Ongoing Standard Developments
Cranberry

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Agenda

- Update: USP Cranberry Standards Development Roundtable (June 22, 2017)
- Progress in Nomenclature and Monograph development
- On going Lab projects
Update: USP Cranberry Standards Development Roundtable (June 22, 2017)
Existing standards for Cranberry

- **USP**
  - Cranberry Liquid Preparation - *(commercial name: 100% Cranberry Juice- 7.5 Brix)*

- **Health Canada**
  - NHPID-Dried Cranberry Juice - *(most comply with the USP Cranberry Liquid Preparation )*
  - NHPID- Cranberry - *(most comply with the USP Cranberry Liquid Preparation )*

- **European Juice Association (AIJN)-Code of Practice**
  - Reference Guideline for Cranberry Juice - *(commercial name: Cranberry Juice Concentrate- 50 °Brix)*
Existing standards for Cranberry…cont.

- **USDA-Commodity Specification Bottled Juices**
  - Cranberry Juice Concentrate (3+1)- *(commercial name: Cranberry Juice Cocktail)*
  - Cranberry Juice Concentrate (55 Gallon Drum) -(commercial name: Cranberry Juice Concentrate- 50 °Brix)

- **Codex General Standard for Fruit Juices and Nectars (CODEX STAN 247-2005)**
  - Botanical Name: *Vaccinium macrocarpon* Aiton, *Vaccinium oxycoccos* L.
  - Fruit’s Common Name: Cranberry
  - Minimum Brix Level for Reconstituted Fruit Juices and Reconstituted Purée: 7.5 ° Brix
  - Minimum Juice and/or Purée Content (% v/v) for Fruit Nectars: 30 (% v/v)
Different type of Cranberry Ingredients
Main Bioactive Compounds in Cranberry

Flavan-3-ols
(-)-Epicatechin (shown here)
(+)-Epicatechin
(-)-Catechin
(+)-Catechin

Proanthocyanidins (PACs)

Flavanoids
Quercetin: R1=R3=OH, R2=H
Myricetin: R1=R2=R3=OH
Kämpferol: R1=R2=H, R3=OH
Quercetin 3-O-galactoside: R1=OH, R2=H, R3=O-galactose

Terpenes
Ursolic acid: R=OH
cis-3-O-p-hydroxyphenyl ursolic acid: R=

Hydroxycinnamic acids
p-coumaric acid: R1=R2=H
Caffeic acid: R1=OH, R2=H
Ferulic acid: R1=OCH3, R2=H
Sinapic acid: R1=R2=OCH3

Anthocyanidins
Peonidin: R1=OCH3, R2=H
Cyanidin: R1=OH, R2=H
Malvidin: R1=R2=OCH3
Delphinidin: R1=R2=OH
Petunidin: R1=OCH3, R2=OH
Pelargonidin: R1=R2=H

Source: AHP monograph

Hydroxybenzoic acids
Benzoic acid: R1=R2=H
p-Hydroxybenzoic acid: R1=H, R2=OH
p-Dihydroxybenzoic acid: R1=OH, R2=H
2,4-Dihydroxybenzoic acid: R1=R2=OH

Source: AHP monograph
<table>
<thead>
<tr>
<th>Monograph requirements for different type of ingredients</th>
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</thead>
<tbody>
<tr>
<td><img src="image_url" alt="Image" /></td>
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</table>

<table>
<thead>
<tr>
<th>Identification</th>
<th>Cranberry Juice Concentrate</th>
<th>Whole berry Powders</th>
<th>Juice-Derived Powders</th>
<th>Juice-Derived Extracts</th>
<th>Pomace Extract</th>
<th>Pomace/skin-Derived powders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic acids (quinic, malic and citric)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td>Absence of Tartaric and Fumaric acids</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>HPLC profile anthocyanins/flavonoids</td>
<td>mod</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<table>
<thead>
<tr>
<th>Composition</th>
<th>Cranberry Juice Concentrate</th>
<th>Whole berry Powders</th>
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<th>Juice-Derived Extracts</th>
<th>Pomace Extract</th>
<th>Pomace/skin-Derived powders</th>
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</thead>
<tbody>
<tr>
<td>Content of Dextrose and Fructose</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td>Content of Organic Acids</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Total Proanthocyanidins (DMAC)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Anthocyanins by HPLC</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td></td>
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<thead>
<tr>
<th>Adulterants</th>
<th>Cranberry Juice Concentrate</th>
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<tbody>
<tr>
<td>Limit of Sorbitol and Sucrose</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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</tbody>
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<thead>
<tr>
<th>Specific tests</th>
<th>Cranberry Juice Concentrate</th>
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<tbody>
<tr>
<td>Refractive Index</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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<tr>
<td>pH</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Limit for Benzoic acid</td>
<td>mod</td>
<td>X</td>
<td>?</td>
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<tbody>
<tr>
<td>Water</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td>Total Ash</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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<tr>
<td>Residual Solvents</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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</table>
Challenges with analytical techniques

<table>
<thead>
<tr>
<th>Test</th>
<th>Determination</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMAC</td>
<td>Quantitative Total PACs&lt;br&gt;DAMC reacts with specific OH groups of terminal units</td>
<td>Unspecific for cranberry PACs&lt;br&gt;Poor accuracy&lt;br&gt;Many analytical parameter affecting the outcome&lt;br&gt;Numerous versions&lt;br&gt;Use of different Reference standards creates confusion</td>
</tr>
<tr>
<td>Butanol-HCl (Bate Smith)</td>
<td>Quantitative Total PACs&lt;br&gt;Depolymerization and conversion to cyanidins&lt;br&gt;Measures soluble and insoluble PACs</td>
<td>Unspecific for cranberry PACs&lt;br&gt;Interference with anthocyanins</td>
</tr>
<tr>
<td>RP- HPLC</td>
<td>Qualitative&lt;br&gt;Quantitative&lt;br&gt;Phenolic acids&lt;br&gt;Individual anthocyanins&lt;br&gt;Flavonol glycosides&lt;br&gt;Monomeric flavan-3-ols, dimeric and trimeric PACs (A and B type)</td>
<td>Not useful for determining polymeric PACs</td>
</tr>
<tr>
<td>Normal Phase -HPLC</td>
<td>Qualitative&lt;br&gt;Quantitative&lt;br&gt;Separation of PACs by degree of polymerization&lt;br&gt;Oligomeric and polymeric PACs</td>
<td>Possible coelution of A-type and B-type PACs&lt;br&gt;Reference standard availability</td>
</tr>
<tr>
<td>Thiolysis-RP-HPLC</td>
<td>Qualitative&lt;br&gt;Quantitative&lt;br&gt;Total PACs&lt;br&gt;Depolymerization and detection of terminal and extension PAC units&lt;br&gt;Average degree of polymerization&lt;br&gt;A-type linkages are not broken</td>
<td>Reference standard availability of thiol derivatives&lt;br&gt;Could be difficult for routine analysis</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>Qualitative&lt;br&gt;Characterization of oligomeric and polymeric PACS&lt;br&gt;Differentiation of A and B-type linkages&lt;br&gt;Molecular weight distribution</td>
<td>Not quantitative&lt;br&gt;Advance instrumentation&lt;br&gt;Not very feasible for routine analysis</td>
</tr>
</tbody>
</table>
DMAC Reaction

- Influence of the degree of polymerization in the response
- Reference Standards
  - Dimeric Procyanidin A2
  - cPAC
    - Frozen cranberry fruit
    - Polymeric fraction isolated by Sephadex LH-20
    - Characterization by MALDI-TOF
- Industry practice of expressing the results in both ways
- Confusion in CoAs

USP Proposal

To use Procyanidin A2 as reference standard together with Correction Factor for the cPAC standard.
## Reference Standard Materials

### Reference Standards

- **Identification**
  - HPTLC anthocyanins/flavonoids
  - HPLC profile anthocyanins/flavonoids
  - Individual anthocyanins (derivatives of cyanidin and peonidin)
  - Cranberry Extract for fingerprinting /chromatographic similarity

- **Composition**
  - Anthocyanins by HPLC
## Possible Adulterants

### Proanthocyanidin (PAC) Fraction
- Red peanut skin (*Arachis hypogaea*, Fabaceae) extract
- Grape seed/skin (*Vitis vinifera*, Vitaceae) extracts
- Pine bark (*Pinus pinaster*, Pinaceae) extract
- Plum (*Prunus domestica*, Roseaceae)

### Anthocyanin (Pigment) Fraction
- Highbush blueberry (*V. corymbosum)*
- Alpine bilberry or bog blueberry (*V. uliginosum)*
- Grape skin (*Vitis vinifera*, Vitaceae) extracts
- Mulberries (*Moris* spp. Moraceae)
- Hibiscus extract (*Hibiscus sabdariffa*, Malvaceae)
- Black rice extract (*Oryza sativa*, Poaceae)
- Purple cornhusks
- Black bean skins (*Phaseolus vulgaris*, Fabaceae)
- Synthetic colorants
Contaminants

- General Chapter <2232> *Elemental Contaminants in Dietary Supplements*
- General Chapter <561> *Articles of Botanical Origin*
- General Chapter <565> *Botanical Extracts*
- Limit for Benzoic acid (should not be added as a preservative)

  - Heavy metals
  - Pesticides
  - Residual solvents
  - Specific test
1. USP should develop new monographs for the different types of cranberry ingredients: juice-derived (spray-dried powders and dry extracts), whole berry powders, pomace extracts and skin-based powders.

2. The modernization of the current USP Cranberry Liquid Preparation monograph is valuable to reveal the current specifications of article of commerce.

1. USP should develop RS materials for the identification and quantification of cranberry ingredients, including Procyanidin A2 and PAC fractions.

2. USP seeks support from stakeholders to gather specification sheets, certificates of analysis, manufacturing process information, analytical methods, and validation reports for different types of cranberry ingredients in order to elaborate new USP standards for cranberry ingredients.

3. Monograph draft proposals will be published in the Pharmacopeial Forum (PF) to receive public comments from different stakeholders.
Progress in Nomenclature and Monographs Development
Sweetened-dried cranberry (SDC) Processing

Cranberry fruit ➔ Fluid-drying ➔ Pressing/Decantation

(1) Juice Concentrate (50 °Brix) USP Cranberry Fruit Juice Concentrate

Absorbent resin ➔ Spray-drying over carrier ➔ IR drying over acacia or guar gum

Green- Titles approved Red- Pending approval

(2) Juice-derived powders USP Cranberry Fruit Dry Juice

(3) Juice-derived extracts USP Cranberry Fruit Juice Dry Extract

Pomace ➔ Pressing ➔ Aqueous Extract

(5) Pomace Extracts

Press cake ➔ (6) Pomace/skin-derived powders

(4) Whole Berry Powders

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On going Projects

Empowering a healthy tomorrow
Lab project - Development of ID Tests

Samples
\( (n=50) \)
Different type of ingredients and production batches

HPTLC
(CAMAG - Switzerland)
- HPTLC Association
- AHP monograph

HPLC-DAD
Different wavelengths
\( \lambda_1 = 520 \text{ nm for anthocyanins} \)
\( \lambda_2 = 365 \text{ nm for flavonols} \)
\( \lambda_3 = 310 \text{ nm for phenolic acids} \)
\( \lambda_4 = 278 \text{ nm for benzoic acids and flavan-3-ols} \)
Next Steps

To publish in Pharmacopeial Forum (PF) the following monographs:

1. USP Cranberry Liquid Preparation - modernization
2. Cranberry Fruit Juice Concentrate - new monograph
3. Cranberry Fruit Dry Juice - new monograph
4. Cranberry Fruit Dry Extract - new monograph

To submit to our Nomenclature Subcommittee new titles/definitions and develop corresponding draft proposals for the following articles of commerce:

1. Juice Soluble Powders
2. Whole Berry Powders
3. Pomace extracts
4. Pomace/skin derived Powders