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Detection of wax coatings on plums by rapid physical methods

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Plums epicuticular wax provides fruit surface water repellency and reduces water loss through the skin. In order to enhance its effect and to prolong the shelf-life of the plums, commercial EU allowed waxes thin coatings are made. Unfortunately in the real practice, some not permitted artificial waxes are also applied. The present study aims at presenting three rapid physical methods for detection and recognition of wax type coatings. Optical microscopy reveals quite different surface morphology of all 10 used waxes, making it possible to detect the wax-treatment existence but not always to identify the type of the wax. Contact angle measurements with polar and nonpolar liquids display clear differences between the two values combinations but this method should be used with caution because of the difficulty in contact angle estimation onto the curved plum surface. Differential scanning calorimetry appears to be the most precise technique since the plum bounded water and the epicuticular wax, both have evaporation and melting peaks far enough from the melting peaks of all investigated waxes, allowing one to detect and identify the type of the wax treatment.

Keywords: *plum, wax fruit coatings, DSC, optical microscopy, contact angle*

INTRODUCTION

The main aim of all fruits producers is to reduce the crop losses and to maintain the quality of the fresh fruits over extended periods of time. From the other side, consumer interest brings together health, nutrition, and food safety combined with environmental concerns. Both points of view meet at protecting fruits with environmentally friendly, nontoxic coatings, which preserve the flavour, nutrition value and visual appearance. Even though many scientific attempts are put on formulation of different kind of coatings [1-3], the most often used coatings are those from the four EU allowed as food additives waxes – beeswax, candelilla wax, carnauba wax and shellac [4]. Such harmless coatings are proved to reduce decay, reduce moisture and weight loss, enhance external appearance, regulate the respiration rate and have antifungal effect [5]. Unfortunately in some cases, because of their lower prizes, some synthetic waxes, not allowed for use in the food products, are also applied for plum coatings. The standard control of the wax coatings is performed by Gas chromatography combined with Mass spectrometry [6]. In the gas chromatograph the evaporated wax components are transported with a carrier gas flow through a capillary column and separated by their different transportation speeds (retention time). However, the components must be vaporisable without decomposition. Wax components with hydroxyl-groups (alcohols, fatty acids) need to be derivatised, which means, the active H-atoms have to be masked and inactivated in order to avoid decomposition or peak broadening. Hence, this method is time consuming, requires specific sample preparation and is not always very effective, for instance if the epicuticular wax and the applied wax have very similar chemical composition.

The present study aims at presenting three rapid physical methods, mainly light microscopy, static contact angle measurements of a small sessile drop, and differential scanning calorimetry (DSC), for detection of plum wax coatings and recognition of wax type. For these purpose 10 different waxes from four categories were used – paraffins, ceresins, palm wax, and beeswax.

EXPERIMENTAL

For the present investigation "Black diamond" plums were bought from the local market. The plums were selected for uniform maturity, size, colour and absence of physical damage or grey. The artificial commercial waxes were supplied by Evricom LTD, Bulgaria. They belong to three different categories – paraffin (E1, E4, E6, E21, E53), ceresin (ECP, EC2, EC3), and palm wax (ES4, ES5). Beeswax was delivered from the local apiarists.

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Two types of samples were prepared: planar wax films and wax plum coatings. The neat wax planar films were prepared by casting the molten wax onto the microscope glass. The plum coatings were prepared at first by fruit dipping into the molten wax and subsequent thinning the coatings in hot air flow. The thinning was performed until the coating became invisible to the naked eye.

The wax morphology was investigated by means of optical (light) microscope type Biorex 3 (Konus, Italy), offering magnification up to 1000x, equipped with CCD camera Microview (Konus, Italy). For the contact angle measurements droplets of 5 μl (for the neat wax films) or 2 μl (for the plum coatings) were inserted onto the surface by means of precise 10 μl micro syringe (Innovative Labor System GmbH, Germany) supplied with steel needle. All measurements were performed at ambient conditions. The final value for the contact angle was obtained as an average of at least 5 drops for each surface. The drops were recorded with CS01-200 Digital microscope (CoolingTech, China) and the contact angle was obtained by image processing with the microscope software. Thermal behavior of the waxes and wax coated plums was examined via Differential scanning calorimeter DSC SETARAM 141. The samples were prepared by fine pilling the plums so that a thin slice consisting of wax and small amount to plum skin was yielded. The samples with masses of several milligrams were first cooled down to 0 °C at cooling rate 5 K/min and then heated at the same rate up to about 150 °C. For the calibration Indium standard was used, having melting temperature of 157 °C and melting enthalpy 28.4 J/g.

RESULTS AND DISCUSSION

As a preliminary study, the planar wax films were investigated under microscope. The images reveal that for all 10 different waxes different types of morphologies occur (see the examples given in Figure 1) as a result of self-assembly of the wax molecules depending on chain-length distribution, functional groups, crystallization conditions and concentration of the major components of the wax [7]. The solid wax films, similar to the epicuticular wax itself, may consist of partially crystalline and partially amorphous or disordered regions [6].

The specific wax films texture is preserved to a great extent even when the waxes were made in the form of plum coatings. The coatings change the initial plum surface morphology, which can be easily established under microscope with appropriate magnification. In Figure 2, as an example, the texture of the natural plum and the

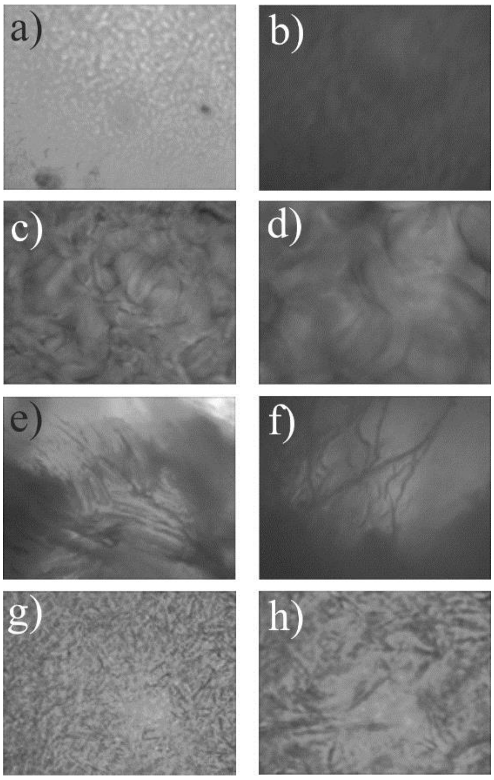

Fig. 1. Microscopic images of different planar wax films and different magnifications: a) beeswax, 40x; b) beeswax, 100x; c) E53, 40x; d) E53, 100x; e) ES5 40x; f) ES5, 100x; g) ECP 40x; h) ECP 100x.

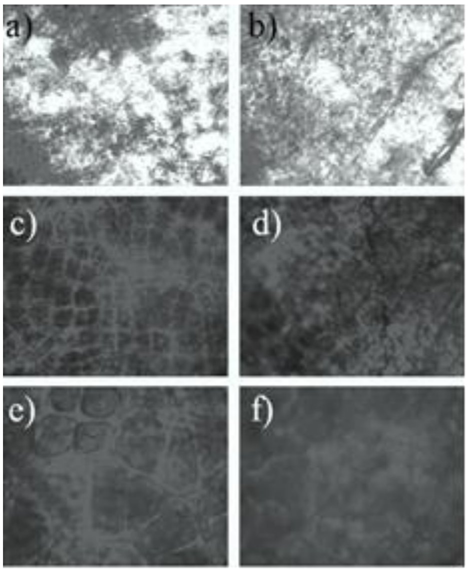

Fig. 2. Microscopic images of plums at different magnifications: a) 10x, natural plum; b) 10x, plum covered with beeswax; c) 40x, natural plum; d) 40x plum covered with beeswax; e) 100x, natural plum; d) 100x, plum covered with beeswax.

Table 1. Results for the contact angle measurements of different waxes with distilled water, θ_{DW}, and methylene iodide, θ_{MI}, wax melting enthalpy, ΔH_m, melting temperatures T_m^W of the neat wax and T_m^{PC} of the plum coating, and temperature shifts $\Delta T=T_m^{PC}-T_m^W$. The values in bold are the peaks of the bounded-water evaporation.

Sample	Type of the surface	θ_{DW} [deg]	Θ_{MI} [deg]	ΔH_m [J/g]	T_m^W [°C]	T_m^{PC} [°C]	ΔT [°C]
Natural plum	Smooth	66	30	-	-	86	-
						118	
Beeswax	Smooth	106	60	215	66.5	65.1	-1.4
						106.0	
ES 4	Rough	65	51	149	-	53.8	-
					55.3	58.9	3.6
					67.5	68.5	1
						121.0	
ES 5	Rough	92	52	259	66.3	58.5	7.8
					-	**120.8**	
E 6	Smooth	106	59	270	47.1	-	-
					65	63.6	-
						129.8	1.4
EC 3	Smooth	106	64	254	49.3	44.4	5.3
					73.3	60.9	8.1
						113.1	
E 53	Smooth	116	65	252	45.4	44.4	-1.0
					63.8	60.9	-2.9
						113.1	
ECP	Smooth	108	67	247	59.7	61.5	2.5
						118.9	
E 1	Smooth	120	70	219	38.7	59.6	-1.5
					61.4	**122.0**	
EC 2	Smooth	122	72	242	57.2	54.4	-2.8
						123.4	
E 21	Smooth	74	72	256	36.2	-	-
					57.2	57.1	-0.1
						77.4	-
						116.1	

beeswax coated plum is presented. The beeswax was chosen since its surface was the smoothest one and it was expected to be the most difficult to be recognized as the plums were coated with it. At magnification 10×, both textures look quite similar (Figure 2a, 2b) but going to magnification 40× the dissimilarities start to appear (Figure 2c, 2d). At such magnification the cuticle layer with separate cells is clearly seen in the images without wax coverage, whereas smeared images without clear features are seen for the coated plums. The magnification 100× offers the best resolution for the requested distinction.

As it is clearly visible (Figure 2e, 2f), the separate cells of the natural plum are very well seen, whereas for the treated plum mostly the wax coating without any specific texture is seen. According to the results, light microscopy, as one of the mostly available and simplest experimental methods in the laboratories, can provide information about the wax treatment of the fruits. Although one should keep in mind, that the final morphology of the coating depends not only on the type of the wax but also on the surface roughness of the plums and hence, the microscopy can give reliable information mostly about the existence of wax-treatment but not on the type of the wax.

Contact angles have been used for a long time as an indicator of surface wettability in the food and plant science [2, 8-11]. The well-known Young equation describes the balance at the three-phase contact of solid, liquid and vapor: $\gamma_{sv} = \gamma_{sl}+\gamma_{lv}.\cos\theta_Y$, where the interfacial tensions, γ_{sv}, γ_{sl} and γ_{lv}, form the equilibrium contact angle of wetting, often referred to as the Young contact angle, θ_Y. Because of the existing difference in the structure of all artificial waxes, one could expect that the equilibrium contact angle will be different, depending on the liquid used, wax type and the surface roughness. The results for a contact angles, measured onto the planar wax films surfaces are listed in Table 1. As it is seen, the combination of the two contact angles – that for distilled water (polar liquid), θ_{DW}, and that for methylene iodide (nonpolar liquid), θ_{MI}, appear to be unique for each wax coating. When the contact angle was measured onto the curved wax plum coatings, the results differ in most of some cases with ±1°. The largest difference of about 6° was observed for ES5 wax coating, and it would be attributed to the significant roughness of the wax surface morphology (see Figure1). The apparent contact angle in this case is very sensitive to that morphology, because the size of the drop (less than 1 mm) is comparable with the size of the roughness.

DSC is a standard method for investigation of the thermal behaviour of many materials and it is also applied in the field of food science [12, 13]. In the present investigation, two sets of DSC curves were recorded – waxes only and the second one plum with coatings. The results are shown in Table 1 and in Figure 3. As it was pointed out by some authors [6] the term ''wax'' is used for a variety of natural or artificial commercial products that contain fatty materials of various kinds. According to the chemical definition, waxes consist of aliphatic compounds with discrete molecules (no polymers), are solid and meltable without decomposition normally below 100 °C. Most of the plant waxes fit to these criteria but they contain other components as well, which lead to higher melting temperatures of those waxes [6]. This is the main reason one can distinguish via DSC the artificial wax coatings from the natural ones. The results show, that the wax coatings melting temperatures shift with respect to that of the bulk waxes, but the main features of the DSC endotherms remain unchanged. In some cases, the enthalpy of the multicomponent peaks changes, which can be attributed to the polymorphism of those waxes, which does not affect the number of the melting peaks. The highest temperature peaks in each DSC run of the plums with coatings is attributed to the bounded water evaporation. Additional experiments (not shown) prove that the melting temperature of the epicuticular plum wax was 86 °C. The obtained results show that DSC analysis of the melting enthalpies and melting temperatures is able to reliably distinguish not only the wax-treatment existence but the type of the wax used as well.

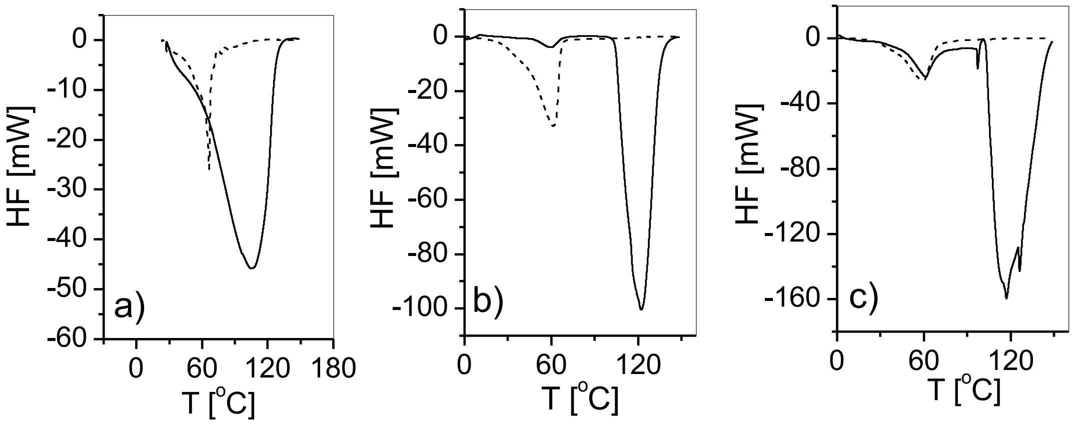

Fig. 3. DSC thermograms of bulk waxes (dashed line) and wax plum coatings and plums (solid line) with: a) Beeswax; b) E1 wax; c) ECP wax.

CONCLUSION

The optical microscopy reveals quite different surface morphology of all waxes used, so it is possible to detect the wax-treatment existence but not always to identify the type of the wax. Contact angle measurements of planar wax films as well as plum wax-coatings, measured with polar (distilled water) and nonpolar (diiodomethane) liquids display clear differences between the two values combinations but this method should be used with caution because of the difficulty in contact angle estimation onto the curved plum surface. Differential scanning calorimetry appears to be the most precise technique. The bounded water in the plums and the plum epicuticular wax, both have evaporation and melting peaks far enough from the melting peaks of all investigated waxes. This allows one to observe the peculiarities of the melting peaks for all different waxes and to identify the type of the wax employed.

REFERENCES

1. E. Velickova, E. Winkelhausen, S. Kuzmanova, V. D. Alves, M. Moldão-Martins, *LWT-Food Sci. Technol*., **52**, 80 (2013).
2. O. Skurtys, P. Velásquez, O. Henriquez, S. Matiacevich, J. Enrione, F. Osorio, *LWT - Food Sci. Technol*., **44**, 1449 (2011).
3. H Karaca, M. B. Pérez-Gago, V. Taberner, L. Palou, *Int. J. Food Microbiol*., **179**, 72 (2014).
4. EUROPEAN PARLIAMENT AND COUNCIL DIRECTIVE No 95/2/EC, on food additives other than colours and sweeteners, http://ec.europa.eu/food/fs/sfp/addit_flavor/flav11_en.pdf.
5. F.P. Gonçalves, M.C. Martins, G.J. Silva J., S.A. Lourenço, L. Amorim, *Postharvest Biol. Tech*., **58**, 211 (2010).
6. K. Koch, H.-J. Ensikat, *Micron*, **39**, 759 (2008).
7. H.J. Ensikat, M. Boese, W. Mader, W. Barthlott, K. Koch, *Chem. Phys. Lipids*, **144**, 45 (2006)
8. R. Andrade, O. Skurtys, F. Osorio, *Food Res. Int*., **54**, 397 (2013).
9. K. Koch, K. D. Hartmann, L. Schreiber, W. Barthlott, C. Neinhuis, *Environ. Exper. Botany*, **56**, 1 (2006).
10. C. Ribeiro, A. A. Vicente, J. A. Teixeira, C. Miranda, *Postharvest Biol Tec*, **44**, 63 (2007).
11. D. Quéré, *Physica A*, **313**, 32 (2002).
12. E.J. Lee, J.K. Park, Y.-S Lee, K.-H. Lim, *Korean J. Chem. Eng*., **27**, 524 (2010).
13. K.R. Nikolova, I. Panchev, D. Kovacheva, S. Pashova, *JOAM*, **11**, 1210 (2009).

ОТКРИВАНЕ НА ВОСЪЧНИ ПОКРИТИЯ ВЪРХУ СЛИВИ ЧРЕЗ БЪРЗИ ФИЗИЧНИ МЕТОДИ

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(Резюме)

Епикутикулярният восък при сливите осигурява отблъскващ нежеланите вещества ефект и намалява загубите на вода през повърхността им. В практиката, за подсилване на този ефект, както и за удължаване на времето за съхранение в магазинната мрежа, се изработват покрития от разрешени от Европейската общност восъци/парафини. Целта на настоящата публикация е да представи три бързи физични метода за откриване на съществуването на такива покрития и за разпознаване на типа им. Оптичната микроскопия разкрива различаващата се морфология за всички 10 изследвани покрития, което прави възможно откриването на третиране, но не винаги е възможно идентифицирането на типа на покритията. Измервания на контактния на ъгъл на омокряне, с полярна и неполярна течности, показва явни различния между двойките ъгли за всички изследвани покрития, но методът следва да се използва внимателно, като се има предвид възникващата от закривената повърхност на сливите възможна неточност в определянето на ъглите. Диференциалната сканираща калориметрия се оказва най-прецизният метод, тъй като пиковете на изпарение на свързаната вода, както и този на топене на естествения епикутикулярен восък са достатъчно отдалечени от пиковете на топене на всички изследвани восъци/парафини, което позволява откриването на съществуващо покритие и идентифицирането на типа му.