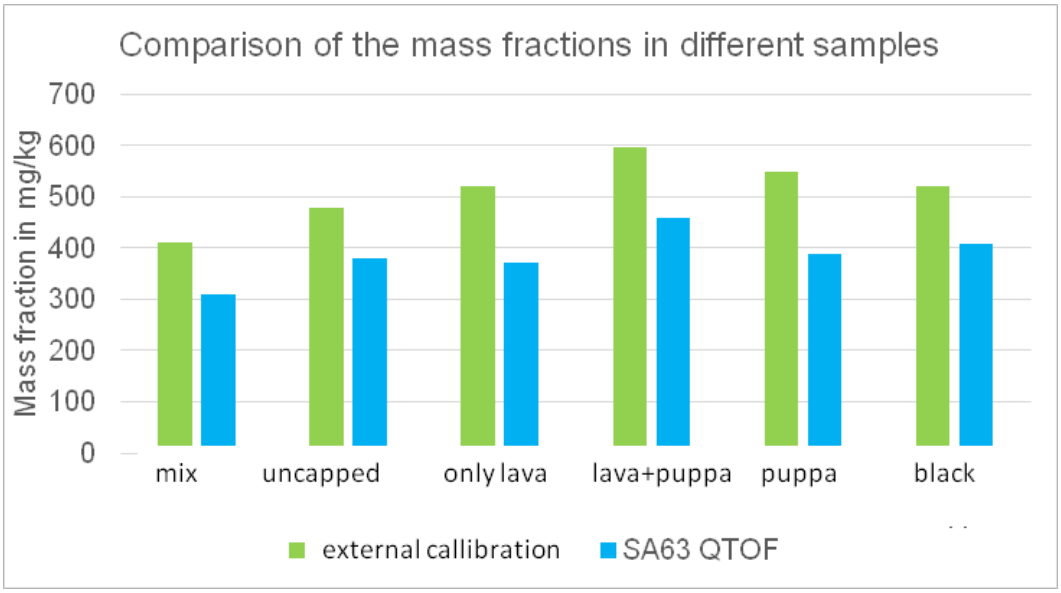

Figure 3: Comparison of the mass fractions in different samples

Combs were removed out of the hive and immediately shock frozen and then freeze dried. Samples were picked out of similar stage developed drones and a number of cells and mixed in order to get a representative sample [4].

In all three methods, the content was highest in the “larva+pupa” sample and was 459 mg/kg when quantified using the addition method (Fig. 3).

Sample	β (Spd) in mg/L	Ø w (Spd) in mg/kg	SD in mg/kg
mix	2,58	411	2
	2,56		
uncapped	2,97	478	4
	3,01		
only larvae	3,28	521	7
	3,23		
larvae+puppa	3,77	596	11
	3,68		
puppa	3,48	549	11
	3,38		
Black puppa	3,25	519	1
	3,24		

Table 2: Mass fractions of spermidine in the respective samples with internal calibration
Legend:
β (Spd) in mg/L: Beta (Spd) concentration in mg/L (Indicates a derived or calculated spermidine concentration in milligrams per liter.)
Ø w (Spd) in mg/kg: Mean spermidine content (by weight) in mg/kg (Shows the average spermidine concentration per kilogram of sample weight.)
SD in mg/kg: Standard Deviation in mg/kg (Represents the variation or dispersion of spermidine content measurements per kilogram.)

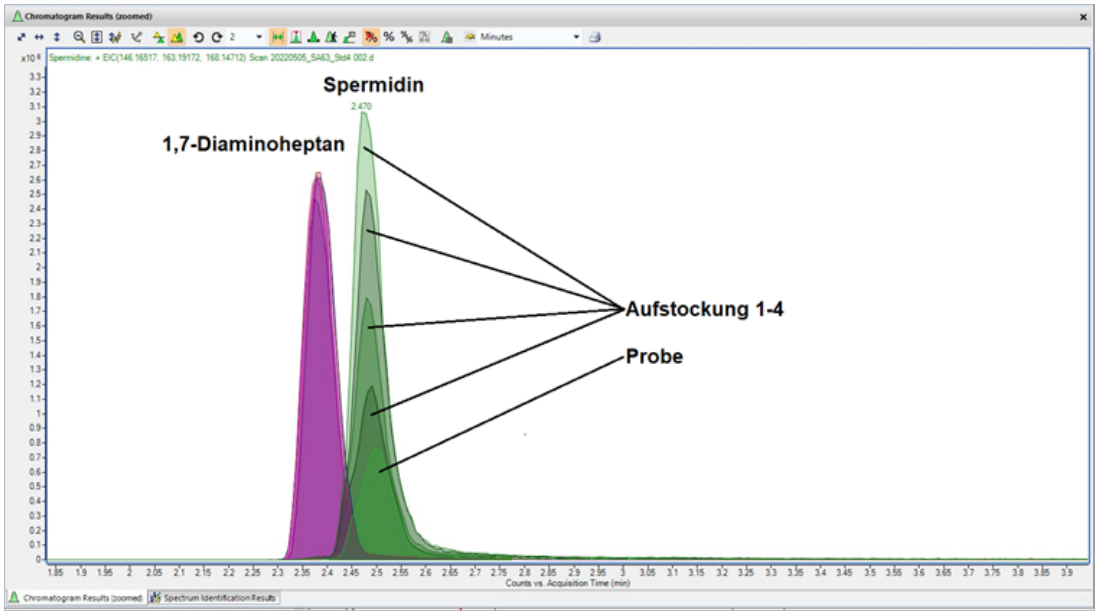

Figure 4: Overlays of the chromatograms of the standard addition method (QTOF)

The external calibration carried out here has the disadvantage that matrix components of the sample have a negative effect on the determination of the spermidine content. In order to take the matrix effects into account during the determination, the standard addition method was used in the following.

The spermidine was quantified using an external calibration with an appropriate standard and the standard addition method.

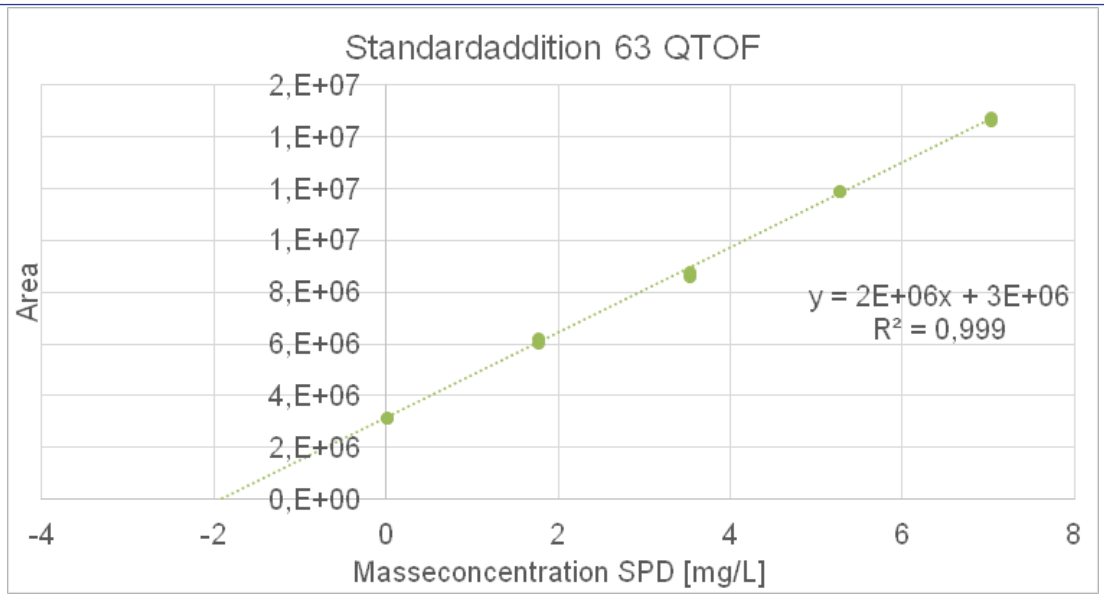

Figure 5: Regression of the standard addition of spermidine

sample	m Apilarnil in mg	Area Spd mit Masse	β (Spd) in mg/L	Ø w (Spd) in mg/kg	SD in mg/kg
mix	25,0	3,18E+06	1,95	310	2
		3,15E+06	1,92		
uncapped	25,1	3,88E+06	2,37	380	0
		3,88E+06	2,37		
only larvae	25,1	3,81E+06	2,33	371	2
		3,78E+06	2,31		
larvae+puppa	25,0	4,65E+06	2,84	**459**	6
		4,73E+06	2,89		
puppa	25,1	3,96E+06	2,42	388	1
		3,98E+06	2,43		
black puppa	25,1	4,14E+06	2,53	407	2
		4,17E+06	2,55		

Table 3: Mass fractions of Spermidine (QTOF) with external calibration

The quantification with the external calibration resulted in higher mass fractions of spermidine in the samples than the quantification with the standard addition. These differences are probably due to matrix effects, which are avoided by the standard addition method for quantification. Therefore, the lower values are considered more credible than the values obtained by external calibration (see table 3).

Apilarnil in different stages of larva development age contains Spermidine in higher concentrations as in wheat germs. Furthermore, Apilarnil contains other bioactive amines and polyamines besides Spermidine like the diamines cadaverine, putrescine and spermine. All could be identified by their exact mass (see fig 6).

In addition, evidence of agmatine, isopropylamine and phenethylamine was detected. However, these peaks of biogenic amines have low signal-to-noise ratios.

DISCUSSION

In this project the presence of Spermidine in Apilarnil has be proven and quantified. It is present in fresh and freeze-dried material of all larva-stages. Whereas the Spermidine content reaches its highest values in the end of the lava stage.

The highest spermidine content has been measured in wheat germ to date and is around 325 mg/kg [5]. The quantification of spermidine in the drone samples revealed a higher spermidine content of 459 mg/kg, even with the addition method.

The spermidine content is another column for explanation the various health applications for Apilarnil in apitherapy [6] and is most likely directly related to its health benefits.

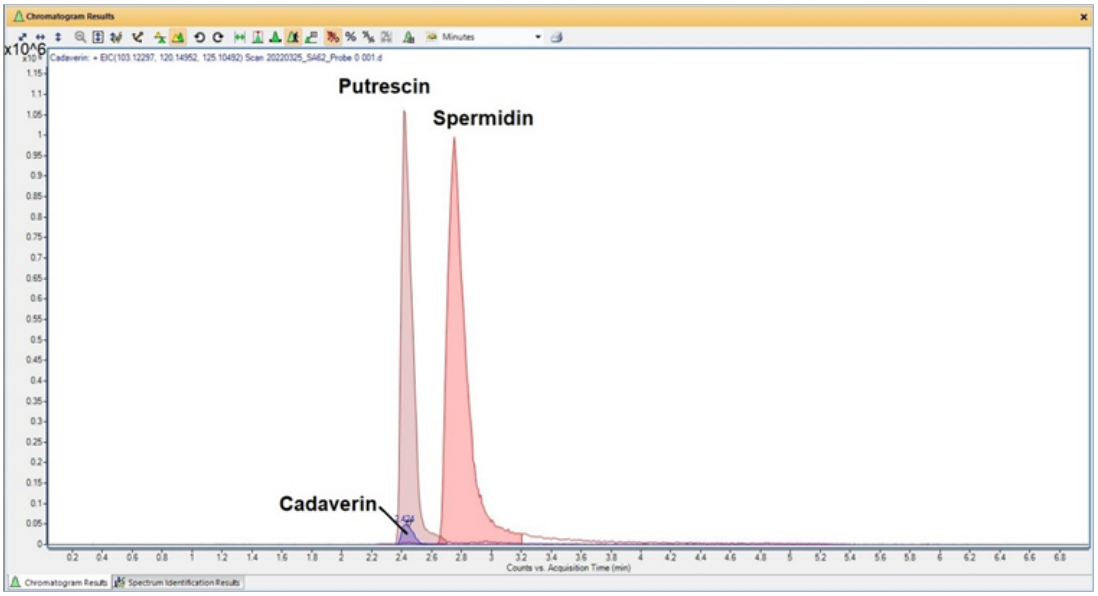

Figure 6: Chromatogram of biogenic amines detected

It will be a further challenge to reveal the molecular mechanisms especially the synergies in the presence of spermidine and the other bioactive ingredients of Apilarnil like the hormones and choline.

From traditional medicine it is known that in mixtures of Apilarnil with other hive products like Royal Jelly, propolis or pollen develop beneficial health synergies. These contain e.g. a lot of flavonoids. In the case of resveratrol, which is a known polyphenol, which is also found in red wine but also in bee products a synergy could be proven in literature: In the experimental study spermidine and resveratrol trigger [7] a certain level of autophagy if they are applied alone. Together applied only 1/10 of both triggers the same effect. This could be a good model how we can understand that low level concentrations of certain bioactive molecules can trigger reasonably sound effects, as we see them in the applications of drone milk (Apilarnil).

The sources of spermidine in human blood are the sum from own synthesis in the body, synthesis in the gut microbiome and external food like Apilarnil or wheat germs. Therefore, the effects derive from a complex matrix and are also influenced by other highly bioactive molecules like the flavonoids, or the hormones present in Apilarnil.

CONFLICT OF INTEREST

GT is producer of Apilarnil (ApiDrohn®) and member of Api-Zentrum Ruhr.

TI: no conflict of interest.

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