

USP Dietary Supplements Stakeholder Forum
Tuesday, May 15, 2018

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)



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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)*

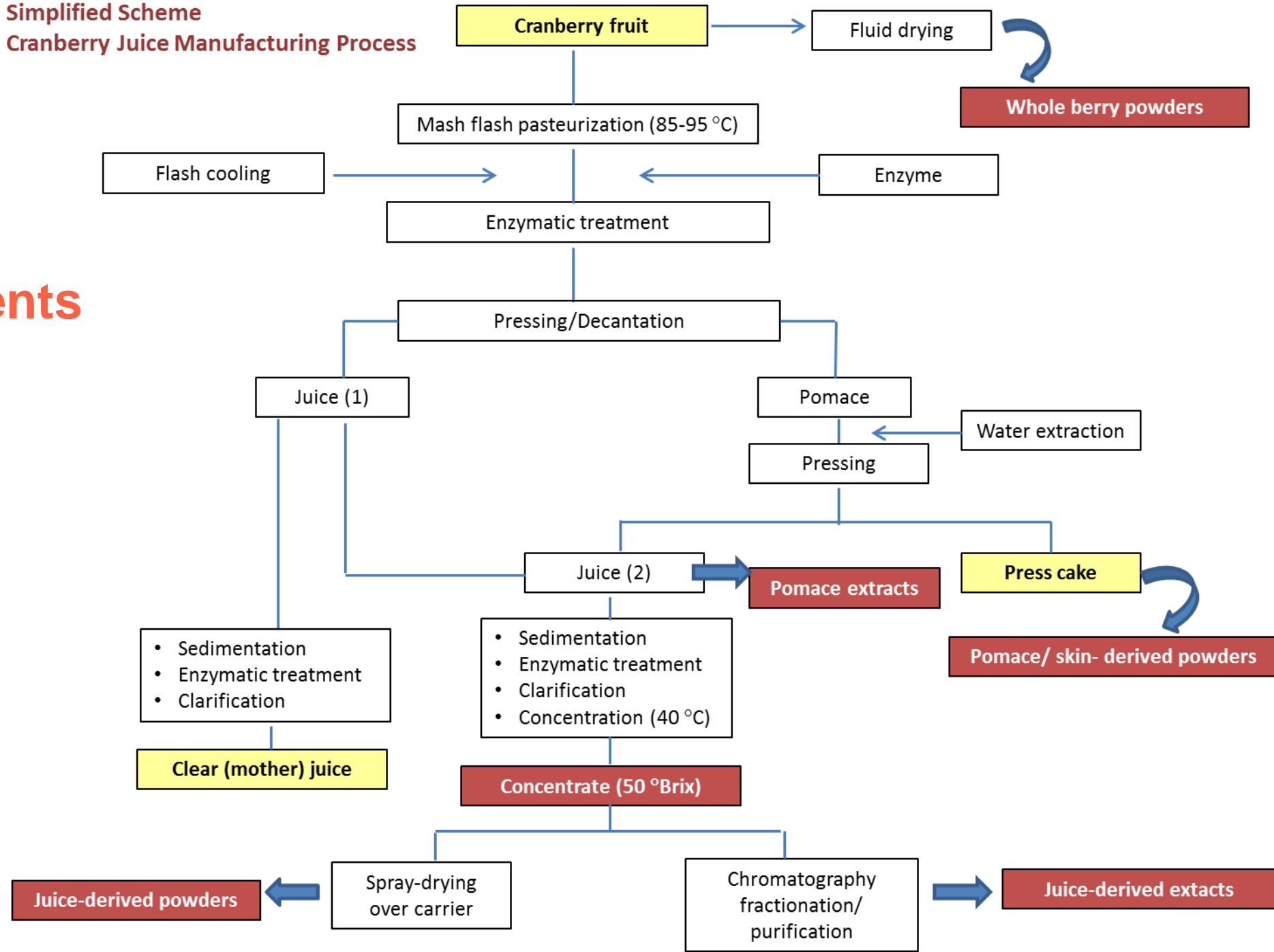
▶ **USDA-Commodity Specification Bottled Juices**

- 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)**

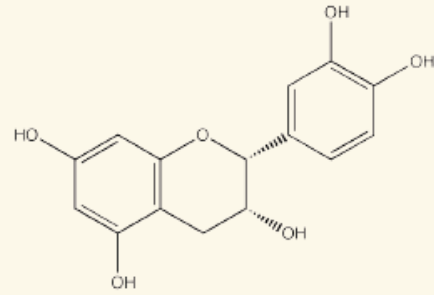
- Botanical Name: *Vaccinium macrocarpon* Aiton, *Vaccinium oxycoccos* L.
- Fruit's Common Name: Cranberry
- Minimum Brix Level for Reconstituted Fruit Juices and Reconstituted Purée: 7.5 ° Brix
- Minimum Juice and/or Purée Content (% v/v) for Fruit Nectars: 30 (% v/v)

**Simplified Scheme
Cranberry Juice Manufacturing Process**

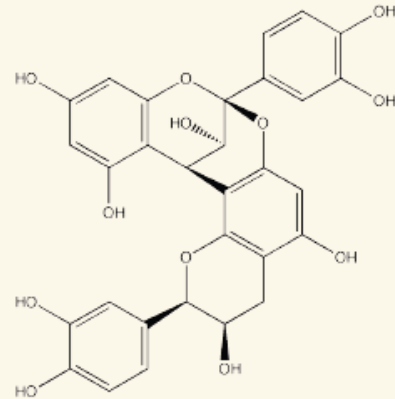


Different type of Cranberry Ingredients

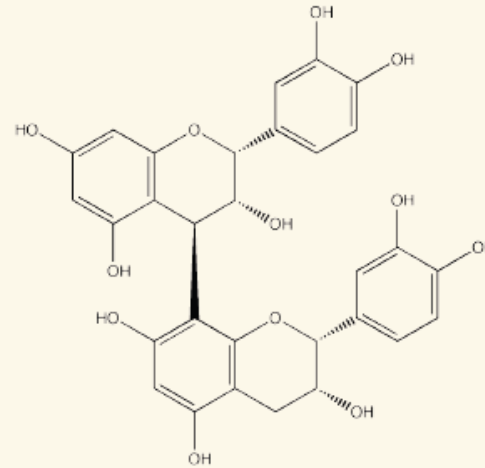
Main Bioactive Compounds in Cranberry



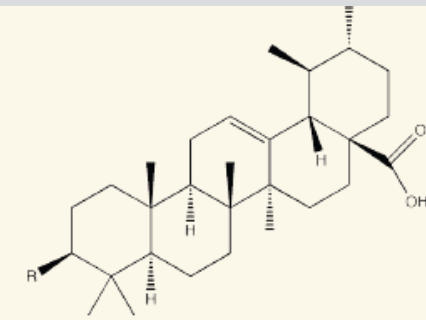
Flavan-3-ols
 (-)-Epicatechin (shown here)
 (+)-Epicatechin
 (-)-Catechin
 (+)-Catechin



Procyanidin A2

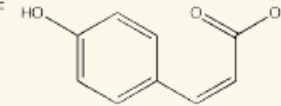


Procyanidin B2

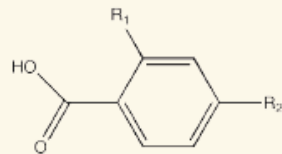


Terpenes

Ursolic acid: R=OH
cis-3-*O*-*p*-hydroxycinnamoyl
 ursolic acid: R=

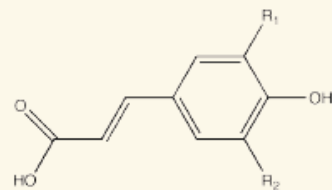


Proanthocyanidins (PACs)

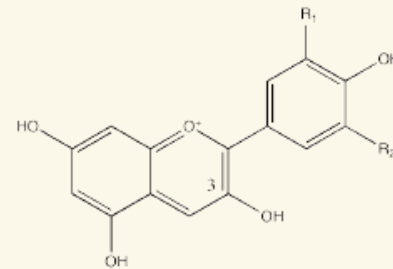


Hydroxybenzoic acids
 Benzoic acid: R1=R2=H
p-Hydroxybenzoic acid: R1=H, R2=OH
o-Hydroxybenzoic acid: R1=OH, R2=H
 2,4-Dihydroxybenzoic acid: R1=R2=OH

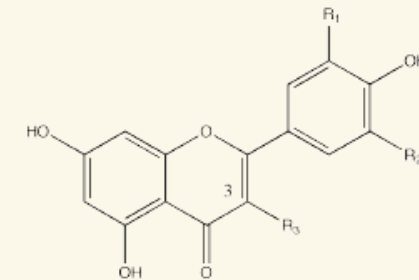
Source: AHP monograph



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



Flavonols
 Quercetin: R1=R3=OH, R2=H
 Myricetin: R1=R2=R3=OH
 Kaempferol: R1=R2=H, R3=OH
 Quercetin 3-*O*-galactoside: R1=OH, R2=H, R3=O-galactose

Monograph requirements for different type of ingredients



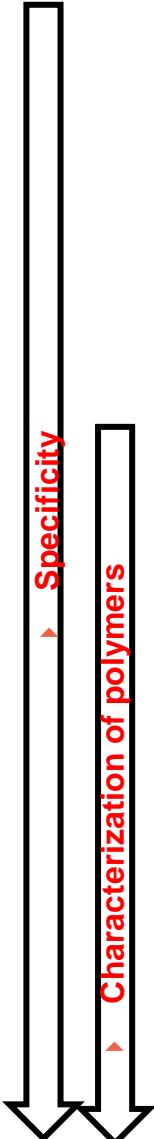
	USP Monograph Cranberry Liquid Preparation	Cranberry Juice Concentrate	Whole berry Powders	Juice- Derived Powders	Juice- Derived Extracts	Pomace Extract	Pomace/skin- Derived powders
• Identification							
Organic acids (quinic, malic and citric)	X	X	X	X			
Absence of Tartaric and Fumaric acids	X	X	X	X			
HPTLC anthocyanins/flavonoids		?	?	?	X	X	X
HPLC profile anthocyanins/flavonoids	mod	X	X	X	X	X	X
MALDI-TOF proanthocyanidins					?	?	
• Composition							
Content of Dextrose and Fructose	X	X	X	X			
Content of Organic Acids	X	X	X	X			
Total Proanthocyanidins (DMAC)		X	X	X	X	X	X
Anthocyanins by HPLC		?	?	?			
• Adulterants							
Limit of Sorbitol and Sucrose	X	X	X	X			
• Specific tests	X	X	X	X			
Refractive Index	X	X	X	X			
pH	X	X	X	X			
Limit for Benzoic acid	mod	X	?	?			
Water					X	X	X
Total Ash					X	X	X
Residual Solvents					X	X	X

Challenges with analytical techniques

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

Specificity

Characterization of polymers

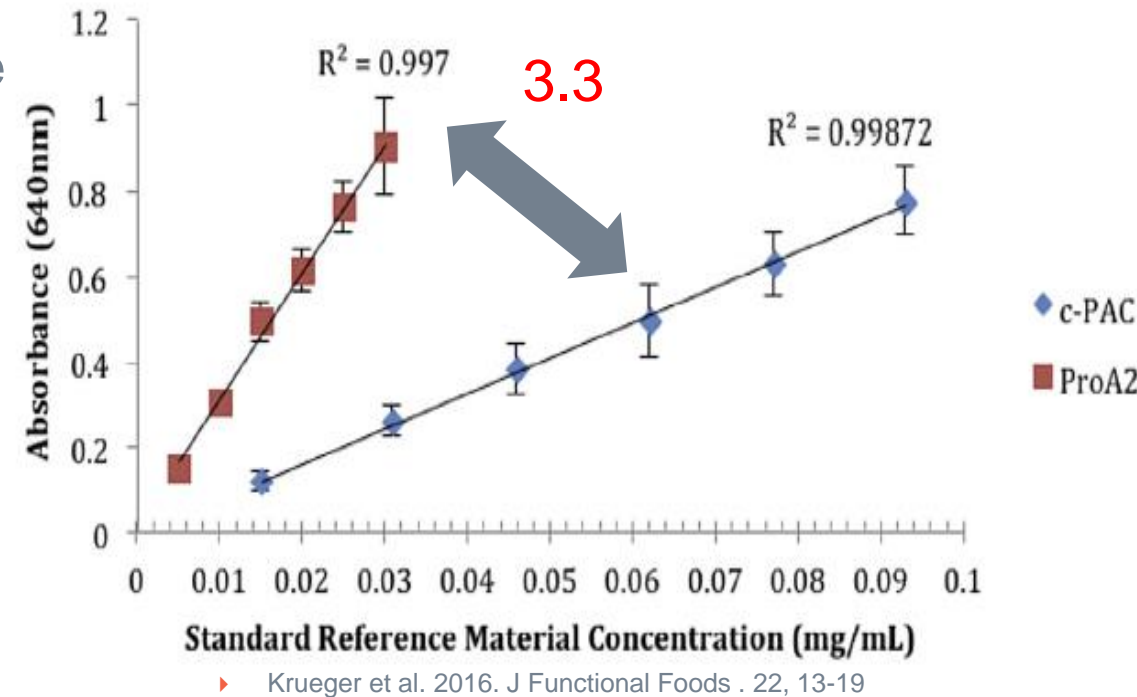


Reference Standard Materials



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

- **Composition**

Anthocyanins by HPLC

Individual anthocyanins (derivatives of cyanidin and peonidin)

Cranberry Extract for fingerprinting /chromatographic similarity

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 (*Morus* 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

Summary of Recommendations and Next Steps

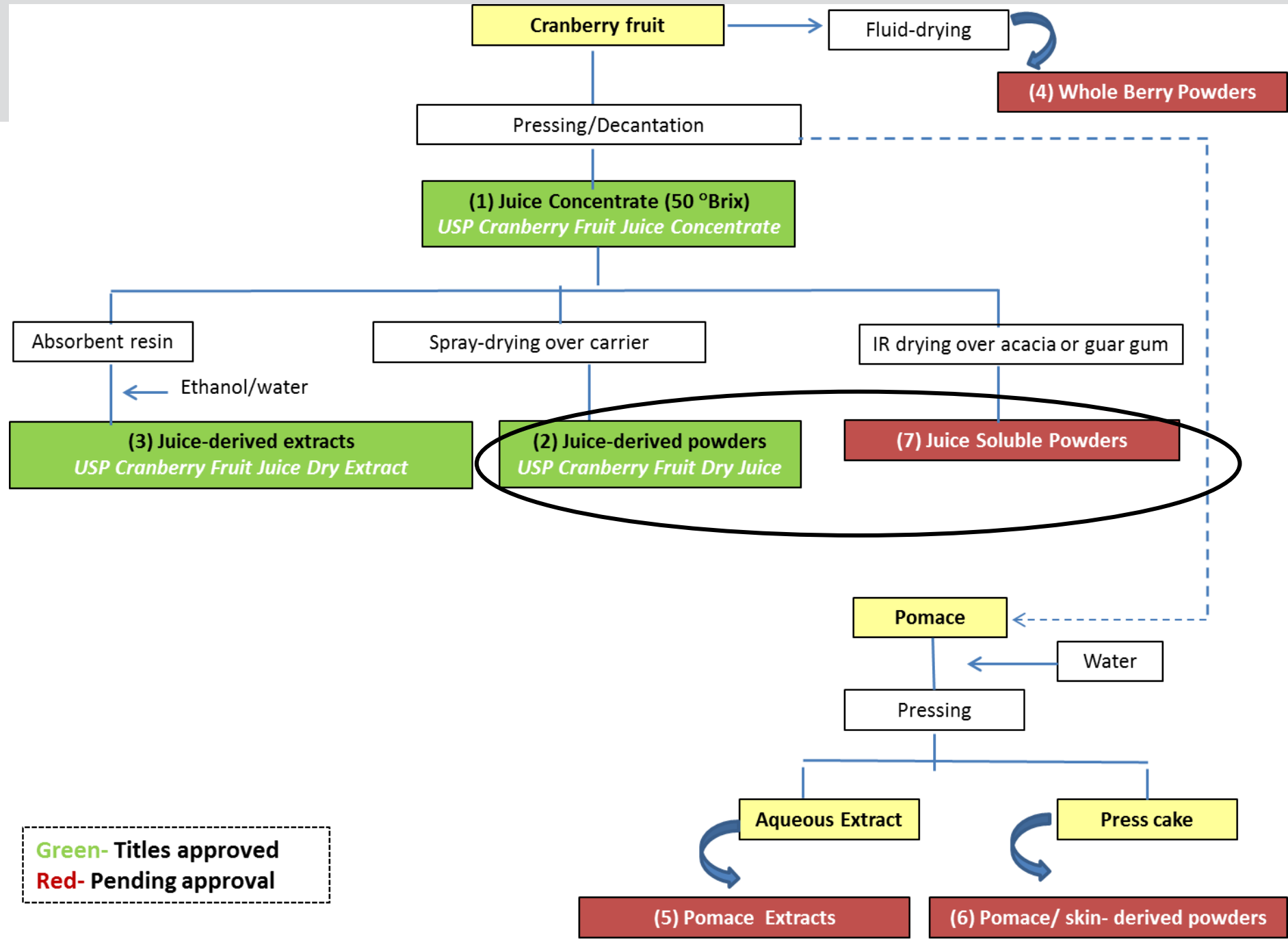


1. USP should develop new monographs for the different types of cranberry ingredients: juice-derived (spray-dried powders and dry extracts), whole berry powders, pomace extracts and skin-based powders.
2. The modernization of the current USP Cranberry Liquid Preparation monograph is valuable to reveal 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

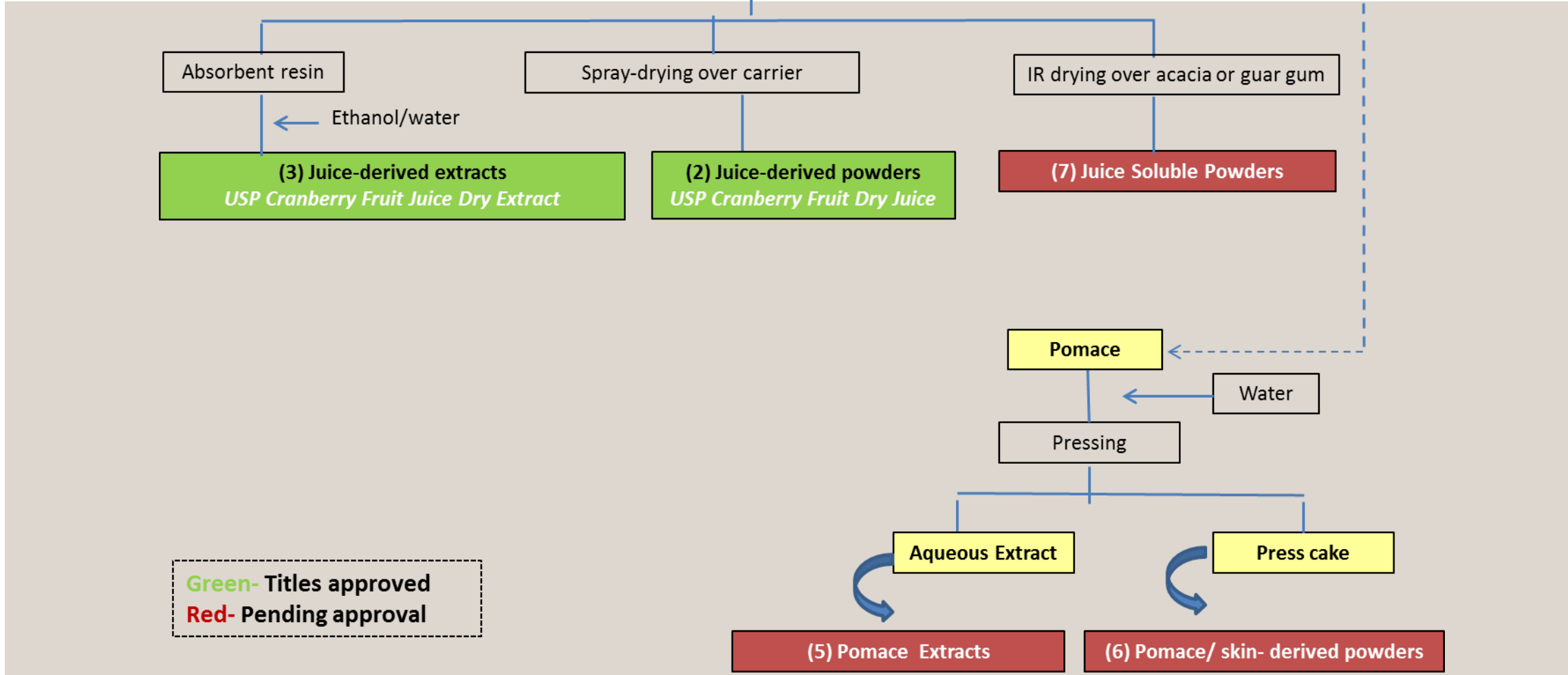
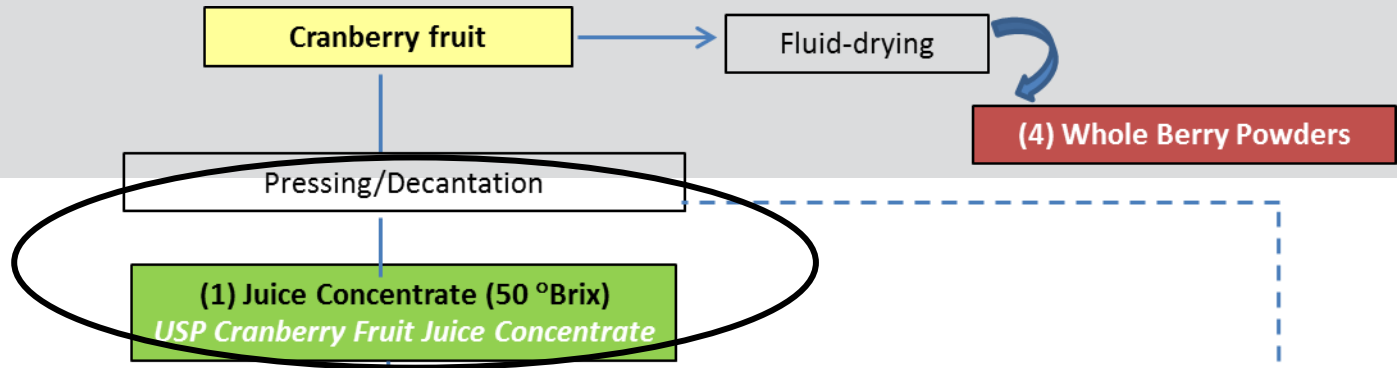


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Green- Titles approved
Red- Pending approval

Sweetened-dried cranberry (SDC) Processing



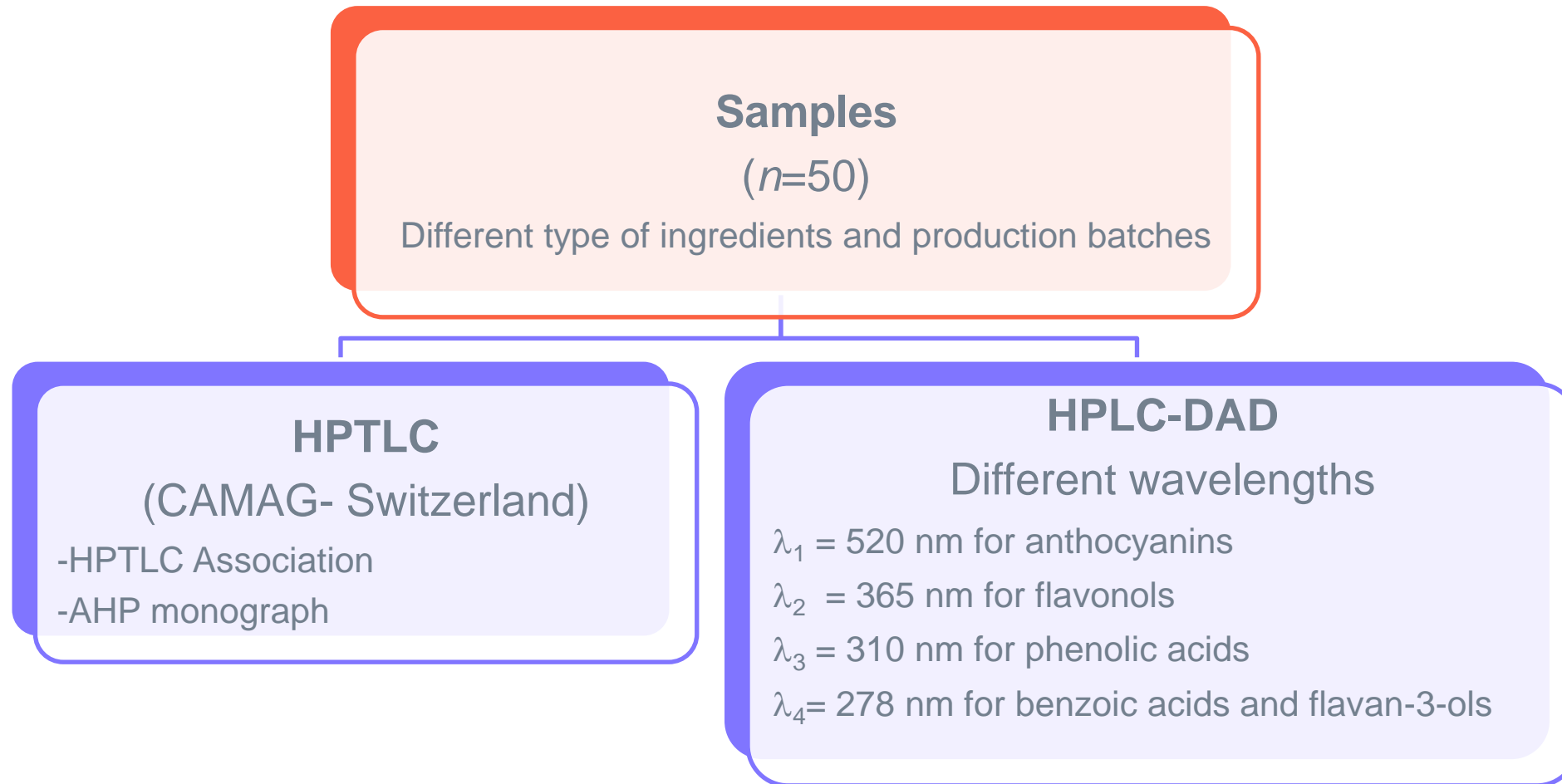
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On going Projects



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Lab project- Development of ID Tests



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