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Healthy chocolate? – UHPLC determination of ingredients in dark chocolate

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SUMMARY

Chocolate … a healthy food? Recently, polyphenols have gained more attention, due to their antioxidant capacity and their possible beneficial implications in human health [1]. As more studies associate an increasing number of health benefits to its high antioxidant content, dark chocolate has been labelled as "superfood". But not all ingredients are considered to have health-beneficial properties. In this application, 14 chocolate relevant compounds were separated in under 1.5 minutes.

INTRODUCTION

Cocoa products contain many physiologically active compounds. The high level of fat contributes to the high energy content of the cocoa bean. Despite its high nutritional value, however, the presence of caffeine and theobromine alkaloids may limit its potential as a nourishing food. The determination of the levels of methylxanthines and polyphenols in cocoa products is becoming increasingly important in the light of recent concern about the health effects of these compounds and their widespread consumption by the public. Methylxanthines such as theobromine and caffeine are typical compounds present in coffee, tea, chocolate, and products made of them [2, 3]. Beside polyphenols and methylxanthines this application allows the simultaneous determination of common preservatives, sweeteners, and flavoring substances that can be also present in dark chocolate.

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SAMPLE PREPARATION

The sample preparation of chocolate included the following steps.

1. Defatting: 1 g of a chopped chocolate sample was defatted with 3 x 10 ml hexane. For this, 10 ml hexane were added to the sample, sonicated, and centrifuged. The hexane supernatant was decanted and discarded each time.

2. Drying: The chocolate residue was dried overnight. Faster drying is possible using a slight nitrogen gas stream.

3. Extraction: The dried sample was then extracted with a mixture of methanol and water 80:20 (v/v). 5 ml of the extractant was added to the dried sample, sonicated, and finally centrifuged. The supernatant was collected. The extraction procedure was repeated two more times, to receive in total 15 ml of extracted supernatant.

4. Concentration/filtration: The extract was concentrated to a volume of 1 – 2 ml. Afterwards the concentrated extract was diluted with water to a volume of 10 ml. Last, the sample was filtered through a syringe filter with a pore size of 0.45 µm.

5. Injection: 2 µl of the prepared sample were injected to the HPLC system.

RESULTS

In **Fig. 1** a mixed standard containing acesulfame K, theobromine, saccharin, theophylline, caffeine, chlorogenic acid, catechin, epicatechin, 4 hydroxybenzoic acid, vanillin, guaiacol, sorbic acid, methyl paraben, and propyl paraben was separated in 1.5 min. The presence of all components in one sample matrix is not common but the separation is technically feasible. As seen in figure 1 the peaks are all baseline separated except guaiacol and sorbic acid. However, resolution is sufficient to clearly identify and quantify all peaks. Furthermore, two different chocolate samples were prepared. Both samples were dark chocolates with 75 % cocoa solids and 85 % cocoa solids, respectively. **Fig. 2** shows the overlaid chromatogram traces of the extracted samples with 75 % cocoa (blue) and 85 % cocoa (red). As expected because of their natural occurrence in cocoa plants, a high amount of theobromine and caffeine was measured. But also, epicatechin was detected. Referring to the higher cocoa amount the values for caffeine and theobromine in the sample with 85 % cocoa are higher. Furthermore, **Fig. 3** displays the overlay of the mixed standard and the chocolate sample containing 85 % cocoa.

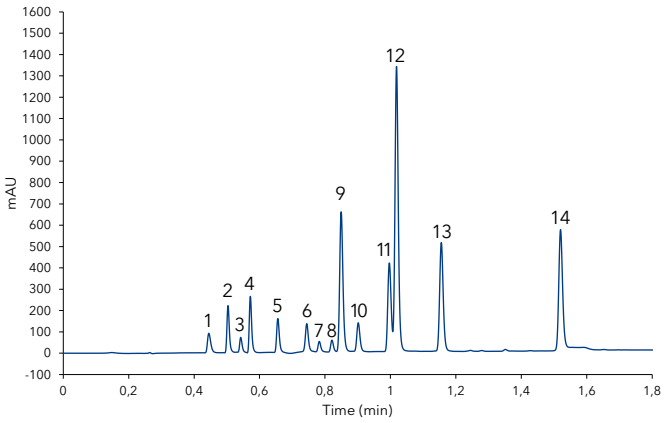

Fig. 1 Chromatogram of mix standard: 1) acesulfame K, 2) theobromine, 3) saccharin, 4) theophylline, 5) caffeine, 6) chlorogenic acid, 7) catechin 8) epicatechin, 9) 4-hydroxybenzoic acid, 10) vanillin, 11) guaiacol, 12) sorbic acid, 13) methylparaben, 14) propylparaben

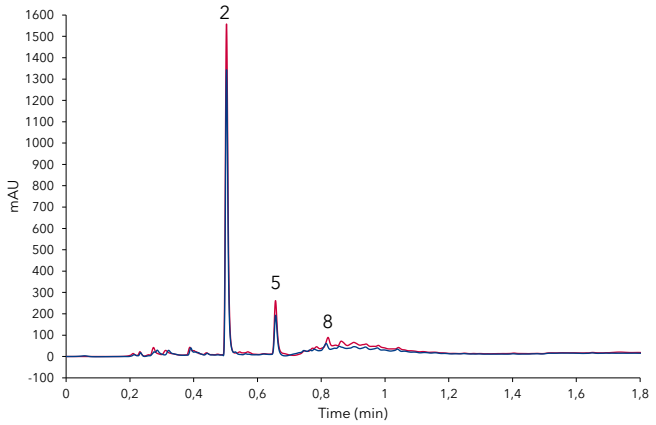

Fig. 2 Overlay of extracted chocolate samples (85 % cocoa – red, 75 % cocoa – blue); 2) theobromine, 5) caffeine, 8) epicatechin

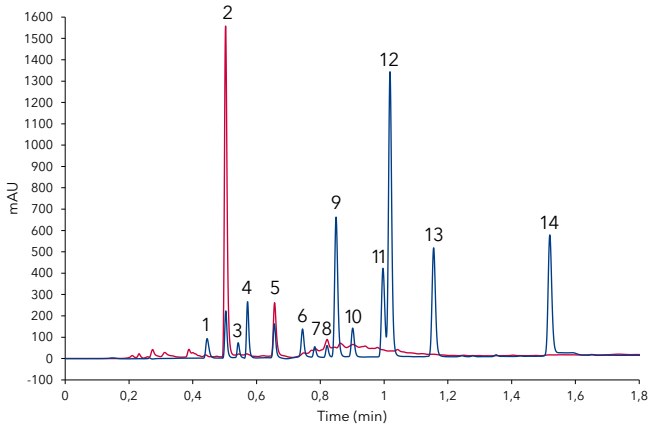

Fig. 3 Overlay of mixed standard (blue) and chocolate sample with 85 % cocoa (red) 1) acesulfame K, 2) theobromine, 3) saccharin, 4) theophylline, 5) caffeine, 6) chlorogenic acid, 7) catechin, 8) epicatechin, 9) 4-hydroxybenzoic acid, 10) vanillin, 11) guaiacol, 12) sorbic acid, 13) methylparaben, 14) propylparaben

Parameter	Description		
Eluent A	10 mM ammonium formate pH 2.8 in water		
Eluent B	10 mM ammonium formate in acetonitrile: water 90:10 (v/v)		
Gradient	**Time [min]**	**% A**	**% B**
	0	100	0
	1.50	100	0
	2.00	0	100
	2.02	100	0
	5.00	100	0
Flow rate	1.2 ml/min		
Temperature	42 °C		
Detection	254 nm		
Data rate	100 Hz		
Time constant	0.01 s		
Injection volume	2 µl		

CONCLUSION

This method allows a robust, sensitive, and fast determination of several ingredients present in dark chocolates. Because of the variety of determined compounds from sweeteners up to polyphenols and methylxanthines the method is suitable for quality control even in a complex matrix. The relatively time-consuming sample preparation is mandatory but tolerable due to the fast analysis time.

MATERIAL AND METHODS

System configuration

Instrument	Description	Article No.
Pump	AZURA P 8.1L	APF45PA
Autosampler	AZURA AS 6.1L, 1240 bar	AAA10AA
Detector	AZURA DAD 6.1L	ADC11
Flow cell	Standard KNAUER LightGuide UV	AMC19XA
Thermostat	AZURA CT 2.1L	ATC00
Column	ACE Excel 2 C18-Amide, 100 x 2.1 mm ID	–
Software	OpenLAB CDS EZChrom Edition - Workstation	A2600-1
Software	OpenLAB CDS EZChrom Edition – PDA/3D UV	A2611-1

REFERENCES

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