

Ingredient interactions with EXBERRY® in low pH non-alcoholic beverages

NAB Category Team

GROWING COLORS

EXBERRY[®]





Possible interactions of EXBERRY® with beverage ingredients



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Possible interactions of EXBERRY® products with beverage ingredients and important parameters of the beverage base

- 1. pH value
- 2. Ascorbic acid
- 3. Sulphur dioxide
- 4. Quinine
- 5. Sweeteners & Acidifiers
- 6. Flavors / Essential oils
- 7. Other ingredients



EXBERRY[®]

Influence of pH value (1)





Influence of pH value (2)

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EXBERRY[®] Shade Vivid Red Recipe A Recipe B Demineralized water Mineral water + 0.05 % EXBERRY[®] Shade Vivid Red + 0.05 % EXBERRY[®] Shade Vivid Red + 0.07 % Citric acid + 0.07 % Citric acid pH 3.0 pH 5.0 pH 4.7 pH 6.7

- With addition of citric acid or another food acid the pH value decreases.
- Changing the pH value can significantly change the color shade of red, pink and purple EXBERRY[®].

Influence of pH value (3)

EXBERRY[®]

EXBERRY[®] Shade Vivid Red

Recipe A

Demineralized water

- + 0.05 % EXBERRY® Shade Vivid Red
- + 0.07 % Citric acid
- + 0.04 % Trisodium citrate

Recipe B

Mineral water

- + 0.05 % EXBERRY® Shade Vivid Red
- + 0.07 % Citric acid
- + 0.10 % Citric acid





- The final pH value of a beverage depends not only on the amount and type of acids but on the overall matrix.
- In practice, the specified range for the acid content [g/L or g/kg] is much narrower than the specified range for the pH value.
- However, for the color shade of EXBERRY[®] the pH value is more important than the acid content!

Influence of pH value: Red / pink / purple EXBERRY® (4)

Appearance of RED, PINK & PURPLE EXBERRY® at different pH values

0.03 % EXBERRY[®] Shade Vivid Red

0.03 % EXBERRY[®] Shade Brilliant Pink

0.03 % EXBERRY[®] Shade Purple Plum



EXBERRY® products made from red fruits and vegetables that contain anthocyanins are pH dependent.

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Influence of pH value: Red / pink / purple EXBERRY® (5)

Anthocyanin-based EXBERRY[®] products at pH levels between 2.7 and 3.9





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0.03 % EXBERRY® Shade Vivid Red

In practice, the specified range for the total acid content [g/L or g/kg] is much narrower than the specified range for the pH value

EXBERRY[®]

However, for the color shade of EXBERRY[®] the pH value is more important than the acid content!



0.03 % EXBERRY® Shade Brilliant Pink

Influence of pH value: Red / pink / purple EXBERRY® (6)

- OH OH HO O-gly O-gly O-alv Quinonoidal Base A: PURPLE Flavylium Cation AH+: ORANGE/RED/PURPLE (pH 7) (pH 1) Chalcone C: COLORLESS/YELLOW 0.03 % EXBERRY® pH 5 pH 4 pH 6 pH 3 pH 7 Shade Brilliant Pink pH value
- Transformation of anthocyanins at different pH values
- The color shade of anthocyanins depends on the pH value of the application.
- 4 molecular anthocyanin configurations exist in equilibrium:
 - Flavylium cation AH⁺: RED
 - Quinonoidal Base A: PURPLE
 - Carbinol Pseudo-Base B: COLORLESS
 - Chalcone C: COLORLESS-YELLOW
- All forms are present at the same time. The relative amount of each form at equilibrium varies with pH value and anthocyanin structure.
- At acidic pH value (< 2) the red flavylium cation is dominant.
- At increasing pH conditions (3-6), colorless carbinol pseudo-base and chalcone structures are formed.
- At neutral pH value (7) the blue quinonoidal base is dominant.
- At pH 4–6, an anthocyanin solution has very little hue due to the small amount of flavylium cation and quinonoidal base!

Influence of pH value: Red / pink / purple EXBERRY® (6)

OH OH HO O-gly O-gly O-aly Quinonoidal Base A: PURPLE Flavylium Cation AH+: ORANGE/RED/PURPLE (pH 7) (pH 1) + H₂O Hydration – H[‡] R₁ OH 0 HO OH O-gly O-gly O-gly O-alv Ö Chalcone C: COLORLESS/YELLOW Carbinol Pseudo-Base B: COLORLESS (pH 4.5) (pH 4.5) 0.03 % EXBERRY® pH 5 pH 3 pH 4 pH 6 pH 7 Shade Brilliant Pink pH value

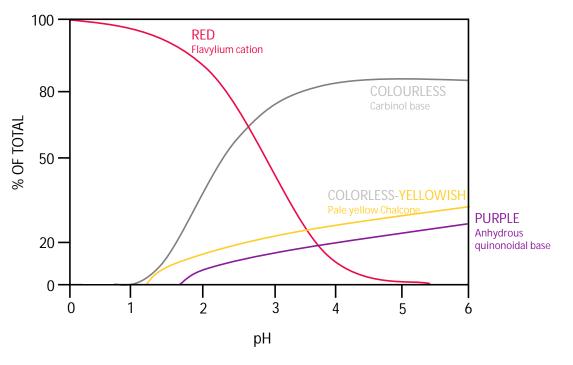
Transformation of anthocyanins at different pH values

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Influence of pH value: Red / pink / purple EXBERRY® (8)



EXBERRY[®] Shade Brilliant Pink



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Influence of ascorbic acid

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Ascorbic acid oxidation bi-products can irreversibly bleach • anthocyanin-based EXBERRY® products

> Oxidation Ascorbic Acid Dehydroascorbic Acid Hydrogen Peroxide Color Loss Bleaching of Anthocyanins

Reference Store light

0.03 % EXBERRY® Shade Vivid Red

Product parameters: 8° Brix pH 3.0 Cold preserved



Ascorbic acid protects carotenoid-based EXBERRY® products from oxidation







With Ascorbic Acid

Reference Store light

Influence of sulphur dioxide

EXBERRY[®] Shade Vivid Red

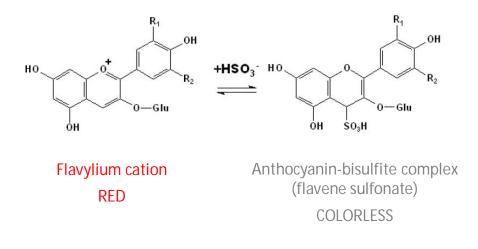
Recipe A

Demineralized water

- + 0.05 % EXBERRY® Shade Vivid Red
- + 0.07 % Citric acid
- + 0.04 % Trisodium citrate
- + 0.02 % Sodium metabisulfite



 Sulphur dioxide forms colorless complexes with anthocyanin-based EXBERRY[®] products



Influence of quinine

- The presence of quinine can cause color loss of anthocyanin-based EXBERRY® products
- The degradation reaction is light driven and substantial in clear bottles when exposed to store light or UV light
- This reaction is not reversible!

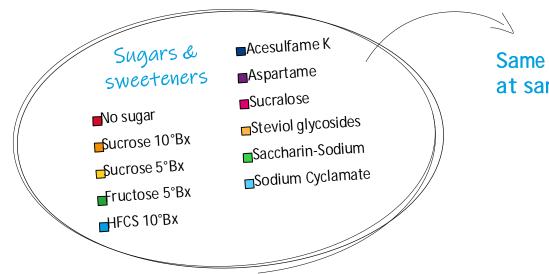


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Influence of the sweetening system



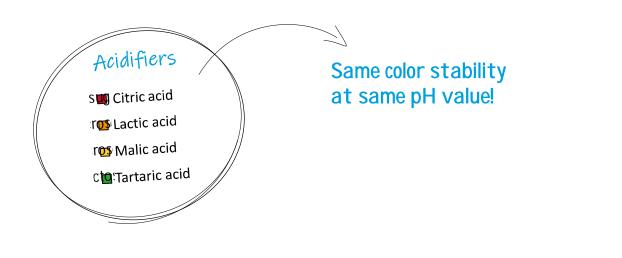
- There are no significant differences in color retention whether a beverage is unsweetened or contains sugars or high intense sweeteners
- The sweetening system in soft drinks can be easily changed without consideration of significant color fading as long as the pH value remains the same



Same color stability at same pH value!

Influence of acidifier type

- There are no significant differences in color retention whether citric acid or any other fruit acid is used
- The acidifier in soft drinks can be easily changed without consideration of significant color fading as long as the pH value remains the same



Current studies running on influence of acidifier <u>dosage</u> at same pH value!

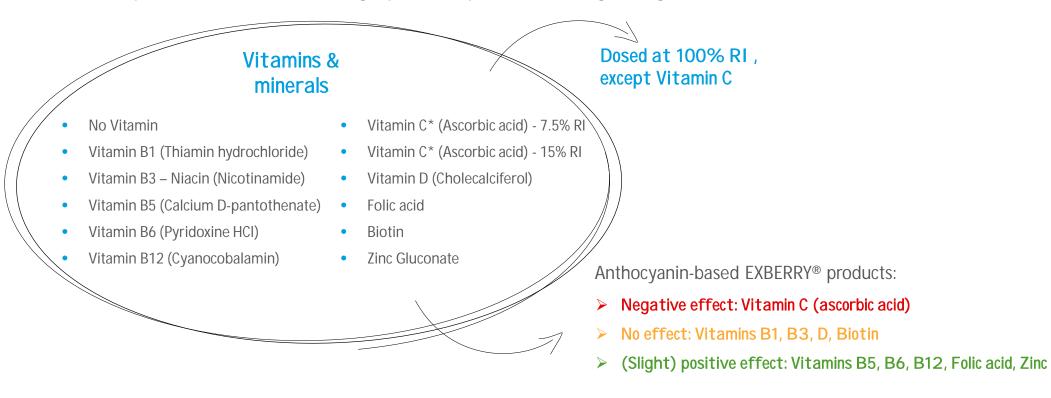
EXBERRY[®]

Complete study



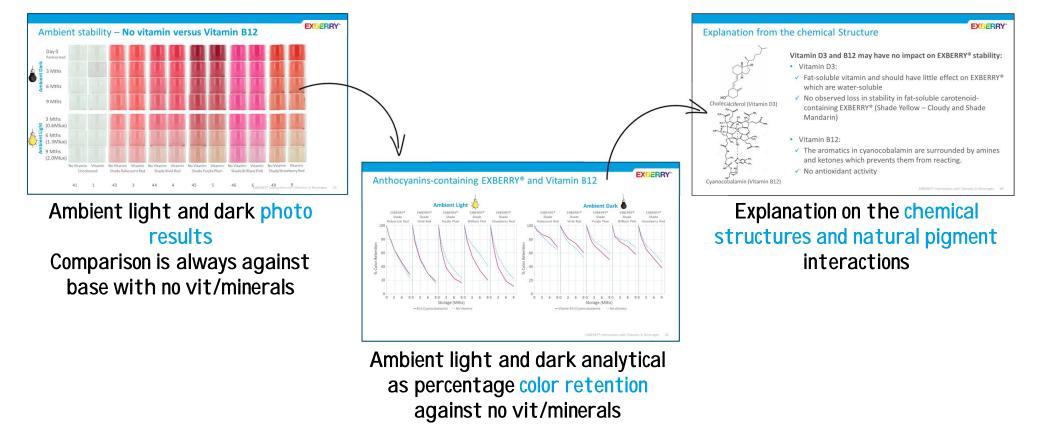
Influence of vitamins and minerals

• Internal project with results of vitamin/mineral interactions on a rainbow EXBERRY[®] performance with photos, color retention graphs and performance grading tables



Study to evaluate vitamin/mineral interactions in NAB

Anthocyanin based red, pink and purple EXBERRY® with Vitamin B12



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Influence of preservatives

* Maximum dosages in soft drinks according to Regulation (EC) No 1333/2008 on food additives

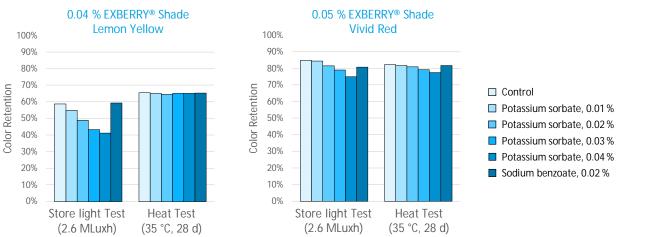
EXBERRY[®]

Potassium sorbate

- Negative impact on the color stability of EXBERRY® Shade Lemon Yellow, but only under light stress
- Slight negative impact on the color stability of anthocyanin-based EXBERRY® products under light and heat stress
 - Color retention for these EXBERRY[®] products decreases with increasing dosage of sorbate (100-400* ppm)

Sodium benzoate

- No significant impact on EXBERRY[®] performance (180* ppm)



Influence of cleaning agents

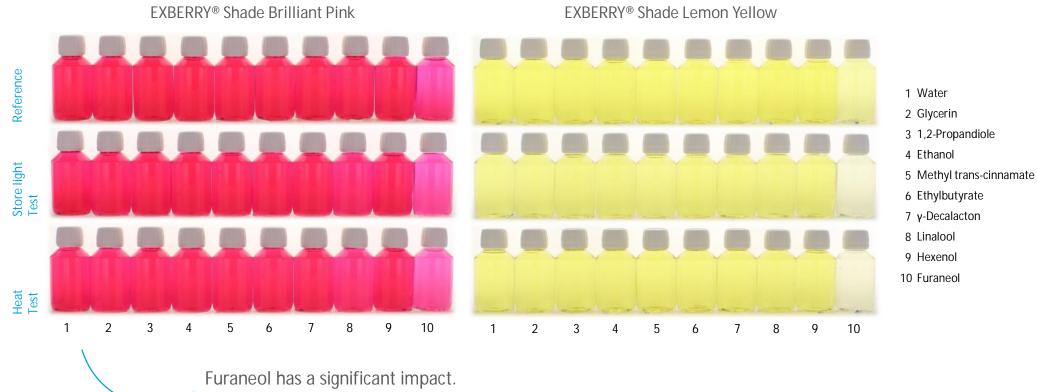
- Residual acidic or alkaline cleaning agents in pipes
 or tanks
- Residual sterilization fluids in filling process
 - > Influence colour shade and stability of EXBERRY®



Study planned to evaluate impact of cleaning agents

Influence of flavors and essential oils (1)

• Some flavoring's component can impact EXBERRY® stability.



Linalool can also have a slight impact.



Influence of flavors and essential oils (2)

- EXBERRY[®] Shade Mandarin has an orange cloudy color shade in beverages. Its crystalline pigments (carotenoids) are insoluble in water.
- These crystals can be dissolved in oil (e.g. in citrus oil in a flavoring), resulting in a more yellow color shade.
- High shear forces (intensive mixing, homogenization) or pasteurization promote this process.



- > Crystalline form \rightarrow No solubility in water \rightarrow ORANGE appearance
- > Carotenoids dissolve in oil \rightarrow YELLOW-ORANGE appearance

Influence of other ingredients

Proteins

 Haze can develop following interaction between EXBERRY[®] and proteins in beverages

Minerals

- Some metals can form complexes with anthocyanins and influence color stability.
- The most common ones are copper (Cu), iron (Fe), magnesium (Mg), tin (Sn) and potassium (K)

Flavorings and botanical extracts

- Certain flavorings and botanical extracts may also cause interactions with EXBERRY®



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Tips and tricks on how to optimize EXBERRY® performance



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Tip 1: Choose an adequate EXBERRY® dosage!

• The higher the EXBERRY[®] concentration, the more intense the color.

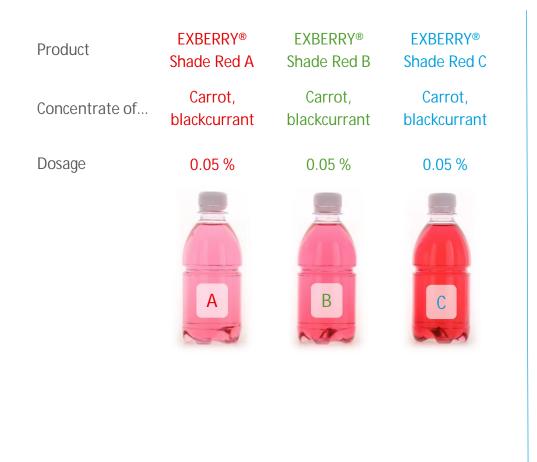


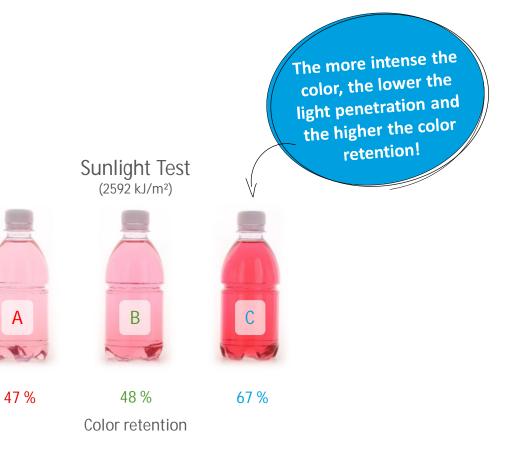
0.020 % 0.033 % 0.050 %

EXBERRY® Shade Brilliant Pink The more intense the color, the lower the light penetration and the better the color protection!

Test if increasing the EXBERRY[®] dosage by 20-30 % will solve stability issues! Always evaluate color stability, especially for very low EXBERRY[®] concentrations!

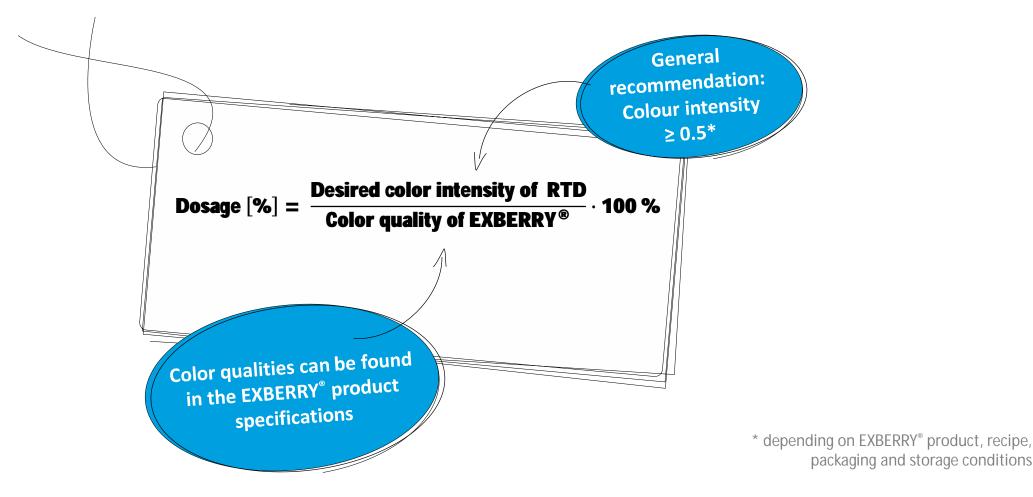
Color quality: Non-adapted dosage



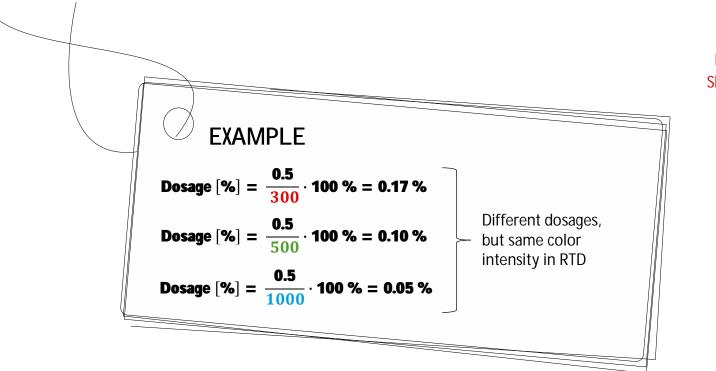


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Color quality: Adapted dosage (1)



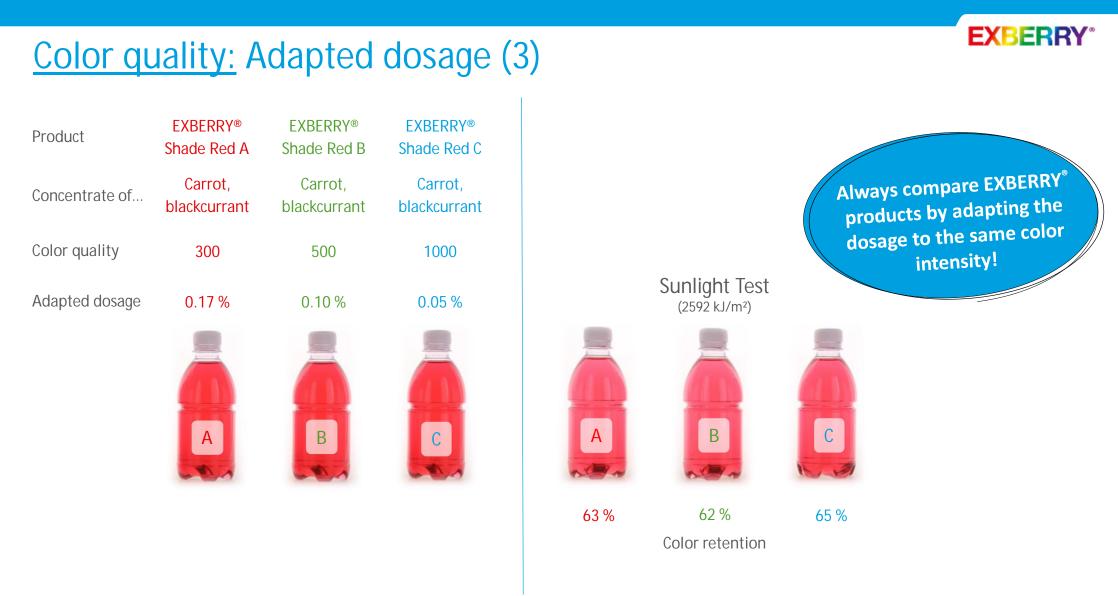
Color quality: Adapted dosage (2)



Color qualities from specification

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	EXBERRY [®] Shade Red A	EXBERRY [®] Shade Red B	EXBERRY [®] Shade Red C	
osages, olor nRTD	300	500	1000	



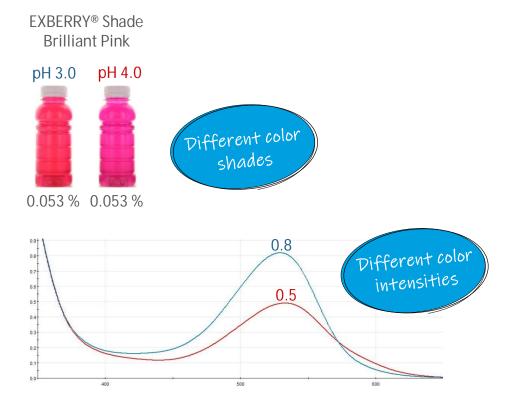
<u>Tip 2:</u> Choose an adequate pH value or adapt dosages! (1) Red, pink and purple EXBERRY®

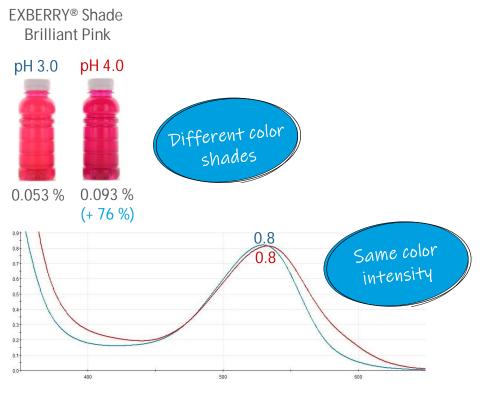
Keep pH as close to <u>pH 3.0</u> as possible for red, pink and purple EXBERRY[®] products (which naturally contain anthocyanins)!

> When you change a recipe and the pH value increases, also <u>increase</u> the EXBERRY[®] dosage!

<u>Tip 2:</u> Choose an adequate pH value or adapt dosages! (2) Red, pink and purple EXBERRY®

Example: Influence of pH value on color intensity

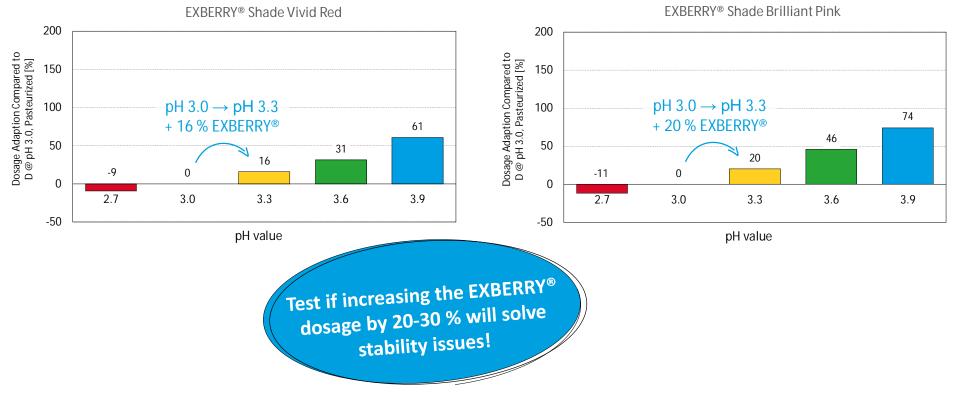




EXBERRY[®]

<u>Tip 2:</u> Choose an adequate pH value or adapt dosages! (3) Red, pink and purple EXBERRY®

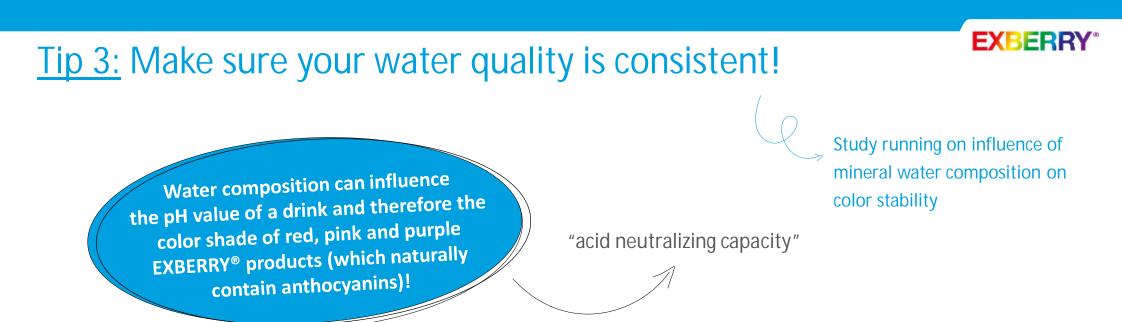
Example: Dosage adjustments for equal color intensities after pasteurization



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EXBERRY[®]

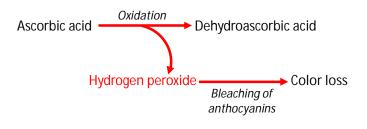
Complete study



- Adjust the pH value when you alter the water quality.
 - > Ensure that the water quality during product development is like the one in the factory.
 - > Ensure that the water quality in the plant is consistent over time (recipe transfer from one factory to another).

<u>Tip 4:</u> Best practice when working with ascorbic acid (1)

Ascorbic acid oxidation products cause irreversible bleaching of anthocyanins.





With Ascorbic Acid



0.03 % EXBERRY[®] Shade Vivid Red

EXBERRY

- When using ascorbic acid and anthocyanin-based EXBERRY[®] in a drink:
 - 1. Keep the technological necessary dose of ascorbic acid as low as possible (GMP).
 - 2. Avoid very low EXBERRY® levels.
 - 3. Remember that ascorbic acid can also be added via fruits (e.g. acerola, citrus).
 - 4. Avoid high vitamin C claims. Ascorbic acid is often overdosed to ensure that the claimed dosage is still present at the expiration date.
 - 5. Shorter shelf lifes are preferred to longer ones.
 - > Heat, time and oxygen level are the main factors promoting color degradation.
 - > Vegetable based EXBERRY[®] concentrates are more stable than fruit juices (e.g. elderberry).

<u>Tip 4:</u> Best practice when working with ascorbic acid (2)

- Ascorbic acid protects carotenoid-based EXBERRY® from oxidation.
 - Carotenoid losses are influenced by both storage temperature and light exposure and are driven by <u>oxidative reactions</u>.
 - The addition of ascorbic acid, which degrades via an oxygenconsuming pathway, has a protective effect on carotenoids.

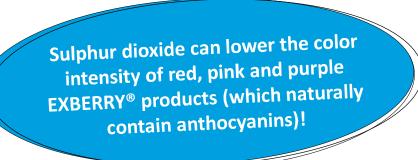
- When using ascorbic acid and carotenoid-based EXBERRY[®] products in a drink:
 - 1. Ascorbic acid is needed, especially when using clear packaging or storage temperatures are high.
 - 2. The amount of ascorbic acid depends on factors as selected EXBERRY® product, oxygen level and intended shelf life.
 - 3. Recommended dosage levels for EXBERRY® Shade Yellow and Shade Mandarin: 200-300 ppm
 - 4. Reducing the amount of oxygen in the beverage (e.g. modified atmosphere by nitrogen flush or carbonization, oxygen scavengers, low head space, hot filling) might significantly reduce the amount of needed ascorbic acid.



0.13 % EXBERRY[®] Shade Mandarin

Planned study on influence of oxygen on color stability.

Tip 5: Best practice when working with sulphur dioxide (1)



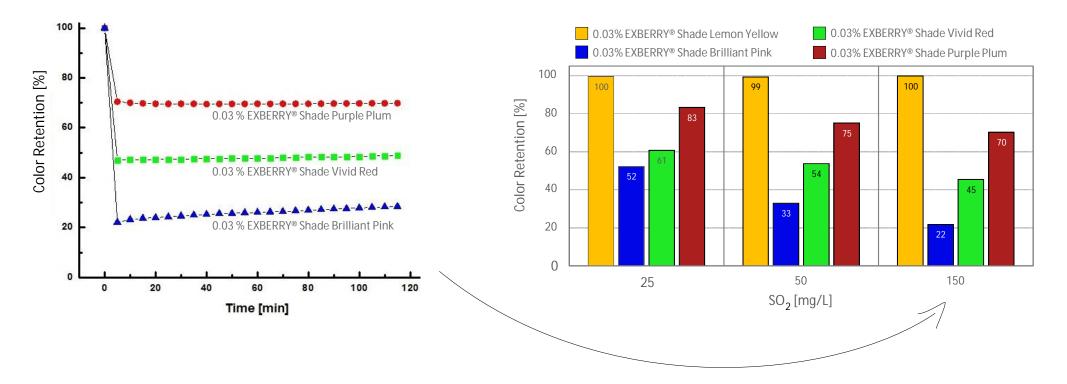
- > Free SO_2 forms a colorless complex with anthocyanins.
- > Different EXBERRY[®] raw materials show different affinities to react with SO₂.
- > The most crucial factor for is the concentration of EXBERRY[®]. Initial loss can be mitigated by increasing the dosage.
- > Reducing the amount of SO_2 will lead to a higher initial color intensity.

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<u>Tip 5:</u> Best practice when working with sulphur dioxide (2)

- Impact of SO₂ addition (150 ppm) on color intensity of different EXBERRY[®]
- Impact of increasing SO₂ levels on the color intensity of different EXBERRY[®] products.

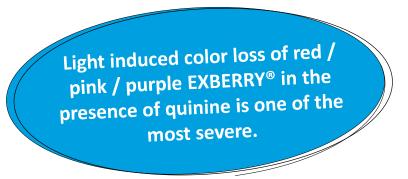
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<u>Tip 6:</u> Best practice when working with quinine (1)

- The quantity of quinine has a significant influence on color stability
- Higher EXBERRY[®] dosages show better color stability in the presence of quinine
- The degradation reaction is light driven so interactions should be minimum in canned products but substantial in clear bottles when exposed to store light or UV light



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<u>Tip 6:</u> Best practice when working with quinine (2)

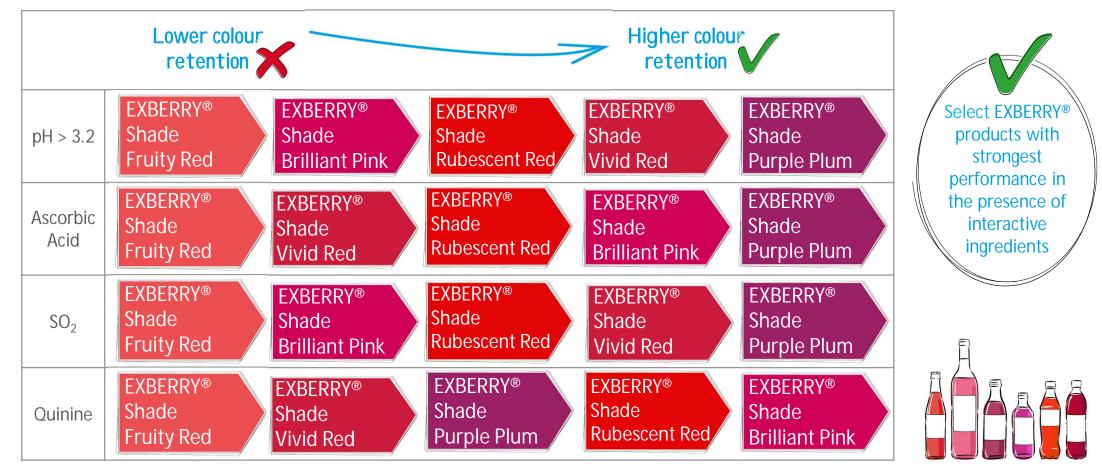
Observations:

- Store light and sunlight testing over-accelerate color loss in samples containing quinine:
 - Effect of UV light
 - Effect of incandescent light + heat
- Due to non-correlation of quinine containing samples, store light and sunlight testing is not advised to predict color stability in tonic waters.

Study running on influence of non-quinine based bitter flavors and quassia extract on EXBERRY®



Performance of red, pink and purple EXBERRY® products under different ingredient impacts



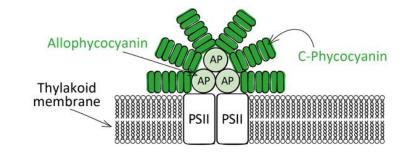
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EXBERRY[®]

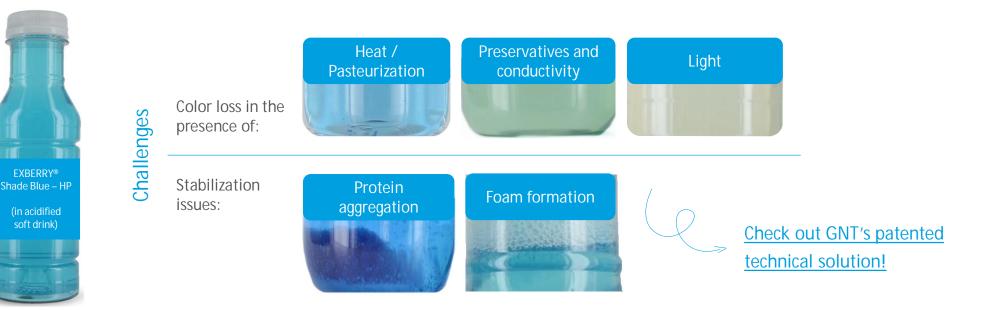
Tip 7: Best practice when working with Blue and Green (1)

Considerations for blue and green NABs

- Spirulina's pigment phycocyanin has a protein backbone
- Several ingredients, recipe parameters, process and storage conditions can lead to color loss and/or aggregation



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Tip 7: Best practice when working with Blue and Green (2)

Considerations for blue and green refrigerated smoothies and juices:

- Keep the processing conditions short to minimize temperature impact.
- Some color loss is expected. EXBERRY® should be overdosed (typically at 150 %).
- Sedimentation depends on the beverage composition.
- Consider increasing the viscosity to reduce sedimentation and/or homogenize to ensure a consistent particle size.
- Background color is important when creating a blue product. Make sure the base is as white/colorless as possible.



Tip 8: Best practice when working with cloudy EXBERRY® (1) EXBERRY® Shade Mandarin

- Without the addition of a suspension agent, EXBERRY® Shade Mandarin will fall out completely within few days.
- Xanthan gum is recommended to be added to the beverage formula:
 - > Xanthan must be hydrated with most of the recipe water.
 - Once xanthan is hydrated, it must be blended with EXBERRY[®] Shade Mandarin first. Then all other ingredients should be added.
 - Recommended final dosage is xanthan is 0.01-0.03 %. Higher levels will impart mouthfeel and increase viscosity. Maximum recommended use level for syrups is 0.05 %.
 - Viscosity decreases with mixing or shaking and will increase when it is stationary, making xanthan a great suspension agent.
- Ascorbic acid addition might be necessary to improve color stability.



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Tip 8: Best practice when working with cloudy EXBERRY® (2) EXBERRY® Shade Yellow

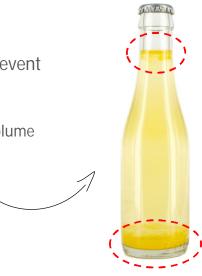
- Yellow carotenoid-based EXBERRY[®] products need a stabilizing system to prevent defects over shelf life. Those can be:
 - Aggregation, where large clusters form, often floating throughout the beverage volume
 - Sedimentation, where solids sink down to the bottom
 - Neck-ringing, where oil floats to the top of the beverage to form a ring

Option 1:

EXBERRY® Shade Yellow - Cloudy Powder

- Spray dried with Gum Arabic (\rightarrow hydrocolloid with emulsifying properties)
- Minimized ringing properties
- Can be used on its own if some sedimentation is acceptable
- Xanthan can be added to minimize sedimentation
- Dry blending (e.g. sugar) recommended for easier dispersion and hydration during formulation





Tip 8: Best practice when working with cloudy EXBERRY® (3) EXBERRY® Shade Yellow



Option 2:

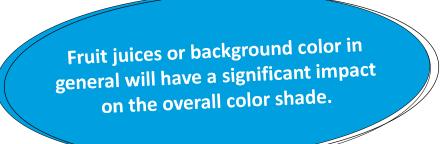
EXBERRY[®] Shade Yellow*

- First step should be making a "color compound" (45 % water + 5 % Gum Arabic* + 50 % EXBERRY®) to delay ring formation if this is a critical quality feature
- Color compound requires homogenization (240 bar, 3 passes, target size: D50 < 0.5 μm)
- Xanthan should be added to reduce sedimentation



*Other hydrocolloids can be used

Tip 9: Consider background color and juice browning!



- The overall color shade is always the sum of background color plus EXBERRY[®].
- Fruit juice color is often not standardized and might change from lot to lot.
 - > Ensure the EXBERRY[®] dosage is high enough to mitigate the color shift.
- Browning:
 - > Consider how the base color from fruit will brown or yellow over shelf life.
 - > Ascorbic acid browning can also be an issue.
 - > EXBERRY® can be used to cover browning to a certain extent.

Clear Base



0.05% EXBERRY® Shade Brilliant Pink

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30% Apple Juice



0.05% EXBERRY® Shade Brilliant Pink





Summary



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Conclusion

- Every beverage is unique! Color stability needs to be assessed with reference to its properties.
- There are INTERNAL factors (like pH value and ingredients) and EXTERNAL factors (like packaging, processing and storage conditions) that need to be considered.
- The selection of the right EXBERRY[®] products and dosages are important to get the desired color shade and color stability over shelf life.



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