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Influence the extraction acidity level on the amount and chemical composition of essential oil from *Rosa damascena* Mill.

A. Dobreva*

Institute of Roses, Aromatic and Medicinal Plants, 49 Osvobozhdenie, 6100 Kazanlak, Bulgaria

Abstract. *It was raced the influence of acidity rate (pH=4 and pH=6) on the quantity and chemical composition of essential oil of Rosa damascena Mill. It was established that the yield is ranging 80 ÷ 110 % compared with control and the amount of the main terpene alcohols (citronelol, geraniol and nerol) increased on average by 16 %.*

Keywords: *Rosa damascena* Mill., essential oil, acidity, yield, chemical composition

Abbreviations: TA – terpene alcohols, HC – hydrocarbons

Introduction

Essential oil in *Rosa damascena* Mill. is localized primarily in the epidermal cells of petals in the flower (Mihailova et al., 1977; Bergougnoux et al., 2007). It's amount is minimal and varies between 0,009 ÷ 0,062% (Kovacheva, 2007). It was found that the biosynthesis of aromatic components is part of the metabolism of the plant and carried out with active participation of enzymatic systems (Dudareva et al., 2004).

Much of the TA and some micronutrients present in the rose flower in the form of glycosidic or ester structures - serving as a precursor or the result of catabolic transformations (Bugorskii and Beznishenko, 1984; Dunphy, 2006; Oka et al., 1999). The conjunctived forms can not be extracted by distillation, because they aren't volatile and ejected of with the exhausted flowers, but also represent a solid reserve, which can increase the yield of essential oil. Given the nature of the links in these complexes, the most appropriate method for the release of aromatic compounds is hydrolysis-acid or enzyme (Staikov and Zolotovich, 1961; Koupenov, 1984). The technologies for complete assimilation of waste flowers used both types of degradation (Koupenov, 1984; Papanov et al., 1986), but there are not enough sources for the pre - hydrolysis of fresh material in literature. The maceration of flowers in hydrochloric acid solution with pH = 2 for 15 ÷ 24 h, give the yield rising by 24,3 ÷ 44,4 % (Staikov and Zolotovich, 1961). According to Loghmani-Khouzani et al. (2007), the distillation of rose flowers in a 10 % solution of sulfuric acid eliminates the harmful aliphatic amines in the essential oil. However, the amount of TA significantly reduced, and geraniol and nerol are not even registered.

Our studies with *Rosa alba* L. show, that the level of acidity is important for the changes occurring in the oil (Dobreva, 2008; Dobreva and Stoyanova, 2009). At pH = 2 water distillation yield increases to 39% compared to control, but the main terpene alcohols tsitronelol + nerol and geraniol decreased by 36% and 34%. The mild conditions (pH = 4 and pH = 6) lead to an increase in yield to 47 % with increasing content of terpene alcohols to 53 % . These results focused purpose of the study to test the same conditions in *Rosa damascena* Mill.

* e-mail: anadobreva@abv.bg

Material and methods

As a raw material used pink in a population of *Rosa damascena* Mill. plantation of the Institute of Rose, Kazanlak. The flowers are picking up early morning (6 ÷ 8 p.m.), with a fully opened and half-opened calyxes. Distillation is conducted in Clevenger apparatus with the following parameters: hydroratio 1: 4; speed at 3 ÷ 4 ml / min and duration - 150 min. Acidification of water is done with orthophosphoric acid (Merck), to pH levels and pH = 4 - 6. The flowers are soaked in the prepared water for different times experience is divided into 5 options: variant 1 (control, without acidification and waiting), variant 2 (without waiting), variant 3 (with standing for 0,5 h), variant 4 (with standing for 1 h), variant 5 (with standing for 2 h). As a criterion for extraction yield is used the essential oil content in raw material, %(v/w). The experiments were repeated three times and treated statistically.

The chemical composition of the oils was determined by gas chromatography apparatus PYE UNICAM with a flame ionization detector under the following operating conditions: capillary column CARBOWAX 20M with length 50m, internal diameter 0,2 mm and film thickness of 0,20 µm.; temperature program: 65 ° C to 230 °C at 6 °C/min.; injector temperature - 230 ° C; detector temperature - 250 ° C, carrier gas - hydrogen; flow rate - 1,3 ml/min. In chromatograms were identified and quantified representative and characteristic ingredients, according to ISO 9842 (Rose oil). For greater accuracy in identifying authentic references were used.

Results and discussion

Table 1 presents data on the extraction of essential oil. It is evident that the level of acidity is important for the quantity of derived oil (pH = 4) has better results in this respect. It can be explained by

Table 1. Oil content by different treatment protocols

Variants	Essential oil content, %(v/w)	
	Ph = 4	Ph = 6
1- control	0.038 ± 0.010	0.040 ± 0.007
2	0.035 ± 0.005	0.032 ± 0.010
3	0.042 ± 0.008	0.035 ± 0.005
4	0.038 ± 0.008	0.039 ± 0.006
5	0.038 ± 0.006	0.038 ± 0.006

the course of the hydrolysis process due to the acidic environment and of the other point of view - by the action of specific enzymes at concrete pH. Figure 1 presented a comparison of essential oil yields obtained with the control. In direct distillation there was a decline to 92 % and 80 % compared to control. At 0,5 h soaking, the process of hydrolysis compensate the losses and variations of pH = 4 reached the maximum increase of 11 %. At pH = 6 this effect is weaker. Standing 1 h extraction returns to the level of control. At 2 h soaking, the level of production remains the same as the previous - in the weaker acid, with a minor decrease. The former our study shows that pH = 2 and variants of the same experience was reported only increase the yield within 25 – 43 %, when the maximum point is with standing 1 h. (Dobreva, 2008). In the case of *Rosa alba* L. the optimum level of acidity is pH = 4 (Dobreva, 2008; Dobreva and Stoyanova, 2009). In his additional quantities flavoring agents reached 47 % after 2 h standing in solution. The amount of terpene

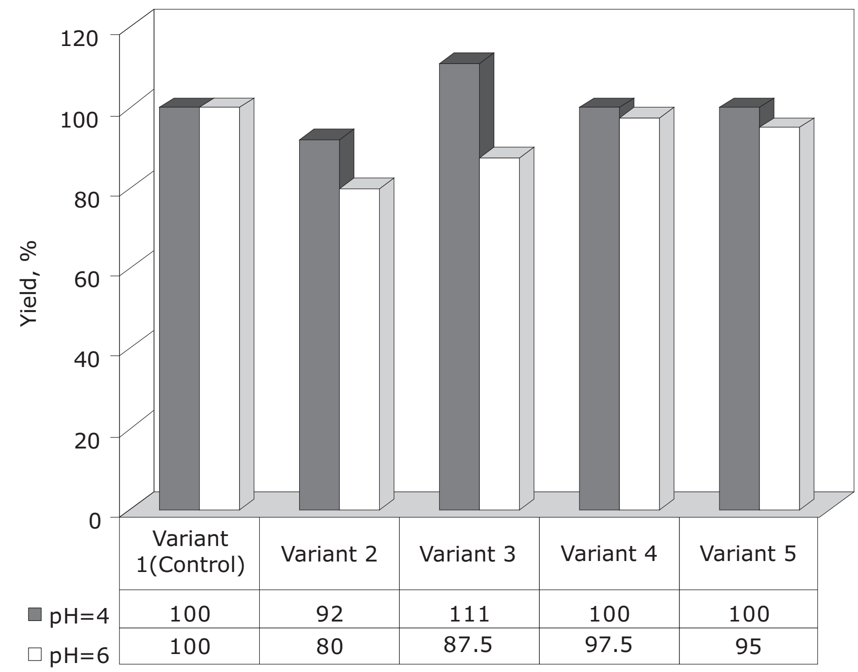

Figure 1. Impact of an acidity rate on the relative content (yield) of R. damascena Mill. essential oil

Table 2. Effect of acidity rate on the essential oil composition of Rosa damascena Mill

Acidity rate	Variants	Typical ingradients of the rose oil, %														
		Ethanol	Linalool	Cis-rose oxide	Trans-rose oxide	Phenylethyl alcohol	Citronelol	Nerol	Geraniol	Geranial	Geranyl acetate	Eugenol	Metyleugenol	Heptadecane C17	Nonadecane C19	Heneicosane C21
Ph = 4	1	0.20	0.53	0.21	0.11	0.36	23.84	9.78	16.05	0.21	1.17	0.22	1.21	1.67	10.46	5.23
	2	0.37	0.61	0.13	0.06	0.35	27.97	10.51	17.35	0.11	*	0.10	1.79	0.83	4.68	0.68
	3	0.13	0.73	0.09	0.02	0.30	24.18	13.51	19.08	0.46	*	0.54	1.75	1.04	4.91	1.21
	4	0.18	0.67	0.12	0.08	0.52	23.92	9.54	17.83	0.18	*	0.33	1.42	3.20	4.99	1.11
	5	0.28	0.51	0.14	0.03	0.89	27.78	10.50	18.54	0.17	*	0.23	1.25	2.58	1.23	1.23
Ph = 6	1	0.67	0.42	0.17	0.07	0.36	13.76	8.34	23.16	0.21	0.84	0.16	0.75	1.40	7.32	3.45
	2	0.49	0.34	0.16	0.04	0.88	31.10	8.00	15.31	0.19	0.63	0.21	1.30	2.80	6.72	2.08
	3	0.34	0.47	1.17	0.04	0.54	39.45	8.56	15.71	0.12	*	0.26	1.03	0.71	5.15	1.6
	4	0.43	0.46	0.13	0.02	0.55	21.92	7.81	13.98	0.20	0.55	0.12	1.20	0.82	6.32	3.55
	5	0.20	0.41	0.16	0.07	0.58	32.93	8.84	14.87	0.14	0.46	0.15	1.28	0.67	1.39	2.54

alcohols in the oil increased on average by 53 % without a significant impact on olfactory characteristic. The difference in the behavior of both species can be explained by the high level of carbohydrate metabolism in the white rose and the high activity of its enzymes amylolitic (Bugorskii and Beznishenko, 1984).

For *Rosa damascena* Mill. with increasing the pH increased and yields of essential oil (Dobreva, 2008), but not less important is its impact on the qualitative composition (Table 2). Data indicate that variations in pH = 4 content of the main terpene alcohols is higher than that of control. Citronelol, nerol and geraniol increased by 9 %, 13 % and 38 %. This fact proves that they exist like bounded forms in the flowers. The latter are unstable complexes that can be easily destroyed. The greatest increase is for the phenylethyl alcohol (200 %) - circumstantial evidence that this element has a large latent quantity. It is an important component of the native flower oil (Oka et al., 1999).

At pH = 6 the effect of acidity of the medium has rather negative effects. The main terpenes citronelol marks only weak growth, and other alcohols reported losses of up to 30 %. Phenylethyl alcohol has a lower rate of variations of pH = 6. The level of acidity influence negative even for microcomponents. Save them, the hydrocarbones reported declines. Our previous study reported a drastic decline (50 %) of the same components at pH = 2. This shows that the average pH = 4 is the most suitable level of acidity to release related aromatic compounds.Probably the result is a consequence of suitable complex conditions for the action of enzyme systems and purely chemical acid hydrolysis of primary metabolites.

Conclusion

Acidification of water distillation in *Rosa damascena* Mill. causing the release of additional quantity essential oil. Optimal results are achieved at pH = 4, the yield and the main terpene alcohols rised at averages 11 %.

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