Hard Resins: The Complimentary Bitter Fraction Present in Hops, Pellets and Ethanol-Extract

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Wöllmer hop fractionation

soluble in methanol: total resins

soluble in hexane:
soft resins

approx. 90 % (w/w)

alpha-acids
beta-acids
humulinones
hulupones
?

insoluble in hexane:
hard resins

approx. 10 % (w/w)

xanthohumol
?
?
?
?

Recent publication on hard resin components

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Sensomics Analysis of Key Bitter Compounds in the Hard Resin of Hops (*Humulus lupulus* L.) and Their Contribution to the Bitter Profile of Pilsner-Type Beer

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Supporting Information

ABSTRACT: Recent brewing trials indicated the occurrence of valuable bitter compounds in the hard resin fraction of hop. Aiming at the discovery of these compounds, hop's ε-resin was separated by means of a sensory guided fractionation approach and the key taste molecules were identified by means of UV/vis, LC-TOF-MS, and 1D/2D-NMR studies as well as synthetic experiments. Besides a series of literature known xanthohumol derivatives, multifidol glucosides, flavon-3-on glycosides, and *p*-coumaric acid esters, a total of 11 bitter tastants are reported for the first time, namely, 1″,2″-dihydroxanthohumol F, 4′-hydroxytunicatachalcone, isoxantholupon, 1-methoxy-4-prenylphloroglucinol, dihydrocyclohumulohydrochinone, xanthohumols

J. Agric. Food Chem. 2015, 63, 3402-3418

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Investigations of Dresel et al.*

- ✓ sensory evaluation of the total hard resin fraction
- ✓ isolation of all the flavour active components
- ✓ identification of their chemical structures
- ✓ characterization of their sensory profiles (receptor tests)
- ✓ determination of their human threshold concentrations
- ✓ recombination experiments to verify their taste contributions
- ✓ quantification in different hop products and beers

** J. Agric. Food Chem. 2015, 63, 3402-3418*

Components of the hard resin fraction*

component	(bitter) taste threshold (mg/l)	concentration in a beer / example (mg/l)
xanthohumol	**3.5**	**< 0.1**
isoxanthohumol	5.6	1.9
desmethylxanthohumol	**5.4**	**< 0.1**
8-prenylnaringenin	2.8	0.1
6-prenylnaringenin	**3.5**	**0.1**
xanthohumol B	9.2	< 0.1
xanthohumol C	**2.1**	**< 0.1**
dihydro-xanthohumol C	2.8	-
dihydro-isoxanthohumol C	**2.1**	**< 0.1**

X 0.3

…. 19 additional taste active prenylflavonoids were identified

** Dresel et al., 2015*

Other hard resin single components*

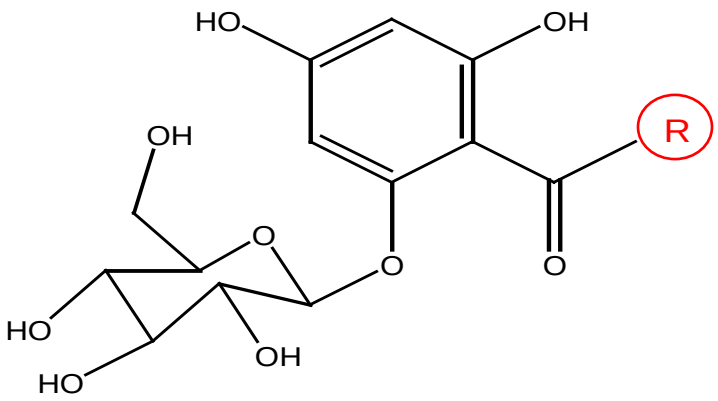
component	(bitter) taste threshold (mg/l)	concentration in a beer / example (mg/l)
coumaric-acid	**3.6**	**< 0.1**
coumaric-acid ester	**35**	**< 0.1**
n-trans-feruloyltyramine	**3.1**	**0.2**
glycosidically bound polyphenols:		
quercetin-glucoside	**13.0**	**1.6**
kaempferol-glucoside	**13.0**	**5.3**
kaempferol-malonyl-glucoside	**18.7**	**0.2**
co-multifidol-glucoside	**1.8**	**1.4**
ad-multifidol-glucoside	**3.7**	**0.3**

X 0.8

especially co-multifidol-glucoside seems to be relevant for bitter taste

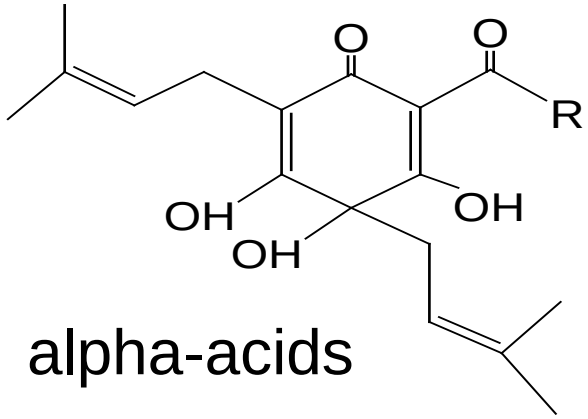
** Dresel et al., 2015*

Multifidol-glucosides



R=	$CH_2CH(CH_3)_2$	n-
	$CH(CH_3)_2$	co-
	$CH(CH_3)CH_2CH_3$	ad-

discovery in hops and structure identification: Bohr et al., 2005



alpha-acids

multifidol-glucosides are intermediate products of the alpha-acids biosynthesis

Conclusions from the paper of Dresel et al.

- ✓ abstract: "… additive contribution of iso-alpha-acids and the identified hard resin components to be truly necessary and sufficient for constructing the authentic bitter percept of beer"

- ✓ therefore, hop products containing hard resins are preferable (i.e. pellets, ethanol-extract)

Components of hard resins in hop products*

component	pellet	ethanol-extract	carbon dioxide extract
xanthohumol	**+++**	**+++**	**-**
other prenylflavonoids	**+++**	**+++**	**-**
multifidol-glucosides	**+++**	**++**	**-**
other glycoside bound polyphenols	**+++**	**+**	**-**

some components are only partially transferred to ethanol-extract

** Dresel et. al, 2015*

Questions for consideration

- ✓ is ethanol-extract an equivalent replacement for pellets?
- ➢ direct comparison of both hop products in brewing trials

- ✓ are there any (potential) negative aspects of hard resins?
- ➢ brewing trial with a hop extract strongly enriched in hard resins

Our own investigations: trial design

- ✓ production of beers with different hop products
- ✓ one single hop addition at start of boil (60min)
- ✓ goal: 20 ppm iso-alpha-acids (lager beer)
- ✓ 6 hl scale (Technical University Munich-Weihenstephan)
- ✓ each hop product was tested in duplicate
- ✓ special analysis using LC-MS/MS
- ✓ sensory: two trained panels (TU and Hopsteiner)

Production of a hard resin enriched extract

ethanol-extract

↓

secondary extraction with supercritical CO_2

↙ ↘

CO_2-extract:

- total soft resins
  - ✓ alpha- and beta-acids
  - ✓ further (unspecified) components
- essential oil

extraction residue:

- total hard resins
  - ✓ prenylflavonoids
  - ✓ glycosides
- residual (iso-)alpha

Composition of the hop products*

all products from the variety Hall. Taurus, crop 2013

%	pellet type 90 P90	ethanol-extract EX		hard resin extract EXH
iso-alpha-acids	**-**	**1.7**		**8.2**
alpha-acids	**14.9**	**42.4**		**5.3**
cohumulone (% rel.)	**24.4**	**25.9**		**23.1**
beta-acids	**4.7**	**13.1**		**0.5**
xanthohumol	**0.8**	**2.7**	→	**10.6**
total resins	**29.7**	**89.3**	**X 3.5**	**100**
hard resins (% rel.)	**11.5**	**12.5**	→	**39.2**
total oil (ml/100 g)	**1.4**	**4.1**		**-**

** according to (modified) methods of Analytica-EBC*

Dosage of the hop products

per hl, at begin of wort boiling

trial nr.	product	g	g alpha	g hard resins	g xanthohumol
1	P90	46.2	6.9	1.6	0.42
2	P90	46.2	6.9	1.6	0.42
3	EX	13.5	6.0	1.4	0.37
4	EX	13.5	6.0	1.4	0.37
5	EXH	30.8	4.2	12.1	3.9
6	EXH	30.8	4.2	12.1	3.9

X 9

exceptionally high hard resin dosage with hard resin extract!

Results of beer analysis* -1-

bitter compounds (HPLC-UV)

trial /	product	bitter units	iso-alpha (mg/l)	alpha (mg/l)
1	P90	19.5	18.9	3.2
2	P90	19.5	19.1	2.2
3	EX	17.8	18.3	1.3
4	EX	18.0	17.0	2.6
5	EXH	25.9	16.8	1.2
6	EXH	27.2	17.7	1.1

very consistent iso-alpha concentrations: solid basis for sensory analysis

** according to methods of Analytica-EBC*

Analysis methods for lead components of the hop hard resin fraction

- ✓ xanthohumol / isoxanthohumol
  - ➢ analysis by HPLC-UV (Hopsteiner in-house method)
- ✓ co-multifidol-glucoside, quercetin- and kaempferol-glucoside
  - ➢ analysis by LC-MS/MS (Hopsteiner in-house method)

Results of beer analysis -2-

single components from hard resin fraction (mg/l)

trial	product	xanthohumol	isoxanthohumol	co-multifidol-glucoside
1	P90	< 0.1	0.4	0.6
2	P90	< 0.1	0.5	0.7
3	EX	< 0.1	0.5	0.3
4	EX	< 0.1	0.5	0.3
5	EXH	0.1	3.6	2.1
6	EXH	0.2	4.0	1.8

X 7 X 7

verification: successful transfer of single components from hard resins

Results of beer analysis -3-

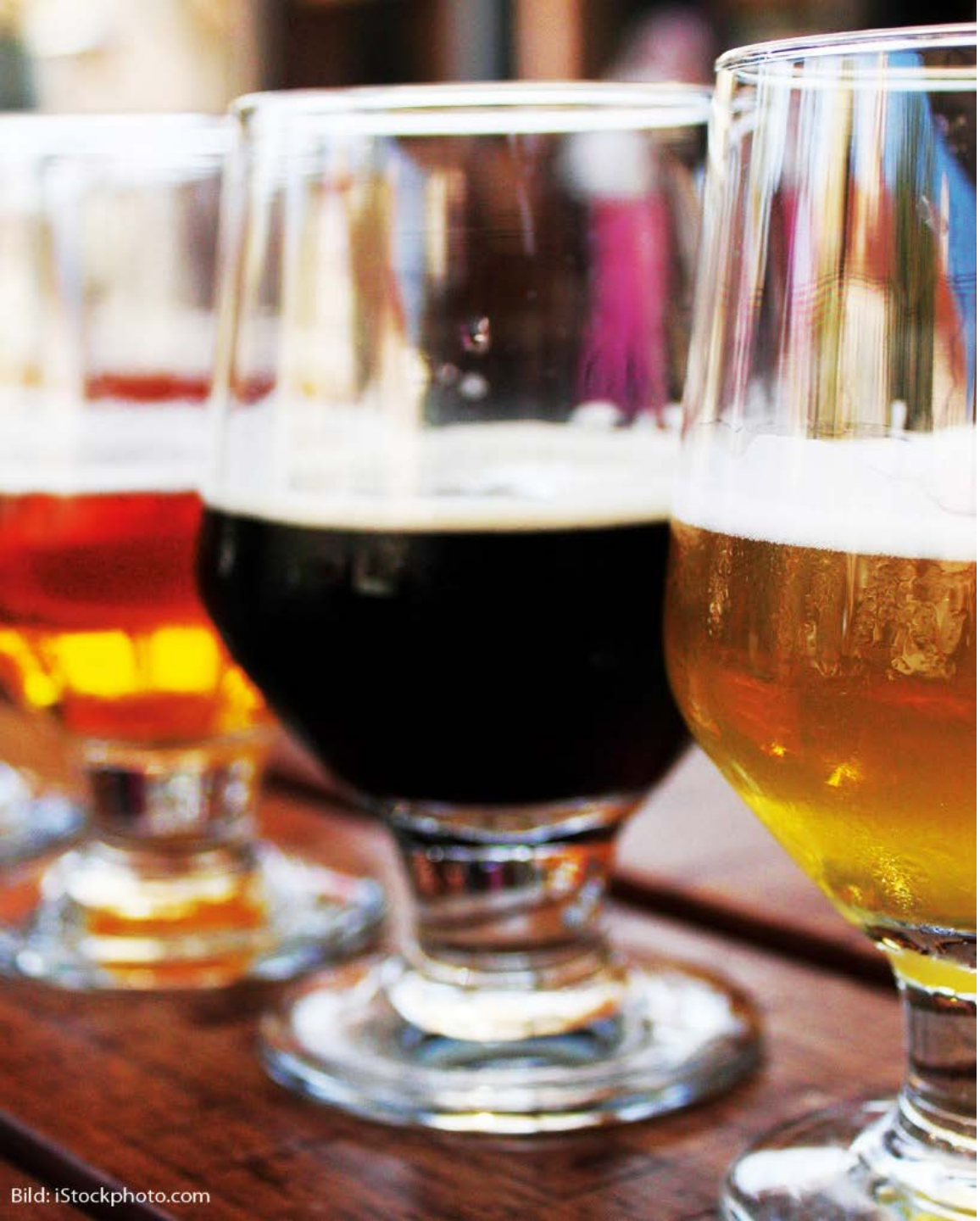
single components from hard resin fraction (mg/l)

trial	product	co-multifidol-glucoside	quercetin-glucoside	kaempferol-glucoside
1	P90	0.6	0.3	0.1
2	P90	0.7	0.3	0.1
3	EX	0.3	< 0.1	< 0.1
4	EX	0.3	< 0.1	< 0.1
5	EXH	2.1	0.3	0.1
6	EXH	1.8	0.3	0.1

X 2

X > 3

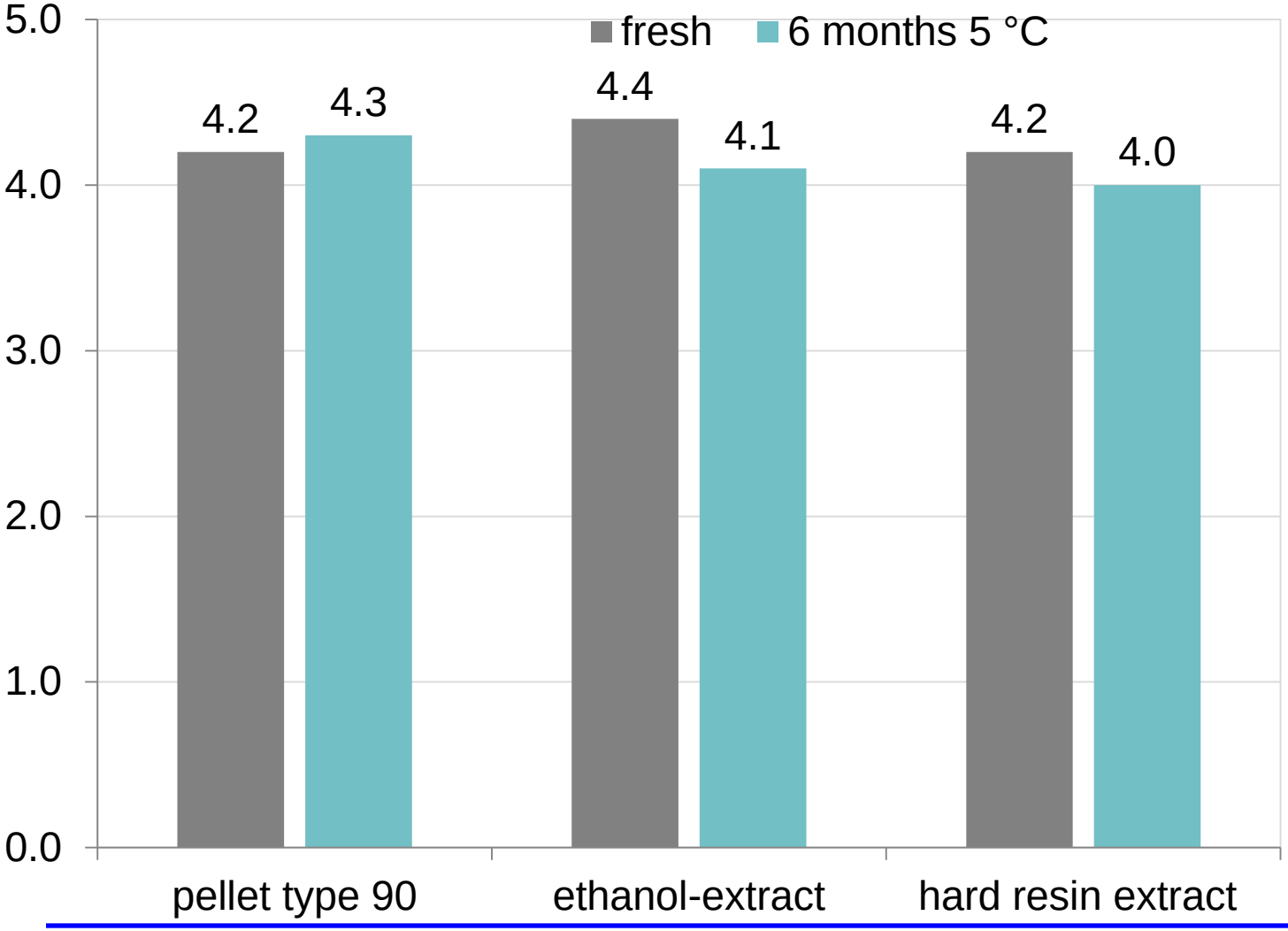
verification: low concentrations of quercetin/kaempferol-glucosides via EX



Tasting results

Ranking

DLG* Tasting Panel at Technical University



** DLG = Deutsche Landwirtschaftsgesellschaft*

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Duo-Trio Test

Mebak – Methods of Sensory Analysis, 2014

3.1.2 Duo-Trio Test (According to DIN EN ISO 10399:2010-06)

Application, Purpose

This method can be employed to reveal slight differences between two samples (water, wort, beer), e.g. type and expression of specific attributes or the overall impression.
This method is especially appropriate for a trained tasting panel, if a known product can be employed as a control sample (e.g. beer from a current production run). With an untrained tasting panel and/or unknown samples, one of the pair of samples is used as a control sample. With samples possessing a lingering aftertaste, the paired comparison is more suitable.

Principle

The uncoded sample is first placed before a minimum of nine, but preferably 20 or more, members of a tasting panel. Afterwards, two coded samples are given to the tasting panel, one of which is identical to the control sample. The tasting panel is asked to identify which of the two samples is different from the first one.

Procedure

- prepare the same number of each sample in the several possible combinations and by drawing lots, distribute them among the members of the tasting panel
- distribute reference samples and both test samples among the members of the tasting panel
- taste the samples from left to right; repeated sampling is permitted
- record results in the tasting form

Example

Evaluation Form			
Name:		Date:	
Test Sample:			
Purpose:	Please decide whether the samples 419 and 284 differ from sample 328 (reference sample); if necessary, guess which sample is different.		
	328	419	284

MEBAK – Sensory Analysis – 3 Methods of Sensory Analysis

Evaluation

Determine the number of correct answers; disregard answers of "no difference". A difference in the samples is detectable by the members of the tasting panel (significant), if the number of correct answers with respect to the total number of answers is greater than or equal to the value in table 3 at a specified level of significance α (minimum).

Table 3: Significance with a one-tailed paired comparison tests [a)] (p = 0.50 with n answers) – according to DIN EN ISO 5495:2007

Number of Answers	Minimum number of right or yes answers at a significance level of α = 0.05	α = 0.01	α = 0.001
7	7	7	-
8	7	8	-
9	8	9	-
10	9	10	10
11	9	10	11
12	10	11	12
13	10	12	13
14	11	12	13
15	12	13	14
16	12	14	15
17	13	14	16
18	13	15	16
19	14	15	17
20	15	16	18
21	15	17	18
22	16	17	19
23	16	18	20
24	17	19	20
25	18	19	21
30	20	22	24
35	23	25	27
40	26	28	31
45	29	31	34
50	32	34	37
60	37	40	43
70	43	46	49
80	48	51	55
90	54	57	61
100	59	63	66

Duo-Trio Test

- ✓ This method can be employed to reveal slight differences between two samples (e.g. expression of overall impression)
- ✓ One (uncoded) sample is first placed, afterwards two coded samples are given to the tasting panel, one of which is identical to the uncoded sample
- ✓ The tasting panel (preferable 20 or more members) is then asked which of the two samples is different from the first one

Duo-Trio-Test* with fresh beers

✓ beer **A (P90)** distinguishable from beer **B (EX)**?

fresh beers	Hopsteiner / Technical University: 22 panelists
correct assignment	15 panelists
significant difference*	no
preference (A / B / no)?	6 / 9 / 0

** MEBAK – Methods of Sensory Analysis, 2014*

Duo-Trio-Test* with fresh beers

✓ beer **A (EX)** distinguishable from beer **B (EXH)**?

fresh beers	Hopsteiner / Technical University: 22 panelists
correct assignment	15 panelists
significant difference*	no
preference (A / B / no)?	8 / 4 / 3

** MEBAK – Methods of Sensory Analysis, 2014*

Conclusions of our investigations

- ✓ is ethanol-extract an equivalent replacement for pellets?
  - ➢ yes
- ✓ are there any (potential) negative aspects of hard resins?
  - ➢ no

Last but not least …

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Original article

American India Pale Ale matrix rich in xanthohumol is potent in suppressing proliferation and elevating apoptosis of human colon cancer cells

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Summary American India Pale Ales (IPAs) with and without addition of dark/roasted malts (DRM) and dry hopping (DP) were analysed to determine whether these processes will increase total phenolic content (TP), antioxidant capacity (AA) and the levels of bioactive compounds (xanthohumol, XN and isoxanthohumol, IX). In addition, bioactivity of whole beer matrices, that is, the 'phytochemical team approach,' was compared to isolated compounds, the 'silver bullet approach,' by measuring antiproliferative and pro-apoptotic properties using HCT 116 human colon cancer cells. DP and addition of DRM elevated the XN, IX, TP and AA. Dark malts reduced losses in XN and TP due to filtration. Xanthohumol content

components from hard resin fraction improve the positive image of beer