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Mini Review

Bamboo Shoot Processing in India

Deepti Dabas, PhD[1]*; Krishna Kumar, MSc[2]

[1]Worked as Scientist-D in National Mission on Bamboo Applications (NMBA) now renamed as NECTAR, Department of Science & Technology, Government of India, New Delhi 110016, India
[2]Bamboo & Allied Activities, Department of Science and Technology, Government of India, NECTAR, 2nd floor, Vishwakarma Bhawan, ShaheedJeet Singh Marg, New Delhi 110016, India

Corresponding author
Deepti Dabas, PhD
Worked as Scientist-D in National Mission on Bamboo Applications (NMBA) now renamed as NECTAR, Department of Science and Technology, Government of India, Vishwakarma Bhawan, ShaheedJeet Singh Marg, New Delhi-110016, India; E-mail: dabasd@gmail.com

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INTRODUCTION

Bamboo is a woody perennial grass belonging to the Poaceae family, which is found in tropical and sub-tropical regions of the world. More than 1250 species are known worldwide that belong to 75 genera of bamboos.[1] Of these, 125 species are found in India – of which only 30 are of commercial value. Bamboos play an important role in the daily life of rural people, especially in tribal regions. It is used as a wood substitute, for making industrial products; e.g., charcoal production, and for various structural applications due to its strength.

India has 677,010 sqm of forest area, of which around 16.8% is under bamboo cultivation – while only around half of this area is under bamboo cultivation in China.[2] In India, out of this total bamboo cultivating area, around 28% is located in the North Eastern region. This area is provides nearly 66% of bamboo resources in the country, where the climate is conducive to bamboo growth.[3] Almost all the states in this region have bamboo growing in the forest – as high rainfall, high humidity, and optimum temperatures favor bamboo growth.

It is estimated that the global bamboo market is at $12000 million, of which India has a mere share of 4.5% despite having 31.1% of the total bamboo growing area in the world. China leads the market by having a market share of up to 50%.[2] In India, the market potential was estimated to be 20,000 crores INR (equivalent to 3 billion US dollars) in 2015.[4]

The edible part of bamboo, which is the freshly sprouting shoot from the ground, is relished by the local people. We at the North East Centre for Technology Application and Research (NECTAR) worked on developing the bamboo shoot sector in the country using different approaches – developing the shoot processing technology as discussed in this article, demonstrating this technology to user groups including providing assistance to set up processing facilities, supporting bamboo shoot plantation, developing package of practices for bamboo shoot cultivation,[5] collaborating with organizations to carry out more research on shoot processing and studying their properties, and harvesting and increasing visibility of this commercially-important food. This mini-review is focused on bamboo shoot processing and ways to exploit the yet-to-be commercialized potential of bamboo shoot in India.

BAMBOO SHOOTS

Out of 125 species prevalent in India, the commonly edible bamboo species are *Bambusa pallida, Bambusa tulda, Bambusa polymorpha, Bambusa balcooa, Dendrocalamus hamiltonii, Dendrocalamus giganteus and Melocanna bambusoides.*[1] The economic value of bamboo shoots is governed by their edible content. The sheath needs to be removed before consumption and the edible portion is 14-45% (data obtained by the authors while at NECTAR), depending on the species and the size of bamboo shoots. The bamboo shoot emerges from ground having the final diameter of the culm. If allowed to grow too much, the bamboo shoot develops into culms and it hardens in form, which can no longer be eaten. Thus, there is a short window of time (approximately a couple of weeks) during which the newly-growing shoot should be harvested, typically before it is a foot long.[5] Also, the shelf life of bamboo shoots is very limited due to its high moisture content (around 90%).[6] Most of the bamboo shoots grow in deep forest areas that are not easily accessed, which makes it harder to harvest and bring out for sales

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or processing.

Bamboo shoots are an intricate part of the cuisine of people living in North East India. In Manipur bamboo shoots are consumed as *ushoi* which is produced by taking the sheath off, and chopping the inner soft portion of the shoot into thin slices and treating them in water for 3-4 hours. It is primarily used in the preparation of different ethnic dishes and can also be preserved for off-season by drying. The properly-processed shoots are used to make a variety of traditional local chutney called *iromba* and also are cooked as a vegetable with meat. In Arunachal Pradesh, young bamboo shoots are boiled and cut into pieces and used as a vegetable for preparation of traditional dish called *kupe*. Shoots obtained from *Bambusa balcooa, Bambusa nutans*, and *Dendrocalamus strictus* are bitter and need to be boiled before consumption.[7]

Plantations dedicated to growing bamboo shoots have started to develop recently. As an example, the *Dendrocalamus asper* species has been introduced in some regions. We at North East Centre for Technology Application and Research (NECTAR) visited one such plantation at Garh Mukteshwar (Hapur, Uttar Pradesh, India). Harvesting from such plantations can begin for bamboo shoots in its third year onwards. Some bamboo shoots must be left to grow into culms so that the plant continues to grow and produce new shoots in the next season.

In recent years (December 2016-December 2017), around 61 million Kg of canned bamboo shoots was imported into the USA from different countries: China, Thailand, Taiwan, Japan, and India – with China being the biggest player. Bamboo shoot is also a part of cuisines in many Asian countries and the demand is rapidly growing. India has exported bamboo shoots worth only $37,885 to various countries.[8] China, despite having a lower area under bamboo cultivation compared to India, manages to process and export larger quantities of bamboo shoots. This is due to the dedicated plantation for shoots, their cultivation practices and presence of processing facilities near shoot-growing areas. Thus, there is a huge unexploited potential in India which can be tapped by using a certain package of practices.

FUNCTIONAL PROPERTIES OF BAMBOO SHOOTS

Bamboo shoot is not only known for providing an exotic taste appeal, but has also been explored for their functional and health promoting attributes. It has been screened for antioxidant and anti-inflammatory effects, antimicrobial and anti-fungal effects, protection of neurons from oxidative stress, anti-apoptotic effects due to pyrolysates, as a supplement for ischemic injury treatment and for its fatigue and cholesterol reducing properties.[6] Recently, its dietary fibre has also been studied for its functional properties.[9]

BAMBOO SHOOT PROCESSING

There are various forms in which bamboo shoots are made available in the market: canning or retorting are common methods of processing the shoots. Canned bamboo shoots stay stable for a few years and are commercially sterile. The vacuum processing the bamboo shoots in nylon-based packages is another way of processing. This method preserves the bamboo shoots for a few months, during which they can either be consumed fresh or further canned. Fermentation and drying of the bamboo shoots are some other ways in which they can be preserved and are relished in many parts of the country; however, they change the taste and texture of the shoots and hence these products have limited applications. In contrast, fermented products are however desired and enjoyed by local people. *Mesu, soibum, ekung* and *heccha* are examples of the fermented foods[10] relished in different North Eastern states of India.

We at NECTAR have developed a unique method of processing bamboo shoots specific to the North East region of India and other bamboo trees growing in the remote areas of the country. This method can be considered as the minimal processing of bamboo shoots, as it preserves the original taste and texture of the shoots. Minimal processing has been defined broadly as the 'least possible treatment to achieve a purpose'. A more specific definition describes a minimal process as those which minimally influence the quality characteristics of a food while giving the food sufficient shelf life during storage and distribution. An even more precise definition which situates minimal processing methods within the context of more conventional technologies that not only preserve the food but also retain, to a greater extent, their nutritional quality and sensory characteristics by reducing the reliance on heat.[11]

Bamboo shoots are prone to spoilage if not preserved soon after harvesting. A high humidity and storage in the open in an environment which has a temperature of 20-30 ^{o}C are factors for their quick deterioration. Microbial action, transpiration and respiration are some of the reasons leading up to spoilage in fresh bamboo shoots. In this method developed by NECTAR, bamboo shoots can be preserved for up to 9 days (if preserved in water) or 23 days (if preserved in brine).[5] Since the process is very simple, it does not require considerable equipment, land or investment, and can be set up very close to the site of the shoot growing areas. Due to its simple nature, it can tap the hitherto the unexploited bamboo shoot sector.

METHODOLOGY USED

The shoots are harvested at an opportune time – this could be between 7-14 days after their emergence, depending on the species. The shoots are washed and peeled and are soaked in water overnight. This ensures a considerable reduction of hydrocyanic acid, a cyanogen which is produced in the shoots upon harvesting. However, for complete removal of cyanogens, heat processing like boiling is crucial. This step can be carried out during subsequent processing of shoots that are packaged using this method.[12] Afterwards, they are cut into small or desired shapes and placed inside nylon-based packages. It is desirable to use thicker nylon packages, at least 90 microns in thickness. The package is then filled with clean potable or preferably, soft water and is heat-sealed. Instead of water, brine can also be used.[5] These packages can then be transported and either sold to markets for fresh consumption or can be used for further industrial processing – either under vacuum or can-based.

ADVANTAGE OF THIS NOVEL PROCESSING METHOD

The biggest advantage of this process is that it can preserve the shoot close to the place of harvesting without the need of low storage temperatures or by using preservatives. This protects the fresh shoots from degradation that could occur if they were to be kept outside under unhygienic environment. It must be noted that bamboo grows under high humidity, varying temperature conditions (15-35 °C) and this kind of environment encourages microbial degradation. Since the processed shoots stay preserved for multiple days, this gives the processors to sell their products to far off places or to take to processing facilities.

Other studies have worked on minimal processing of bamboo shoots. Low temperature and packaging have been used to reduce transpiration losses occurring in bamboo shoots stored in the open and at prevalent temperatures (which can range from 20-30 °C). Discoloration is the major cause of quality loss of shoots.[13] Fungicides have also been suggested to preserve their shelf life. Wang and He reported that addition of fungicide to bamboo shoots packaged in polyethylene film extended their shelf life to 62 days at 0 °C.[14] Kleinhenz et al attempted a combination of different packaging and low temperatures to increase the shelf life of bamboo shoots – polyvinyl chloride (PVC) gave the best results followed by low-density polyethylene (LDPE) and then microperforated LDPE. They were able to get a shelf life of 28 days when kept at 1-2 °C.[13]

CONCLUSION

We believe that this technology can be used to preserve bamboo shoots and can minimize the post-harvest losses, as well as effectively exploit bamboo shoot sector in India, leading to an increase in production and hence export of processed bamboo shoots. This processing does not require refrigeration or cold chain hence it is successful for use in areas where only minimal facilities are available – thus, this processing can be of use in forest areas where bamboo shoots are commonly harvested from.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Original Research

Lipid Oxidation Inhibition by Natural Tocopherols Increases the Nutritional Value of Tuna Salami

Rita Sousa, MSc; Rui Pedrosa, PhD; Maria M. Gil, PhD*

MARE - Marine and Environmental Sciences Centre, ESTM, Instituto Politécnico de Leiria, Campus 4, Santuário Nossa Senhora dos Remédios, Apartado 126, 2520-641 Peniche, Portugal

Corresponding author
Maria M. Gil, PhD
MARE – Marine and Environmental Sciences Centre, ESTM, Instituto Politécnico de Leiria Campus 4, Santuário Nossa Senhora dos Remédios, Apartado 126, 2520-641, Peniche, Portugal; E-mail: maria.m.gil@ipleiria.pt

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ABSTRACT

Introduction
The quality attributes of fish products deteriorate due to the lipid oxidation during processing and storage. Tuna salami is highly susceptible to oxidation, which can precipitate health hazards and economic losses in terms of inferior product quality.

Methods
The effect of adding different natural antioxidants on the lipid oxidation, colour and fatty acid profile of tuna salami after nine days of storage at 4 ºC was assessed. Three different commercial mixes of tocopherols were added during the production of the tuna salami and samples were sliced and refrigerated for 9 days.

Results
The lipid oxidation increased from 0.98 to 4.03 mg of malondialdehyde/kg. The oxidation process was inhibited by DLT-100 and VIT-100; however, RNX10 did not blunt the oxidative process. VIT and dose-limiting toxicity (DLT) reduced the total unsaturated fatty acid reduction. The *RNX* did not prevent the reduction of the a^* value.

Conclusion
It was concluded that tocopherols supplementation kept the nutritional value of tuna salami mainly by lipid oxidation inhibition.

Keywords
Tuna salami; Fatty acids profile; Thiobarbituric acid-reactive substances (TBARS); Antioxidants; Colour changes.

Abbreviations
DLT: Dose-Limiting Toxicity; TBARS: Thiobarbituric Acid-Reactive Substances; PUFA: Polyunsaturated Fatty Acids; MDA: Malondialdehyde; ROS: Reactive Oxygen Species; FAME: Fatty Acid Methyl Esters; GC: Gas Chromatography; HPLC: High-Performance Liquid Chromatography; ANOVA: One-Way Analysis of Variance.

INTRODUCTION

Tuna salami is a new food product developed by a small Portuguese food producer "*Conservas Dâmaso*" (Vila Real de Santo António, Algarve). This product is a type of dry sausage made from tuna meat that is ground up and mixed with oil and spices. Tuna salami is normally sliced thinly when served and often used for sandwich filling (salami sandwich), for pizza toppings and for viand as well. Nowadays, there is a growing interest in tuna products due to the beneficial effects on human health, because of its high polyunsaturated fatty acids (PUFA) content. However, food products with high content of PUFA are highly susceptible to lipid oxidation and therefore the shelf-life of these products are short.[1,2] Therefore, the reduction of lipid oxidation during storage has been of major importance to the food process industries.

Lipid oxidation encompasses a series of reactions between oxygen and unsaturated lipids to form lipid peroxides, often

readily identified by sensory changes, such as generation of off-flavour and off-odours.[2-4] This oxidative spoilage releases secondary products. Malondialdehyde (MDA) is one of the most important products of oxidation that can be followed as a marker of lipid oxidation.[1,5] Simultaneously, heme-pigments (myoglobin and haemoglobin) also oxidize in a coupled lipid-pigment reaction.[6] The myoglobin is a globular heme protein localised in red muscle fibres and the major giver to the colour of muscle.[7-11] Changes in fish colour are usually influenced by intrinsic factors such as muscle pH, redox potential, metmyoglobin reductase activity, oxygen-consuming reactions, and susceptibility to lipid oxidation. However, as extrinsic factors such as light exposure and storage temperature also produce changes in the fish colour. Therefore, colour changes are also an important factor that influences quality and acceptability by the consumers of tuna products (colour quality attribute strongly influences consumers' preferences). In order to quantify colour changes objectively, the visual observing components needed to be taken into account are the object, light, and the observer.[12] Colour can be measured by a tristimulus colorimeter, represented by three coordinates in the colour scale. The L parameter represents lightness (L=0 black; L=100 white), a^* (red to green) and b^* (blue to yellow).[12-14]

Vacuum packages are normally used in this type of fish food products in order to prevent lipid oxidation and colour changes and consequently increase the products' shelf-life. However, once the package is opened, the contact with the oxygen triggers immediately the lipid oxidation process. The lipid oxidation of fatty acids like omega-3 can be reduced by adding natural or synthetic antioxidants. This process extends the products' shelf-life and moreover enhancing the health benefits of food products.[2] Vitamin E can be found naturally in some foods, which can be added to others and is available as a dietary supplement. Vitamin E is the collective name for a group of fat-soluble compounds with distinctive antioxidant activities, the tocopherols, that stops the production of reactive oxygen species (ROS) formed when fat undergoes oxidation.[15]

The aim of this work was to study the effect of adding different natural antioxidants (tocopherols mix with and without rosemary extract) on the lipid oxidation and colour changes of tuna salamis during nine days at 4 ºC exposure to the air (real situation at consumers home).

MATERIALS AND METHODS

Antioxidants and Tuna Salami

Tuna salami was supplied by *Conservas Dâmaso*. Three different commercial mixes of tocopherols were added during the production of the tuna salami: DLT-100 (100 mg of tocopherols/100 g of sample) and VIT-100 (100 mg of tocopherols/100 g of sample), from different suppliers (not identified) and RNX 10 (10 mg of tocopherols+3.89 rosemary extract/100 g of sample). The supplier provided one lot of each DLT, VIT and RNX samples. Tuna salami samples were sliced (thickness=2 mm) and stored in a refrigerator (4 °C) and unpacked (forcing the oxidation process and simulating the consumer's utilization) and analysed. Tuna salami without antioxidant was used as a control sample.

Chemical Composition

Tuna salami samples were analysed for fatty acid composition, thiobarbituric acid-reactive substances (TBARS) and colour. All measurements were performed in triplicate and samples were obtained from cross sections of full-size salami.

Fatty acid composition determination: Fatty acid methyl esters (FAME) of tuna salami were performed according to Lepage & Roy.[16] 300 mg tuna salami were homogenised with 5 mL acetyl chloride-methanol mix (98% acetyl chloride, *Acros*; methanol, *Panreac*) for 30 seconds and placed in a water bath at 80 ºC for 1 h and cooled to room temperature. Then, 1 mL of water for high-performance liquid chromatography (HPLC) (*Fisher Scientific*) and 2 mL of *n*-heptane (*Fluka*) were added and samples were homogenised for 1 minute and centrifuged at 1500 g for 5 minutes. All samples were analysed in gas chromatography (GC) (Finnigan Trace GC Ultra, Thermo Electron Corporation, Waltham, MA, USA).

TBARS determination: The TBARS values in tuna salami were determined according to the procedure described by Vyncke,[17] Lemon,[18] Robles-Martinez[19] with slight modifications. Fifteen grams of tuna salami were mixed with 30 mL of 75% trichloroacetic acid solution (TCA with propyl 3,4,5-trihydroxybenzoate, *Merck*), trichloroacetic acid *(Schlarlau*), ethylenediaminetetraacetic acid (EDTA, *Panreac*) for 2 minutes in homogeniser; sample was then transferred to an Erlenmeyer by filter (*Whatman* n.º1) and centrifuged at 2000 rpm for 5 min. The extract of this solution was removed into 1-5 mL and fixed volume until 5 mL of the 75% TCA solution; adding 5 mL of TBA reagent (4,6-dihydroxy-2-mercapto pyrimidine, *Acros*) and maintained in boiling water bath for 40 min. The calibration curve was determined by same proportions and reagents. The sample was replaced by the standard 1,1,3,3-tetraethoxypropane (TEP, *Acros*). Finally, absorbance was read at 530 nm in a spectrophotometer (Helios α, Thermo Electron Corporation, Waltham, MA, USA). The results were expressed as milligrams of MDA per kg of product. For each assay were used three different samples of tuna salami with triplicate measures.

Colour measurement: Colour was evaluated in terms of L^*, a^* and b^* values using a tristimulus colorimeter (Chroma Met er CR-400, Konica Minolta, Inc., Ramsey, NJ, USA). Measurements were performed in the Commission Internationale de bÉclairage (CIE) L^*, a^*, b^* system, using illuminant D65 and 2º observer. The colorimeter was calibrated against standard white tile (L^* 95.37; a^* 13.10 and b^* 7.94). The colour behaviour of tuna salami was described for L^*, a^* and b^* values. Measurements were taken in triplicates with ten readings each (ten slices of each salami were measured at three distinct positions on each slice).

Statistical Analysis

One-way analysis of variance (ANOVA) was used to analyse data among samples, followed by Dunnett test (when applicable) to discriminate significant differences between samples and controls. These analyses were performed using GraphPad Prism 5 for Win-

dows. Results are presented as mean±standard error of the mean (SEM). The significance level was inferred at $p<0.05$ for all statistical tests.

RESULTS AND DISCUSSION

Secondary oxidation products are a suitable index of lipid oxidation due to the fact that they are odour-active and stable compounds, in comparison with primary products (hydroperoxides) which are colourless, flavourless, and usually labile compounds.[20] One of the most extensively employed methods to detect oxidative deterioration in foodstuffs is the TBA test. This procedure is based on the formation of MDA), during the autoxidation of PUFAs, followed by reaction with TBA to form a pink complex that is measured spectrophotometrically.[21,22] The extent of oxidation is stated as the TBA value and is reported as milligrams of MDA equivalents per kilogram of sample or as micromoles of MDA equivalents per gram of sample. Nevertheless, TBA is not selective to MDA and can also react with many other compounds such as aldehydes, carbohydrates, amino acids and nucleic acids leading to overestimation and variability in the results attained by the TBA method.[23] For this reason, this method is also known as thiobarbituric acid reactive substances (TBARS) test. The oxidative stability of vacuum packaged tuna salamis was evaluated during 60 days at room temperature and exposure to artificial light. Non-significant differences ($p>0.05$) in TBARS levels were found during the storage time of samples, showing an oxidative stability of packaged tuna salami (data not shown). This all agree with the view that the vacuum packages prevent the lipid oxidation of this type of food product. Moreover, no significant differences were obtained for pH and moisture content of the samples during the storage time (data not shown).

To study the effect of adding different natural antioxidants on the lipid oxidation mimicking the consumer's use, tuna salami samples were sliced and refrigerated unpacked (4 ºC) during those 9 days.

The fatty acid composition of tuna salami slices for days 0 and 9 is shown in Table 1. Fatty acids composition is strongly influenced by the fat quality used during the food processing and may vary during products storage. Additionally, adding antioxidants can also influence the initial fatty acid composition. In this study, supplementation of tuna salami with DTL-100 and VIT-100, generally did not affect the fatty acid composition, when compared to control for day 0 ($p>0.05$). Interestingly, RNX10 characteristics resulted in a low C18:1, n-9 fatty acid content for both days 0 and 9, when compared to control, DLT-100 and VIT-100 (Table 1).

The fatty acid profile is also highly marked by the presence of monounsaturated fatty acid (MUFA). Oleic acid (18:1) is one of the most abundant MUFA and frequently abundant in this type of product. Comparatively to the control, DTL-100 showed an increase in oleic acid content, from 71.86% to 79.65% with the statistically significant difference ($p<0.05$) for day 9, meaning that DLT-100 can prevent lipid oxidation. Similarly, C22:6, n-3 presented higher values for day 9 for samples treated with DLT-100 (6.09%) but also for RNX10 (11.45%). Furthermore, control samples showed the highest unsaturated fatty acids content variation ($p<0.05$) when compared with the samples supplemented, revealing the importance of the addition of antioxidants to prevent their oxidation. Besides this, no significant differences were found for lipid content between days 0 and 9. Nevertheless, one can be concluded differently that commercial mixes of tocopherols, can lead to different antioxidant activity.

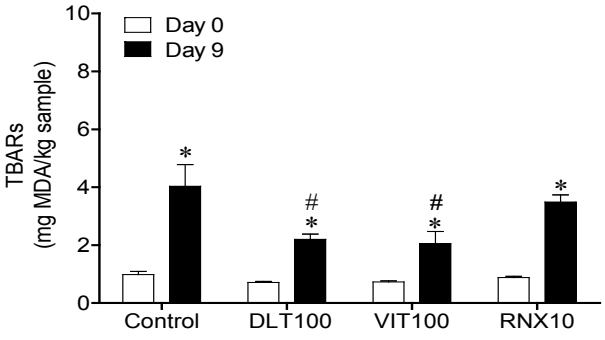
As mentioned before, TBARS assay is one of the most widely used indices of food quality as a critical index in lipid oxidation. Figure 1 presents the TBARS values of sliced tuna salami in the absence or in the presence of three different commercial mixes of tocopherols. Results showed clearly that lipid oxidation occurs during the storage time in tuna sausages products as observed in

Table 1. *Fatty Acid Composition (% of Total Fat) of Slices of Tuna Salami (Day 0 and Day 9; Stored at 4 °C) in the Absence or in the Presence of Three Different Commercial Mixes of Tocopherols, DLT-100 (100 mg of Tocopherols/100 g Sample), VIT-100 (100 mg of Tocopherols/100 g of Sample), RNX10 (10 mg of Tocopherols+3.89 Rosemary Extract/100 g of Sample)*

Fatty Acid	Samples							
	Control	Control	DLT 100	DLT 100	VIT 100	VIT 100	RNX 10	RNX 10
	Day 0	Day 9	Day 0	Day 9	Day 0	Day 9	Day 0	Day 9
16:0	14.63±1.12	14.46±1.68	13.88±1.73	12.26±0.30	11.80±0.19	14.46±2.07	21.54±0.68#	21.60±0.57#
18:1, *n*-9	73.55±2.66	71.86±0.41	76.41±3.00	79.65±0.79#	78.67±0.53	74.16±4.10	63.76±1.10#	62.53±0.83#
20:4, *n*-6	0.66±0.07	0.65±0.10	0.43±0.18	0.45±0.18	0.77±0.01	0.78±0.16	0.75±0.02	1.38±0.44
20:5, *n*-3	1.43±0.25	1.43±0.31	1.19±0.19	1.02±0.05	1.01±0.06	1.34±0.29	2.07±0.08#	1.98±0.10
22:6, *n*-3	8.66±1.31	5.38±1.58	7.09±1.07	6.09±0.35	7.16±0.27	8.59±1.63	11.07±0.36	11.45±0.39#
Σ Unsaturated	84.31±1.04	79.32±1.52*	85.12±1.65	87.20±0.31#	87.60±0.21	84.87±2.23#	77.65±0.66#	77.34±0.61
Σ *n*-3	10.09±1.55	6.81±1.28	8.28±1.25	7.11±0.39	8.17±0.32	9.93±1.90	13.15±0.44	13.43±0.49#
n-3+*n*-6	10.75±1.62	7.47±1.18	8.71±1.38	7.56±0.48	8.94±0.33	10.72±2.01	13.89±0.45	14.81±0.37#
n-3/*n*-6	15.15±0.67	11.72±2.87	12.69±1.30	9.68±0.18*	10.67±0.33#	13.16±1.89	17.62±0.43	11.68±3.02*
DHA/EPA	6.11±0.16	4.73±1.49	5.99±0.08	5.94±0.12	7.12±0.37#	6.54±0.71	5.35±0.06#	5.80±0.13*

Values are means±SEM of 3 to 5 independent experiments. Significantly different from control values (#$p< 0.05$) or from day 0 (*$p< 0.05$).

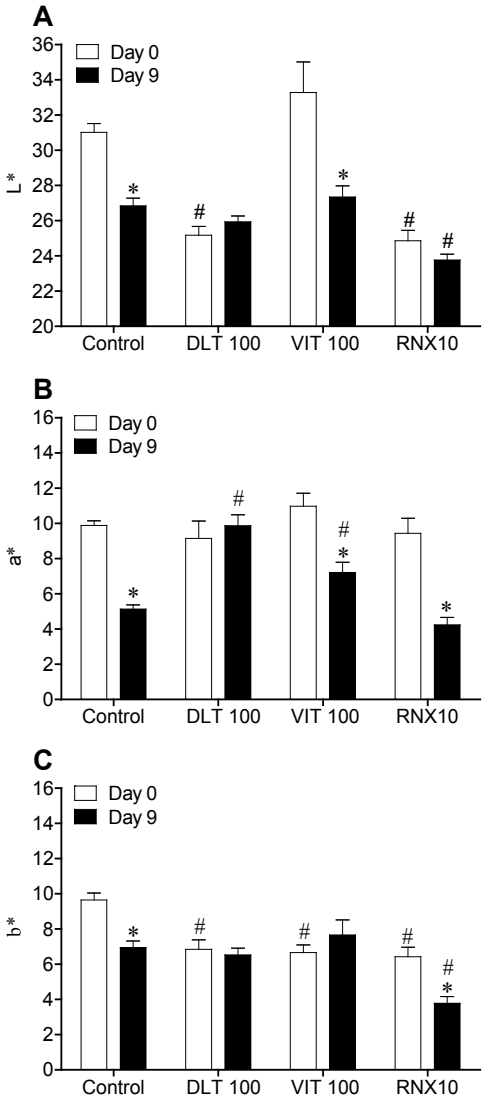
Figure 1. *TBARs Values (mg of MDA/kg of sample) of Slices of Tuna Salami (Day 0 and Day 9; Stored at 4 °C) in the Absence or in the Presence of Three Different Commercial Mixes of Tocopherols, DLT-100 (100 mg of Tocopherols/100 g Sample), VIT-100 (100 mg of Tocopherols/100 g of Sample), RNX10 (10 mg of Tocopherols+3.89 Rosemary Extract/100 g of Sample). Each Column is the Mean of 6 to 14 Separate Experiments; Vertical Lines Indicate SEM. Significantly Different from Control Values (#p<0.05) or from Day 0 (*p<0.05)*



control. In this study, all samples (at time zero) showed lower values of TBA than the proposed limits (5 mg of malonaldehyde equivalents kg-1 of tissue). The level of lipid oxidation of freshly caught fish is typically between 3 and 5 mg of MDA equivalents per kilogram flesh.[2] However, according to Fernández & Rodríguez,[24] these values are insufficient for the sensorial detection of rancidity.[21] It was also observed that, TBA greatly increased during the 9 days of storage in all samples ($p< 0.05$) (Figure 1). These results indicated that, although the tocopherols should reduce lipid oxidation supplementation was not enough. Nevertheless, samples treated with VIT-100 and DLT-100 antioxidants present lower level of lipid oxidation than control on day 9 (lower level of TBARS – 2.1969 and 2.0441 for DLT-100 and VIT-100, respectively). Previous studies have found that Rosemary extracts present efficient antioxidant activity. Besides this, in the present work, for tuna salami supplemented it seemed that the prevention of lipid oxidation was not increased by the presence of rosemary extract. This can be explained due to a number of active compounds that can act as antioxidants and are present in VIT-100 and DLT-100; therefore, it is possible that synergism occurs. Additionally, it can be also explained by the higher content of tocopherols compared to RNX10 (100 mg of tocopherols/100 g of sample and 10 mg of tocopherols/100 g of sample, respectively).

The most important indices by which consumers evaluate the freshness and quality of foods are colour and flavour. The colour is commonly used by the consumer as an indication of the freshness of the product. In general, food colour should remain unaltered upon the addition of additives and during storage. Colour parameters are presented in Figure 2. In Figure 2A small changes on L^* value can be observed for samples treated with DLT-100 and RNX10 at day 0 and day 9, respectively, showing that samples became darker. The dark colour formation in tuna salamis can be explained by the browning and the exposure to the light. Papadima & Bloukas[26] also referred that high-levels of fat resulted in higher L^* values in Greek sausages.[22] Furthermore, sample darkening coincides with higher oxidation values; whereas, low TBARS are nor-

Figure 2. *Colour Parameters L (A), a* (B) and b* (C) of Slices of Tuna Salami (Day 0 and Day 9; Stored at 4 °C) in the Absence or in the Presence of Three Different Commercial Mixes of Tocopherols, DLT-100 (100 mg of Tocopherols/100 g Sample), VIT-100 (100 mg of Tocopherols/100 g of Sample), RNX10 (10 mg of Tocopherols+3.89 Rosemary Extract/100 g of Sample). Each Column is the Mean of 30 Separate Experiments; Vertical Lines Indicate SEM. Significantly Different from Control Values (#p<0.05) or from Day 0 (*p<0.05)*



mally associated with higher L* values.[6] However, in this study, this correlation was not observed. The VIT treatment was the only that did not change the L* colour at day 0. After 9 days the L* value was similar in all the samples. However, the DLT and RNX presented the same darkening parameter at day 0 and day 9 (Figure 2A).

The occurrence of lipid oxidation in tuna salami-like products can be shown by the decrease of a* value (product less red). The difference of red tint over the time occurs due to oxidative process of transfer of myoglobin to metmyoglobin, which is associated with the reduction of reddish pigmentation.[6] The negative change of this parameter influences the acceptability of the product by the consumer, which is of major concern to food producers. Figure 2 presents the a* values at days 0 and 9, for samples treated with and without antioxidants. The a* value showed a significant (p<0.05) decreasing trend with time (Figure 2B), except for samples treated with DLT, that non-significant differences (p>0.05) were observed after the 9 days period. However, the a* value reduction was also lower on the VIT samples. These results confirm the occurrence of lipid oxidation in control samples and are well correlated with TBARS values. During the dry processing of tuna muscle, changes in colour, from bright red to dark red, are the result of the conversion of myoglobin and oxymyoglobin oxidation to brown metmyoglobin1.[2,6] The view that VIT and DLT supplementation partial and total the decrease on reddish colour, respectively, are in line with the TBARS observation and the stability of unsaturated fatty acids levels. Moreover, the results are in agreement with those reported by Sánchez et al[27] who reported that myoglobin and oxymyoglobin oxidation to brown metmyoglobin is associated with reduction in reddish colour and lower a*. Dark fish, like tuna, contain considerable amounts of myoglobin and haem, which makes them susceptible to discoloration and lipid oxidation.[28] In respect to day 0, non-significant differences were observed among samples which mean that no colour change is developed by the addition of antioxidants. Moreover, non-significant differences were observed among samples treated with RNX and control.

The b* value showed changes between days 0 and 9, for samples treated with RNX and for the control i.e. samples became more blue as time increased. According to Bozkurt,[29] browning reactions explained those results. Pérez-Alvarez et al[30] who reported a decrease in b* value for Spanish sausages, refers that it is correlated with oxygen consumption by microorganisms. Samples treated with DLT and VIT present stability over time in respect to b* value

CONCLUSION

The antioxidants added to tuna salami demonstrated effects in delaying both lipid oxidation and darkness of the samples. Moreover, the reduction of the unsaturated fatty acids levels observed in the control samples was by the presence of VIT and DLT antioxidants. During 60 days, the tuna salami preserved on a vacuum package did not show significant changes in lipid oxidation levels, pH, and colour. However, the experiment with slices of tuna salami (mimicking the consumer experience) evidenced a significant increase in the lipid oxidation after 9 days at 4 ºC. This evidence was accompanied by decrease in unsaturated fatty acids and decreases on the red colour of the tuna salami. The supplementation with DLT (100 mg of tocopherols/100 g sample) and VIT-100 (100 mg of tocopherols/100 g of sample) inhibited the lipid oxidation, the reduction of the reddish colour and also the unsaturated fatty acids reduction. On the other hand, the 10 fold reduction of tocopherols on the RNX antioxidant samples, which is constituted by rosemary, being the factor of differentiation to the others, showed higher values of darkness in slices during the period of storage. Moreover, the prevention of the oxidation process and the reduction of the reddish colour were not observed with the RNX supplementation.It is concluded that tocopherols supplementation (100 mg/100g of sample) enhance the nutritional value of tuna salami mainly by lipid oxidation inhibition.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Medical Rice: A New Wax-Free Brown Rice and its Protein Reduced Rice

Shaw Watanabe, MD, PhD[1]; Azusa Hirakawa, PhD[1,2]; Norihiro Takei, PhD[3]; Shigeru Beppu, PhD[3]; Hiroyuki Hashimoto, PhD[4]; Keiji Saika, PhD[2,5]

[1]*Lifescience Promoting Association, Tokyo, Japan*
[2]*Ootsuma Womens' University, Tokyo, Japan*
[3]*Forica Foods Co., Ltd, Niigata, Japan*
[4]*Tsuno Food Industrial Co. Ltd, Wakayama, Japan*
[5]*Tokyo University of Agriculture, Tokyo, Japan*

[*]***Corresponding author***
Shaw Watanabe, MD, PhD
Life Science Promoting Association, Daikyo-cho, 25-3-1004, Shinjuku, Tokyo 160-0015, Japan; E-mail: watashaw@lifescience.or.jp

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ABSTRACT

Introduction
Removal of wax layer from brown rice keeping nutrients rich bran layer was necessary for improving palatability of brown rice. Further removal of rice protein yielded low protein brown rice which is beneficial for chronic kidney disease patients.

Methods
Removal of wax layer was elaborated by the new machine. Extraction of protein from wax free brown rice (WFBR) was performed by the enzyme method. Six differently processed rice, i.e. brown rice, WFBR, low protein extracted wax free brown rice (LPBR), half polished brown rice, *kinme* polished white rice and bran grind (BG) rinse-free white rice, from the same lot of brown rice (*koshihikari* and *tsuyahime*) were boiled and served for analysis. Major and minor nutrients, amino acids, functional ingredients, and antioxidant activity were measured for calculation of actual intake.

Results
WFBR and its low protein reduced brown rice (LPBR) were successfully made by the new processing method and technology. Nutrients of WFBR were almost the same to those of brown rice, and LPBR was characterized by the low protein, low phosphate, low magnesium and trace of potassium. Vitamin B1 and B2 and folic acid were also decreased. Dietary fibers remained in both rice. Half polished and BG rinse-free white rice decreased lipid about half. Although energy contents were the same in all these 6 different rice, vitamins and minerals were almost lost in polished rice. Nearly 80% of brown rice amino acids remained in all rice, especially in BG rinse-free white rice. Gamma oryzanol reduced half by wax removal, but antioxidant activity was kept after removal of wax layer.

Conclusion
WFBR had the same nutritional values with brown rice. The palatability was comparable to polished white rice. Its protein depleted rice decreased 70 % rice protein, two third phosphate and almost all potassium that were toxic for chronic kidney disease (CKD) patients, so LPBR could be available for patients with renal insufficiency.

Keywords
Brown rice; Wax free brown rice; Low protein brown rice (LPBR); *Kinme* white rice; Rinse-free white rice; Nutrition; Gamma oryzanol; Antioxidant; Medical rice.

INTRODUCTION

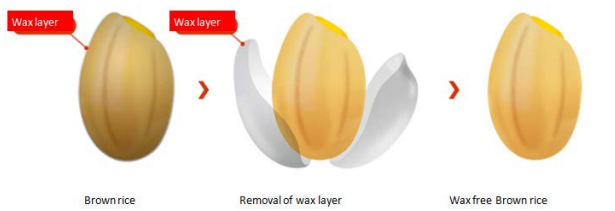
In many countries, rice contributes to health by supplying dietary energy, protein and fat.[1,2] The rice bran was originally known to have a rich amount of vitamins and minerals. In addition, various functional substances such as γ-oryzanol, sterols and phytic acid, etc. are included. The influence of brown rice on health is extremely large, so it could be called "medical rice" for keeping health and preventing various diseases.[3]

According to our research, macrobiotic practitioners consumed more magnesium, iron, vitamin E, vitamin Bs and dietary fiber although their energy intake was less than that of average Japanese.[4] Their Body Mass Index (BMI), blood pressure and low-density lipoprotein (LDL) cholesterol levels were mostly found to be low, and HbA1c revealed within normal levels.[4,5] The macrobiotic dietary pattern of eating brown rice seemed to contribute to their healthy state.

Recently much attention has been paid to rice bran, because the ingredients of rice bran show various interesting properties, like anti-oxidation.[6] Recent study further clarified many functional ingredients in the rice oil.[7] It is separated to gum, wax, dark oil and scum, and each fraction contained a number of chemicals. Brown rice is made up of three concentric layers, respectively: (i) the outer layer of the endosperm (starch storage cell layer), (ii) the aleurone layer and (iii) the bran layer. Removing the bran layer makes the rice tastier.[8] Proteins, vitamins, and other functional nutrients are mostly present in the bran layer. They are covered by an extremely hard wax layer.

The health benefits of brown rice are well-known, but consumers prefer polished white rice. Brown rice does not become popular, due to its hardness for mastication, and some felt gastric distress. In Japan, soft textures, such as the fast food, are preferred recently by young generation. The wax layer of brown rice disturbs immersion of water when boiling. So, Keiji Saika elaborated to invent the new rice processing machine to remove only surface wax layer (Figure 1).

Figure 1. *Wax Layer of the Brown Rice and Wax Removal*



A low-protein diet (LPD) has long been used for dietary therapy to preserve the renal function of patients with chronic kidney disease (CKD).[9-11] However, low protein rice was made from polished white rice, so almost all nutrients except carbohydrates were lost. Protein extraction from wax free brown rice (WFBR) was also succeeded by Forica Group. Nutritional aspects of this rice in comparison with other processed rice is dealt with in this paper.

The above rice was candidates of medical rice, so precise characterization was necessary.

MATERIALS AND METHODS

The Food Composition Table 2015 precisely described the data of rice, but these are data of raw rice, and their corresponding boiled rice data were calculated by multiplying 2.2 as a cooking water.[11] To know the true amount of nutrients in boiled rice should be directly prepared for the measurement.

To know the changes of nutrients by specific rice processing, the same brown rice was used for different processing method; i.e. removal of wax layer (WFBR), low protein rice of wax free brown rice (LPBR), half polished brown rice, *kinme* polished white rice, and bran grind (BG) rinse free white rice. *Kinme* polishing process remained aponeuronic layer for better taste.

The "bran grind method" (BG method) as an alternative method of rice processing avoiding the use of water.[8] To make rinse-free rice, the viscosity of bran itself is used to remove "skin bran." *Kinme* polishing remained aponeurotic layer for saving amino acids, because the endosperm surface is important to keep the taste by free amino acids.

The LPBR used proteolytic enzymes to digest and remove the protein in WFBR.[9] As proteolytic enzyme, acidic protease approved by the Ministry of Health, Labour and Welfare (MHLW) as a food additive and available on the market was used. In our method, an enzyme mix (EM) consisting of *Aspergillus oryzae*, *Rhizopus niveus*, and *Aspergillus niger* was used. The main component was protease in the aspartic protease family (EC 3.4.23).

The raw LPBR was spread out evenly into a thickness of 30 to 50 mm, then steamed for 4 to 10 minutes using superheated steam of at least 105 °C. Immediately after steaming, the steamed LPBR was separated loosely and dried by warm air so that the moisture level was uniform throughout, at 16% to 20%. It was the continuous production conveyor-system.

The processed 1.0 kg rice grain by a stepwise method was boiled by the pressure induction heating (IH) rice cooker (Tiger JPC-A), according to the instruction. The boiled and immediately frozen rice was sent to the SUNATEC Research Laboratories to measure the following; major nutrients (carbohydrate, protein, saturated and unsaturated fatty acids and neutral fat, ash, water soluble and insoluble dietary fibers), micronutrients (vitamins A, B1, B2, B6, B12, C, D, K, niacin, panthotenic acid folic acid, biotin), minerals (calcium, phosphate, iron, natrium, potassium, magnesium, zinc, copper, selenium, manganate), 28 amino acids and GABA, γoryzanol and antioxidants (AOU-L and AOU-P).[13] γ oryzanol was measured at the Tsuno Food Industrial Laboratory. For an ORAC measurement, 6-hydroxy-2,5,7,8-tetramethylchroman- 2-carboxylic acid (Trolox) was used as a standard

antioxidant, and the antioxidant capacity of the sample was expressed as Trolox equivalent.[14-16]

Some measurement of LPBR was performed at the Nippon Food Analysis Center, Tokyo, Japan.[17] Energy, major nutrients, vitamins, minerals and number of bacilli and heat resistant spores were measured according to the guidelines of the Ministry of Agriculture, Forest and Fishery.

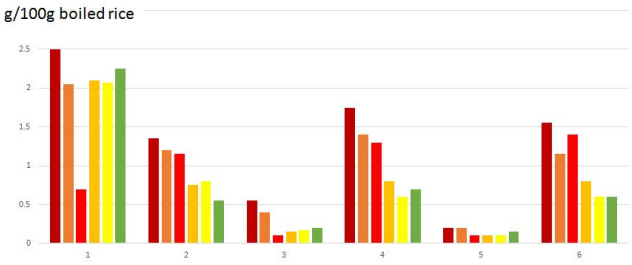
All data were collected in the Excel file and transferred to SPSS file for statistical analysis.[18] Comparison was made by t-test or ANOVA, and significant levels were set, as *<p=0.05, **<p=0.01, ***<p=0.001.

RESULTS

Macronutrients contents are shown in Table 1. Removal of wax layer did not affect the nutritional values of brown rice. Slight reduction of energy source was caused by immersion of cooked water, which made soft palatability like white polished rice (Figure 2). Protein extracted rice showed 70% reduction of rice protein, as well as ash reduction. Dietary fibers remained in both wax removed rice, but it became half in the half polished and polished rice. Water soluble fiber seemed to be more easily solved out than insoluble dietary fiber during the processing. (Figure 3).

Figure 2. *Raw Brown Rice (Upper Left), and Wax Free Brown Rice (Upper Right). Boiled Rice; Brown Rice, Wax Free Brown Rice, and BG Rinse-Free Rice (Lower Half). Brown Rice Particles (Left) are Smaller Compared to the White Rice (Right). Wax Free Brown Rice (Middle) Shows the Same Size of White Rice Due to Good Immersion of Water at Boiling*

Figure 3. *Comparison of Protein, Lipid, Ash and Dietary Fibers of 6 Differently Processed Rice Marked Reduction of Protein and Ash is Noticed, While the Dietary Fibers were Stored*

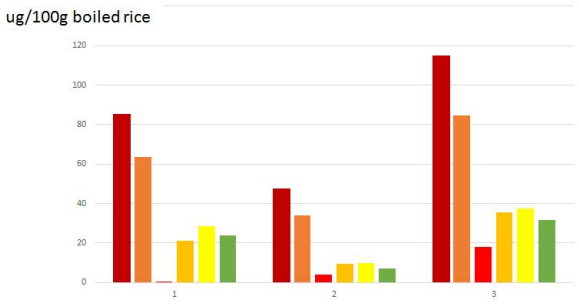


Columns show different rice meal: From left; brown rice, wax free brown rice, wax free and low protein brown rice, Half polished rice, *Kinme* polished rice, BG water free polished rice

As for the micronutrients, vitamin B1 was remained almost 90% in WFBR, but vitamin E, niacin, folic acid, panthotenic acid were lost 30 to 40%. (Table 2). Polished rice lost more, but *kinme* rice seemed to keep better.

Reduction of minerals was characteristic in LPBR. The remaining minerals was only 0.3% in potassium, 8.4% in magnesium, 9.6% in manganese, 15.7% in phosphate, and 39.5% in zinc. BG rinse free white rice also showed low potassium and phosphate. WFBR broadly decreased about 20-30%. (Fig. 4).

Figure 4. *Comparison of Minerals in 6 Differently Processed Rice Potassium is Completely Removed and 84% of Phosphate Decreased*



Columns show different rice meal: From left; brown rice, wax free brown rice, wax free and low protein brown rice, Half polished rice, *Kinme* polished rice, BG water free polished rice

WFBR broadly reduced all 18 amino acids about 20%. Polished rice kept similar level, and BG rinse-free rice remained higher contents. Loss of amino acids was about 5% in i-leucine, valine, tyrosine, phenylalanine, and about 10% of serine, histidine and leucine.

GABA remained 85.7% of brown rice in WFBR, but decreased to half in the polished rice.

Gamma oryzanol content became half in WFBR, and remained only a few percent in the polished rice.

Antioxidant activity of both brown rice and WFBR remained 3-4 unit in the boiled rice, mostly water soluble AOU-

Table 1. *Comparison of Nutrients in 6 Differently Processed Boiled Rice*

Item	Unit	Brown Rice	WFBR	LPBR	HalfBR	KinmeWR	RFWR
Energy	Kcal/100g	149.0±1.4	125.0±1.4**	157.5±0.7*	137.5±2.1	141.0±7.5	147.0±2.8
Water	g/100g	63.9±0.5	69.9±0.4**	62.2±0.5	66.4±0.5	65.5±2.0	63.7±0.8
Protein	g/100g	2.5±0.1	2.1±0.2	0.7±0**	2.1±0.1	2.1±0.2	2.3±0.2
Lipid	g/100g	1.4±0.1	1.2±0.0	1.2±0.4	0.8±0.1	0.8±0.1	0.6±0.1
Carbohyd	g/100g	31.8±0.4	26.5±0.1**	35.4±0.6*	30.7±0.5	31.5±1.9	33.4±0.6
Ash	g/100g	0.6±0.1	0.4±0.0	0.1±0*	0.2±0.1	0.2±0.1	0.2±0.0
DF Total	g/100g	1.8±0.1	1.4±0.1	1.3±0.3	0.8±0***	0.6±0.1**	0.7±0.1***
DF Water	g/100g	0.2±0.1	0.2±0.1	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.1
DF Insoluble	g/100g	1.6±0.1	1.2±0.1*	1.4±0.0	0.8±0***	0.6±0.1**	0.6±0***
Vit.E α	mg/100g	0.40±0.00	0.30±0.14	0.0	0.10±0.00	0.17±0.06	0.10±.
Vit. B1	mg/100g	0.12±0.03	0.11±0.01	0.0	0.10±0.04	0.08±0.02	0.05±0.01
Vit. B2	mg/100g	0.03±0.02	nd	nd	nd	nd	nd
Niacin	mg/100g	2.10±0.00	1.40±0.00	0.0	0.14±0.01	0.26±0.08	0.21±0.01
Folic Acid	ug/100g	11.00±1.4	7.00±0.00	2.0	4.00±0.00	5.33±2.31	4.00±0.00
Panthoteic a	mg/100g	0.41±0.02	0.33±0.01*	0*	0.11±0.00	0.18±0.01	0.15±0.01
GABA	mg/100g	3.50±0.71	3.00±0.00	2.0	nd	2.00	nd
γ oryzanol	mg/100g	10.00±0.00	5.55±0.5**	nd	0.54±0.10	0.42±0.23	0.30±0.15
AOU	unit	3.50±0.00	3.50±0.00	0.0	0.0	0.0	0.0
AOU-L	unit	3.50	3.50	0.0	0.0	0.0	0.0
AOU-P	unit	0.00	0.00	0.0	0.0	0.0	0.0
Na	mg/100g	2.50±0.57	2.50±0.14	1.85±0.21	2.60±0.28	1.93±0.45	2.00±0.57
K	mg/100g	85.30±2.26	63.75±2.05*	0.25±0.35***	21.30±0.57	28.70±0.98	23.95±0.21
Ca	mg/100g	6.00±0.00	5.00±0.00	8.0	4.00±0.00	3.67±0.58	3.50±0.71
Mg	mg/100g	47.55±0.07	34.10±0.57**	4***	9.35±0.07	9.83±0.58	7.25±0.21
Mo	ug/100g	nd	nd	6.0	nd	nd	nd
P	mg/100g	115.00±7.07	84.50±0.71*	18*	35.50±0.71	37.33±1.53	31.50±0.71
Fe	mg/100g	0.40±0.00	0.30±0.00	0.3	0.15±0.07	0.17±0.06	0.10±0.00
Zn	mg/100g	0.76±0.06	0.64±0.06	0.3	0.55±0.03	0.58±0.06	0.61±0.07
Cu	mg/100g	0.10	nd	0.1	nd	nd	0.10
Mn	mg/100g	0.83±0.01	0.60±0.01**	0.1**	0.28±0.02	0.29±0.02	0.27±0.02
Se	0.1ug/100g	nd	nd	0.0	nd	nd	nd
Nacl	g/100g	0.01±0.00	0.01±0.00	0.0	0.01±0.00	0.01±0.00	0.01±0.00
Arg	mg/100g	206.5±26.2	166.0±18.4	na	176.0±12.7	169.7±22.0	181.5±19.1
Lys	mg/100g	92.5±10.6	72.5±7.8	na	71.5±2.1	69.0±6.6	71.0±4.2
His	mg/100g	59.5±4.9	48.0±4.2	na	52.0±4.2	50.0±7.0	53.0±5.7
Phe	mg/100g	119.5±14.8	96.5±12.0	na	105.5±7.8	104.0±12.1	110.0±11.3
Tyr	mg/100g	120.5±13.4	100.0±9.9	na	111.5±10.6	106.7±12.2	114.5±16.3
Leu	mg/100g	195.5±21.9	156.5±17.7	na	169.0±12.7	165.3±19.6	176.0±18.4
I-Leu	mg/100g	80.5±10.6	65.0±9.9	na	72.5±6.4	68.7±12.7	75.5±9.2
Met	mg/100g	69.5±4.9	55.0±1.4	na	61.0±0.0	58.7±4.2	59.5±3.5
Val	mg/100g	115.5±20.5	93.0±18.4	na	103.5±7.8	97.0±21.0	108.5±14. 8
Ala	mg/100g	143.5±14.8	114.0±9.9	na	115.5±6.4	114.0±11.1	119.5±9.2
Gly	mg/100g	118.5±9.1924	95.0±7.0711	na	96.0±5.6569	93.7±9.6	98.5±9.2
Pro	mg/100g	115.0±15.6	92.0±9.9	na	88.5±6.4	89.7±11.2	97.0±5.7
Glu-a	mg/100g	436.0±52.3	350.0±41.0	na	376.0±26.9	375.3±42.2	395.5±44.5
Ser	mg/100g	135.5±10.6	108.5±7.8	na	114.5±6.4	114.3±10.7	120.0±9.9
Thr	mg/100g	92.5±7.8	72.5±6.4	na	75.5±3.5	74.7±6.0	77.0±5.7
Asp-a	mg/100g	236.5±23.3	187.0±17.0	na	190.5±12.0	190.0±19.3	199.0±18.4
Tryp	mg/100g	27.0±2.8	20.5±3.5	na	23.5±2.1	21.0±5.6	24.0±0.0
Cys	mg/100g	75.0±7.1	56.0±8.5	na	73.0±7.1	68.7±3.1	58.5±6.4

Table 2. *Loss of Nutrients, Functional Ingredients and Amino acids from Brown Rice in 6 Differently Processed Rice Meal*

Item	WFBR	LPBR	HalfBR	KinmeWR	RFWR
Energy	16.11±0.15	-6.42±0.48	7.72±0.55	2.69±1.92	1.35±0.96
Water	-9.40±0.29	3.19±0.77	-3.92±0.03	-0.94±0.88	0.32±0.45
Protein	18.11±3.85	70.83±0.00	15.71±10.43	14.10±3.63	10.10±3.40
Lipid	10.99±4.66	17.86±25.25	44.23±8.16	44.51±2.33	59.34±3.11
Carbohyd	16.53±0.48	-12.38±1.80	3.47±0.48	-2.35±1.98	-5.03±0.83
Ash	26.67±9.43	80.00±0.00	73.33±9.43	71.67±16.50	63.33±4.71
DF Total	19.77±11.32	23.53±16.64	54.25±1.85	62.75±5.55	59.80±9.71
DF Water	-66.67±188.56	0.0	33.33±47.14	33.33±47.14	-16.67±117.85
DF Insoluble	25.83±1.18	12.5	48.33±2.36	58.13±2.65	61.25±1.77
Vit.E α	25.00±35.36	100.0	75.00±0.00	62.50±17.68	75.00±.
Vit. B1	9.29±27.27	90.0	22.14±11.11	28.57±40.41	62.14±3.03
Vit. B2	nd	nd	nd	nd	nd
Niacin	33.33±0.00	100.0	93.57±0.34	89.76±1.68	90.24±0.34
Folic Acid	35.83±8.25	83.3	63.33±4.71	63.33±4.71	63.33±4.71
Panthoteic a	19.69±2.46	100.0	72.80±1.42	54.21±4.14	64.10±3.63
GABA	12.50±17.68	60.0	nd	nd	nd
γ oryzanol$_w$	44.50±4.95	nd	94.60±0.99	95.80±2.26	97.05±1.48
γ oryzanol$_d$	34.07±2.73	nd	93.90±1.56	95.40±2.09	96.72±1.42
AOU	0.00±0.00	nd	100.0	100.0	100.0
AOU-L	0.00±0.00	nd	100.0	100.0	100.0
Na	-1.97±17.42	11.90±10.10	-8.05±35.76	22.91±8.01	20.53±4.64
K	25.27±0.42	99.70±0.42	75.01±1.33	66.99±0.46	71.91±0.99
Ca	16.67±0.00	-33.3	33.33±0.00	33.33±0.00	41.67±11.79
Mg	28.29±1.08	91.6	80.34±0.12	80.02±0.03	84.75±0.47
P	26.36±5.14	83.6	69.05±2.52	67.39±0.16	72.58±1.07
Fe	25.00±0.00	25.0	62.50±17.68	62.50±17.68	75.00±0.00
Zn	16.53±2.16	58.3	27.29±9.13	21.88±4.42	19.86±3.34
Cu	nd	nd	nd	nd	nd
Mn	27.28±1.09	90.2	66.65±2.86	64.86±3.13	67.89±2.30
Nacl	0.00±0.00	50.0	0.00±0.00	0.00±0.00	0.00±0.00
Arg	19.53±1.29	na	13.69±17.10	12.39±3.22	11.99±1.91
Lys	21.59±0.58	na	22.06±11.23	21.32±5.20	23.00±4.24
His	19.35±0.42	na	12.00±14.45	10.12±0.84	11.01±2.10
Phe	19.25±0.02	na	10.62±17.62	8.21±2.53	7.83±1.99
Tyr	16.95±1.04	na	6.40±19.24	7.00±0.98	5.14±2.92
Leu	19.95±0.07	na	12.64±16.31	10.65±1.70	9.94±0.69
I-Leu	19.36±1.67	na	8.62±19.95	5.92±4.49	6.15±0.95
Met	20.59±7.69	na	12.01±6.27	16.04±14.12	13.99±11.21
Val	19.63±1.65	na	8.35±23.01	5.27±8.86	5.72±3.88
Ala	20.49±1.33	na	18.85±12.83	16.99±1.69	16.61±2.22
Gly	19.82±0.25	na	18.56±11.09	17.33±0.75	16.93±1.31
Pro	19.85±2.23	na	21.96±16.09	17.70±1.91	15.21±6.55
Glu-a	19.71±0.23	na	12.76±16.63	9.78±1.42	9.25±0.67
Ser	19.91±0.53	na	15.05±11.35	12.19±0.43	11.45±0.37
Thr	21.63±0.29	na	17.93±10.72	16.14±1.70	16.72±0.89
Asp-a	20.90±0.63	na	18.80±13.09	16.03±0.81	15.83±0.53
Tryp	24.34±5.17	na	12.07±17.07	11.17±1.17	10.62±9.36
Cys	24.46±18.44	na	2.68±0.25	10.36±6.57	21.25±15.91

WFBR; wax free brown rice, LPBR; low protein brown rice, HalfBR; half polished brown rice, na; not applicable, nd; not detected. γ oryzanol$_w$; in wet boiled rice, γ oryzanol$_d$; in dried powever.

L. Polished rice did not show antioxidant activity at all.

DISCUSSION

Whole brown rice, compared with white rice, is rich in vitamins, minerals, dietary fiber and various functional chemicals.[3] The effects of eating brown rice have been gaining attention for preventing and treating not only beri-beri and constipation, but also other chronic diseases including symbiosis with enterobacteria.[4,5,19,20] Rice bran is used in a variety of applications such as food, animal feed and fertilizer, but most of the rice bran is discarded at present. So, recommendation of brown rice eating is contributed to both public health concern and environmental issue.[21-23] *Genmai* (brown rice) eating is beneficial to keep the proper body weight.[5,20]

It was said that about 1/3 brown rice (*genmai*) eater abundaned eating *genmai*. Brown rice does not become popular, due to its hardness for mastication, and some felt gastric dyspepsia.[24] Removal of waxy layer succeeded by Keiji Saika to improve texture and palatability of brown rice. The nutrients and antioxidant activity were the same as brown rice after adjusting water content, so it should be a gospel to whom want to eat brown rice for health. It made it possible to use even for *Sushi*.

Newly made protein extracted wax free brown rice (LPBR) could be expected to have wide usage in clinical nutrition.[7,11] CKD patients have a necessity to reduce phosphorus and potassium intake in addition to decrease protein intake. At the same time, there is a need to ensure the patient takes in enough nutrients, particularly energy. LPBR meets all of these requirements as a staple food for CKD patients. Additional benefits should be obtained for the disease by dietary fiber, vitamins, gamma oryzanol, ferulic acid and antioxidant activity. Almost no potassium and low phosphate in LPBR were also beneficial for the patients to prevent hyperkalemia and hyperphosphatemia.[11] Recently we finished a randomized clinical trial in Bangkok by using protein extracted *Indica* rice, and had a report of similar effectiveness for CKD. Dietary fiber intake stimulated growth of intestinal bacteria which produce short chain fatty acids, which are beneficial for gut environment.[18,19]

γ-Oryzanol and GABA are thought to be effective for mental health.[3,25] According to the aging society the increased number of impaired cognitive function becomes serious problem. In Japan it is estimated to be 2 million people, and WHO estimates that 47.5 million people have dementia and there are 7.7 million new cases every year worldwide.[25] GABA and γ-oryzanol are involved in hypothalamic catecholamine metabolism. γ-Oryzanol is known to have anti-stress effects, palliation of menopausal disorders and dysautonomia.[26] The effects on humans, improvement of hypertension, curative effect of Alzheimer's disease, amelioration in muscular fatigue are recently reported.

Large germ brown rice and pre-germinated brown rice contain functional ingredients to prevent dementia, such as GABA, γ-oryzanol, in addition to the nutritional elements such as vitamins, minerals, and dietary fibers. Removal of wax layer could be applicable to large germ brown rice in the near future.

Many phytochemicals contain antioxidant ability which protects damages caused by the free radicals.[13-15] An antioxidant test known as ORAC (Oxidation Radical Absorbance Capacity) has become popular by easy applicability for standardization.[26-28] The US Department of Agriculture measured the antioxidant capacities of 326 food items, and in the case of fruits and vegetables, the ORAC values for the hydrophilic fractions (H-ORAC) were typically much higher than those of the lipophilic fractions (L-ORAC).

However, other multiple assay system such as, DPPH, TRAP, TEAC, etc., have been used to define the antioxidant capacity of food ingredients.[26,27] Consumption of rice is about 500 g a day as a staple food in daily meals should prevent carcinogenesis and diseases caused by free radicals.[29]

CONCLUSION

Health effects of brown rice is empirically well known, and accumulating knowledge about the physiological and pharmacological activity of rice bran strongly support the brown rice meals, but it does not become popular in Japan. Removal of wax layer improved much of the dispute of brown rice. LPBR has many benefits for patients for renal insufficiency by decreasing protein intake, low patassium and phosphate, and functional ingredients and antioxidant activity. These are strong candidates for medical rice.

DECLARATIONS

Dr. S. Watanabe conducted this study and do not have any conflict of interest with lower companies.
Ms. Azusa Hirakawa is a graduate student of Otsuma women's University.
Mr. Keiji Saika were working for Toyo rice Co. Ltd.
Drs. Norihisa Takei and Shigeru Beppu were employee of Forica Foods Co., Ltd.
Dr. Hiroyuki Hashimoto was an employee of Tsuno Food Industrial Co. Ltd.
All study and work has been performed at the Toyo Rice Co. Ltd, Forica Food Industy Co. Ltd, and Tsuno Food Industrial Co. Ltd.

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Review

Colorants: Their Interaction with Flavor in Product Development

Deepti Dabas, PhD*

Lead Scientist and Senior Manager, Kalsec® Inc., 3713 West Main Street, Kalamazoo 49006, Michigan, USA

Corresponding author
Deepti Dabas, PhD
Lead Scientist and Senior Manager, Kalsec® Inc., 3713 West Main Street, Kalamazoo 49006, Michigan, USA; E-mail: dabasd@gmail.com

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ABSTRACT

Color is an important part of product appeal and is a critical criterion in a consumers' ultimate selection of foods and beverages. The color of a food or beverage not only acts as an indicator of quality and freshness, it can also elicit expectations of flavor. The utilizationof naturally sourced colors in food and beverages is increasing at a faster pace compared to synthetic colors. Naturally occurring molecules having a higher molar extinction are favored as colorants due to higher efficiency of use in applications compared to other naturally existing molecules. Flavor is another critical sensory attribute in food and beverages and therefore it is important to study and understand the interaction of color and flavor while developing products. Color needs to be dispersible with the flavor as well as in the application. Ideally color should not alter the flavor profile of the food or beverage, nor should it negatively impact the stability of the flavor system.Ultimately, colors that align well with the flavor and with the application should be selected. Colorant extracted from radish is an example of a color system which imparts flavor and therefore may need special processing. However, different radish sources may have different flavor thresholds. One final consideration when choosing a natural colorant for development into a food or beverage product is that different consumers may have varying degrees of sensitivities to the flavor of these color products.

Keywords
Natural colorant; Color flavor interaction; Beverages; Fruit preparations; Colors imparting flavor.

INTRODUCTION

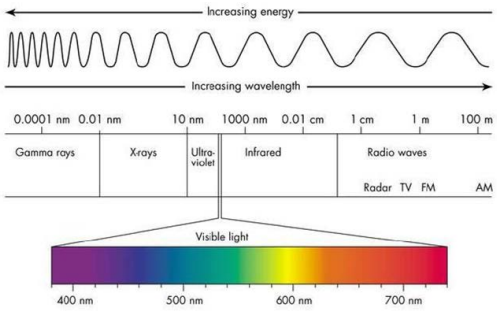
The purpose of this review is to discuss critical aspects and considerations of color/flavor interaction during the development of food and beverage products. This review covers the science of why we see color, why colors are added to food and beverage, and provides a definition of naturally sourced colorants. In addition, color/flavor interaction is described through beverage and fruit preparation applications. Discussions regarding dispersibility, flavor impact and stability of colorants are also included.

COLOR AND VISIBLE LIGHT

Color is the effect on the eye caused by light waves in the visible region. The visible region ranges from 380 nm, higher energy/shorter wavelength violet light, to 700 nm, lower energy/longer wavelength red light. The speed of light being a constant, c, and knowing the speed of light is equal to the product of the frequency (ν) and wavelength of light (λ), $c=\nu\lambda$, red light has a lower frequency wave than violet, which has the highest frequency of light wave in the visible region. Figure 1[1] highlights the visible region of the electromagnetic spectrum. It is of interest to note

that color can be expressed quantitatively by either frequency or wavelength. A dispersive prism is an excellent tool to separate and visualize the color components of white light. White light waves are refracted within the prism, shorter wavelengths of light are refracted more than longer wavelengths, thus separating the light into spectral colors.

Figure 1. *The Relationship of Energy and Wavelength of the Visible Region of the Electromagnetic Spectrum. Reproduced from Cyberphysics.Co.Uk*



Why Do We See Different Objects as Different Colors?

When white light strikes an object, visible light is either absorbed or reflected depending upon the chemical structure of the molecules present in the object. If all visible light is absorbed the object is perceived as black. If all visible light is reflected from the object the object is perceived as white. The appearance of color, say red, occurs when a red light is reflected, and all other colors of the visible region are absorbed by an object, or less frequently, when the complimentary color of red, green, is absorbed and all other colors are reflected by an object. Figure 2 illustrates why we see black, red and white colored objects.

Figure 2. *Why We See Objects as Black, Red and White. Reproduced from clarkscience8.weebly.com*[2]

Aromatic compounds having hetro elements (elements other than carbon and hydrogen) in their structure and those possessing conjugated bonds are typically the best candidates to be colorants. These compounds selectively absorb and reflect wavelengths of visible light to produce their unique colors as described above. Such absorption is associated with energy changes within the molecule. This energy difference between the ground and excited state is proportional to the observed frequency (inversely proportional to wavelength) of absorbed light. When light strikes a compound, the light's energy is used to promote electrons from bonding or nonbonding orbitals to empty anti-bonding orbitals. Each electron transfer utilizes energy from light and bigger jumps (σ^*, sigma anti-bonding orbital) need more energy than smaller jumps (π^*, pi antibonding orbital) - sigma antibonding orbital lies above the pi antibonding orbital in terms of energy. The higher the energy jump, the greater absorption of light at a higher frequency is needed-hence greater the frequency, greater is the energy. Or the larger the energy jump, the lower wavelength of the light absorbed– hence molecules displaying this phenomenon will appear orange or red. As the resonance or resulting delocalization in a molecule increases, it is easier for the electrons to be excited and hence more likelihood of the molecule being colored. For example, beta-carotene has much more delocalization with 11 carbon conjugated double bonds. Thus, it is much more intensely colored than retinol which has only 5 conjugated bonds and is light yellow colored. Hence beta-carotene has a higher molar extinction coefficient (a measure of how strongly a compound absorbs radiation) than retinol.

WHY IS COLOR ADDED?

Color is added to many processed foods and beverages and is an important part of product appeal. Color is the most notable attribute in food evaluation making it to be a critical component of intent to purchase. Appearance and color are the visual elements to which our eyes, minds, emotions and palates are sensitive. The following sections detail additional reasons as to why color plays such an important role in foods and beverages.

Color Acts as Indicator of Quality and Deterioration for Fruits and Vegetables

Naturally-derived colorants are pigments extracted from fruits and vegetables, many of which change as a plant proceeds through maturation and ripening. Inherent pigments imparting color quality are fat-soluble chlorophylls (green), carotenoids (yellow, orange and red), water-soluble anthocyanins (red, blue), flavonoids (yellow) and betalains (red). In addition, enzymatic and non-enzymatic browning reactions may result in the formation of brown, grey and black colored pigments. The enzymes involved in browning reactions include polyphenol oxidase, which catalyzes the oxidation of polyphenolic compounds such as in apple juice.[3] The quality deterioration as responding to various stresses during storage can be evaluated in terms of changes in color (green -chlorophyll, yellow and orange-carotenoids, brown-enzymatic browning). Other parameters like texture (stiffness, firmness and consistency), off flavor soapy-like, stearin-like and paraffin-like, nutrition (antioxidant, polyphenol and ascorbic acid), composition (starch, sugar) and sensory (flavor, taste, color, consistency, appearance and overall attributes) are also affected.[4] An example where color is indicative of deterioration of product quality is a banana whose peel has blackened.

Foods and Beverages Induce Pre-Consumption Expectations about Flavor Identity

Color intensity is a cue for the quality of foods as well as the intensity of taste. Food color also elicits expectations of nutritional value with red colored foods perceived as higher energy density than green colored foods.[5] We associate colors with certain flavors-such as yellow color is generally associated with lemon flavor and red color with berry flavors (raspberry, blueberry and strawberry). Color, therefore, elicits expectations of certain flavors and guides us towards identifying those flavors.

Color Variety Impacts not Only the Perception of Taste but also the Amount Consumed

As an example, studies have shown that multicolored candy is consumed in higher amounts than single colored candy. This phenomenon has also been observed in other food groups, including fruits and vegetables. In one research study the perceived color variety of the meals and its effects on consumption of different food groups (fruits, starches, protein, vegetables, sugar, dairy) was evaluated. The increase in perceived meal variety was associated with an increased proportion of vegetables consumed and reduced the proportion of extra sugary content consumed.[5]

NATURAL COLORS

The Food and Drug Administration (FDA) has not officially defined 'natural colors', however, it is generally understood and an industry standard that natural colorants are defined as pigments extracted and isolated from natural sources. Examples of natural colors are paprika oleoresin extracted from paprika pods, curcumin derived from turmeric rhizome and chlorophyll derived from alfalfa. The polarity of the natural pigment determines the solvent and solvent ratio in the extraction. Residual solvent limits in the final natural colorant product are set by FDA and are in the ppm range. Artificial colors on the other hand are often manufactured from non-sustainable synthetic materials such as coal tar or petroleum derivatives but are generally more stable and of lower cost than natural colors. The food and beverage color market is projected to grow at a compound annual growth rate (CAGR) of 8.40% and reach USD 3.75 billion by 2022; natural colorants are expected to have a 53.6% share of this market (USD 2.01 billion). This rate of growth is higher than that of synthetic colors. The consumer demand for 'clean'labelled productshas resulted in the projection of a higher rate of growth of natural colorants over synthetics,[6] with the largest market of natural colorants expected to be in North America.

CONSIDERATIONS IN MAKING A PRODUCT CONTAINING BOTH COLOR AND FLAVOR

For the purpose of this review, naturally sourced colors and flavors will be discussed. Pigments generally contain higher molecular weight molecules as compared to flavor molecules. Substances responsible for odor perception are volatile (high vapor pressure) and are generally low molecular weight compounds. The structural and property differences between natural colors and flavors are important to know and understand when adding to food and beverage applications.

Dispersibility of Color and Flavor

Due to the difference in their physical and chemical properties, color and flavor may not disperse together. This may call for adding specific additives to disperse them uniformly in the application. Different emulsifiers are commonly used to disperse color or flavor or both into the matrix such as lecithin or polysorbates. The use of a specific emulsifier and its concentration is determined by the properties of the matrix. For example, to attain a strawberry flavor in a beverage application, emulsifiers would be needed to disperse the flavor constituents which are comprised of particular esters, terpenes, and furans;[7] however, a black carrot based colorant may work for a beverage application and will not require any emulsifier for dispersion since it is water soluble.

Color Imparting Flavor

When developing products with both colors and flavors considerations need to be madeaccording to the application. A common concern for scientists developing new products is the odor/flavor system associated with the concentrated color raw material. Most color raw materials are developed with high concentrations to allow use in multiple applications and to save on transportation costs. Because of the high potency of the commercial colorants they may possess noticeable odor and flavor in their concentrated form. However, when the colorant is dosed into the application, it may not cause any flavor impact. Therefore, the formulator should test the product in his desired application before looking for color alternatives.

Color Affecting the Stability of Flavor

Keeping color and flavor together in a food matrix can have its challenges. For example, the stability of one may be affected by the other. Some color pigments may undergo rancidity such as carotenoids. Carotenoids may get oxidized, generate off flavors and lose color; these oxidized products then can cause off flavor in an application. However, carotenoids may also enhance original flavor stability by protecting other constituents including flavor.

Selecting Suitable Color for a Flavor

Lemon flavor is generally associated with yellow color; lemon gets its flavor profile from molecules like citral, geranial, lemonal etc.[3] Colors are then selected based on this association and different yellow colors can be used such as orange carrot extract, turmeric oleoresin, gardenia yellow or safflower. It is crucial to know the properties of these pigments and select the one that aligns with that of flavor product. Factors such as water solubility, light stability and local labeling regulations should be considered. For example, sensitivity of turmeric to light impacts its use in certain application like beverages, while it is quite stable in a hard candy.

Regulatory Landscape

Regional regulations can dictate what color and flavor systems may be utilized in formulating a food or beverage. Most flavors and excipient ingredients in food and beverage products have traversed the generally recognized as safe (GRAS) affirmation route, but color additives are specifically prohibited from being determined GRAS outside of the FDA petition process. Therefore, all food colorings must go through a pre-market review and approval by the FDA to be included on the approved colorant listing. A food ingredient found to be GRAS for one technological function, e.g., as an antioxidant or acidulant, might also be the subject of a color additive petition if it also imparts color into an application. In addition, if an approved or exempt colorant is discovered through research and testing to have an additional function in a food or beverage, e.g., an antioxidant or an acidulant, the colorant may be to subject to an external GRAS affirmation as a food ingredient for this non-color function.[8] It is important to remember that regulatory definition of natural varies for colors and flavors. For colors, the FDA has a regulatory definition that if an ingredient is derived, it may not be called natural universally (example, gardenia blue) and for derived flavors there is a specific definition that allows them to be categorized as natural if they fit the outlined requirements and use approved ingredients (example, maillard reaction products). In Europe, ingredients added as colorants are classified either as color additive (E numbers), or as coloring foods. E numbers are used for additives used to color foods; recently coloring foods have come into the forefront and regulations around them are still being developed and adopted. Although there are no regulations inhibiting the use of artificial colorants, studies like Southampton have discouraged the use of artificial colorants in Europe.[9]

SPECIFIC APPLICATIONS CONTAINING COLOR AND FLAVOR

Beverages

Beverages can be challenging applications as they may require color and flavor added in the form of an emulsion that may be exposed to light, high water activity and other environmental factors. Beverages can be used in different forms such as 'ready to drink' or more concentrated forms where the consumer adds water at 6-8 times the concentration level. Some pigments, such as turmeric, may not remain stable over time in this relatively harsh environment. Colors and flavors may also be formulated with different emulsifiers which can affect performance. For example, protein based emulsifiers and pectin emulsifiers may affect the flavor and color respectively and after addition may precipitate at an approximate pH of 3.[10] This could cause the beverage to coalesce due to oppositely charged particles of these emulsifiers and would mean separation of color and flavor even though flavor precipitation may not be visible.

Generally, flavor oils have much lower density in the aqueous phase which may be a factor causing emulsion breakdown. To prevent this, weighting agents may need to be added to create a stable emulsion system. Flavor consists of not only the flavor molecule but are generally mixtures of flavor oil and non-flavor oils. As an example, citrus-based flavor oils such as orange, lemon, lime and grapefruit fall into this category. These oils are highly complex substances that contain a variety of different kinds of molecules with different structures, polarity and chemical properties. The primary constituents are the hydrocarbon terpenes (monoterpene and sesquiterpenes) like myrcene, limonene, citral, geranial etc., but they also contain other compounds responsible for flavor such as alcohols, ketones, acids and esters.[11] These compounds need to be incorporated into an emulsion as they have limited water solubility. Infact, these latter group of compounds have some water solubility which may lead to Ostwald ripening. Ostwald ripening is the growth of bigger globules at the expense of smaller ones in an emulsion system; if left unchecked it may ultimately lead to creaming and breakdown of the emulsion. Adding different emulsions (flavor and color) into a beverage may also cause creaming based on different particle sizes.[12]. Thus, it is important to keep the particle sizes of both the color and flavor emulsion in a similar range.

For some products, color and flavor may be present in separate phases unlike the oil phase examples discussed above. A raspberry flavored beverage, for example, where the flavor oil may be in the oil phase and the color (typically an anthocyanin) will be in a continuous phase, is less challenging. Anthocyanins can be radish, cabbage, black carrot based-sometimes berry anthocyanins are also used which may not be as stable as vegetable based sources. In most cases, the beverages do need to be pasteurized after adding the colorants-in such cases stability studies with the actual beverage matrix should be conducted before choosing the source and concentration of the color. Vegetable based anthocyanins have more likelihood of surviving the pasteurization conditions; black carrot is a good example.

Fruit Preparations

Fruit preparations are used in many applications such as yogurt, baking and ice-cream where they contribute the color, flavors and texture of the fruit. This application does require water dispersibility and oil soluble colors and flavors may need to be dispersed or emulsified before adding to a fruit preparation. Many similar considerations with respect to emulsifiers apply as referenced in beverage applications. Fruit preparation processing should be done in a vacuum kettle to retain flavor volatiles by processing at lower temperatures to a predetermined level of solids which is typically 65%.[10] At a higher brix level, boiling temperature is generally 5-7 times higher than the boiling pressure of water. This can lead to color loss, so this should be a consideration when colors are added. To avoid this, color should be added at the end of the manufacturing process when possible. It is important to note that the nonvolatile portion of flavor may also degrade on heating.

COLORS BRINGING THEIR FLAVOR TO PRODUCTS

In some cases, unwanted flavors may accompany colorants. Some color pigments are known for their unwelcoming flavor such as

beet and radish pigments. Removal of flavor may require further processing after color extraction. In the case of radish extract, hydrogen disulfide, dimethyl disulfide and other sulfur-based compounds are responsible for this odor and flavor. Radish is known for its off flavor which is derived from glucosinolates.[13] As the vegetable is crushed and prepared for extraction, myrosinase (an enzyme) acts on the vegetable and creates different molecules responsible for the characteristic radish flavor and additional off flavors.

Kalsec® conducted a study on the interaction between flavor and odor in radish pigments. Determination of odor and taste threshold by forced choice ascending concentrations series method of limits was used. Panelists evaluated the products in order of increasing color strength and were asked to complete an entire series of seven stations, increasing in concentrations. Best estimate threshold (BET) was determined and the following observations were reported:

- Each of these two samples had different best estimate thresholds which was expected. The mean of sample 1 was found to be 230 ppm whereas the mean for sample 2 was estimated at 544 ppm. This reflects the challenge of formulating.

- The individual BET for each sample varied immensely among participants. For selected panelists, the range was 39.4 to 1623.5 ppm. Thus, some panelists are very sensitive to these compounds while others may not be able to perceive these compounds.

This study shows that while switching supplies of a naturally sourced colors, the possible impact on flavor must be considered.

CONCLUSION

Color is an important sensory attribute in foods and beverages and can play a key role in a consumers' selection process to purchase a product. There are many reasons colors are added to foods and beverages. Not only are colorants a crucial attribute while selecting food for consumption, they are associated with the quality attributes of the foods and beverages. Colors are also associated with certain flavors and when added, point our senses towards that flavor. For example, red color added to a strawberry flavored candy. Naturally sourced colors are becoming more significant as the demand for fewer artificial ingredients increases. Formulating with naturally sourced colors and flavors can be challenging as they may have very different chemical properties. Also, other ingredients like emulsifiers or diluents are usually added to these colors and flavors and can interfere with the stability of finished product. Therefore, it is important to know the interaction and understand the stability of these compounds before introducing a new product to market. Some colors can bring in their own flavor and this should be evaluated for its impact on the application. This can impact the recommendation of naturally sourced colors chosen for a particular application. As observed in the sensory study, two sources of radish had different BET values and indicates the different concentration of active odor molecules in the two sources. This indicates a thorough comparison of color and its flavor is important when evaluating color sources. Natural sources are expected to have some variation regarding the ratio of different color and flavor components. Food application formulators should consider these aspects when formulating with naturally sourced colors. Liquid color extracts and oleoresins can help to provide reduced variation in formulating.

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CONFLICTS OF INTEREST

The author declares no conflict of interest.

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Review

A Review of Color Flavor Interaction in Food and its Application in Food Product Development

Shane T. McDonald, PhD*

Principal Flavor Chemist, Kalsec® Inc., 3713 West Main Street, Kalamazoo 49006, Michigan, USA

Corresponding author
Shane T. McDonald, PhD
Principal Flavor Chemist, Kalsec® Inc., 3713 West Main Street, Kalamazoo 49006, Michigan, USA; E-mail: SMcDonald@kalsec.com

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ABSTRACT

Humans use all of the senses, especially sight and ortho-nasal olfaction, to create an expectation of what a flavor of a food will be before ingestion. The expectation is confirmed or disconfirmed when the food is placed in the mouth by taste (gustation) and retronasal olfaction. Color is an important constituent of sight. Colors that create the correct expectation of a flavor are called "*congruent*" and colors that create incorrect expectations are termed "*discongruent*". Studies have shown that the ability of people to correctly identify flavor is increased considerably if the color is congruent, or expected, with the flavor. Congruently colored foods have been shown to increase the perception of sweetness in beverages, the richness of orange juice, the pungency of salsa, and the richness of chocolate. Incongruent colored foods may create an incorrect perception of the foods flavor, which many consumers find surprising and unpleasant. For that reason, it is usually best to develop foods that are congruently colored. However, there are opportunities to create excitement and other positive benefits from deliberately coloring foods in a discongruent fashion. More research is needed on this topic, especially in the areas of the effect of minor differences of shades of colors in food flavor perception, and how to successfully implement a strategy of discongruence in food product design.

Keywords
Natural colorant; Color flavor interaction; Beverages; Fruit product design.

INTRODUCTION

"*You eat with your eyes*"? What does that mean? Humans make their first judgements on food based on appearance and confirm with taste and smell. One of the most important visual characteristics of appearance is color.

Flavor is the sensory impression of food and beverage when taken into the mouth. It is mostly determined by the senses of taste and smell. However, all of the senses are involved in the perception of food. As an illustration, you see a hamburger. At the same time, flavor compounds volatilize off the hamburger and escape into the air. The chemicals are swept up into the nose and are detected by receptors in the olfactory nerve, a process called "*orthonasal olfaction*", that is, from the external environment into the nose. This information is used to create a perception in the brain: it expects the food to taste like a hamburger. You then bring the food into your mouth and chew. Non-volatile tastants are perceived by the taste buds as sweet, sour, bitter, salty, and umami. Other tactual senses are noted such as temperature, texture, sound, and chemesthetic effects. Flavor compounds volatilize off the food mass and are swept through the back of the throat into the nose and again detected by the olfactory bulb: this is called "*retronasal olfaction*", from the inside of the mouth into the nose. The sensory information from the mouth and retronasal olfaction confirm or disconfirm the expectation.

As will be discussed, humans are very poor at identifying flavors without visual information. And a very important constituent of visual information is the color of the food.

LITERATURE REVIEW

Food color is well-known to affect the consumer's perception of the flavor of the food.[1] It affects the aesthetics, safety, sensory characteristics, and acceptability of food. Color is a major con-

tributor for initial acceptance/rejection of food. Early in cognitive development, one learns to associate foods with specific colors.

There have been numerous studies on how the color of food affects the perception of flavor. In a famous study by Dubose et al,[2] panelists were asked to taste and identify colored-masked samples of retail, fruit-flavored, non-carbonated beverages. Panelists did a poor job of identifying the flavors by tasting without seeing the color of the beverage. Seventy percent of panelists correctly identified grape, but only 20% identified orange. However, when allowed to see the colors of the beverages, identification was almost 100%.

When the color of the beverage was typical for the flavor (red for cherry, green for lime, orange for orange, and colorless for flavorless), flavor identification was greatest. However, when the color was inappropriate (or incongruent, which means unexpected), flavors were often incorrectly perceived. For example, 33% of panelists thought a red-colored lime-flavored beverage was a red fruit (cherry, strawberry, raspberry). Fourty percent thought a green-colored, flavorless beverage was lemon-lime flavor.

Other studies suggested that color can affect the perceived intensity of sweetness,[3] the intensity of pungency perception in salsa[3,4] the acceptability of fruit juice,[1] and the intensity of chocolate flavor.[4] All pretty amazing considering that the color doesn't change the flavor or wholesomeness of the product. The color of a product gives an indication of the odor, flavor and texture of the food. Through pattern recognition, a consumer associates certain food colors with certain food flavors, so visual clues would influence food choice and acceptability.

Early on, it became apparent in investigating color/flavor interaction that the story was complicated. Many (over 100) studies have been done on this phenomenon.[5] It has been noted that many of these studies have been contradictory. So many, it appears that the simple results of experiments may be only part of the story.

In everyday life and some professional activities (sensory analysis, perfumery and flavor creation, oenology) humans make use of their abilities to identify odors.[6] The odors of wine tend to be grounded by the colors of the wine. In oenological tasting, a single taster can provide a wide variety of comments. Comments are based on analytical descriptions of the visual, olfactory and gustatory properties of the wine. The analysis of wine tasting is well adapted for studying interactions between various sensory modalities. Determination is modified if the color of the wine is obscured by use of an opaque glass. Acceptance of wine is significantly correlated to its color.

Morot[6] study four extensive bodies of tasting wine tasting notes (corpuses). For the most part, red wine descriptors are represented by red or dark objects, while the white wine with yellow or clear objects, which was common for all four corpuses. Red wines had descriptors such as prune, cocoa, raspberry, and tobacco. White wine descriptors included "*melon*", "*citrus*", "*butter*" and "*apple*". It was determined separately that it is difficult to determine if a wine is red or white when tasted in opaque glasses: 70% got it right, which is better than chance but is shows that it is difficult to determine a red wine *vs.* white wine without visual assistance.

The panelists were given two wines. One was a white wine "*W*" and one was a white wine dyed red "*RW*". It was determined separately that the two wines were identical in taste and that the dye did not affect the flavor. Panelists tasted the two sets of wine and overwhelmingly used white wine descriptors to describe W and red wine descriptors to describe RW.

The white wine was perceived to have the odor of red wine when colored red. The wine's color provides significant sensory information, which misleads the subject's ability to judge flavor. The mistake is stronger in the presence that in the absence of access to the wine color.

The subjects smell the wine, make the conscious act of odor determination and verbalize their olfactory perception by using odor descriptors. However, the sensory and cognitive processes were mostly based on wine colors.

The study concluded that the sense of smell is, by itself, unlikely to provide sufficient information to allow for a consciously reasoned decision. The capacity to identify odors could only be an accessory aspect of the olfactory function.

A similar study was performed on beer.[7] They differentiated "*experts*" (people with technical knowledge of beer as well as in-depth sensory exposure), "*trained assessors*" (sensory panelists with several years training in beer attributes, but generally without the technical knowledge of beer) and "*novices*" (beer drinkers but without sensory or technical training). This study involved trained assessors and novices; either sighted or blindfolded evaluation of three styles of beers (blond, amber, and dark) from each of three breweries. It was assumed that when sighted, the trained assessors would discriminate based on brewery and not beer color, unlike the novices who would go by color first.

The results were that, when blindfolded, both groups tended to categorize beers by brewery, detecting the different characteristic qualities of each brewer. When sighted, both groups categorized by color. So, when blindfolded, both groups could categorize by brewery but when sighted they used color? This was even though the trained assessors claimed that they ignored color when they were evaluating the beer. The researchers had two hypotheses on what happened. One, when blindfolded, the panelists concentrated on chemosensory properties such as bitterness, mouthfeel, and aroma. When sighted, they couldn't process both visual and chemosensory information at the same time, so they used visual information and ignored smell and taste information. This is called selective attention. The other theory was that the assessors were able to switch attention from color to chemosensory attributes, but the chemosensory information was driven by visual information. That is, when analyzing the beers,

they were seeking to confirm the visual information rather than analyzing taste and smell information. This is called "*congruence seeking*". This may be why the trained assessors felt they relied more on chemosensory information when in fact they relied on visual information.

Cultural expectations also come to play.[5] What happens when a Taiwanese and a Briton taste a brown liquid which is in fact a grape product? This question was studied using Taiwanese and British panels. The Taiwanese expected grape flavored product and a tart taste. Upon tasting the beverage, their expectation will be confirmed, and the brown color enhanced the flavor of grape. The Briton tasted the beverage expecting the sweet taste of cola. Instead, it was tart, and they received a "*disconfirmation*" of expectation. They could either "*assimilate*", that is, perceive the drink to be sweeter than it is to match expectation, or "*contrast*", the color expectation has no effect on sweetness perception. Assimilation can occur if there is not a great discrepancy between expected and actual, or the stimulus is ambiguous, allowing for the panelist to focus on qualities congruent with expectations, or simply social pressure/experimental demands to align perception with group expectation. Otherwise, contrast will occur.

Zellner,[8] reviewed of color-odor interactions. She contrasted sensations that perceived food "out there" (color and orthonasal olfaction which detects aroma) and food in your mouth (gustation, retronasal olfaction, trigeminal stimulation). Because of the co-occurrence of visual and olfactory inputs, there should be no big surprise that the two senses interact.

People tend to perceive intense colored food as having stronger odors. Some color-odor combinations are quite common, such as brown and caramel and strawberry and pink/red. However, as mentioned by Shankar[5] with their studies of color/flavor perception from different cultures, much color-flavor association is learned.

Color exerts its influence on odor identification, discrimination, intensity and pleasantness through its ability to activate an odor image and indirectly through its ability to facilitate retrieval of odors labelled from memory, which can also active an odor image. So, many odors have been acquired and are stored in memory. As mentioned above, humans tend not to have unique odor words (labels) like taste (sweet, sour, bitter). Rather, the words used are objects that possess the aroma. Think banana, fruity, grilled, smoky, etc. So, with no visual clue, when presented with an aroma without a context, such as a colorless liquid in a jar, upon smelling the odor you discover a match through a "*library*" of retained odor objects. This takes a long time.

However, with a color clue, the mind activates olfactory "*images*" of all odors corresponding to the color and creates a precept that is a fusion of all these images. The odors associated with that color will form a larger portion of the precept. For instance, cherry is a flavor associated with the color red. The color-induced precept will contain more cherry odor than odors not associated with the color.

Also, color will help retrieve an odor label which will help in odor identification. It helps narrow the possible odor labels to those that have been associated with that color in the past. It also fuses the color and label precepts with the actual flavor.

These labels will have the ability to transform the perception of the actual odor into ones that most closely resembles the odor image activated by the label. The color activates a list of odor labels that the mind associates with that color. An appropriate color will increase the likelihood that the correct odor image will be retrieved, and the correct odor image will be chosen as a match for the actual odor. After the label is chosen and an odor image is activated from memory, the label transforms the actual odor perception into one more closely resembling the memory, giving rise to a very strong, clear odor experience. Thus, olfactory intensity, identification, and enjoyment of the odor will all be increased by the presence of an appropriate color.

What if an inappropriate color is used? The wrong odor images will be recalled, which are inconsistent to the aroma smelled. The subject may be unable to find an appropriate label. This will result in the perception of a strong, unpleasant aroma. Alternatively, the aroma can be mis-identified, and the perceived odor judged as unpleasant example of the flavor, or the mind can transform to one more congruent with the color.

Violations of expectations can affect retronasal olfaction. The color causes the aroma (orthonasal) perception to be enhanced. If the perception of the color-enhanced aroma is not met, the retronasal perception can be weaker in a colored solution than in a clear solution. Inappropriate-colored odors may be perceived as unpleasant in the mouth due to violated expectation.

COLOR FLAVOR INTERACTION IN FOOD PRODUCT DEVELOPMENT

In summary, sight and orthonasal olfaction create an expectation of flavor. Taste and retronasal olfaction confirm or disconfirm the expectation. Humans find it difficult to identify odors without visual clues. When the color helps created the correct flavor expectation, the color and flavor are "*congruent*". When they do not, they are "*discongruent*".

When the color and flavor are congruent, a number of advantageous effects occur, including mentioned above and summarized in Table 1.

Table 1. *Positive Effects of Congruent Color Flavor Interaction in Foods*

Increased flavor recognition	Dubose[2]
Increase perception of richness in orange juice	Clydesdale[1]
Increase perception of pungency in salsa	Shermer and Levitan[4]
Increase perception of sweetness in beverages	Roth[3]
Increased chocolate flavor perception	Shermer and Levitan[4]
Assist in recognition of beer style	Lelievre[7]

In most cases, the product developer would want the

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color to create a perception that is accurate and favorable to the flavor of the product. Bear in mind that this color and flavor combination must survive processing and remain congruent through the shelf life of the product.

Color flavor incongruency can lead to a number of negative results, as summarized by Table 2. As descried by Dubose[2] above, incongruecy leads to poor flavor identification.

The risk is surprising a customer, who may then dislike the product as the flavor did not meet the expectation. An example of this was a "*dinner party*" where the attendees ate steak, peas and French fries under color-masking conditions. When half-way through the meal, the lighting was changed to allow the true color of the food, it was revealed that the steak was blue, the peas were red, and the French fries were green. Many of the attendees were physically ill at the unexpected color change.[9]

Another example of discongruency is a cola soft drink (typically brown in color from the use of caramel coloring) that was formulated to be clear and colorless. Many consumers were confused by the cola taste of a beverage that looked like a lemon-lime soft drink.[10,11] As described earlier by Shankar,[5] a color may suggest a particular flavor to one ethnic group and be an example of positive congruency but be incongruent for another with consumer dislike and confusion. Morot[6] described how panelists would incorrectly described white wine when colored red.

Dubose[2] and much of the literature cited above describes very obvious color flavor incongruency (e.g. green and cherry). However, work by Garber[12] indicate that even small differences in the colors used to represent flavors can significantly affect the consumer's ability to correctly identify the flavor as well as the perceived flavor profile and preferences.

Table 2. *Negative Effects of Color Flavor Discongruency in Foods*

Poor flavor identification	Dubose[2] and others
Product dislike as color and flavor do not match	Garber[9]
Confusion on product identity and taste perception in wine and soft drinks	Morot[6] and Murray[11]
Misidentification of flavor due to cultural or ethnic associations	Shankar[5]

However, opportunities exist for deliberately mismatching color and flavor. Because consumers have strong color/flavor associations, deliberately mismatching them can be quite attention getting.[10]

He details three ways to successfully implement this strategy. Once was to teach the consumer a new association. Examples include the brown in colas (from caramel color) and green in mint (mint oil is colorless). A second way is to celebrate the new color, such as certain soft drinks which are colored a vivid green. A third technique is to break the color/flavor association completely so color is not suggestive of the flavor. This can easily be accomplished by opaque packaging. Some companies also use names that are not suggestive of flavor at all (think of "*sunset breeze*" or "*winter chill*").

Examples of positive results in deliberate discongruency of color and flavor in foods is summarized in Table 3. Glass[13] described the efforts of a major food manufacturer to create new colors of catsup. Typically, almost always red in color, this company sought to excite children with green catsup, and later explored other colors. One concern with using novel coloring is that color is that, if successful, color can be easily duplicated by competitors. Also, consumers may initially be excited by the new color, then quickly lose interest and return to the traditional coloring.

Raspberries are usually red, as are strawberries, cherries, and watermelons. To reserve a shade of red just for raspberries, food manufactures used the color amaranth (FD&C Red #2) for raspberry flavored products. When the Food & Drug Administration (FDA) banned the use of Red#2, manufacturers switched to the cheap but relatively unused FD&C Blue #1. The justification for the color change was that this was the flavor of "*Blue Raspberries*" (*Rubus leucodermis*), despite the fact that neither the color nor the flavor was really accurately represented by the blue raspberry flavor. Blue raspberry flavor, with its brilliant blue color, is now common in many confections and frozen desserts.[14]

The perception of different flavors can be accomplished by using the same flavor, but in different colored products.[15] One cereal manufacturer has a product with cereal pieces of different colors, including red, yellow and orange, which are traditionally associated with flavors such as cherry or strawberry, lemon and orange. In fact, the cereal pieces all have the same flavor.

Vegetarian "*burgers*" often try to simulate a beef hamburger in flavor and appearance. At least two companies have gone further by coloring the interior of the burger red to make it look like the bleeding of a rare to medium hamburger.[16,17] The manufactures are not trying to pass their burgers as real meat. They feel that the plant protein composition is a competitive advantage for various reasons. However, the red coloring enhances the perception of the beef flavor in the burger.

Table 3. *Positive Uses for Color Flavor Discongruency*

Excitement from untraditional color in catsup	Glass[13]
New flavor categories (such a blue raspberry)	Conservapedia[14]
Perception of multiple flavors in cereal	Adams[15]
Intensify beef flavor perception in meat-free products	Robertson[16]. Camlee[17]

CONCLUSIONS

Many studies have demonstrated that color of a food will impact the perception of the flavor. Consumers will often let the color override odor in the perception of the flavor of the product. Coloring of food, when it creates the correct perception of the flavor of the food ("*congruency*"), will increase the intensity, character, and intensity of the flavor. When the color creates an incorrect perception of the flavor ("*discongruency*"), the consumer may be

confused and dislike the product. In certain applications discongruency can be beneficial.

Much of the research that has been done in this field has resulted in conflicting results. There is still a lot of research needed to determine the mechanisms and effects of color/flavor interaction. In most cases, food color should be congruent with the flavor. However, much of the research has been done using with obvious discongruency. It is generally obvious that a lemon-flavored food should not be colored red. More work needs to be done on minor differences of shades. What shades of red should cherries, strawberries, and watermelon flavored foods be? How can color be used to distinguish a fresh, wild, ripe, or cooked strawberry flavor? The food product developers can use this information to better design their products.

Another area of interest is how to use discongruent color and flavor interaction. In most cases, this can result in customer dissatisfaction, but there are examples of this concept being used quite successfully. Research on this subject will decrease the risk of this method and result in more successful, exciting and innovative product launches.

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