Formation of Flavor
Is Natural the same as Clean Label?

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“Natural” ingredient manufacturers utilize rules set forth in the Code of Federal Regulations 21CFR101.22. In Europe, European regulation EC 1334/2008, defines the naturalness of food flavors. Studying how these flavor-impact molecules are made in nature, producers use microbes or purified enzymes to facilitate these reactions. Methods now utilized to form “natural molecules” from plant products include fermentation, enzymology, and “soft chemistry” followed by subsequent purification or isolation such as by steam distillation, extraction, chromatography or crystallization.

The drive for “Clean Labels” was born out of consumers desire to read labels and find materials they recognize from daily life. “Clean label” today is where “Natural” was decades ago. Consumers do not necessarily equate clean label to natural; they dislike a “Black Box-Natural Flavors” statement. A primary challenge is that the terms “Natural” and “Clean Label” are regulated and interpreted differently around the world. In this talk we will discuss the formation of flavors by the respective methodology that in turn will help respective attendees determine the best label for their products.
Sensory, trigeminal and psychology along with smell, sight and sound all play a role in enjoying food. Our ancestors on discovering fire, understood the value of putting food on fire – prehistoric grilling and smoking. It became obvious to them it’s a good method of preservation and increase scrumptiousness – **taste**. The dawn of taste science.
Consumers consider food “Natural” when it comes from plant, or animal.

The feed stock and maintenance of “health” methodology has an impact on labeling.

Not using pesticides, weedicides and renewable fertilizers in crop production afford Organic crops.

The use of certified organic feed stocks (grains and grasses) and not using growth promoting hormones or antibiotics afford Organic Animal proteins.

Minimal methods of processing, allows material to be labelled as such. For example HPP (high pressure processing – cold pasteurization by high isostatic pressures)
Plant products are converted to “Natural ingredients” by fermentation.

Understanding the biochemical pathways that form molecules in “Nature”, we can guide/direct cells to mimic these reactions. It's achieved by a few different methodologies and has an impact on the final regulatory label.

Cells grown in Fermenters are rich in enzymes. These can be utilized to transform plant material to useful molecules for food.

These whole cells could be Fungi, Bacteria or yeast. – Single cell proteins (SCP).

One can utilize Biotechnology to introduce useful traits to the whole cells, Fungi, Bacteria or Yeast to produce useful molecules for Food. – Synthetic Biology
Plant products are converted to “Natural ingredients” by fermentation.
1. *Aspergillus oryzae* (Koji filamentous fungi used in Soy fermentation) or *Mortierella* fungi.

The fungi is grown in a stream of oxygen gas and once secondary metabolic growth stage is achieved, Hexanoic acid is fed.

2. Acidified and heated before being steam distilled to obtain the lactone.
Cells grown in Fermenters are rich in enzymes. These can be utilized to transform plant material to useful molecules for food.

These whole cells could be Fungi, Bacteria or yeast. These are also referred to as Single cell Proteins (SCP) -- biomass or protein extract from pure or mixed cultures of algae, yeasts, fungi or bacteria.

Some examples would be:

- Natural Cheese production (Enzymes, Cultures and Fungi etc.)
- Beer (Yeast and specialty Beers contain other cultures)
- Bread (Yeast)
- Yoghurt
- Natural Color for Fish Feed (*phaffia rhodozyma* - astaxanthin -- red yeasts)
One can utilize Biotechnology to introduce useful traits to the whole cells, Fungi, Bacteria or Yeast to produce useful molecules for Food. --- Synthetic Biology

Manus Biosynthesis, Cambridge, MA 02139, USA.
Kaurenoic acid to Rebaudioside(s)

Kaurenoic acid

CH$_2$OH

OH

CH$_3$

H$_3$C

H

H

Engineered Enzymes or
Engineered Microbes

depending on Glycolysation
gives rise to Rebaudioside(s)

Steviol

OW2016073740 Philippe etal
The effort by DSM to produce Rebaudioside by expressing the genes in Yeast

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<tr>
<th>Code</th>
<th>Subject matter</th>
<th>Priority</th>
<th>Filing</th>
<th>Published</th>
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<td>29325</td>
<td>Increased rebaudioside production in 11 different deletion mutants</td>
<td>31 May 2013</td>
<td>2 Jun 2014</td>
<td>04 Dec 2014</td>
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<td>29 Jan 2015</td>
<td>WO2015/011209</td>
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**Figure 1.** Backbone figure of *Stevia* sweeteners.

**Table 1.** R-groups, molecular formulas, molecular weights and potencies of the *Stevia* sweeteners.

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<tr>
<th>Sweetener</th>
<th>Reference Number in Text</th>
<th>R-Groups in Backbone Figure Above</th>
<th>Formula</th>
<th>Molecular Weight (g/mol)</th>
<th>Potency *</th>
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<td>Rebaudioside A</td>
<td>1</td>
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<td>150</td>
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<td>C_{35}H_{48}O_{16}</td>
<td>788.87</td>
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</tbody>
</table>

glc = glucose; rha = rhamnose; xyl = xylose; * Potency from [1,6,7].

Foods 2014, 3, 162-175; Prakash et al.
Engineered microbes make ‘natural’ colorants

Four engineered bacteria cooperate to synthesize possible replacements for artificial colorings

By Stu Borman

Four bacteria (ovals) work together to biosynthesize the anthocyanin callistephin from glucose. Arrows inside bacteria are biosynthetic steps.

Source: Adapted from mBio
Mattheos A. G. Koffas recently published a method to prepare Callistephin an anthocyanin found in pomegranate, strawberries and purple corn. They fed glucose to a combination of four strains of genetically engineered bacteria to afford Callistepsin (mBio. vol 8 no3 6 June 2017).
Single cell Proteins (SCP) –
Biomass or protein extract from pure or mixed cultures of algae, yeasts, fungi or bacteria

Some examples would be:
- Autolyzed yeast Extracts
- Natural Color
  - Fish Feed (*phaffia rhodozyma* - astaxanthin -- red yeasts)
  - Blue Color – Spirulina
- Omega 3 oils from Algae
United States Patent Office

3,212,902
Patented Oct. 19, 1965

RECOVERY OF EDIBLE PRODUCTS FROM SPENT GRAINS AND YEASTS

Vincent S. Marattutto, Whitefish Bay, Wis., assignor to Chas. Pfizer & Co., Inc., New York, N.Y., a corporation of Delaware

7 Claims. (Cl. 99--9)

The principal object of this invention is to provide a method of recovering an edible product from brewery residues such as spent brewers' grain and spent brewers' yeast and from fresh yeast.

Another object of the present invention is to produce an edible enzymatic protein hydrolysate satisfactory for human consumption and for consumption by animals other than ruminants. Said enzymatic protein hydrolysate may, of course, also be used by ruminants.

EXAMPLE I

One ton of wet spent brewers' grain, containing about 20% solids content and about 27% protein based on solids content, and one ton of water are pumped into a jacketed tank equipped with an agitator. The liquid is adjusted to pH 7.5 by the addition of sodium hydroxide and is heated, with agitation, to 100° C. and held at this temperature for one hour. The liquid is then pumped through a heat exchanger to a reaction tank where it is allowed to cool at 50° C. After the contents of the reaction tank reach 50° C., 0.1% by weight of pancreatin (4NF), based on the weight of the wet spent brewers' grain, is added as a suspension to the contents of the reaction tank, and the whole is agitated for 48 hours at 50° C. The reaction mixture is then centrifuged to separate the solid residue from the extract. The solid residue is pressed to recover as much extract as possible. The extracts are combined. The solid residue, containing about 10% protein, is dried and is suitable as an animal feed. The combined extracts are concentrated to near-dryness by means of a multiple effect evaporator and are then vacuum drum-dried to yield 106 pounds (26.6% recovery of solids) of a product having a protein content of 69% (total nitrogen X 6.25).

EXAMPLE IX

Following the procedure of Example VIII, two thousand gallons of wet fresh brewers' yeast containing about 20% solids content are reacted with 0.1% by weight of an enzyme mixture of 95% pancreatin (4NF) and 5%...
Spent Brewers Yeast or Bakers Yeast

- Suspend in water and adjust pH (based on treatment to bust the cell)
  - Busting cell
    - Temperature – Heat to boil or 100°C
    - Add Proteases enzyme (papain, alcalase, peptidase etc.)
    - Acidify to pH 2 and heat to 50°C
    - Add RNAse enzyme
- Centrifuge to separate cell debris collect the supernatant
- Spray dry the supernatant as is or on a carrier
- Concentrate the supernatant to afford a paste
Boiling defatted soya, corn wheat or casein with 6N HCL breaks down the proteins to their amino acids. The resultant is then neutralized with NaOH to afford HVP.

Treating defatted soya, corn wheat or casein with peptidases breaks down the proteins to their amino acids. The resultant is then rich in umami character and referred as enzymatically derived HVP.
Plant material can be converted to “Natural ingredients” by Enzymes. The enzymes used and the protocols used has an impact on the final regulatory label.

Purified enzymes are used, in multiple food processing, to ultimately yield a Food product. The methodology to produce these enzymes has an impact on the final regulatory label.

One uses fruit juices without further purification as a source of enzymes to produce “Natural Ingredients”. The process with the Juices are obtained will have an impact on final regulatory label.
Enzymatic conversion of Dimethyl Anthranilate to Methyl Anthranilate

1. pH 6.0 water, HRP 10mg, DMA 1g, 100μl of 30% H₂O₂, stir at room temp

2. pH 6.0 water, HRP 10mg, DMA 1g, 10mg of Glucose Oxidase, 0.5g of glucose, stir at room temp

HRP -- purified horseradish peroxidase enzyme; DMA – dimethyl anthranilate

Kedderis et al. The Journal of Biological Chemistry 1983, 258, p 8129
Plant material can be converted to “Natural ingredients” by Enzymes. Some examples of using enzymes within microbes to effect these transformations.

The microbes can be seen as immobilized cost effective enzymes. Protocols used has an impact on the final regulatory label.

1. Lipoxygenase from Soya flour
2. Hydroperoxide lyase from Guava juice
3. Bakers Yeast

Muller et al. US Pat. 5464761
One uses fruit juices without further purification as a source of enzymes to produce “Natural Ingredients”. The process with the Juices are obtained will have an impact on final regulatory label.
FIG. 1

Linolenic acid or precursor in hydrolysate form

\[ \text{C}_{13}\text{HDPO} \]

\[ \text{pH} 9-9.5 \quad + \text{soya flour (lipoxynasen) } \]

\[ \text{pH} 7-9 \quad + \text{guava homogenate (lyase) } \]

Isolated 3-(Z)-hexen-1-al → solvent extraction → 3-(Z)-hexen-1-al

Isolated 2-(E)-hexen-1-al → steam distillation → 2-(E)-hexen-1-al

\[ \text{pH} 6.5 \quad + \text{citric acid } \]

+ baker's yeast (reductase)

2-(E)-hexen-1-ol

Steam distillation

Solvent extraction

Isolated 3-(Z)-hexen-1-ol

Isolated 2-(E)-hexen-1-ol
Purified enzymes are used, in multiple food processing, to ultimately yield a Food product. The methodology to produce these enzymes has an impact on the final regulatory label.

Some examples would be:
• Meat tenderizing by Papain or Bromelain
• Natural Cheese by Rennet
• Enzymes like Pectinases used to clarify Juices
Plant products are mixed and during heating a new Natural ingredients is formed. The exact methodology has an impact on the final regulatory label.

Plant products are mixed and the pH increased to alkalinity. It is followed by heating and further purification by different methodologies. The exact methodology has an impact on the final regulatory label.

Plant products are mixed and the pH decreased to acidity. It is followed by heating and further purification by different methodologies. The exact methodology has an impact on the final regulatory label.

“Soft Chemistry” means using methods that is similar to what is available in the Kitchen. It essentially means very limited physical and acid or base treatments. Plant products are converted to “Natural Ingredients”. The end products are not optically equivalent to those found in Nature. Regulatory labelling differ among countries.
Plant products are mixed and during heating a new Natural ingredients is formed. The exact methodology has an impact on the final regulatory label.
Plant products are mixed and the pH increased to alkalinity. It is followed by heating and further purification by different methodologies. The exact methodology has an impact on the final regulatory label.

**Cinnamaldehyde**

CAS# 104-55-2
FEMA 2286
50% of Cinnamon bark oil

\[ \text{4% Na}_2\text{CO}_3 \text{ and heat at reflux} \]

**United States Patent**

Wiener et al.

**Patent Number:** 4,617,419
**Date of Patent:** Oct. 14, 1986

**Abstract**

Described is a process for preparing natural benzaldehyde and acetaldehyde and compositions of matter containing natural benzaldehyde and acetaldehyde as well as products produced thereby and organoleptic utilities therefor, which process comprises the step of contacting with base naturally occurring cinnamaldehyde or a natural product rich in cinnamaldehyde such as Ceylon oil of cinnamon, Ceylon cinnamon bark.
Plant products are mixed and the pH decreased to acidity. It is followed by heating and further purification by different methodologies. The exact methodology has an impact on the final regulatory label.

t-3-Hexenoic acid
CAS# 1577-18-0
FEMA 3170

75% Phosphoric Acid and reflux

γ-Hexalactone
CAS# 695-06-7
FEMA 2556
Acid catalyzed Formation of acetals

Vanillin
CAS# 121-33-5
FEMA 3107

Propylene Glycol (PG)

Vanillin propylene glycol acetal
CAS# 68527-74-2
FEMA 3905
Plant or Animal products are subjected to steeping with solvents or the oils pressed out to produce "Natural Ingredients". It's achieved by a few different methodologies and has an impact on the final regulatory label.

Plant or Animal products contain polar and nonpolar compounds. These oils partition into water and the water insoluble non-polar components separate. Washed oils are valuable flavoring material and the methodologies used determine the final regulatory label.

Expressed oils are Plant or Animal products are pressed to expel the inherent oil they contain.
Plant or Animal products are pressed to expel the inherent oil they contain, and these are expressed oils.

Numerous glands and sacs in the outer colored layer of the peel contain the citrus oil. It's best to separate, juice and peel.

Two main industrial equipment – Brown oil extractor and the FMC (now JBT) extractor. In the Brown method submerged rollers cut the peel under water to release the oils. In the FMC method, while the peel is been cut water is introduced at high pressure.

The resultant water oil mixture is filtered and chilled to separate the water and the oil. Since no heat is used in the process it’s referred to as “cold pressed” oil production.
Citrus Washed Oils

Plant or Animal products contain polar and non polar compounds. These oils partition into water and the water insoluble non-polar components separate. This washed oils are made. These are valuable flavoring material and the methodologies used determine the final regulatory label.

Citrus Oil -- 25%
Ethanol -- 25%
Water -- 50%

• Stir very well, and store at 4°C.
• Oxygenated compounds in the citrus oil migrates to the water / ethanol.
• The bottom water layer is harvested and is used as a water soluble flavor ingredient.
• Plant or Animal products extracted with Organic solvents are extracts.

• When Ethanol is used as the solvent -- Extracts are a tincture

• When the solvent used in the extracts is evaporated – the product is an oleoresin

These are valuable flavoring material and the methodologies used determine the final regulatory label.
Standard of Identity

The information on this page is current as of April 1 2016.

For the most up-to-date version of CFR Title 21, go to the Electronic Code of Federal Regulations (eCFR).
From --- 21CFR 169.175 (shown in an earlier slide)
Vanilla extract may contain one or more of the following optional ingredients:
(1) Glycerin.
(2) Propylene glycol.
(3) Sugar (including invert sugar).
(4) Dextrose.
(5) Corn syrup (including dried corn syrup)
14.7 Preparation of Process Flavorings

Process flavorings are prepared by processing together raw materials listed under 14.5 as follows: 14.7.1 The product temperature during processing shall not exceed 180°C. 14.7.2 The processing time shall not exceed ¼ hour at 180°C, with correspondingly longer times at lower temperatures, i.e., a doubling of the heating time for each decrease of temperature by 10°C. 14.7.3 The pH during processing shall not exceed 8. 14.7.4 Flavorings, (14.6.1) and non-flavoring food ingredients (14.6.2) shall only be added after processing is completed, unless otherwise specified.

**Protein nitrogen sources**
- Foods containing protein nitrogen (meat, poultry, eggs, dairy products, fish, seafood, cereals, vegetable products, fruits, yeasts) and their extracts
- Hydrolysis products of the above, autolyzed yeasts, peptides, amino acids and/or their salts.

**Reducing Sugars**
- Examples: Maltose Syrup, glucose, fructose, galactose

**Fat or fatty acid sources**
- Foods containing fats and oils
- Edible fats and oil from animal, marine or vegetable origin
- Hydrogenated, transesterified and/or fractionated fats and oils
- Hydrolysis products of the above.

**Maillard Reaction** --- the reaction of amino acids with reducing sugar named after Louis Maillard - yield a variety of compounds.

**Strecker Degradation** --- the reaction of α-amino acid with a dicarbonyl compound named after Adolph Strecker - yield an aldehyde and an amine.
Decomposition of Amadori Compounds

Mottram, D.S. Volatile Compounds in Food Beverage, 107, 1991
Plant or Animal products are subjected to heating and the volatiles compounds are trapped using a cold condenser. The resultant material is a much valued “Natural Ingredient”. Its achieved by a few different methodologies and has an impact on the final regulatory label.

Plant or Animal products on subjecting to heat, the volatiles are allowed to reflux and are collected at different temperatures. In general the these temperature coincide with the components boiling points. Therefore the techniques affords a method of purification of liquids. The resultant material is a purified compound of a normal distillation product. Its achieved by a few different methodologies and has an impact on the final regulatory label.

Plant or Animal products are added to water and steam is passed into it. The escaping excess steam is trapped on a cold condenser. Organic compounds forms azeotropes with steam and are carried away from the feed stock and is collected with the distillate. On cooling the oil and water separate affording a valuable “Natural Ingredient”. Its achieved by a few different methodologies and has an impact on the final regulatory label.
Plant or Animal products are added to water and steam is passed into it. The escaping excess steam is trapped on a cold condenser. Organic compounds forms azeotropes with steam and are carried away from the feed stock and is collected with the distillate. On cooling the oil and water separate affording a valuable “Natural Ingredient”. Its achieved by a few different methodologies and has an impact on the final regulatory label.

If we look at the production of Cinnamon oil Cinnamon bark would be placed in the flask with warm water.

Steam would be passed into it.

The distilled liquid will have water and oil in the receiver.

The cinnamon oil will be decanted to afford a valuable ingredients for the flavor industry.
Plant or Animal products on subjecting to heat, the volatiles are allowed to reflux and are collected at different temperatures. In general the these temperature coincide with the components boiling points. Therefore the techniques affords a method of purification of liquids. The resultant material is a purified compound of a normal distillation product. Its achieved by a few different methodologies and has an impact on the final regulatory label.

Many plant and Animal products are mixtures of organic compounds. Most of them have boiling points and are safely distilled with decomposition. Industrially one would distill at reduced pressure, decreasing the boiling point and further ensuring non decomposition.

The fractionator may contain anywhere between 0 to over 100 theoretical plates.

Citrus oils would be subjected to fractional distillations to afford single ingredients like citral.
Armed with the new building blocks generated by Maillard Reactions / FEMA GRAS molecules and extracts, one can successfully compound safe flavors.

The question is: Are these Clean Label Flavors?

Claims do help sales, they need to be Authentic and Transparent.

Consumers are looking at simple names that they can relate to on the labels and reduced number of items.

Cold-Pressed, minimal processing, fruit and vegetables, artisan, “free from”

There is no Law or statement in the CFR on Clean label.

The question is: Are these Clean Label Flavors?
In the recent past many have talked about Clean Label, invariably most have equated “Natural” to Clean Label

Based on published information
Consumers want to be able to relate to the labels, they are running away from “Black Box – Natural and Flavors”

• It is not clean eating
• In today’s 140 / 280 character world None peer reviewed, big discovery daily, could be fact or fiction -- to provide balance in a world of extremes
• These none peer reviewed material give rise to more irrational non fact based concerns -- provide a discreet relief from current confusion
• Customers are looking to prevent allergies and balance diets --- complex diets Vs refined ingredients. – Personal control of one’s own health and protection from hidden harm
Thank you.

Questions?