White Paper (ADPI Cheese Color Task Force): Annatto usage - an emerging problem

Over the last few months the ADPI Cheese Color task force has gathered, collected and distributed information on annatto and alternative colors. Two major suppliers of alternative colors have contributed information to help understand their products better. The goals of the task force were to:

1. Assess the impact of the use of Annatto cheese color on whey product quality and acceptability in the global market
2. Determine the acceptability of alternative colorants

The following challenges with the use of annatto as a cheese color will be addressed in this paper:

a) Color carry-over into the whey and the need for bleaching
b) The impact of bleaching on whey quality
c) Limitations on the marketability of whey products as ingredients in infant formula and other products due to the carry-over of annatto and/or compounds from bleaching

Annatto:

Annatto is the yellow/orange pigment used to color cheese. Annatto is an extract of the pericap or fruit wall of the shrub *Bixa orellana* and is among the oldest colorant known to man. The shrub is approximately 2 to 5 meters tall and is native to the American tropics. Many tropical countries such as Bolivia, Brazil, Ceylon, Dominican Republic, Ecuador, Guyana, India, Jamaica, Mexico, Peru and Surinam now grow *Bixa orellana*. The fruit is found in a burr-like pod containing 10 to 50 seeds about the size of grape seeds. The seeds are covered by a thin layer of soft, sticky bright red pulp. The pulp contains the annatto pigment. Annatto extracts may be water or oil soluble or suspensions of the pigment in oil. In general, mechanical friction in conjunction with solvents such as vegetable oil, fats, alkali and alcoholic solutions are used to leach the color from the pulp. The extract then is further refined depending on the final application. Precipitation with acids, recrystallization and spray drying in either oil or water soluble forms are possible processes. The major pigments comprising annatto are the bixin and norbixin in the cis and trans forms. Cis and trans refers to the isomers forms of norbixin. Isomers are compounds that have the same molecular formula but different molecular structure. Cis and trans refers to the position of groups around a double bond. Although isomers have the same chemical formula the compound can have very different properties depending on whether the reactive groups are in the Cis or trans position. The major pigment (+80%) of the seed coat is bixin. Stable forms of bixin were first isolated in 1913. Bixin is soluble in fats and oils at < 0.1% by weight while the potassium or sodium salt of norbixin is soluble in water. The color of bixin is dependent on pH and ranges from yellow orange to pink at lower pHs. pH does not affect stability of the color. Bixin is stable at temperatures less than 100°C (212°F) but relatively unstable at temperatures greater than 125°C (257°F). The pigment also is unstable to light. *Cis*-bixin is orange in color and insoluble in vegetable oil. Heating converts *cis*-bixin into the isomer *trans*-bixin which is red and soluble in oil.

Norbixin is formed when the methyl group of bixin is saponified under alkaline conditions. When the saponified form is placed in an acidic environment the molecule can convert to an insoluble form and precipitate. Emulsifiers may be used to prevent precipitation. Calcium in hard water or cheese also can cause precipitation of the pigment. Norbixin also will react with protein to form a peach-red color oxidation of annatto is very important to the whey industry. Oxidation leads to loss of color by annatto.
Oxygen is required and light acts as a catalyst for the reaction. Higher temperatures, presence of metal ions, greater intensity of light and greater availability of oxygen increase oxidation of annatto and loss of color. Contact with air is not considered very effective for oxidizing annatto. The presence of an antioxidant such as ascorbyl palmitate protects annatto from loss of color in the presence of lig Carotenoids are known to combine with proteins to stabilize the carotenoid molecule. In addition, annatto will react with carboxyl groups (-COOH). Because norbixin contains a carboxyl group the molecule can complex with divalent metal ions. Norbixin then is able to bind with the carboxyl group of another molecule thereby forming a stable complex. Such a complex can protect norbixin from oxidation and help retain the original color. While such a reaction may be desired for some products it may be a problem when color removal by bleaching is desired. Annatto can produce a pink color in cheese. The pink color may be the result of hydrogen sulfide causing precipitation of norbixin. The pink color may be protected from further color change by phospholipids and -casein. The pink color is stable to oxygen, light and pH changes. Treatments to the whey and brand of annatto used are believed to be important in determining whether the defect occurs.

Although annatto in cheese largely is combined with casein, a portion of the annatto will be present in the resulting whey. Research has indicated that 18 to 26% of the bixin added to cheese milk is present in the resulting whey. Lower levels of bixin addition had slightly higher percentages of bixin in the whey versus higher bixin addition levels. In general, approximately 20% of the bixin added to cheese milk partitioned into the whey. There is a lack of information on the status of norbixin in whey. Although norbixin will combine with casein it is not known whether norbixin has affinity for any specific whey components. Annatto in whey powder is believed to be associated with the whey proteins.

Summary:

- Annatto is an extract prepared from the seeds of the annatto tree (*Bixa orellana*) and is commonly used in colored cheddar cheese.
- The annatto pigment (bixin and norbixin) present in cheese color preparations, associates with casein and gives cheese a desirable yellow/orange color
- Bixin is hydrophobic whereas Norbixin is hydrophilic.
- Water/oil-dispersible annatto colors typically contain both pigments and food-grade emulsifiers.
- These extracts can be used to color food products, such as in cheese, which contain both aqueous and lipid phases
- Annatto is not stable
- Can produce a pink color during storage
- Annatto also can cause a pink color in secondary products
- Factors such as pH, heat treatment and brand of annatto used contribute to pinking defects
Cheese color (Annatto) use and its impact on whey product quality and acceptability in the global marketplace:

- Annatto (norbixin) added to cheese milk carries over into fluid whey, imparts undesirable color to spray dried product
- 18-26% of norbixin added to cheese milk partitions into the whey stream
- Norbixin plays no direct role in flavor of whey or whey protein
- To minimize the impact of colors in whey products ranging from dried whey to whey protein isolates bleaching must occur. Bleaching impact several sensory properties in finished products. Flavor Considerations in Manufacture and Use of Dairy Ingredients always must be a consideration for ingredient selections
- In the case of whey ingredients, functionality and nutrition are targets and so is flavor
- No flavor – no flavor carry through is the desired target
- Lipid oxidation and sulfur degradation products are the primary sources of off flavors in WPC80 and WPI
- Where do these compounds come from and how can we minimize? Three flavor sources: Cheesemake and starter, Whey protein (ingredient) processing, end user processing
- Influence of processing -- several sources. A few include: Storage of fluid product, Storage of dried product and instantization, Bleaching, Solids and pH
- Bleaching applied to fluid whey or retentate. Two approved chemical agents in U.S. Hydrogen peroxide (HP) Benzoyl peroxide (BP), Enzymatic bleaching Lactoperoxidase, Commercial peroxidase, Non-specific oxidation processes

Bleaching:

Hydrogen peroxide and benzoyl peroxide are the only compounds currently allowed in the United States for bleaching whey. Codex regulations permit the use of both hydrogen and benzoyl peroxide for bleaching. Previously only hydrogen peroxide was allowed.

Hydrogen peroxide is a clear, colorless liquid having a slightly pungent odor. Food grade hydrogen peroxide typically has a concentration of 30 to 50%. Hydrogen peroxide is safe and stable under recommended storage and handling conditions, although hydrogen peroxide will decompose by exothermic reaction when exposed to soil and other foreign materials. Hydrogen peroxide use as a bleaching agent is covered by 21CFR 184.1366. Hydrogen peroxide may be used at a rate of <500 ppm (<0.05%) and is considered effective at all temperatures and total solids levels.

Benzoyl peroxide or dibenzoyl peroxide is a colorless, crystalline solid. Benzoyl peroxide has a faint odor of benzaldehyde, is insoluble in water. The dry concentrated form of benzoyl peroxide is a highly reactive dangerous oxidizing material that may spontaneously explode. Commercial products may contain 15 to 35% benzoyl peroxide. Benzoyl peroxide use is permitted under 21CFR 184.1157 for
removing color in whey both from naturally occurring colored compounds and annatto addition except in whey products for infant formula. Benzoyl peroxide for bleaching has no use rate limitation other than that dictated by good manufacturing practices. A typical use rate for benzoyl peroxide is <20 ppm (< 0.002%). Most effective use conditions are 60°C (140°F) for 15 minutes at pH 6 to 7. Longer holding times are required if lower temperatures are used. Use of benzoyl peroxide in products for export can be a concern. Although CODEX regulations recently have changed to permit benzoyl peroxide as a bleaching agent some countries such as Japan still may be concerned about the use of benzoyl peroxide in imported products.

Hydrogen peroxide can alter the functionality of whey proteins. The susceptibility of whey proteins to hydrogen peroxide depends on the specific protein, concentration of hydrogen peroxide, temperature, time and pH. Hydrogen peroxide is known to inhibit browning in milk systems. Additional information on the mechanism of browning inhibition is lacking although it is likely hydrogen peroxide alters the ability of reactive groups on the proteins to interact with sugars thereby limiting the Maillard reaction.

Benzoyl peroxide combined with heat can affect whey proteins. Beta-lactoglobulin, α-lactalbumin, proteose peptone, serum albumin and immunoglobulin’s were altered by benzoyl peroxide addition of 10,000 and 50,000 ppm (1 and 5%). The effect of benzoyl peroxide on casein was less apparent. Very limited information is available on the effect of benzoyl peroxide on functional properties of whey. The foaming properties of whey protein isolate produced from sweet whey is not affected by either the annatto addition or bleaching by benzoyl peroxide although there may be slight differences in non protein nitrogen, true protein and ash content. Benzoyl peroxide can oxidize milkfat resulting in tallowy, oxidized flavors. Flavor problems are more apparent with increasing temperature, contact time and benzoyl peroxide. International concerns center on the use of benzoyl peroxide. Many Asian and European countries do not like the use of benzoyl peroxide although CODEX recently approved both hydrogen and benzoyl peroxide for use in bleaching whey. The presence of benzoic acid is an issue for certain markets. The major decomposition product of benzoyl peroxide is benzoic acid. The safety of benzoic acid and its derivative benzoates has been widely studied. Benzoates and the related salicylates are widely distributed in food plants and are present in prunes, tea, cloves, cinnamon and many berries. Benzoic acid and benzoates have been used as preservatives in food and beverages for approximately 100 years and are among the most commonly used additives.

Originally it was believed that benzoic acid related compounds did not cause adverse reactions when consumed. It is now apparent that a small percentage of the population is sensitive to such compounds. People with adverse reactions to benzoic acid related compounds typically have underlying diseases such as asthma. Asthmatics often are intolerant to aspirin, also known as 2-acetoxybenzoic acid and Oacetylsalicylic acid, which is very similar in structure to benzoic acid. The mechanism of the intolerance does not appear to be an allergy type but rather a pseudo-allergic response that relies on enzymes rather than an immunological reaction. Adverse reactions to benzoic acid related compounds appear to be relatively rare. An exception would be asthmatics with aspirin intolerance. Reactions generally are mild with life threatening reactions extremely rare.

Chemical or enzymatic bleaching of whey is a common practice in the industry used to remove the yellow-orange color in cheese whey.

Whey bleaching reduces the nutritive and functional value of proteins.
Bleaching can also generate off flavors.

Bleaching impact on **Flavor**:

- Hydrogen Peroxide (HP) bleaching has the biggest impact on off-flavor development
- Benzoyl Peroxide (BP) bleaching creates a product more similar to no bleach treatments

Bleaching impact on **Functionality**:

- HP bleaching alters heat stability and foam stability
- BP bleaching increases foam stability, same results observed with **WPC80** and **WPI**.

**A recap of bleaching concerns associated with the use of annatto**

- **Processing Issues**
  - Corrosive & hazardous chemical handling
  - Difficult to control resulting in variable and inconsistent color
  - Negative Impact processing

- **Quality Issues**
  - Negative impact on natural & pure image of Whey Proteins
  - Lipid Oxidation and Flavor problems.
  - Protein denaturation and functionality impairment

- **Regulatory Issues**
  - Prohibited in infant formula applications
  - Permissible by codex.
  - Increasingly unacceptable by many local authorities.

- **Economic Issues**
  - Restricted market for whey products
  - Equipment fouling
  - Capital and operational costs of bleaching
Limitations on the marketability of whey products as ingredients in infant formula and other products due to the carry-over of annatto and/or compounds from bleaching:

**Carry-over principle**

- Most countries have food regulations that allow for the inclusion of a food additive in a food, not as a result of direct addition, but as a result of it being included in an ingredient in the food – this is called ‘carryover’.

- In general the regulations allow for carryover of an additive as long as it meets certain criteria such as:
  - That the additive does not have a technological function in the food
  - That the amount of the additive doesn’t exceed the max level allowed for such a food
  - That the level of additive is no more than would be present under good technological conditions and GMPs.

- The principle relating to the carry-over of food additives into foods (the "Carry-Over Principle") addresses the presence of additives in food as a result of the use of raw materials or other ingredients in which these additives are used. The Codex Alimentarius Commission at its 17th Session (1987) adopted a revised statement of the principle as a Codex Advisory Text. The Text is printed in its entirety in Codex Alimentarius, Second Edition, Vol. 1 (General Requirements), pp. 85-88, 1992.
**Compliance with the Carry-over Principle**

- Other than by direct addition, an additive may be present in a food as a result of carry-over from a food ingredient, subject to the following conditions:
  - The additive is permitted in the raw materials or other ingredients (including food additives) according to this General Standard;
  - The amount of the additive in the raw materials or other ingredients (including food additives) does not exceed the maximum amount so permitted.
  - The food into which the additive is carried over does not contain the food additive in greater quantity than would be introduced by the use of the ingredients under proper technological conditions or manufacturing practice; and
  - The food additive carried over is present at a level which is nonfunctional, i.e. at a level significantly less than that normally required to achieve an efficient technological function in its own right.

**Non-Compliance with the Carry-Over Principle**

- An additive carried over into a particular food in a significant quantity or in an amount sufficient to perform a technological function in that food as a result of the use of raw materials or other ingredients in which the additive was used, be treated and regarded as an additive to that food, and shall be provided for according to the general principles of this Standard.

**Carry-over Principle exemption not applied to Infant Formula**

Some additives may be present in a food because they were contained in one of the ingredients. They need only be indicated in the list of ingredients if they perform a significant technological function in the final food. Whether or not the additive performs a technological function in the final product will depend both on the ingredient containing the additive and the food to which it is added. There is also a provision in legislation for what is known as reverse carry-over. In this instance, an intermittent ingredient can contain an additive that it would not normally be permitted to contain, on the basis that the additive is permitted for use in the final foodstuff and that the intermittent ingredient is used solely for the final foodstuff. The carry-over principle does not apply to infant formula, follow on formulae or weaning foods except where specifically provided for in legislation. The warning label for six colors, often referred to as the ‘Southampton colours’, was included in Article 24 of Regulations 1333/2008 on food additives. The requirement applies to all products placed on the market from 20th of July 2010 onwards, whereas foods placed on the market or labeled before this date can continue to be marketed until their best-before date.

Most countries have food regulations that allow for the inclusion of a food additive in a food, not as a result of direct addition, but as a result of it being included in an ingredient in the food – this is called ‘carryover’. In general the regulations allow for carryover of an additive as long as it meets certain criteria such as that the additive does not have a technological function in the food, that the amount of the additive doesn’t exceed the max level allowed for such a food, that the level of additive is no more than would be present under good technological conditions and GMPs. The principle relating to the carry-over of food additives into foods (the "Carry-Over Principle") addresses the presence of additives in food as a result of the use of raw materials or other ingredients in which these additives are used. The

Some infant formula manufacturers in the EU are informing suppliers that the EC Regulation n.1333/2008 on food additives confirms the prohibition of carry-over of food additives, including food colorings in foods for infants and young children, except where specifically provided for, and therefore:

- will not tolerate the presence of annatto in all the sweet whey derivatives they purchase
- for beta-carotene, which is naturally occurring in milk, request a level below the limit of 40 mg per kg of fat, reflecting a natural concentration in milk

**Annatto – Infant Formula Regulations:**

**US**

Annatto is not an approved food additive for Infant formula
All food ingredients in IF need to be GRAS
The carry over principle is allowed in foods
No specific exemption for infant formula.

**EMEA**

Annatto is not on the list of approved additives
Carryover principle allowed in foods, however it is not allowed for Infant formula

**Approved Additives Infant Formula:**

<table>
<thead>
<tr>
<th>E No.</th>
<th>Additive name</th>
<th>Maximum limit, restrictions / exceptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>E 270</td>
<td>Lactic acid</td>
<td>quantum satis only (†)-form</td>
</tr>
<tr>
<td>E 304</td>
<td>Fatty acid esters of ascorbic acid</td>
<td>ML = 10 mg/kg</td>
</tr>
<tr>
<td>E 306</td>
<td>Tocopherol-rich extract</td>
<td>ML = 10 mg/kg</td>
</tr>
<tr>
<td>E 307</td>
<td>Alpha-tocopherol</td>
<td>ML = 10 mg/kg</td>
</tr>
<tr>
<td>E 308</td>
<td>Gamma-tocopherol</td>
<td>ML = 10 mg/kg</td>
</tr>
<tr>
<td>E 309</td>
<td>Delta-tocopherol</td>
<td>ML = 10 mg/kg</td>
</tr>
<tr>
<td>E 322</td>
<td>Lecithins</td>
<td>ML = 1000 mg/kg</td>
</tr>
<tr>
<td>E 330</td>
<td>Citric acid</td>
<td>quantum satis</td>
</tr>
<tr>
<td>E 331</td>
<td>Sodium citrates</td>
<td>ML = 2000 mg/kg</td>
</tr>
<tr>
<td>E 332</td>
<td>Potassium citrates</td>
<td>...</td>
</tr>
<tr>
<td>E 335</td>
<td>Phosphoric acid</td>
<td>ML = 1000 mg/kg</td>
</tr>
<tr>
<td>E 339</td>
<td>Sodium phosphates</td>
<td>ML = 1000 mg/kg</td>
</tr>
<tr>
<td>E 340</td>
<td>Potassium phosphates</td>
<td>...</td>
</tr>
<tr>
<td>E 412</td>
<td>Guar gum</td>
<td>ML = 1000 mg/kg, only where the liquid product contains partially hydrolysed proteins</td>
</tr>
<tr>
<td>E 471</td>
<td>Mono- and diglycerides of fatty acids</td>
<td>ML = 4000 mg/kg</td>
</tr>
<tr>
<td>E 472c</td>
<td>Citric acid esters of mono- and diglycerides of fatty acids</td>
<td>ML = 7500 mg/kg, only when sold as powder.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ML = 9000 mg/kg, only sold as liquid where the products contain partially hydrolysed proteins, peptides or amino acids</td>
</tr>
<tr>
<td>E 473</td>
<td>Sucrose esters of fatty acids</td>
<td>ML = 120 mg/kg, only products containing hydrolysed proteins, peptides or amino acids</td>
</tr>
</tbody>
</table>
**China:**

Annatto is not an approved food additive for infant formula  
The carry over principle is allowed in foods  
  • No specific exemption for infant formula.

**Annatto Alternatives:**

**Alternative Colors: Beta-Carotene**

• **Beta carotene** is a fat-soluble yellow pigment and antioxidant found in grass. After a cow chews the cud, beta-carotene dissolves into the animal's fat stores and ends up in fat globules in its milk.

• Carotenoids are a family of more than 600 pigments found in nature. This includes cheeses, where the sumptuous oranges and yellows of a mature cheddar or gouda can make the difference between a consumer buying them, or not...

• We are one of the world's largest producers of carotenoids, most of which are nature-identical - which means that their chemical structures and properties cannot be distinguished from the carotenoids found in plants or animals.

• In cheeses, our carotenoids are natural coloring and stabilizing agents that help the product retain its appearance while withstanding direct sunlight.

• Beta-carotene is probably the most prominent of carotenoids and aside from imparting beautiful color into a cheese also plays an important role as a precursor to vitamin A – an essential vitamin for the body especially when combined with vitamins E and C.

• When combined there is strong evidence to suggest they can prevent degenerative diseases such as cardiovascular disease or cancer

• Color 1:1 match to traditional annatto

• No effect to cheese composition (Moisture, TS, Fat)

• No impact to cheese flavor

• No bleeding in bi-colored cheese (e.g. Colby Jack)

• Consistent color development regardless of conversion time

• Stability to oxidation – e.g. exposure to lighting

• Stability in packaging (MAP, vacuum, natamycin)

• Color stability over time (cheese matured for >2 years)

• No pinking/pH effects

• Suitability for use in processed cheese
**Alternative Colors: Paprika Oleoresin**

- Paprika is manufactured from the dried and ground sweet pepper pods of *Capsicum annum*. The colorings that impart the characteristic yellow to orange hue of paprika are *capsanthin* and *capsorubin*.

- Paprika, a red spice, imparts flavor and color to food. Paprika, the ground, dried fruit of *Capsicum annuum*, has been used as a color and/or spice for centuries as the raw ground powder in foods such as chili, chorizo, and goulash. Paprika color compounds can also be solvent extracted to produce paprika oleoresin, a purified form of the coloring compounds.

- The colorants found in paprika and paprika oleoresin are: *capsanthin*, *capsorubin* and *beta-carotene*, all of which are carotenoids. Paprika and paprika oleoresin are both stable to heat but sensitive to light and alkaline conditions. The pigments are naturally insoluble in water, especially the oleoresin. Food coloring manufacturers circumvent this through emulsification, allowing paprika to be used in both aqueous and lipid products. Food and beverage companies commonly use the oil soluble form of paprika oleoresin for coloring.

- Paprika is readily available and delivers a range of hues from light yellow to a unique dark orange. Paprika is very popular in Japan. The European Union requires labeling as a color additive E160c if the carotenoid content is greater than 7%. Paprika is oil soluble but it is not water soluble so it must always be emulsified to be water dispersible.

- SO-TEC Natural Cheese color (NCC22000) is a patent pending proprietary color formulation that was developed for use in Cheddar cheese manufacture by Socius Ingredients Inc.

  - Blend of red and yellow fat soluble carotenoid colors of paprika and beta-carotene.
  - Claimed to be selectively entrapped within the cheese curd.
  - Produce color free whey that will eliminate the need for a bleaching treatment of the whey.
  - It is an opaque, orange-red liquid that is dispersible in water or milk
  - Natural Cheese color is prepared by blending the red and yellow fat soluble carotenoid colors of paprika and beta-carotene to match the orange color shade of annatto.
  - This fat soluble blend is then encapsulated as a fat globule within a caseinate matrix and dispersed in an aqueous medium
  - SO-TEC Natural Cheese color produced cheese with a color similar to annatto colored cheese.
  - Whey produced from SO-TEC Natural Cheese color has a color identical to uncolored cheese whey, and would not require a bleaching treatment.
  - SO-TEC Natural Cheese color was resistant to pink discoloration during storage under fluorescent light
  - Unlike annatto, using of BC orange colorant produced uncolored whey stream during cheese manufacture.
• BC Orange colorant produced cheese with a color similar to annatto colored and no colored cheeses.
• BC orange cheese colorant was resistant to pink discoloration during storage under fluorescent light.

Beta Carotene – Infant Formula Regulations

• US
  – Approved food additive, CFR 73.95
  – Allowed for use in infant formula, CFR 184.1245
  – Beta-carotene may be used in infant formula as a source of vitamin A in accordance with section 412(g) of the Federal Food, Drug, and Cosmetic Act or with regulations promulgated under section 412(g) of the act.

• EMEA
  – Approved Food additive E160(ii)
  – Not an approved additive for infant formula
  – No carryover allowed in infant formula

• China
  – Not an approved Food additive
  – Approved for nutrition fortification - GB 14880 2012
  – Not an approved additive for infant formula
  – Ingredients of Vitamin A shall only include preformed retinol. When calculating or claiming activities of Vitamin A, no carotenoids ingredient shall be included’.
**Alternative Colors**

### Yellows
- **Beta Carotene**
- **Turmeric**

### Oranges
- **Annatto**
- **Paprika / β - Carotene**
- **Apo Carotenal / β - Carotene**
- **Paprika / Turmeric**

### Reds
- **Paprika**
- **Apo Carotenal**

**Clear Whey-Socius**
- Identical Color & Shade Match to Annatto – no delayed development
- No Color Transfer to Whey
- Greater Cheese Color Stability than Annatto – does not pink
- Age Stability – more than 3 years
- Fully Compliant with all global food legislative standards including CFR
- Fully Certified – Kosher, Halal, 3rd party audit, etc.
- Fully Commercial
- Color Stability

**White Whey (DairyMax) - Chr. Hansen**
- Color 1:1 match to traditional annatto
- No effect to cheese composition (Moisture, TS, Fat)
- No impact to cheese flavor
- No bleeding in bi-colored cheese (e.g. Colby Jack)
- Consistent color development regardless of conversion time
Stability to oxidation – e.g. exposure to lighting
– Stability in packaging (MAP, vacuum, natamycin)
– Color stability over time (cheese matured for >2 years)
– No pinking/pH effects
– Suitability for use in processed cheese

Clear Whey (Socius Ingredients)
Composition & Functionality

• Fat soluble colors, are encapsulated as fat droplets within a matrix and dispersed in an aqueous medium.
• The specific color pigment(s) are chosen to meet desired color shade.
• Readily dispersed in milk.
• Selectively entrapped within curd.
• 0% Transfer to whey.
This responds to your request for our opinion regarding a new process used to color cheese without impacting the color of whey – a byproduct of the cheese making process – which may be used by downstream customers in applications such as infant formula. Specifically, you have asked for our opinion on the regulatory implications of the new process in the United States, the European Union, and China.

**SUMMARY OF OPINION**

As discussed in greater detail below, it is our opinion that the new process is defensible in all three jurisdictions, assuming that the encapsulation materials and other substances used in the process have an appropriate regulatory status, and residual levels of such substances have no technical or functional effect in the whey or other whey-derived ingredient. This opinion is based on our understanding of the facts as provided to us, our examination of the relevant authorities in all three jurisdictions, and our general experience in this area of the law.

**BACKGROUND**

As you know, seasonal variations in ingredients and other factors can impact the color of finished cheese. Accordingly, manufacturers of cheese frequently add color – annatto, beta carotene, and others – to the cheese so that it meets consumer expectations as to physical appearance. Indeed, colors are permitted optional ingredients in a number of standardized cheeses.\(^1\) However, this often results in the presence of significant levels of residual colors in the byproducts of the cheesemaking process, including whey, which itself is further processed and used in a number of foods, including infant formula. The presence of these colors in infant formula in particular raises regulatory issues, particularly in the European Union and China.

To address this, the industry has developed two different color additive technologies that essentially encapsulate the color additive and impart color in the cheese but not in the whey. The first color additive technology is produced by Socius® under the trade name Clear Whey™.\(^2\) Socius describes the color additive as an encapsulated fat soluble color. We understand that the color additives that are encapsulated are beta-carotene and paprika. Socius has also confirmed

---

1. See, e.g., the Food and Drug Administration Standard of Identity for Cheddar Cheese (21 CFR §133.113(b)(3)), Edam Cheese (21 CFR §133.138(b)(3)), and Mozzarella Cheese (21 CFR §133.155(b)(3)).

that no residual level of color additive will be transferred to the whey produced as part of the cheesemaking process.

The second color additive technology is produced by Chr. Hansen under the trade name DairyMax™ or WhiteWhey™. We understand that the Chr. Hansen ingredient is also an encapsulated color additive technology and that low levels of residual color additive may remain in the whey. We further understand that DairyMax™ is encapsulated beta carotene. Chr. Hansen has stated that the residual level of beta carotene in the whey is “comparable to the naturally occurring levels typically found in milk today.”

DISCUSSION

1. Regulatory Status of New Color Additives – United States

Based on the information we have regarding the color additives described above, it is our opinion that there are no regulatory issues associated with the use of these color additives in cheese as long as the color additives that are encapsulated have an approved regulatory status in the U.S. and the material used to encapsulate the color additive has an appropriate regulatory status for use in food. The color additives that will be encapsulated are beta-carotene and paprika, which are both recognized as approved color additives in the U.S. –beta-carotene at 21 CFR 73.95, paprika at 21 CFR 73.73.340, and paprika oleoresin at 21 CFR 73.345. We have no additional information regarding the encapsulating agents.

---

3 Chr. Hansen WhiteWhey™, http://www.chr-hansen.com/news-media/singlenews/whitewheyTM-is-the-right-way.html; described as having an 85-95% reduction in color transfer to the whey.

4 Chr. Hansen has also stated that infant formula companies view beta-carotene as an acceptable alternative to annatto or bleaching agents and that Danone Baby Nutrition has set a limit of beta carotene in sweet whey derivatives (for use in EU) as below the limit of 40 mg per kg of fat, reflecting a natural concentration in milk and other suppliers, such as Friesland Campina, which have set the same limit. See email from Dan Meyer to Rick Mann dated May 22, 2015.

2. Regulatory Status in Infant Formula of Whey that is a Byproduct of the Cheese Making Process that Uses the New Color Additive Technology

A. United States

We see no regulatory issues with the use of the whey that is a byproduct of the cheese that is produced with the use of the encapsulated color additives in infant formula. We do recommend confirming that the encapsulating agents used in the new color additive technology have an approved regulatory status for use in food, especially for the Chr. Hansen color additive where residual levels end up in the whey product.

B. European Union

As discussed further below, it is our opinion that the color additive technology that results in no residual level of color additive in the whey (Socius®) would be acceptable in the EU and the color additive technology that may result in residual levels of color additive in the whey (Chr. Hansen) may be acceptable assuming: (1) the residual level does not endanger the health of infants; (2) the residual level must not impart any color to the whey, even unintentionally; (3) the residual level of beta-carotene must be consistent with the levels that are naturally occurring in cow milk; and (4) there are no physiochemical differences between the residual beta-carotene and the beta-carotene that is naturally occurring in cow milk. We also recommend confirming that the encapsulating agents have an appropriate regulatory status in the EU if they are part of the residual level that ends up in the whey.

First, there is no legal definition of “whey” under EU law. It is included in the definition of a “milk product” per the COM Regulation, but it is not subject to any specific legislation with regard to its manufacturing process and composition. As a byproduct in the manufacture of cheese, it is well known to vary in characteristics with the type of cheese from which it originates. Accordingly, it is our opinion that the fact that the whey that may contain residual levels of the encapsulated color additives does not raise any issues with regard to a standard for whey.

To be lawfully used in the manufacturing of infant formula, the addition of the whey must not result in infant formula that is out of compliance with the specific compositional requirements set in


\[\text{Reference} \text{\footnote{http://www.food.gov.uk/sites/default/files/multimedia/pdfs/milkproductguide.pdf}}\]
Directive 2006/141/EC. In particular, it must not contain any substance in such quantity as to endanger the health of infants and young children.\(^8\)

Regarding the use of whey that is a byproduct of the cheese making process that uses beta-carotene and/or paprika as a color additive, the main regulatory issue relates to the presence of those colors in the whey due to transfer during the curd formation and straining (whey removal). In short, the first technology having no residual level transferred in the whey will not raise any regulatory issues and the second technology may raise some issues depending on the nature, technological properties and level of the residues that remain in the whey.

The use of beta-carotene (E 160a(i) or (iii)) and paprika (E160c)\(^10\) is permitted in ripened orange, yellow and broken-white cheese like cheddar at quantum satis level\(^11\) per the Food Additives Regulation 1333/2008.\(^12\) However, it is not permitted in infant formula\(^13\) (including by virtue of the carry-over principle).\(^14\) That being said, the residual presence of beta-carotene in whey that results from the presence of this color additive in the manufacturing of cheese from which it is a byproduct is arguably unintentional. It must be distinguished from the intentional use of beta-carotene in whey that would be carried-over in the infant formula. In our opinion, while beta-carotene is not permitted as a food additive in infant formula per EU law, its unintentional presence in whey, which is used as an ingredient in infant formula does not suffice to be considered unlawful per the EU Food Additives Regulation. The

---

\(^8\) Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC Text with EEA relevance, OJ L 401, 30.12.2006, p. 1–33. “Infant formulae” is defined as “foodstuffs intended for particular nutritional use by infants [children under the age of 12 months] during the first months of life and satisfying by themselves the nutritional requirements of such infants until the introduction of appropriate complementary feeding.” See Article 2(c) of Directive 2006/141/EC.

\(^9\) Article 4 of Directive 2006/141/EC.


\(^11\) ‘quantum satis’ shall mean that no maximum numerical level is specified and substances shall be used in accordance with good manufacturing practice, at a level not higher than is necessary to achieve the intended purpose and provided the consumer is not misled.


\(^13\) Article 16 and Annex II to Regulation 1333/2008

\(^14\) Article 18(2) of Regulation 1333/2008
critical element for determining if the presence of beta-carotene at residue level is in breach of the EU Food Additives Regulation is whether it performs a technological function in whey. If it imparts color to the whey, it may be difficult to rebut the presumption that beta-carotene is an unauthorized color additive in infant formula carried over from the whey. However, it is our understanding that any residual color additive would not impart color to the whey, thereby not triggering the restriction under EU regulations.

We also note that it is our understanding that beta-carotene is naturally occurring at low levels in cows’ milk and therefore in the whey from which it is derived. Accordingly, there should be plausible arguments to mitigate the enforcement risk associated with the residual presence of beta-carotene in whey used in infant formula provided:

1) Such quantity of beta-carotene does not endanger the health of infants, for example, it does not negatively impact the estimated intake of beta-carotene ingested by infants as a result of the regular consumptions of foods (restricted diet of infants) in which it occurs naturally.

   - Beta-carotene is not authorized for addition to infant formula – by contrast with processed cereal-based foods and baby foods for infants and young children\(^{15}\) - as a source of vitamin A owning to the lack of knowledge on the bioconversion of carotenoids in infants\(^{16}\).

   - Per EFSA\(^ {17}\), the use of beta-carotene as a food colour is not a safety concern, provided that the estimated combined intake from its use as a food additive and as a food supplement is not more than the amount likely to be ingested as a result of the regular consumption of foods in which it occurs naturally (5–10 mg/day). This would ensure that the exposure to beta-carotene from its use as a food additive and a food supplement would remain below 15 mg/day, the level of supplemental intake of beta-carotene for which epidemiological studies did not reveal any increased cancer risk.

2) There is no difference in the physicochemical characteristics between the beta-carotene added during the manufacturing of cheese and the one naturally occurring in milk.


\(^{17}\) EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2012a. Scientific Opinion on the re-evaluation of mixed carotenoids (E 160a (i)) and -carotene (E 160a (ii)) as a food additive. EFSA Journal, 10(3):2593, 67 pp
Since we have no additional information regarding the encapsulating agents, we are not able to opine on the related regulatory issue. Anticipating that encapsulating agents may be regarded as food additives under EU law (carriers), their status under the EU Food Additives Regulation needs to be assessed to determine if their presence in infant formula raises any issues.

C. China

As discussed further below, it is our opinion that as long as the encapsulated color additives are permitted for use in cheese, the cheese’s byproduct –whey– may be used in infant formula which may or may not contain naturally occurring levels of color additive.

First, the national food safety standard (GB2760-2014) provides the use of approved food additives in various food categories. The regulatory status of annatto, beta-carotene and paprika is as follows:

a) Annatto (INS 160(b)) is permitted as a color additive in ripened cheese and processed cheese.

b) Beta carotene (INS 160(a)) is permitted for use in unripened cheese, ripened cheese, processed cheese and cheese analogues.

c) Paprika orange and Paprika red are permitted color additives; however, they are not approved for use in any cheese categories. Paprika oleoresin (INS160c) is permitted for use in processed cheese only.

Second, “whey powder” and “whey protein powder”, produced from whey, are regulated by the national standard of GB 11674-2010. Thus, a whey product which complies with GB 11674-2010 may be sold and used in China.

Third, Article 3.4 of GB2760-2014 permits the carry-over of food additives through a food ingredient (including food additive) where:

a) the food additive is permitted to be used in the food ingredient;

b) the use level of the food additive in the ingredient shall not exceed the maximum permissible use level;

c) the ingredient is used under normal manufacturing conditions and the presence level of the additive in food shall not exceed the level carried over by the ingredient; and

d) the presence level of the additive in food carried over by the ingredient is obviously lower than the usual level where the additive is directly added in food.

GB2760-2014 does not make a distinction between infant formula and foods intended for other populations. Accordingly, it is our opinion that, in the absence of a clear regulation, a food additive may be carried over into infant formula provided the prescribed conditions are met.
We consulted with one of the officials who shepherded the drafting of this standard, GB2760-2014, who agreed with our view this regard.

Fourth, GB10765-2010 is the food safety national standard for infant formula products. Article 4.1 of this standard requires that “ingredients shall comply with applicable safety standard and/or relevant regulations in order to ensure safety of infants, satisfy nutrition needs, and shall not use any substances that endanger nutrition and health of infants.” Accordingly, if a whey product is added to infant formula, the whey must comply with the applicable standards, which in this case would be GB 11674-2010 for whey powder and whey protein powder, as discussed above. Further, if any food additives are used in the whey, such use also must be consistent with the food additive standard, GB2760. We understand that here, the color additives are not being added to the whey products although in some cases some residual levels may end up in the whey products as a result of their use in the cheese making process.

As set forth in GB2760-2014, beta carotene and some paprikas are authorized for use in different cheese categories. For example, since beta carotene is approved for use in ripened cheese, its presence in the byproduct of ripened cheese, i.e., whey, may be justified based upon the carry-over principle. Subsequently, residual levels of beta carotene in infant formula as a result of its presence in the whey, an ingredient of infant formula, may also be acceptable. This would not be the case if the color additive is not permitted in the cheese itself. Accordingly, it is our opinion that approval of the color additive in a cheese category would be essential to support its presence at residual levels in whey used in infant formula.

We also suggest continued monitoring of developments in the regulation of infant formula. Many new requirements and policies for infant formula are driven by international regulatory practices, consumers and media coverage. While we do not see any regulatory issues associated with use of whey containing residual levels of colors in infant formula under the circumstances outlined above, this could be affected by new regulations or new interpretations of existing regulations.\textsuperscript{18}

3. Ingredient Declaration for the New Color Additive Technology in Cheese – United States

The color additives that will be encapsulated and used to produce cheese are all approved color additives that can be used in foods generally in amounts that are consistent with good manufacturing practices (GMPs) and may be used to color standardized foods if the standard of identity authorizes the use of added color. As noted above, a number of cheese standards of identity expressly permit the addition of colors. Accordingly, there are no issues associated with the use of the color additives beta-carotene or paprika in such standardized cheeses.

\textsuperscript{18} For example, we note that the industry has long taken the position that enzymes approved in GB2760 in general food also permit their use in infant formula. However, a recent discussion on an ingredient in infant formula suggest that officials are considering the need for specific petitions for enzymes intended for infant formula due to special safety concerns. This signifies the trend of the Chinese authorities to enhance management of infant formula related issues.
You have also asked how the new color additives would be declared on the cheese label in the U.S. The color additives used, beta-carotene and paprika, are not subject to certification. Thus, they may be declared as:

- “artificial color,”
- “artificial color added,”
- “color added,”
- or by an equally informative term that makes clear that a color additive is present in the food.

The color additives may also be declared as “colored with ____” or “_____ color,” with the blank filled with the specific name of the color additive.\(^\text{19}\) Finally, we note that alternative color additive labeling (e.g., “may contain annatto, paprika, beta-carotene”) is technically not permitted in the U.S.

The regulatory citations that support the use of “color added” when β-carotene and paprika oleoresin are added to a standardized cheese such as a cheddar cheese:

• The relevant provisions are found in the regulations that identify the labeling requirements for color additives 21 CFR 101.22(k) and the cheddar cheese standard of identity at 21 CFR 133.113. We start first with the cheese standards of identity because they control the ingredients that may be added to cheese.

• Each cheese standard of identity must be reviewed to determine whether a color lawfully may be added to the cheese and whether it is subject to any special labeling requirements. Many of the cheese standards allow for the addition of colors. For example, the standard of identity for cheddar cheese lists “coloring” as an optional ingredient and then goes on to require that “[e]ach of the ingredients used in the food shall be declared on the label as required by the applicable sections of parts 101 and 130 of this chapter.” 21 CFR 133.113(b)(3)(i); 133.113(d). For cheddar cheese, the added color must be declared as required by the relevant provision in 21 CFR Part 101.

• The ingredient labeling requirements in 21 CFR 101.22 identify the manner in which colors must be declared in the ingredient statement. The color labeling requirements state in relevant part: (2) Color additives not subject to certification and not otherwise required by applicable regulations in part 73 of this chapter to be declared by their respective common or usual names may be declared as “Artificial Color,” “Artificial Color added,” or “Color Added” (or by an equally informative term that makes clear that a color additive has been used in the food). Alternatively, such color additives may be declared as “Colored with _____” or “_____ color,” the blank to be filled in with the name of the color additive listed in the applicable regulation in part 74 of this chapter. When a coloring has been added to butter, cheese, or ice cream, it need not be declared in the ingredient list unless such declaration is required by a regulation in part 73 or part 74 of this chapter to ensure safe conditions of use for the color additive. Voluntary declaration of all colorings added to butter, cheese, and ice cream, however, is recommended. 21 CFR 101.22(k) (emphasis added).

• The alternate colors provided are exempt from certification under 21 CFR §§ 73.95 (β-carotene) and 73.345 (paprika oleoresin). Because these colors are exempt from certification, they must be labeled in the cheddar cheese consistent with the requirements in 21 CFR 101.22(k), which allows them to be labeled as “colored with β-carotene and paprika oleoresin,” “artificial color,” or “color added,” or an equally informative term. Any of these labeling options is appropriate. Under 21 CFR 101.22(k)(2), the colors, therefore, can be identified by the term “Color Added.

Additional Regulatory Issues Regarding Annatto Color Use in Cheese (Keller & Heckman):

Regulations in the U.S., China and the EU regarding the use of color additives in cheese which may, in turn, be present as carryover ingredients in whey and in infant formula (IF).
In summary, for all regions, there is no prescribed method of analysis for annatto in IF; further, the carryover color would generally have to be declared as an ingredient of the whey if it imparts color but would be exempt from declaration if it does not have a technical or functional effect in the whey (does not impart color).

UNITED STATES

1) What are the testing requirements/detection limits for Annatto in IF for US?

There are no specific prescribed methods of detection for annatto in IF in the US. There are specifications for annatto in the color additive regulation at 21 CFR 73.30 but the regulation does not discuss a method of detection for annatto. See http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=73.30.

We located one paper that discusses the use of an HPLC method of detection for annatto in whey but we did not quickly find anything regarding the method of detection in IF. See http://www.ncbi.nlm.nih.gov/pubmed/24377797. We would be happy to conduct additional research in the scientific literature to locate methods or follow up with our contacts at various labs to see if they would suggest a certain method of detection for annatto in an IF matrix.

2) Are there any labeling requirements for whey products with carryover of color (either annatto or beta carotene / paprika)?

In the US, section 403(i) of the Food, Drug and Cosmetic Act (FD&C Act) requires the label of a food (including food ingredients such as whey) to list all ingredients. “Color additives” are considered ingredients that are defined as “a dye, pigment or other substance . . . when added or applied to a food . . . is capable (alone or through reaction with other substance) or imparting color thereto.” See Section 201(t)(1) of the FD&C Act. The only exemption from labeling declaration of ingredients is for incidental additives that are “present in a food at insignificant levels and do not have any technical or functional effect in that food.” See 21 CFR 101.100(a)(3), http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=101.100. One of the examples of an incidental additive is a substance that has “no technical or functional effect but are present in a food by reason of having been incorporated into the food as an ingredient of another food, in which the substance did have a functional or technical effect.” As such, if the color that carries over to the whey as a result of the cheese making process has no technical or functional effect in the whey, then the color would be exempt from declaration; however, if the carryover color imparts color to the whey, it is difficult to take the position that it has no technical or functional effect. Thus, where the carryover color imparts color to the whey we believe it would be considered an ingredient that must be declared in the whey.
**CHINA**

1) What are the testing requirements/detection limits for Annatto in IF for China?

China routinely provides test methods as part of specific food safety standards; however, there does not appear to be any methods of detection for annatto in IF in China. In the event the government authority needs test for a substance, based on our previous experience, they usually refer to methods recognized by international organizations or other food national authorities.

Having said that, the authority is working on a draft test method to determine bixin and norbixin (annatto in China is also known as bixin and norbixin) in some imported foods by using liquid chromatography. Cheese is the only dairy product included in the method. Infant formula is not mentioned. The proposed detection limit in cheese in the draft method is 1 mg/kg. The method of detection has not yet been published.

We understand that you haven’t inquired about the detection limit for beta carotene, but we thought you may be interested. China has developed a national standard (GB 5413.35-2010) to determine beta carotene in infant formula. The detection limit is 2 μg/100g.

2) Are there any labeling requirements for whey products with carryover of color (either annatto or beta carotene / paprika)?

According to the food labeling standard (GB7718-2014) in China, where a food additive is carried over into a finished food but does not function in the finished food, the food additive is exempted from labeling on the package of the finished food, assuming the carry-over principle prescribed in the food additive standard (GB2760-2014) is also satisfied.

The memo we previously sent to you outlined the criteria of food additive carry-over in GB2760-2014 and discussed an example of beta carotene and ripened cheese. In that example, we took the position that the presence of residual level of beta carotene in the byproduct of ripened cheese may be justified in terms of carry-over. Therefore, based on the carryover principle, the label on the whey need not declare beta carotene based on the above labeling exemption in GB7718-2014.

However, it is important to note that the issue of technical or functional effect in the ingredient must be addressed as well. We understand that the whey is a byproduct of the ripened cheese and that the color additive is used to impart color to the cheese but incidentally may impart color to the whey. Per GB7718-2014, the label of a food shall declare the ingredients used during the manufacturing process of the food. Here, the color is used indirectly in the manufacture of the whey. If the whey is colored as a
result, we believe that it would be considered an ingredient in the whey, and must be declared. If there is no color imparted to the whey, or it is removed, then the color would not have to be declared.

EUROPEAN UNION

1) What are the testing requirements/detection limits for Annatto in IF for EU?

While annatto is permitted for use in various food commodities, it is not authorized in IF under EU law (including by virtue of the carry-over principle). Thus, there is no method of detection for annatto in IF in the EU.

Under EU law, the specifications for Annatto (E160b) – as set in Regulation 231/2012 – only refer to spectrometry for identification purpose of two main colored compounds: Bixin (maximum in chloroform at ca. 502 nm) and Norbixin (maximum in dilute KOH solution at ca. 482 nm).

The availability of methods used for analysis of annatto in the various food matrices is not prescribed by law. Typically, EU regulators/assessors and national enforcement authorities refer to published methods and methods recognized/validated internationally to determine the presence of EU-permitted additives in foods.

Of course, there is also a need for analytical methods capable of detecting very low levels of annatto in food ingredients and commodities in which it is not permitted but the developments in that respect are primarily driven by non-compliance records. Unlike spices, sauces, oils, fruit nectars, surimi sticks where non-permitted annatto was detected many times by national enforcement authorities in the past years, there is no reported incident on non-permitted annatto in IF. We anticipate that it also results from the specific nature of IF and the very strict quality systems in place in the baby food industry to ensure compliance, especially in a proactive manner and through the adoption of a ‘zero tolerance’ approach as applied to the monitoring of illegal colors. As a direct consequence, we have not been able to identify any official document with regard to the detection of annatto in IF. That being said, we anticipate that the available methods for annatto are currently used by the baby food industry to ensure compliance.

The recent investigation report on the availability of methods used for analysis of annatto in the most relevant food matrices by EFSA and the Joint Research Centre (JRC) gives the following overview of the state of art of the methods for the analysis of annatto in different foodstuffs:

*Published methods for annatto are adequately sensitive for the levels of annatto added for colouring purposes. HPLC methods must be capable of detecting and quantifying all of the main bixin and norbixin isomers. However, as for other natural colorants, the access to well characterised reference materials for the main colouring components is crucial (Scotter, 2011).*
Methods for the determination of annatto in a variety of foodstuffs are reasonably well established and have been validated for a number of different sample types (M.2.2.19, M.2.2.21). Conditions for the extraction and cleanup are sample dependent and require refinement to widen the scope of the methods to all foodstuff covered by EU regulations. The methods reported by Scotter et al. (2002) M.2.2.17, Breithaupt (2004) M.2.2.22, and Noppe et al. (2009) M.2.2.21 could be considered as a basis for future method development and validation.

We note that the various reviews carried out by MJ Scotter from the UK Food and Environment Research Agency are commonly referred to by EU regulators and assessors when it comes to methods for the determination of EU-permitted added natural colors in foods. Of particular interest, the data evaluated and the specific recommendations made by Scotter to the UK authorities in its 2010 report gives a good overview of the extraction and analysis conditions for a selection of available methods for annatto that can be used by EU enforcement authorities. We have extracted the following summary table from the report (table 4) for the dairy products:

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Applications</th>
<th>Typical extraction conditions</th>
<th>Typical analysis conditions</th>
<th>Validation</th>
<th>LOQ/LOD</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bixin and norbixin cis/trans isomers</td>
<td>Candies, cheese, butter and margarine</td>
<td>Sample size 20g. Extraction / partition using ethanolic aqueous ammonia, pet ether, hexane and CHCl3.</td>
<td>RP-HPLC with UV-VIS detection at 500nm</td>
<td>Recoveries from cheese 92.6%, butter 93.2%, candies 88%.</td>
<td>LOQ ca. 5 ng/g</td>
<td>Lancaster and Lawrence, 1995</td>
</tr>
<tr>
<td>Norbixin and bixin cis/trans isomers + other isomers</td>
<td>165 composite + 2 single foods – cheese, margarine, fish, edible ices, snacks, bakery wares, desserts, compound foods</td>
<td>Five different extraction regimes depending upon sample type. Either variant of extraction / partition using ethanolic aqueous ammonia, hexane with Celite; partitioned CHCl3/HOAc; or Biphasic partition CH3CN/hexane. Antioxidant added</td>
<td>RP-HPLC with PDA detection at 455nm x 10nm BW</td>
<td>Single lab validation with IHRMs. Recoveries 61-96% for 12 different commodities spiked at 1.7 – 27.7mg/kg.</td>
<td>LOQ 0.1 mg/kg</td>
<td>Scotter et al., 2002</td>
</tr>
<tr>
<td>Bixin and norbixin cis/trans isomers</td>
<td>Cheese, butter and cream</td>
<td>Sample size 5g. Extraction with acetone/HCl (cheese) or ethanolic aqueous ammonia, pet ether (butter and cream) ,centrifugation, filtration, SPE</td>
<td>RP-HPLC with UV-VIS detection at 460nm</td>
<td>Recoveries 80-100%</td>
<td>LOD 0.15 mg/kg</td>
<td>Bareth et al., 2002</td>
</tr>
</tbody>
</table>
2) Are there any labeling requirements for whey products with carryover of color (either annatto or beta carotene / paprika)?

We note that the use of certain colors is permitted in ripened orange, yellow and broken-white cheese like cheddar per the Food Additives Regulation 1333/2008. However, color is not permitted in infant formula (including by virtue of the carry-over principle as defined in the Regulation).

According to the EU Food Labelling Regulation 1129/2011, the list of ingredients shall include all the ingredients of the food, in descending order of weight, as recorded at the time of their use in the manufacture of the food.

The term ‘ingredient’ is defined as any substance or product, including flavourings, food additives and food enzymes, and any constituent of a compound ingredient, used in the manufacture or preparation of a food and still present in the finished product, even if in an altered form; residues shall not be considered as ‘ingredients’. (emphasizes added)

The intentional use of color in whey would be regarded as use of a food additive in the manufacture of whey and therefore as an ingredient of whey that would be ‘carried-over’ in the meaning of the Food Additive Regulation in IF. However, as further detailed in our draft memorandum, the residual presence of color in whey that results from the presence of this color additive in the manufacturing of cheese from which it is a byproduct is arguably unintentional. Although unintentional, if the color has an impact on the color of the whey it will be difficult to take the position that it is a residue because it has a technical or function effect on the finished whey and, thus, the color would be considered an ingredient (that needs to be approved under the Food Additives Regulation) and declared in the whey. If the color is present at such low levels that it does not impart color to the whey we believe you can take the position that it is a residue and would not have to be declared.
### FDA COLOR REGULATION

<table>
<thead>
<tr>
<th>“Subject to Certification”</th>
<th>“Exempt from Certification”</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Artificial Colors”</td>
<td>“Natural or Nature Identical Colors”</td>
</tr>
<tr>
<td>Broadly equates to “Artificial Colors”</td>
<td>Broadly equates to “Natural Colors”</td>
</tr>
<tr>
<td>Term “Artificial” has no legal status as color descriptor</td>
<td>Term “Natural” has no legal status as color descriptor</td>
</tr>
<tr>
<td>Includes chemically synthesized Lakes &amp; Dyes</td>
<td>Includes Annatto, Beta Carotene &amp; Paprika</td>
</tr>
</tbody>
</table>

“Nature Identical” is a term used commercially (again term has no legal status) to describe colors that are synthetic versions of a naturally occurring color.

Annatto & Paprika only available in “natural forms”. (Apo Carotenal may be considered a nature identical form of Paprika)

Beta Carotene is available as both “natural” and “nature identical / synthetic” form

---

http://www.fda.gov/ForIndustry/ColorAdditives/ColorAdditiveInventories/ucm115641.htm

---

**Chr Hansen clarification on additional questions:**

Apologies for the delay, but we just wanted to confirm with our colleagues in The Netherlands about their market.

* In the presentations at ADPI a couple questions were presented to Chr. Hansen on Annatto testing and Beta Carotene testing limits in IF.

* LOD for HPLC methods is dependent on lab, method of extraction and method/column dependent. Accuracy is influenced by concentration – low levels may give more variability. In our experience with various labs, the range of detection limit is between 0.001 and 0.03ppm.

* Another question was acceptance of beta carotene as a substitute by the IF companies.

* The IF companies have communicated an acceptance of BC as an acceptable alternative to annatto or bleaching agents used to remove annatto color from whey.
* Danone Baby Nutrition has set a limit for beta-carotene for their suppliers of sweet whey derivatives (for use in their products for the EU Infant Milk Formula market) as below the limit of 40 mg per kg of fat, reflecting a natural concentration in milk.
* FrieslandCampina has set the same limit
* Other suppliers have set similar limits.

* We also understand the Dutch have switched to Beta Carotene. Is that true?

* Yes that is true. Where color is used, the entire Dutch Cheese industry with the exception of one medium size cheese producer that is not processing the whey has switched to the DairyMAX WhiteWheyTM solution.

<table>
<thead>
<tr>
<th>Color Projects</th>
<th>Estimated Ingredient Costs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DairyMax (White Whey)</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Ingredient availability**

**Current cost of color and color bleaching**

<table>
<thead>
<tr>
<th></th>
<th>Regular annatto</th>
<th>$H_2O_2$</th>
<th>Total cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost per Gallon</td>
<td>$22,000</td>
<td>$3,948</td>
<td></td>
</tr>
<tr>
<td>Usage per vat (Gal)</td>
<td>0.49136</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Cost per vat</td>
<td>$10,810</td>
<td>$1,421</td>
<td>$12,23120</td>
</tr>
<tr>
<td>Cheese (lbs) per vat</td>
<td>6300</td>
<td>6300</td>
<td></td>
</tr>
<tr>
<td>Cost per lb of colored cheese</td>
<td>$0.00172</td>
<td>$0.00023</td>
<td>$0.00194</td>
</tr>
</tbody>
</table>

**If switched to Beta-Carotene**

<table>
<thead>
<tr>
<th></th>
<th>Beta-Carotene</th>
<th>$H_2O_2$***</th>
<th>Total cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost per Gallon</td>
<td>$49,020</td>
<td>$3,591</td>
<td></td>
</tr>
<tr>
<td>Approx usage per vat (Gal)</td>
<td>1.2284</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Cost per vat</td>
<td>$60,216</td>
<td>$0,000</td>
<td>$60,21617</td>
</tr>
<tr>
<td>Cheese (lbs) per vat</td>
<td>6300</td>
<td>6300</td>
<td></td>
</tr>
<tr>
<td>Cost per lb of colored cheese</td>
<td>$0.00956</td>
<td>$0.000000</td>
<td>$0.009560.0076</td>
</tr>
</tbody>
</table>

More expensive than current ingredient costs

**Additional Product Information on DairyMax (Chr Hansen) and Clear Whey (Socius Ingredients) is available upon request**