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The effect of pasteurisation and storage on aroma compounds in lager

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Abstract

Why was the work done: To investigate the impact of pasteurisation and storage in bottle on aroma compounds in pale lager beer.
How was the work done: Pale lager beer was produced at an industrial scale with 100% pilsner malt and a bottom fermenting yeast. Samples were taken of unpasteurised beer from bright beer tank, after flash pasteurisation and six months after packaging in amber glass bottles.
What are the main findings: Post pasteurisation, a marked increase was found in the concentration of 2,3-pentanedione (50%) and diacetyl (33%), presumably reflecting the decomposition by heat of precurser acetohydroxy acids. There was also a marginal increase in dimethyl sulphide (6%) with little or no change in other aroma compounds. Storage for six months in bottle, also resulted in an increase in the level of 2,3-pentanedione, diacetyl and dimethyl sulphide. The linear (Pearson) correlation was > 0.8 for both dimethyl sulphide and diacetyl, and 2,3-pentanedione and diacetyl. Accordingly, it was concluded that the levels in beer of dimethyl sulphide and 2,3-pentanedione are proportionally related to diacetyl.
Why is the work important: This work provides an insight into the effects on flavour and aroma of lager of flash pasteurisation and subsequent storage in bottle. The inter-relationship between aroma compounds in beer suggests that such synergies may undermine the sensory perception of threshold levels and identification of specific aromas.

Keywords:

aroma compounds, bottled beer, unpasteurised beer, pasteurisation, malt pale lager beer, storage

Introduction

Beer has a long history dating back to at least 5000 BC (De Gaetano et al. 2016). It is the most popular alcoholic beverage globally and the third most popular drink after water and tea (Salanță et al. 2020). The quality of beer over its shelf life has become an important issue, due to the complexity of beer composition and external environmental factors. Beer is composed of more than 800 chemical compounds derived from raw materials (malt, yeast, water and hops), with many formed during maturation and storage (Meilgaard 1982). The physicochemical properties of beer are constantly changing over time (Vanderhaegen et al. 2003; Jaskula-Goiris 2018; Gagula et al. 2020). The instability of beer flavour is a ongoing concern in the brewing industry and a limiting factor in extending product shelf life (Mascia et al. 2016), as changes in aroma compounds have negative effects on product quality. In addition, beer is susceptible to changes in sensory characteristics during its shelf life, caused by storage, transportation, and serving conditions (Jaskula-Goiris et al. 2018; de Lima et al. 2023; Gagula et al. 2023). The reaction of consumers to the flavour and aroma of beer is important both for acceptance and demand for a particular style in the market (Lerro et al. 2020).

The objective of this study was to compare the content of aroma compounds (acetaldehyde, dimethyl sulphide, ethyl acetate, diacetyl, propanol, 2,3 - pentanedione, isobutanol, isoamyl acetate, isoamyl alcohol, higher alcohols, and esters) in unpasteurised beer from bright beer tank, after pasteurisation and in bottled beer during storage over six months.

The main idea in this research is that changes in the aroma compounds in beer are influenced by pasteurisation. This was determined by comparing the content of the compounds in beer from the bright beer tank and in beer from the bottles immediately after the flash pasteurisation. Two hypotheses were tested. The first was that there are differences in aroma compounds in beer from bright beer tank and in bottled beer. The second was that there is a correlation between some aroma compounds, leading to a predictable paired change.

Materials and methods

Brewing, fermentation and bottling

Malt pale lager beer was produced with 100% pilsner-type malt (wort 11.47°P) and a bottom fermenting yeast *Saccharomyces pastorianus* (Saflager^{TM} W-34/70, Fermentis) pitched at 0.75 kg per hL wet weight (1400 hL wort). During the fermentation in a cylindrical-conical tank (total volume 1700 hL) the pressure increased from 20 kPa to 100 kPa. Primary fermentation was for 12 days at 12.5°C. Lagering (cold stabilisation) was for a week in a tank (Cr/Ni steel; 1500 hL) at -1°C. The beer was filtered (Kieselguhr vertical filter; capacity 300 hL/h) into a bright beer tank (Cr/Ni steel; 1080 hL), flash pasteurised (capacity: 250 hL/h) at 24 PU (a temperature of 72°C and a retention time of 27 seconds) and bottled (nominal capacity 50,000 bottles/h) into amber glass bottles (0.50 L with crown cap).

Returnable glass bottles were cleaned with 1.8% (w/v) sodium hydroxide and disinfected with 1% (v/v) chlorinated water. After rinsing, microbiological testing and pH test showed satisfactory results. The European Brewery Convention (EBC) Technology and Engineering Forum (1995) recommend a range of PUs for pilsner and lager beer: a minimum of 15 and a maximum of 25 PU.

Bottled beer was stored at a temperature of 20 ± 1°C, in the absence of sunlight and UV radiation. The typical time between analyses was 29 days, with deviations in the frequency caused by non-working days in a month.

Analysis of beer

The alcohol content (by volume) and CO_2 content in beer were determined using methods 2.9.6.2 and 2.26.1 (MEBAK 2013). Dissolved oxygen, headspace air and total package oxygen (TPO) in bottle were determined immediately after packaging using methods 9.37, 11.2.1, and 11.5.2 (European Brewery Convention 2010). Volatile compounds (Table 1) were analysed (EBC 9.39 and 9.24.2) in beer from bright beer tank, after bottling and monthly thereafter for six months.

Table 1.

Analysis of aroma compounds in beer.

Compound	Abbreviation	Unit	EBC Method
acetaldehyde	ACE	mg/L	9.39
dimethyl sulphide	DMS	µg/L	9.39
ethyl acetate	EAC	mg/L	9.39
diacetyl	DIA	µg/L	9.24.2
propanol	PRO	mg/L	9.39
2,3-pentanedione	PEN	µg/L	9.24.2
isobutanol	IBU	mg/L	9.39
isoamyl acetate	IAC	mg/L	9.39
isoamyl alcohol	IAA	mg/L	9.39
higher alcohols	HA	mg/L	9.39
esters	EST	mg/L	9.39

EBC Method 9.39 - Dimethyl sulphide and other lower boiling point volatile compounds in beer by gas chromatography (European Brewery Convention, 2010)

EBC Method 9.24.2 - Vicinal diketones in beer: gas chromatographic method - (European Brewery Convention, 2010)

Aroma compounds were determined by gas chromatography (Perkin Elmer Clarus 580 Waltham, MA, USA) coupled with a head space sampler (Turbomatrix 40. Perkin Elmer, USA), a flame ionisation detector (FID) and electron capture detector (ECD). A capillary analytical column (Elite-WAX ETR, 60 m x 0.32 mm, 0.5 µm) was used, with a calibration curve performed for each run (R^2 = 0.99). Operating conditions were: oven temperature at 75°C for 9 min, increased to 110 °C at the rate of 25°C/min, and held for 4 min; FID detector temperature was 250°C and ECD detector temperature was 190°C. Beer samples (5 mL) were added to vials (10 mL), closed immediately, and analysed with the data integrated with Total Chrom VS6.3.2. software. The results are the average of two samples. Microsoft® Excel 2013 was used for the data analysis.

To exclude the possible influence of microbiological contamination on the concentration of aroma compounds, microbiological analyses were performed according to Analytica Microbiologica (European Brewery Convention 2005) as follows: general aerobic count (4.3.2.1), general anaerobic count (4.3.2.2), and *Lactobacillus* and *Pediococcus* (4.2.4.2). These analyses confirmed that there was no microbiological contamination, thereby eliminating any concern as to the integrity of the data.

Results and discussion

Unpasteurised and pasteurised pale lager

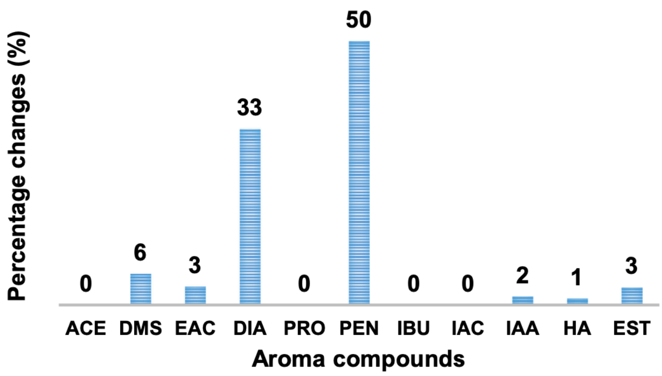
Heat treatment using pasteurisation is a widely applied and effective method for assuring the microbiological safety of beverages packaged in glass bottles (Horn et al. 1997). Beer is typically filtered and pasteurised to remove primary yeast and other microrganisms so as to stabilise the beer (Humia et al. 2019).

The beer in this work had an original extract of 11.47°P, 4.72% ABV and 5.30 g/L CO_2. Acceptably low values were found for dissolved oxygen (50 µg/L), headspace air (0.21 mL) and total packaged oxygen (TPO) (72 µg/L). This is important as oxygen causes a deterioration of flavour of packaged beer (Vanderhaegen et al. 2006).

The aroma compounds in bottled beer after pasteurisation and after six months of storage are presented in Table 2. The change of aroma compounds before and after pasteurisation is reported as a percentage (Figure 1). Marked changes were found after pasteurisation with an increase in 2,3-pentanedione (50%) and diacetyl (33%) presumably reflecting the decomposition of precurser acetohydroxy acids (Wainwright 1973). Dimethyl sulphide increased marginally (6%) with little or no change in the other aroma compounds.

Figure 1.

Aroma compounds pre- and post pasteurisation.



ACE - acetaldehyde, DMS - dimethyl sulfide, EAC - ethyl acetate, DIA - diacetyl, PRO - propanol, PEN - 2,3-pentanedione, IBU - isobutanol, IAC - isoamyl acetate, IAA - isoamyl alcohol, HA - higher alcohols, EST – esters

Table 2.

Aroma compounds in beer - unpasteurised, post pasteurisation and during storage over six months

Time of analysis	ACE	DMS	EAC	DIA	PRO	PEN	IBU	IAC	IAA	HA	EST
	mg/L	µg/L	mg/L	µg/L	mg/L	µg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Unpasteurised beer											
Immediately before pasteurisation	1.7	51.0	8.6	9.0	12.0	6.0	10.0	0.5	63.0	85.0	9.1
Pasteurised beer											
Immediately after packaging	1.7	54	8.9	12.0	12.0	9.0	10.0	0.5	64.0	86.0	9.4
Storage - Month 1	2.5	61	8.5	16.0	12.0	13.0	9.0	0.5	63.0	84.0	9.0
Storage - Month 2	2.7	60	8.5	14.0	12.8	10.1	9.7	0.4	63.9	87.0	8.9
Storage - Month 3	2.7	85	8.6	25.0	12.0	16.0	10.0	0.5	64.0	86.0	9.1
Storage - Month 4	2.8	69	8.7	19.0	13.0	16.0	10.0	0.5	65.0	88.0	9.2
Storage - Month 5	2.7	64	8.9	23.0	13.0	20.0	10.0	0.5	64.0	87.0	9.4
Storage - Month 6	2.1	65	8.4	20.0	16.0	16.1	9.8	0.5	62.2	88.0	8.9
Avg	2.5	66	8.6	18.4	13.0	14.3	9.8	0.5	63.7	86.6	9.1
SD	0.40	9.8	0.21	4.66	1.42	3.85	0.37	0.02	0.89	1.40	0.21
CV %	16.3	14.9	2.4	25.3	10.9	26.9	3.8	4.6	1.4	1.6	2.3

Avg - average (pasteurised beer), SD - standard deviation (pasteurised beer), CV - coefficient of variation (pasteurised beer)

Aroma compounds. ACE - acetaldehyde, DMS - dimethyl sulphide, EAC - ethyl acetate, DIA – diacetyl, PRO - propanol, PEN - 2,3-pentanedione, IBU - isobutanol, IAC - isoamyl acetate, IA - isoamyl alcohol, HA – higher alcohols, EST – esters

Pasteurised lager during six months storage in bottle

Clapperton (1976) showed that visible haze formation in beers with high headspace air contents occurred after six to eight weeks of storage and that excessive contact of beer with air must be avoided because of the risk of flavour deterioration.

After pasteurisation and over six months of storage in bottle showed a coefficient of variation of more than 20% for diacetyl and 2,3-pentanedione. This is linked to pasteurisation as well as storage in glass bottles. Gagula et al (2023) reported an almost two-fold increase in diacetyl over a six months in glass bottles and stainless steel kegs.

Diacetyl (2,3-butanedione) and pentanedione (2,3-pentanedione) or 'vicinal diketones' (VDKs) are produced by amino acid metabolism during fermentation. Both α-acetolactic acid and α-acetohydroxybutyric acid leak from the yeast cell and are spontaneously decarboxyated forming diacetyl and 2,3-pentanedione in the fermenting wort (Ferreira and Guido 2018). Diacetyl and 2,3-pentanedione are taken up and reduced by yeast to acetoin and 2,3-pentanediol, compounds with higher flavour thresholds during fermentation and maturation (Olaniran et al. 2017). Above their flavour threshold, VDKs are responsible for off-notes contributing 'butter' and 'honey' aromas (Dzialo et al. 2017).

Recommended values for free diacetyl in lager beer range up to 25 µg/L (via gas chromatography) with vicinal diketones (via distillation) up to 100 µg/L. High CO_2 pressure in fermenter results in reduced production of higher alcohols and esters but higher formation of diacetyl (Knatchbull and Slaughter 1987). The concentration of 2,3-pentanedione was found to be unstable during storage with a tendency to increase Table 2). The increase of diacetyl in packaged beer has been reported previously (Pavlečić et al. 2012) as increasing with pasteurisation, age and in the presence of oxygen.

Aldehydes are formed during the brewing process, notably during malting and mashing (Moreira et al. 2022). During fermentation, aldehydes increase to a peak value and then decrease at the end of fermentation (Liu et al. 2018). Moreira et al (2022) also reported that the concentration of aldehydes of relevance to the ageing process increase throughout storage. These aroma compounds are generally considered as off-flavours (Rossi et al. 2014; Marconi et al. 2016). The presence of acetaldehyde above the threshold (10-20 mg/L) results in pronounced aroma (Lodoloet al. 2008; Olaniran et al. 2017) reported as 'green grass', 'apple' and 'ribes-like' flavours (Lehnhardt et al. 2018). Table 2 showed a coefficient variation of concentration for acetaldehyde as 16.3% indicating a change in concentration during storage.

Dimethyl sulphide is a sulphur compound with the usual values in the beer range of 14-144 µg/L and a sensory threshold of 30-45 µg/L (Meilgaard 1982). The measurements showed a coefficient variation of dimethyl sulphide concentration of 14.9%, which is in agreement with the findings by Peppard (1978), who reported that the reaction between methane sulphonic acid and hydrogen sulphide leads to the production of dimethyl sulphide during beer storage.

Esters are formed during primary fermentation by the enzymatic chemical condensation of organic acids and alcohols (Pires et al. 2014; Schneiderbanger et al. 2016). The presence of different esters is a positive flavour attribute in fresh beer, which can have a synergistic effect on beer flavour acting below their individual threshold concentrations (Meilgaard 1975). Table 2 showed an average ester concentration of 9.1 mg/L with little change during the six months of storage based on a coefficient variation of 2.3%.

Ethyl acetate is important to the aroma of estery wheat beers (Schneiderbanger et al. 2016). Concentrations of ethyl acetate in beer above the flavour threshold (> 160 mg/L) are characterised as ‘solvent’ or ‘nail polish’ (Holt et al. 2019). During storage, the concentration of some esters can drop below the threshold values with the result that the fruity flavour of the beer is diminished (Schneiderbanger et al. 2016). The results reported here showed a low coefficient of variation of 2.4% (Table 2), which may be explained by the absence of wheat in the grist. The average value of 8.6 mg/L of ethyl acetate is below the threshold level of 21-30 mg/L (Verstrepen et al. 2003) or 25-30 mg/L (Pires et al. 2014).

The threshold of isoamyl alcohol in beer is 50-65 mg/L (Pires et al. 2014) and gives 'banana-like', 'wine', and 'alcoholic' flavours to beers (Hughes and Baxter 2001; Preedy 2011; Pires and Brányik 2015; Olaniran et al. 2017). If the content of amyl alcohol increases, the flavour of beer is described by sensory analysis as ‘heavier’ (Kucharczyk et al. 2020). Based on the results in Table 2, concentrations of isoamyl alcohol were in the above the threshold level, but stable during pasteurisation or storage.

The ester isoamyl acetate was stable during beer storage at a value below the threshold of 0.6-1.2 mg/L (Verstrepen et al. 2003; Kobayashi et al. 2008) or 1.2-2 mg/L (Pires et al. 2014). The ester was stable over six months of storage with a coefficient of variation of 4.6%. This is in agreement with the observations of Gagula et al (2020), in a study over six months of storage of beer in various packaging formats. Where isoamyl acetate is above the threshold level in beer it is reported as being fruity-fresh with ‘banana’, or ‘peardrop’ aromas (Olaniran et al. 2017; Verstrepen et al. 2003).

The quality and aroma of beer are affected by the content of higher alcohols and esters (Cui et al. 2021), and the combination of esters with higher alcohols is crucial to producing balanced beers of good quality (Pires et al. 2014). Higher alcohols are formed by yeast during fermentation and are 'generally recognised as safe' (Lechenmeier et al. 2008). Higher alcohols or 'fusel alcohols' have more than two carbon atoms and include n-propanol, isobutanol, 2 and 3 methylbutanol, isoamyl alcohol and 2-phenyl ethanol (Humia et al. 2019). These compounds have both a positive and negative impact on the aroma and flavour of beer. Higher alcohols >300 mg/L in beer result in strong, pungent aroma and taste, whereas optimal levels contribute desirable characteristics (Olaniran et al. 2017). In this work (Table 2) the total higher alcohols were below the upper threshold at 84-88 mg/L. Other work (Vesley et al. 2014) showed that changes in the levels of higher alcohols during beer ageing had little relationship with pasteurisation.

It has been reported that higher alcohols decrease during beer storage, possibly because of the formation of the corresponding aldehydes, via the oxidation of melanoidins (Cao et al. 2011). Here, the coefficient of variation is 1.6% (Table 2) indicating the stability of higher alcohols in bottled beer during six months of storage. With a coefficient variation of 3.8% (Table 2), the concentration of isobutanol was stable during six months of storage in an amber glass bottle. The higher content of isobutanol is undesirable for beer quality (Kobayashi ey al. 2008). Well controlled process parameters minimise the content of higher alcohols responsible for undesirable flavour or aroma (eg 'alcohol', 'sweetness', or 'solvent'). While the formation of isobutanol is increased by oxygenation during fermentation, isoamyl alcohol does not appear to be significantly affected by oxygen availability in beer (Vidal et al. 2015).

Correlation between aroma compounds

Based on a one-sample t-test, no difference was found between esters ($p = 0.736$) and ethyl acetate ($p = 0.662$) during the six months of storage (Table 3) or their concentration in bright beer tank (Table 2). Isoamyl acetate showed almost unchanged values during storage for six months. Consequently, these parameters were not subject to further correlation analysis.

Analysis of aroma compounds in bottled lager during storage (Table 3) showed a Pearson correlation coefficient of 0.82 for diacetyl and dimethyl sulphide and 0.88 between diacetyl and 2,3-pentanedione. Haukeli et al (1973) reported a high correlation between diacetyl and 2,3 pentadione in 91 samples of all malt beer from 18 Norwegian breweiries.

Diacetyl (2,3-butanedione) and 2,3-pentanedione are important compounds in the flavour profile of beer and - depending on the style - are normally required to be present at levels beneath their flavour threshold. Although dependent on the

Table 3.

Pearson linear correlation matrix for aroma compounds in pasteurised beer.

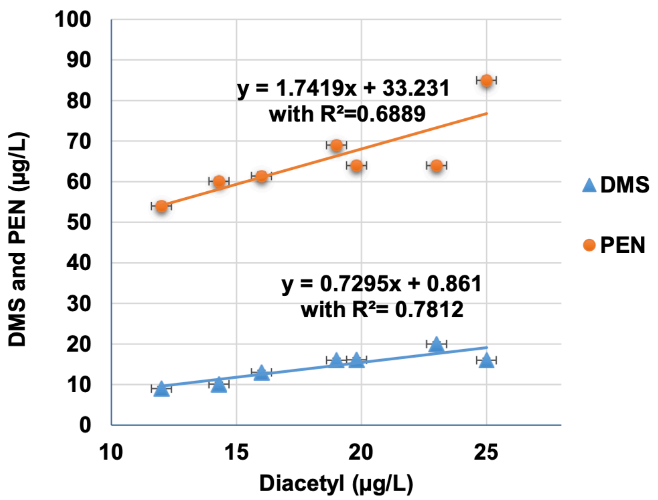
	ACE	DMS	DIA	PRO	PEN	IBU	IAC	HA
ACE	1							
DMS	0.57683	1						
DIA	0.57191	0.82999	1					
PRO	-0.17035	-0.08833	0.18236	1				
PEN	0.53219	0.51659	0.88385	0.34062	1			
IBU	-0.04505	0.26924	0.33231	0.13373	0.26197	1		
IAC	-0.21718	0.23679	0.39163	0.05338	0.48270	0.10296	1	
HA	0.11281	0.071534	0.23342	0.66657	0.34214	0.70090	0.13525	1

Aroma compounds. ACE - acetaldehyde, DMS - dimethyl sulphide, DIA - diacetyl, PRO - propanol, PEN - 2,3-pentanedione, IBU - isobutanol, IAC - isoamyl acetate, HA - higher alcohols beer style, the flavour threshold reported for diacetyl in lager beer is 0.1-0.2 mg/L (Krogerus and Gibson 2013), about ten fold lower than for 2,3-pentanedione (0.9-1.0 mg/L) (Wainwright 1973; Meilgaard 1975). Given the results (Table 2) and linear regression analysis (Figure 2), diacetyl and 2,3 pentadione are inter-dependent. Theoretically, increasing diacetyl to a threshold value of 0.1 mg/L or 0.2 mg/L results in an increase in 2,3 pentanedione to 0.93 mg/L or 1.0 mg/L.

Low levels of dimethyl sulphide are required in lager as the compound contributes to the signature aroma. However, excess dimethyl sulphide results in an unpleasant taste and aroma described as 'cabbage-like' or 'cooked vegetable' (Scarlata and Ebler 1999), 'cooked sweet corn' or at higher levels 'blackcurrant-like' (Anness and Bamforth 1982). Dimethyl sulphide concentrations can diminish over time, due to the formation of complexes with other compounds (Anness and Bamforth 1982). Based on linear regression (Figure 2) diacetyl and dimethyl sulphide are inter-dependent. An increase in diacetyl from the threshold of 0.1-0.2 mg/L relates to an increase of dimethyl sulphide to 33.4-33.6 µg/L, which is above the threshold level of 30-45 µg/L reported by Meilgaard (1982).

Figure 2.

Linear regression - diacetyl v DMS and PEN



Diacetyl - DMS and Diacetyl - PEN in pasteurised bottled beer. DMS – dimethyl sulfide, PEN – 2,3-pentanedione

Conclusions

The expectation of consumers is for beer to have a stable flavour and aroma. The first question in this work was were there are differences in aroma compounds in bright beer tank and after pasteurisation. Analysis showed an increase in the concentration of 2,3 - pentanedione, diacetyl and, and to a lesser extent, dimethyl sulphide post pasteurisation. These compunds also increased during storage in bottle. Other aroma compounds were stable post pasteurisation and during storage.

A second question was whether there was any correlation between individual aroma compounds, leading to a predictable paired change. The Pearson linear correlation between dimethyl sulphide and diacetyl, and between 2,3 - pentanedione and diacetyl were both greater than 0.8. Based on their linear dependencies it was demonstrated that the threshold levels of the aroma compounds was not significant but, relative to diacetyl, both dimethyl sulphide and 2,3 - pentanedione are in proportion. Should the concentration of diacetyl be at the flavour threshold, both 2,3 - pentanedione and dimethyl sulphide would likely to be at or above their respective threshold levels.

Recommendations for further research include (i) the influence of residence time in bright beer tank before pasteurisation and (ii) the stability (and predictability) of aroma compounds in beer during shelf-life under different conditions of storage.

Author Contributions

Goran Gagula: conceptualisation, data curation, validation, methodology.
Dragica Durđević-Milošević: writing (original draft, review and editing).
Thembekile Ncube: writing (review and editing).
Damir Magdić: supervision, writing (review and editing).

Conflict of interest

The authors declare there are no conflicts of interest.

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