

Content of aroma substances in apple juice processed by pressing or extraction

Indhold af aromastoffer i æblemost fremstillet ved presning eller ekstraktion

K. KAACK

Summary

Experiments were carried out with the aim of evaluating the steps of apple juice processing by pressing or extraction.

The content of aroma substances was determined by gas chromatographic analyses of aroma distillates.

If apples are heated to 60°C before processing lipoxygenase, esterases, and alcohol dehydrogenases are inactivated. This means that no aldehydes are released from lipoxydation of unsatu-

rated fatty acids. Furthermore no hydrolysis of esters or formation of alcohols from reduction of aldehydes takes place.

At 60°C apple aroma substances are quite stable. To obtain reasonable aroma substance yields by water extraction at 60°C six to eight extraction steps are necessary. The extraction may be described by exponential mathematical models.

Pomace from pressing of apple juice has a fairly high content of important aroma substances.

Key words: *Malus domestica*, apple, juice, pressing, extraction, aroma substances.

Resumé

Der er blevet udført forsøg med henblik på bestemmelse af, hvorledes de enkelte procestrin under fremstilling af æblemost ved presning eller ekstraktion påvirker aromastofindholdet.

Til analyse for indholdet af aromastoffer blev fremstillet aromadestillater, der blev analyseret ved brug af gaskromatografi.

Hvis æblerne opvarmes til 60°C før presning eller ekstraktion, sker der inaktivering af enzymer, som katalyserer dannelse, nedbrydning eller omdannelse af aromastoffer. I uopvarmede og formalede æbler bevirker enzymet lipoxygenase dan-

nelse af aldehyder ud fra umættede fedtsyrer. Esteraser bevirker hydrolyse af estere, og alkoholdehydrogenase katalyserer omdannelse af aldehyder til alkoholer.

Ved 60°C er aromastofferne forholdsvis stabile, hvorfor der ikke sker væsentlige ændringer, selv om der anvendes relativt lange holdetider.

Ved trinvis ekstraktion kan ekstraktionsforløbet for aromastoffer beskrives ved brug af eksponentielle funktioner. Ved 60°C opnås fuldstændig ekstraktion efter seks til otte ekstraktionstrin. Presseresten efter normal presning har et betydeligt indhold af vigtige aromastoffer.

Nøgleord: *Malus domestica*, æble, æblemost, presning, ekstraktion, aromastoffer.

Introduction

A great number of alcohols, carbonyls, esters, and carboxylic acids have been identified in the apple aroma (5,6,8,12,13,17,21,22,27,28,31,32,33). Apples may also contain primary aroma compounds such as farnesene, eugenol and damascenone (20,23). Secondary aroma compounds such as benzaldehyde and 2,4-dienals are produced during processing by cyanogenic glycolysis or enzyme catalyzed oxidation of unsaturated fatty acids respectively (23). 4-methoxyallylbenzene is a compound, which may contribute a spicy or anis-like note to apple flavour (35). Aroma compounds with apple like flavour (character impact aroma substances) are hexanal, 2-hexenal, ethyl propionate, ethyl butyrate, butyl acetate, ethyl 2-methylbutyrate, hexyl 2-methylbutyrate (11,16,23,25). A synthetic apple juice odour containing three alcohols butanol, 2-methylbutanol, 1-hexanol), three aldehydes (hexanal, hexenal, benzaldehyde) and three esters (ethyl butyrate, pentyl acetate, ethyl 2-methylbutyrate) has been developed (7).

Apple juice is processed either by pressing or by extraction followed by pasteurization. Either before pressing or after extraction enzyme treatment often is carried out. During processing the primary aroma substances present in the raw apples are suspect to changes during processing. Also secondary aroma substances may arise mainly due to enzyme catalyzed processes.

The aim of this paper is to study the changes of aroma substance concentrations during processing of apple juice and to evaluate differences between juice obtained by pressing and by extraction.

Materials and methods

Raw material

Mature fruits of the apple cultivars 'Aroma', 'Cox Orange', 'Elstar', 'Ingrid Marie', 'Jonagold', and 'Spartan' were harvested at the Department of Pomology. Apples of each variety were picked at the optimum harvest time and stored at 3°C until processing.

Processing

3 kg of apples were cut into cubes (0.5·0.5·0.5 cm) immediately before processing.

The pressing was carried out by use of a Tincture Press. Samples of 240 g of raw apple cubes were pressed by increasing the pressure linearly to 200 bar within one hour. With the aim of obtaining a possibility for comparison of samples from extraction the juices processed from pressing of 240 g of apples were diluted to 2000 g with water.

Extraction was carried out by use of glass jars with 240 g of distilled water equilibrated to 60°C in a water bath. For each experimental level 240 g of apples were successively extracted several times with fresh water at 60°C. All the extraction liquids were combined and diluted to 2000 g with water.

Aroma solutions were processed by vacuum evaporation of 400 g of liquid from 2000 g diluted juice or extract. The evaporation was carried out at 45°C by use of a Rotavapor with the distillation flask in a water bath at 70 °C and the condenser cooled to 0°C by circulating cooling liquid. By this procedure with evaporation of 20 w/w% of the liquid all aroma substances are transferred to the distillate (18).

For each experimental level three samples of 100 g aroma solution were mixed with 1 ml 0.5 w/w% malic acid solution and pasteurized by heating for 15 minutes in a water bath at 85°C. After cooling of the jars in running tap water (13°C) they were stored at 3°C until analysis.

Analyses

For each experimental level three determinations of the content of aroma substances, soluble solids, and titratable acids was carried out. The content of soluble solids were determined by refractometry using a Bausch & Lomb refractometer. The content of titratable acids was determined by titration with 0.1000N NaOH to pH 8.1 using an automatic titrator (Mettler DL 40).

Equipment and the settings for the determination of aroma substances are shown in Table 1. A standard solution (see Table 2) was applied for determination of the retention times (Rt). The response factors were determined by use of solutions containing 1 ml of the standard aroma substance solution, diluted with 9 ml water. 200 microliter internal standard solution containing 5000 ppm cyclohexanone were applied.

Table 1. Equipment and settings for determination of the aroma substances in aroma solutions.

Analyseudstyr og indstillingen af dette ved bestemmelse af aromastofindholdet i aromaopløsninger.

Hewlett Packard 7675A head space sampler:

Sample size	10 ml
Prepurge time	5 min
Purge gas	N ₂ 30 ml/min
Purge time	10 min
Precolumn	Tenax GC
Precolumn temperature	20°C
Eluting temperature	200°C
Eluting time	5 min
Cleaning temperature	250°C
Cleaning time	5 min

Hewlett Packard 5840A gas chromatograph:

Column	6 m stainless steel 1/8 inch i. d.
Carrier material	80/100 mesh Chromosorb
Stationary phase	Carbowax 20 M, high temp.
Injector temperature	200°C
Carrier gas	N ₂ 20 ml/min
Column temperature	30–180°C
Rate	0.75°C/min for 100 min and then 1.5°C/min
Detector temperature	350°C
Paper velocity	0.25 mm/min
Slope sensitivity	From 0.5 to 6 micro-volt/sec.
Attenuator	2(EXP(6))
Internal standard	Cyclohexanon (50 µl/10 ml)

With a content of aroma substances of x micro-litre/5 l (Table 2) the amount of aroma substance (Y) in 10 ml standard solution was:

$$Y = 1000x/5000 = 0.2x \quad 1)$$

For the standard solution (IS=internal standard) and the diluted juices or extracts the response factors bs and b were determined:

$$bs = \frac{(\text{peak area of aroma substance} \cdot 1000)}{(\text{peak area of IS})} \quad 2)$$

$$b = \frac{(\text{peak area of aroma substance} \cdot 1000)}{(\text{peak area of IS})} \quad 3)$$

Because the sample of diluted juice or extract corresponded to m gram of raw apple the content (c) of aroma substances in the apples was calculated:

$$c = (0.2 \cdot bs \cdot 1000) / (m \cdot bs) \text{ } \mu\text{g/kg} \quad 4)$$

Experimental design

The design of experiment 1 encompassed three treatments:

- I. 120 g of apple cubes were mixed with 880 g of boiling water in a glass jar. After one hour and cooling of the jar in cold tap water 200 g distillate was produced by evaporation in the Rotavapor.
- II. 120 g of apple cubes were treated in a Waring blender with 880 g of cold water at 20°C and 200 g of aroma solution was produced by evaporation within 20 minutes after the mixing.
- III. 120 g of apple cubes were treated in a Waring blender with 880 g of cold water at 20°C and 200 g of aroma solution was produced after six hours.

The design of experiment 2 encompassed four treatments:

- I. Two pressings of 240 g of apple cubes were carried out using of the Tincture Press. The combined apple juices were diluted to 4000 g with water and 800 g of the liquid was evaporated of in the Rotavapor.
- II. The combined pomaces from the pressings were mixed with 2000 g of water treated in a Waring blender, then 400 g distillate was produced by distillation.
- III. Two series of eight successive extractions of 240 g apple cubes were carried out at 60°C by use of water. The 3840 g of extraction liquids were combined, diluted to 4000 g with water and 800 g of aroma solution was produced by distillation.
- IV. 480 g of apple cubes and 480 g water were treated in a Waring blender and diluted to 4000 g with water. 800 g aroma solution was then processed immediately by evaporation.

Experiment 3 encompassed processing of juice and extract of six apple cultivars: 'Spartan', 'Aroma', 'Elstar', 'Cox Orange', 'Ingrid Marie' and 'Jonagold'. For each sample of apple cubes four successive extractions were carried out.

In experiment 4 580 ml glass jars with 240 g apple cubes were kept in a water bath at 60°C for up to five hours. The content of aroma substances, soluble solids, and titratable acid in the filtrates was determined.

Experiment 5 was carried out as described for experiment four but the content of aroma substances in the distillates from the whole sample (apple+extraction liquid) were determined.

In experiment 6 the content of aroma substances in each of six successive extractions of 240 g of apple cubes were determined.

Results and discussion

Results from determination of retention time (Rt), and response factors by analyses of a standard solution is shown in Table 2, where x is the concentration of the aroma substances. The obtained standard deviations are very satisfactory because an average value of retention time or peak area normally is determined by average standard deviations $s(\text{avg}) = s/(\text{SQR}(n))$, where n is the number of replications.

The concentrations of ethyl acetate, 1-butanol, propyl acetate, butyl acetate, and hexyl acetate were high in 'Golden Delicious' (3). 'Cox Orange', 'Jonathan', and 'Golden Delicious' also has high concentrations of butyl acetate, pentyl acetate, hexyl acetate, 1-butanol and 1-hexanol (6,14). The cultivar 'Schone van Boskoop' is an alcohol type of apple with high concentrations of alcohols (ethanol, 1-propanol, 1-butanol, and 1-hexanol) and only one noteworthy ester (ethyl butyrate) (10).

The results from experiment 1 with and without enzyme inactivation is shown in Table 3. After

heating of the apples a low concentration of the aldehydes hexanal and 2-hexenal were found. This is because the enzyme lipoxygenase which after tissue disintegration normally catalyze the formation of aldehydes from unsaturated fatty acids (3,10,14,24) is inactivated by the heating. The aldehydes contribute with a fresh »green« note to the flavour of apple juice (11).

lipoxygenase
unsaturated fatty acids ----- > aldehydes 1)

As found earlier (19,26,29) the concentration of the esters and aldehydes decreased during the holding time after blending. These changes may be explained by biochemical processes catalyzed by esterases and alcohol dehydrogenase by which hydrolysis of esters and dehydrogenation of aldehydes respectively takes place (1,2,4,8,9,14,15, 19,29,31,34). The esters contributes with fruity notes to the flavour of apple juice (12,23).

esterases
esters ----- > alcohol + acid 2)

alcohol dehydrogenase
aldehydes ----- > alcohols 3)

Table 2. Retention time (Rt) and response factors (bs) from analyses of a standard solution containing 1 ml standard aroma solution, 9 ml water and 200 microliter of the internal standard solution. s(Rt) and s(bs) are standard deviations of retention time (Rt) and response factors (bs) respectively.

Retentionstid (Rt) og responsfaktorer (bs) fra analyse af en stamløsning af aromastoffer, der indeholder 1 ml aromaopløsning, 9 ml vand og 200 mikroliter af den interne standard opløsning. s(Rt) og s(bs) er standardafvigelse på henholdsvis retentionstid og responsfaktor (bs).

Substance Aromastof	conc. (x) µl/5l	Rt Min.	s(Rt) Min.	Response factor	
				bs	s(bs)
Ethyl 2-methylbutyrate	10	45.07	0.06	522.20	19.90
Butyl acetate	10	48.68	0.05	337.69	31.63
Hexanal	10	49.94	0.05	288.64	6.93
i-butanol	100	53.32	0.06	243.71	6.80
Amyl acetate	10	57.11	0.06	647.34	30.04
1-butanol	100	65.64	0.35	172.18	6.66
2-hexenal	110	75.45	0.19	1178.50	106.55
1-pentyl alcohol	100	82.72	0.45	221.03	18.45
Hexyl acetate	10	84.40	0.48	684.66	61.11
Cyclohexanone	-	89.40	0.17	-	-
1-hexanol	100	100.53	0.21	366.86	17.62
Hexyl 2-methyl butyrate	10	111.49	0.19	785.62	96.39

After 6 hours the concentration of butyl acetate, pentyl acetate, hexyl acetate and hexyl 2-methyl acetate had decreased to about half of the concentration in the aroma solution processed with enzyme inactivation. The concentration of hexanal and 2-hexenal decreased by 42 and 57% after 6 hours compared to the concentration obtained by processing immediately after blending. The concentration of 1-hexanol increased in the aroma solution processed after 6 hours. Enzyme catalyzed ester hydrolysis or alcohol dehydrogenation may be the explanation of these changes.

Table 4 shows the results from experiment 2. Except for 1-hexanol and hexyl 2-methyl butyrate the total content of aroma substances in the apples (IV) was higher than in anyone of the solutions processed by other methods. Apple pomace contained considerable amounts of important aroma substances (II).

Even by combination of the products obtained from pressing and processing of pomace a lower concentration was obtained for all aroma substances except 1-hexanol and hexyl 2-methyl butyrate (I+II).

By comparison to pressing a lower concentration of aroma substances was obtained by extraction. In apple juice stored without enzyme inactivation the concentration of esters and aldehydes decreased and the content of alcohols increased

Table 3. Results from experiment 1 with enzyme inactivation (I) and evaporation of aroma substances after 20 minutes (II) or 6 hours (III) without enzyme inactivation. Cultivar 'Jonagold'.

Resultater fra forsøg med enzyminaktivering (I) og aromastofdampning efter 20 minutter (II) eller 6 timer (III) uden enzyminaktivering. Sort 'Jonagold'. Forsøg 1.

Aroma substance <i>Aromastof</i>	$\mu\text{g/kg}$			
	Treatment <i>Behandling</i>			
	I	II	III	LSD
Butyl acetate	6528	5124	2772	504
Hexanal	0	7126	3017	621
Pentyl acetate	385	329	172	36
1-butanol	12429	10130	8729	1209
2-hexenal	3076	4945	2805	406
Hexyl acetate	2807	2441	1020	188
1-hexanol	2001	2729	3710	167
Hexyl 2-methyl butyrate	145	76	72	15

because of enzyme activities (32). Similar reactions may take place during extraction resulting in a lower concentration of esters and aldehydes and a higher concentration of alcohols as explained in details in the reaction schemes shown above.

Table 4. Results from experiment 2 with determination of the content of aroma substance concentration after pressing of raw apples (I), after evaporation of aroma substances in the pomace from pressing (II), in the extract from eight successive extractions (III) and in the raw untreated apples (IV). Cultivar 'Jonagold'.

Resultater fra forsøg 2 med bestemmelse af aromastofkoncentrationen efter presning af rå æbler (I), destillation fra presserester efter presning af rå æbler (II) fra ekstraktion (III) og i de rå, ubehandlede æbler (IV). Sort 'Jonagold'.

Aroma substance <i>Aromastof</i>	$\mu\text{g/kg}$				LSD
	Treatment <i>Behandling</i>				
	I Pressing juice <i>Pressesaft</i>	II Pomace <i>Pressek.</i>	III Extract <i>Ekstrakt</i>	IV Total <i>Total</i>	
Butyl acetate	2002	166	1660	5093	244
Hexanal	2190	1930	0	8182	493
Pentyl acetate	105	26	94	405	53
1-butanol	11612	1882	7081	15015	1473
2-hexenal	2083	782	104	6274	372
Hexyl acetate	544	223	347	2211	156
1-hexanol	3602	2869	2091	3159	339
Hexyl 2-methylbutyrate	49	113	38	92	23

A lower content of aldehydes and esters in extraction juices has been reported earlier (31). The content of alcohols was the same in the juices processed by pressing and extraction.

Table 5 shows the results from processing of juice by pressing and extraction of apples of six

cultivars. The content of all aroma substances was lower in the juice processed by extraction. The reason for this is probably the low (four) number of extraction steps. Lower amounts of carbonyls and esters, but higher contents of alcohols in extraction juice has been reported earlier (31).

Table 5. Content of aroma substances in juice after pressing (P) or extraction (E) of juice from six apple cultivars. Butyl acetate (BuAc), hexanal (Hex), pentyl acetate (PeAc), 1-butanol (BuOl), 2-hexenal (2Hex), hexyl acetate (HeAc), 1-hexanol (HeOl). Experiment 3.

Indhold af aromastoffer i saft fremstillet ved presning (P) eller ekstraktion (E) af seks æblesorter. Butyl acetate (BuAc), hexanal (Hex), pentyl acetate (PeAc), 1-butanol (BuOl), 2-hexenal (2Hex) hexyl acetate (HeAc), 1-hexanol (HeOl). Forsøg 3.

Cultivar	Method	$\mu\text{g}/\text{kg}$						
		BuAc	Hex	PeAc	BuOl	2Hex	HeAc	HeOl
'Aroma'	P	2658	3573	279	30433	6296	556	5598
»	E	1873	6	161	20911	864	412	3673
'Cox Orange'	P	9675	1641	127	14445	9422	1333	4465
»	E	3901	11	53	8964	18	855	1676
'Elstar'	P	6663	8381	132	11503	11293	1208	4542
»	E	2000	0	0	4832	0	635	1962
'I. Marie'	P	3523	3365	82	9047	13616	878	3134
»	E	2288	0	56	6734	364	447	3821
'Jonagold'	P	6283	2593	602	9543	8746	922	1833
»	E	1818	0	202	5360	52	500	1132
'Spartan'	P	1418	4367	450	11856	8196	620	2945
»	E	494	10	127	6245	188	354	2006
100 (E/P)% average		41	0.1	36	61	3	58	63

Table 6. Content of aroma substances, soluble solids and titratable acid in filtrates with treatment of apple cubes at 60°C in a water bath. Cultivar 'Jonagold'. Experiment 4.

Indhold aromastoffer, opløseligt tørstof og titrerbar syre i filtrater fra forsøg med forskellige holdetider ved ekstraktion. Sort 'Jonagold'. Forsøg 4.

Substance Aromastof	$\mu\text{g}/\text{kg}$						
	Holding time, hours Holdetid, timer						
	0.5	1	2	3	4	5	Avg.
Butyl acetate	2454	2212	2191	2102	2356	2222	2256
Hexanal	0	0	0	0	0	0	0
i-butanol	54	64	71	97	65	70	70
Pentyl acetate	53	43	46	47	27	43	43
1-butanol	2841	3048	2918	2911	2712	3772	3034
2-hexenal	7	6	57	73	27	15	31
Hexyl acetate	412	308	270	280	261	264	299
1-hexanol	818	1049	1020	1051	973	1276	1031
$\text{g}/100 \text{ g}$							
Soluble solids	3.0	4.5	5.0	5.7	6.4	6.2	5.8
Titratable acid	0.09	0.11	0.14	0.14	0.14	0.14	0.14

Results from determination of the content of aroma substances, soluble solids and titratable acid in filtrates from apple cubes stored in glass jars at 60°C are shown in Table 6.

Equilibrium between apples and liquid was obtained after 0.5 hour and the concentration of aroma substances did not change during the holding time. From this it may be concluded that the aroma substances are fairly stable at 60°C. By blanching of apples the biochemical system in the apple peel which produces esters is inactivated (15).

Hexanal was not detected and the concentration of 2-hexenal was very low. This indicates that lipoxygenase was inactivated at 60°C. The concentration of soluble solids and titratable acids in the filtrates increased with time until about 2 hours. Similar conclusions may be drawn from the results obtained by determination of the content of aroma substances in distillates from apple cubes and extraction liquid (Table 7).

Table 7. Effect of holding time on the content of aroma substances in distillates processed from samples of apple cubes kept in extraction liquid at 60°C for up to four hours. Cultivar 'Jonagold'. Experiment 5.

Virkning af holdetid under ekstraktion på indholdet af aromastoffer i destillater fra prøver af æbleterninger i ekstraktionsvæske på vandbad ved 60°C. Sort 'Jonagold'. Forsøg 5.

Substance Aromastof	µg/kg			
	Holding time, hours Holdetid, timer			
	0.5	1	3	4
Ethyl 2-methyl butyrate	5	3	2	6
Butyl acetate	3320	4192	3742	3793
Hexanal	1	18	26	66
i-butanol	153	149	122	135
Pentyl acetate	48	91	112	110
l-butanol	7393	7879	7368	8240
t-hexenal	67	44	59	45
l-pentanol	24	36	40	102
Hexyl acetate	1070	1339	1226	1293
l-Hexanol	2543	2700	2441	2765
Hexyl 2-methylbutyrate	24	35	33	47

Typical results from experiment 6 are shown in Figs 1, 2 and 3. The concentration of soluble solids, titratable acid, butyl acetate, pentyl acetate,

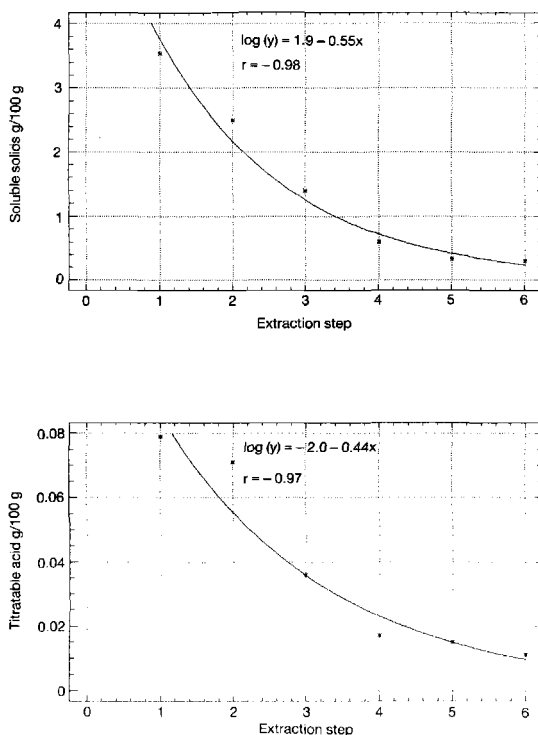


Fig. 1. Concentration of soluble solids and titratable acids in the extraction liquid as a function of the successive extraction steps.

Koncentration af opløseligt tørstof og titrerbar syre i ekstraktionsvæsken som funktion af antal ekstraktionstrin.

hexyl acetate, l-butanol and l-hexanol in the extraction liquid decreased with the number of extractions. Fig. 1 shows that all aroma substances may be extracted. This requires that the number of extraction steps is six to eight. Some of the low yield of aroma substances obtained by extraction in experiment 3 can be explained by the low number of extraction steps applied.

From Fig. 1 it is quite clear that as found earlier (30) considerable amounts of aroma substances remain in the apples after four extraction steps.

The results could be fitted by use of mathematical models as shown in Figs 1-3. The fitness may be evaluated by use of the correlation coefficients.

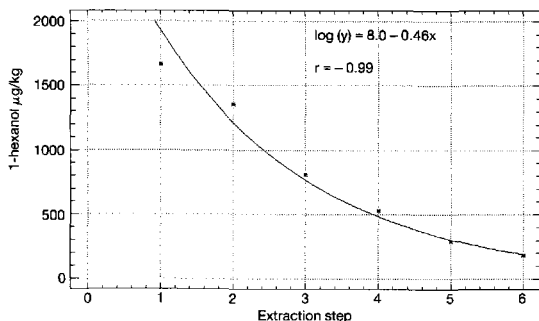
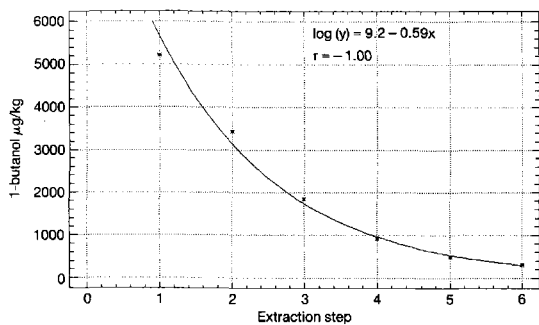


Fig. 2. Concentration of acetate esters in the extraction liquid as a function of the successive extraction steps.

Koncentration af eddikesyreestere i ekstraktionsvæsken som funktion af antal ekstraktionstrin.

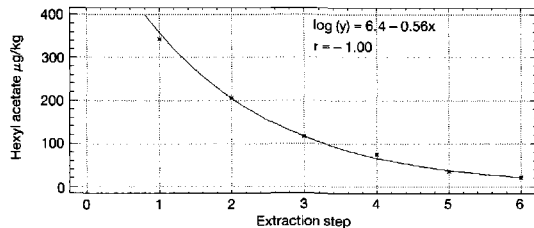
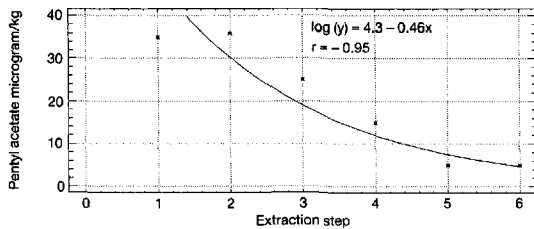
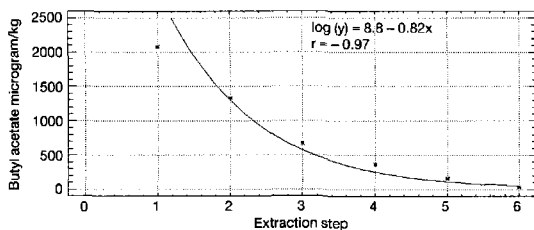


Fig. 3. Concentration of 1-butanol and 1-hexanol in the extraction liquid as a function of the number of successive extraction steps.

Koncentration af 1-butanol og 1-hexanol i ekstraktionsvæsken som funktion af antal ekstraktionstrin.

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