Caramel color with low content of 4-Methylimidazole for the food industry.

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Figure 1 Headquarters of SECNA NATURAL INGREDIENTS GROUP in Spain.

1. Introduction. The caramel color and its elaboration.

Caramel color or also known as E150 is one of the dyes most used in food, with a history of use dating back to the late nineteenth century. The caramel color has a good microbiological stability since it is manufactured at very high temperatures, high acidity, high pressure and low water activity. This food dye is currently used in countless products and applications, which has allowed it to gradually become an alternative to the use of synthetic dyes in food applications of all kinds. The caramel color is totally miscible in water and contains colloidal aggregates that represent the majority of its coloration. Its hue ranges from an amber color, through a wide range of browns to a very dark brown with red hues. Caramel is of a complex polymeric nature since during its production hundreds of compounds are produced, the volatile substances and the group of non-volatile products, coming from oligo-condensation, being the best known. Caramelization reactions occur when sugars are heated and dehydrated, to subsequently produce condensation and polymerization reactions, resulting in compounds of increasing complexity and variability. It is during this phase that the typical caramel smell and taste develops. If the reaction lasts over time, compounds of increasing molecular weight, darker colors and typically bitter flavors of burnt sugar will appear.

The first study on the chemistry of caramel coloring appeared in 1858 and is due to the French chemist M.A. Gelis. Gelis' work indicated that caramelized sucrose contains three main products: a dehydration product, which he called caramelan $C_{12}H_{18}O_9$; and two polymers, which are called caramelen $C_{36}H_{50}O_{25}$ and caramelin respectively $C_{96}H_{102}O_{51}$. In the type c and d colored caramels, as we will see later, the presence of ammoniacal N induces the generation of melanoidin, composed with an intense dark color, which is why ammonia caramels have a greater coloring capacity.

Table 2 Coloring and flavoring compounds of caramel

S. No	Coloring compounds of caramel (Non volatile compounds)
1	$C_{24}H_{36}O_{18}$ (caramelan)
2	$C_{36}H_{50}O_{25}$ (caramelen)
3	$C_{96}H_{102}O_{51}$ (caramelin)
	Flavoring compounds of caramel (Volatile compounds)
1	Acetylfuran
2	furfurol,
3	5-hydroxymethylfurfural
4	3-hydroxy- 2-acetylfuran
5	3-hydroxy-2(5H)-furanones
6	4-hydroxy-3(2H)-furanones
7	4-pyrone derivatives

Table No. 1. Main components of caramel color

Compared to other dyes of natural origin, caramel is stable at high temperatures and pressures, which makes it ideal for the production of food products subject to extreme processing conditions.

In addition to its properties as a dye, some studies in soft drinks have shown that caramel has other beneficial properties such as its antioxidant activity. Specifically, Schwarz and his team reported an increase in the antioxidant activity of sherry brandy as a result of its incorporation.

Caramel color is used to make beers, carbonated beverages, pastry and biscuits, to make sauces, prepared dishes and to make distillates and different types of alcoholic beverages. According to some estimates, E150 dyes represent worldwide 75 to 85% by weight of dyes for food use, or what is the same, about 200,000 tons per year.

This dye is obtained from different sugars (glucose and / or fructose syrups, sucrose ...), by heating, under controlled pressure and temperature, in which, in addition, and according to its type, acids and bases are used, and other type of substances. In general terms, the polymeric material of caramel is produced from the condensation reactions of the aldehydes and ketones that the heating of the sugars generates in the presence of different bases or acids. But, there is not a single caramel color, but a large number of products are manufactured within this denomination (SECNA NATURAL INGREDIENTS GROUP produces more than 70 different references of this type of products). Each of these caramel colors has a different manufacturing process, and gives rise to products with different physicochemical and functional properties that ensure compatibility with a specific product and avoid undesirable effects such as the presence of mists, matrix flocculation and phase separations, with a characteristic behavior in the presence of acids, electrolytes, and tannins depending on their manufacturing process. An issue of special importance is the value of the isoelectric point (PI), since it determines the applicability of caramel. Caramel colors can therefore be classified as positive (PI 5.0–7.0), negative (PI 4.0–6.0) or "spirits" (PI <3.0). The average molecular weight of the compounds in electropositive caramel colors is 5 kDa, while in electronegative caramel color average molecular weight is 10 kDa. Other important properties are pH, water solubility, tone, as well as taste and aroma. But of all of them, the intensity of color is by far the most important, so its measurement is perfectly standardized. According to international standards, color intensity is defined as the absorbance measured at 610 nm, in a 0.1% solution of solid caramel (coloring weight / volume of distilled water) in a 1 cm thick cell.

Figure nº 2. Deposits of raw material. Headquarters SECNA NATURAL INGREDIENTS GROUP. Chiva facilities. Spain.

In any case, and for normative purposes an international classification is used in four generic categories according to their manufacturing mode, namely: Caramel color type I (E150a) or caustic caramel, type II (E150b) sulfite caramel, type III (E150c) or ammonium caramel and type IV (E150d) or sulfite and ammonia caramel. In type I caramel, sulfite and ammonium compounds cannot be used. In type II sulphites are used, but not ammoniacal compounds. Type III uses ammoniacal compounds, but not sulphites. And finally, in type IV caramel colors both sulfites and ammoniacal compounds are used. In the case of these last two classes, the reactions are not simple caramelization reactions, but Maillard reactions occur. During the Maillard reaction, the carbonyl groups of the sugars, aldehydes and ketones react with the nitrogen-containing compounds, to form brown pigments called melanins, with a greater coloring power. The following table summarizes the main characteristics and uses of each of the types of caramel color.

Table No. 2. International classification of the UN Committee of Experts on Food Additives for Agriculture and Food and WHO on Food Additives.

Code	Description	Characteristics	Most common applications
E150a	Caustic caramel	Yellow-brown-reddish color; stable in alcohol, tannins and salt-rich media	High-grade spirits, cookies, cereals, bakery products and juice
E150b	Sulfite caramel	Yellow-brown-reddish color; stable in alcohol	Tea, wines, spirits, vinegars and pastry
E150c	Ammonia caramel	Dark reddish-brown color; Stable in environments rich in alcohol and salt.	Beer, licorice, confectionery, sauces
E150d	Sulfite and ammonia caramel	Dark brown color; Stable in acidic media.	Carbonated drinks, balsamic vinegar, coffee, syrups, sauces, soups and condiments.

2. Applications of caramel color in the food industry.

One of the most frequent applications of caramel color is in the formulation of carbonated soft drinks. In fact, caramel color is the most widely used dye in the production of this type of beverage. Carbonated beverages are characterized by a low pH, so you should use E150d type caramel colors due to their stability in acidic media. Caramel provides its characteristic color to soft drinks and improves the foaming effect and aroma. Secna Group produces different references of type E150d “single strength” and “double strength” (also including references with 4-methylimidazole contents below 10 mg / kg) suitable for this application. The single and double force describes the relative color intensity of the caramel color. The double force (typical of the caramel colors used in the coke) refers to the E150d with greater color intensity. Another common application of caramel color is its use in standardization and improving the intensity of color and tone of teas. In this case, the use of intense yellow tones is recommended. Taking this into account and considering the presence of tannins, and the acidity of these products, either burnt sugar or type E150b can be used. Regarding the use of caramel color and burnt sugar in the field of bakery and pastries, its main purpose is to increase and homogenize the brown color in the final product obtained after cooking. In this case, both burnt sugar and E150c and E150d are recommended options. On the one hand, the E150d gives a dark brown tone to the final product, while E150c gives a more reddish-brown tone. If, however, a burnt sugar is chosen, a yellowish-brown color will be added to the final product, in addition to a very pleasant peculiar taste. In all these cases, its application can be in both liquid and powder, depending on the choice, which is necessary or not, produced under anhydrous conditions. Another sector in which caramel color is frequently used is in the ice cream industry. As in the rest of the applications, there are several possible alternatives. On the one hand, E150d caramel colors are frequently used because of their high coloring intensity, but burnt sugar is also added to chocolate, coffee and toffee ice cream. Burnt sugars are always a very interesting possibility due to their especially attractive tone, their intensity of color and especially because their use is adapted to clean label strategies increasingly demanded by the consumer. Burned sugars are also frequently added to ice cream cones to enhance and stabilize their color. Regarding the use of caramel color in drinks with a high milk content, it should be considered that, due to its neutral pH, the caramel color used must have a higher pH than usual. Among the products of the Secna Group portfolio, the reference E150d SC-5 is specially developed for this application, precisely because the original recipe has been modified to adapt to this circumstance. Aromatic caramels are also used in the preparation of dairy desserts. In this case they are used in layers and toppings to confer flavor and improve the appearance of the product, some references made especially interesting to avoid migration between the different phases of the dairy product. The diversity of needs in this case is so great that, in general, Grupo Secna, in addition to offering a wide range of aromatic caramels, usually develops customized products on demand. Another application also related to dairy products is the use of E150b caramel to improve the color of cheese crusts, in addition to providing double functionality, as said caramel acts as a product protector against unwanted microbiological growth. Another widespread application of caramel color is its use in the formulation of balsamic vinegars in order to enhance its color. Due to the acidic environment, the double concentration E150d caramel color is the best solution in this application. The addition of E150d also serves as a vinegar stabilizer and helps prevent foaming. Sometimes some vinegar producers prefer to use the caramel color E150a since it is also valid for this application and gives the product some differential characteristics; However, E150a does not have such a high resistance to acids, so that differences in the composition of organic acids from different sources of raw materials used in vinegar production can affect the stability of the final product. Therefore, to ensure a successful formulation, this type of caramel must be tested in order to avoid precipitation derived from ionic interactions with vinegar raw materials. Caramel colors are also used in the meat sector, mainly to improve the color and attractiveness of meat and some meat preparations such as sausages, and to increase the smoked or roasted effect. For this type of applications, caramel colors E150c and E150d are the most suitable. In general, the E150d is designed to penetrate the protein matrix, while the E150c is best suited to color the outer surface. If the final product also has a high salt content, the E150c is the best solution. On the other hand, caramel powder colors are a very suitable option for coloring mixtures of meat spices. Another of the numerous applications of caramels is their use in the preparation of sauces, liquid caramels being the most used, however, powder formats can also be used if required. Usually, E150c or E150d work well, and the choice depends on the desired tone (redder when using E150c, more dark brown with E150d) and the salt content of the sauce, since a high percentage of salt (above the 20-25%) requires an E150c caramel. Another application of caramel color is in the candy and confectionery industry. A wide range of burnt sugars and heat-stable caramel colors are available for this type of product. In this case, one of the main selection criteria is the desired tone. Thus, you can opt for the use of burnt sugar, caramel color type E150b and type E150c, which respectively and gradually give the final product from a yellowish tone to a reddish brown. But if what is desired is a dark brown tone, then the type E150d is frequently used. Powdered caramel colors are generally used in the coating of comfits and in the case of black licorice the caramel color E150d double strength is recommended. Caramel dyes are also used regularly in the alcoholic beverage industry for their high performance, stability and low cost. Its use is common in the preparation of rum, whiskey, brandy and other spirits, but it is also used in the preparation of lower-grade beverages such as cider, beer, vermouth and other wine-based drinks.

Generally, type a, b and d caramel colors are used in high-grade alcoholic beverages, while type c is especially suitable for use in beers. Among the products of the SECNA NATURAL INGREDIENTS GROUP portfolio, the MO-7 reference is specially developed for the production of ciders, wine liquors and other beverages derived from it, since it has a pH between 2.3 and 3.4 and provides a brown hue to the Final product. The MO-7 in general lines is suitable for drinks that do not exceed 15° of alcohol content.

Table nº 3. SECNA caramel color references and their recommended use in alcoholic beverages

TIPO	SECNA GROUP REFERENCE	Beer	Cider	Brandy	Liquor Wines	Alcoholic Drinks	° alco
BS	Burnt Sugar BS-100			X	X	X	> 40°
BS	Burnt Sugar BS-8			X	X	X	40-65°
BS	Burnt Sugar BS-B					X	<40°
BS	Burnt Sugar BS-7			X	X	X	40-65°
BS	Burnt Sugar BS-50					X	<40°
BS	Burnt Sugar BS-111					X	<40°
BS	Burnt Sugar BS-37					X	<40°
BS	Burnt Sugar BS-37A					X	<40°
BS	Burnt Sugar BS-40					X	<40°
BS	Burnt Sugar BS-100M					X	<40°
BS	Burnt Sugar BS-75					X	<40°
BS	Burnt Sugar BS-I					X	<40°
BS	Burnt Sugar BS-L					X	<40°
BS	Burnt Sugar BS-200			X	X	X	40-65°
a	Caramel Color BS-50-5					X	<40°
a	Caramel Color AZ-16			X	X	X	40-65°
a	Caramel Color A-15					X	40-65°
a	Caramel Color DE-16A			X	X	X	40-65°
b	Caramel Color SA-60					X	25-30°
b	Caramel Color AZ-30					X	25-30°
b	Caramel Color AZ-30/70					X	25-30°
b	Caramel Color S-30					X	25-30°
b	Caramel Color N-35					X	25-30°
b	Caramel Color SA-30					X	25-30°
b	Caramel Color SL					X	25-30°
b	Caramel Color AZ-20					X	25-30°
b	Caramel Color S-35					X	25-30°
c	Caramel Color DE-P300	X					NA
c	Caramel Color DE-P170	X					NA
c	Caramel Color P-13S	X					NA
c	Caramel Color P-13L	X					NA
c	Caramel Color P-13B (low 4-Mel)	X					NA
c	Caramel Color DE-P100	X					NA
c	Caramel Color P-9	X					NA
d	Caramel Color SA-12					X	<40°
d	Caramel Color AZ-15			X	X		<40°
d	Caramel Color SC-5					X	<40°
d	Caramel Color DE-7R			X	X		<40°
d	Caramel Color SA-6V					X	<40°
d	Caramel Color MO-7		X		X		<40°
d	Caramel Color DS-10SA (Passover)					X	<40°
d	Caramel Color DE-5R			X	X		<40°
d	Caramel Color DS-7					X	<40°
d	Caramel Color DS-7SA					X	<40°

On the other hand, caramels type E150c, due to their positive colloidal load, are usually used at different stages of brewing, since they prevent precipitation of proteins in suspension and achieve a standardization of color and greater transparency. But, in the case of these products with low alcohol content, what we want is to obtain lighter colors and a characteristic taste of burnt sugar, caramelized syrups of the Burnt Sugar type will be used.

The reference of SECNA DE-5R (a type d caramel color) for example is widely used in the manufacture of brandies, wine-based spirits and spirits. The product has a pH between 3.0 and 4.0, optimal for this type of products. The DE-5R, unlike the MO-7, results in reddish hues in the final application. In any case, when we are faced with the development of a new product and we must choose a suitable caramel color, we must consider factors such as, the solubility in hydroalcoholic medium, its isoelectric point, its pH, its color intensity, its tone and its stability to sunlight. For example, when we must formulate a high-grade alcoholic beverage, its solubility in alcohol is a determining factor. Type a caramel colors are suitable for alcoholic beverages with about 50°, type b caramel colors are applied in liquors with 20° and those of type d for 40° liquors. Therefore, type a and b caramels are the most suitable for this type of beverage, as they have high solubilities in hydroalcoholic mixtures. However, it is always advisable to perform alcohol stability tests, especially when faced with new applications, since even the raw material of origin in the manufacture of caramel color strongly influences stability. In fact, caramel colors made from sucrose base are usually more stable against higher proportions of alcohol than the rest.

Another variable to consider is, logically, the desired tone in our product. The ranges of yellows - amber are used in brandy and whiskey, while in the manufacture of rums reddish tones are demanded. But type d caramel colors are also used in some special applications and Burnt Sugar when its main purpose is to provide flavor and aroma.

Figure 3. Examples of high-grade alcoholic beverages.

Another factor to take into account is the stability of the color against ambient light. While this problem can be largely avoided by protecting the product by staining the glass and using cardboard boxes, keep in mind that not all dyes respond equally to different types of light. In general, type a caramels are more resistant to the effect of ambient light, while type d caramels degrade more rapidly resulting in changes in intensity and perceived tone. Table 3 can serve as a first approximation when selecting the type of caramel according to its application in alcoholic beverages. To conclude with this brief description of the innumerable applications of caramel colors in the food industry, we will mention their use in the development of pet food. Caramel colors are used both in pet food in dry format, as wet or in the preparation of treats, in order to improve their color, smell, taste and make them more palatable. For this type of applications there is a wide range of alternatives, from different burnt sugars, caramel colors type E150c and type E150d. The selection will depend on the desired tone and whether or not a "clean label" strategy is relevant to the company. In this case the options are numerous, since all our carmels are stable under the usual temperature conditions during the processing of pet food.

3. Presence of 4-Methylimidazole in caramel color.

4-Methylimidazole (4-MeI) is a heterocyclic organic chemical compound derived from imidazole by replacing the hydrogen atom with a methyl group at position four of the ring. Purified and in environmental conditions it is a yellowish solid, with a density of 1.02 g / cm^3, and with melting and boiling points of 47 and 263 ° C respectively.

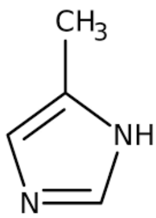

Figure 4. 4-Methylimidazole molecule

4-MeI can be produced by different routes, by heating sugars through Maillard reactions, fermentatively and synthetically in industrial applications such as the manufacture of drugs and chemicals.

In our case, 4-MeI is usually produced during the heating of carbohydrates in the presence of nitrogen compounds when the carbonyl groups of the sugars react with the nitrogen compounds. It is usually present in roasted foods, such as grilled meats, in coffee and in some c & d - color caramels, but in general it is important to highlight that it is generated naturally when cooking foods of all kinds and therefore it is found in many foods cooked in our homes, and humans have consumed it for generations.

The presence of this substance in commercially available c & d-type caramels is usually parts per million (not counting additional dilution of the dye in the target matrix), but the greater or lesser content of 4-MeI in c & d caramels It depends on many factors, including the pH, temperature and recipe.

Figure 5. Vials for chromatographic determination of 4-MeI

Due to doubts about its possible toxicity, in recent years multiple studies have been carried out by different organizations and international public health authorities aimed at clarifying its effect on human health. These studies have been specially oriented at knowing the average consumption doses, the maximum recommended doses, and the levels of presence in caramel and in foods from which there is some risk in their consumption.

In addition, these studies have been carried out by different research groups using different species of animals, evaluating the levels of 4MeI in plasma and in different organs of the animal, as well as the ability to remove the substance from the organism. Thus, for example, in experiments with rats, 90% of 4MeI was excreted in just 5 hours, and the results of similar experiments using sheep allowed us to observe that in relatively short periods 98% of the substance was eliminated as urine.

However, the controversy persists, due to the publication of other studies that appear to show toxic effects in model animals in the presence of high doses of 4-MeI.

As of today, the current regulatory specifications applicable to these caramel dyes (FDA and EFSA, among others) allow such dyes to contain up to 250 mg / kg of 4-MEI based on color. This is so, given that, according to health authorities indicate, there is not enough scientific evidence to state that their presence at this level or lower, carries risks to human health.

However, and while the situation is clarified, SECNA NATURAL INGREDIENTS GROUP has endeavored to optimize the production process to obtain caramel colors with much lower levels of 4-MeI, meeting consumer demand and a policy of maximum requirement as far as food security is concerned.

3. Regulations applicable to the preparation of caramel color.

Although the presence of 4-MeI in the dyes E150c and E150d is usually of the order of mg/kg, for some decades the interest in its effects on health has been reactivated as a result of a situation, which originally was not related to food.

Figura nº 6. Diferentes tonalidades de muestras de caramelo color diluidas

As we have advanced previously, 4MeI is synthesized for industrial use (electronic and pharmaceutical) in a high degree of purity and concentration, so, given its high concentration in these work environments, a first study was initiated a decade ago to evaluate the human health risk against exposure. This study was addressed within the framework of the US National Toxicology Program (NTP). This two-year study showed evidence of carcinogenic activity in male and female mice based on a higher incidence of alveolar / bronchiolar neoplasms (NTP, 2007).

However, these results were and continue to be questioned by different research groups and companies, so they have been challenged for considering them unusual and insufficient.

Despite all this, this study has been the origin of addressing the presence of 4-MeI in Proposition 65 of California (OEHHA, 2011) and in the classification of this substance as 2B by the International Agency for Research on Cancer (IARC) (IARC, 2013).

Given this situation, on the one hand, it was possible to ensure that there is consensus in the EFSA and the FDA that the current regulations are enough and that the color of type c and d caramels can be used safely in food. But, on the other hand, consumer associations and other social agents have shown their concern in this regard and ask that further investigation to be carried out in this field until the existence of any risk is unequivocally clarified. The CSPI and the US Consumers Union being the most active and demanding in the measures they propose.

Although neither the FDA nor the EFSA have changed their guidelines or the previously existing regulations as a result of these studies and the pressure of these organizations, they did respond to consumers, indicating that dietary changes were not necessary on the basis of the evidence currently provided (FDA, 2014). In any case, it is expected that more news and results of new works related to this topic will continue to appear over the next few years.

In conclusion, with regard to the regulations applicable in Europe on the presence of 4-MeI in caramel colors, the reference standard establishes a maximum limit of 4-MeI between 200 and 250 mg/kg on an equivalent basis at an intensity colored (0.1), in caramel colors E150c and E150d respectively (COMMISSION REGULATION 231/2012, 09 March 2012).

For those who wish to learn more about these issues, we recommend reading the work of A. Vollmuth (2018), from which some interesting conclusions can be drawn: 1. Caramel color has been used in food and beverages for more than 160 years , its use being perfectly regulated worldwide, not having reported health problems in all this time. 2. Toxicokinetic data show that much of the ingested color is not absorbed, when it is excreted by the body within 24 hours after its intake. 3. No adverse effects are observed in toxicity studies at the highest doses tested. 4. The normal intake of caramel colors is below the ADI (allowable daily intake) established. 5. However, control of suspicious substances is recommended to ensure safe levels of use.

4. Current trends, beyond the regulations.

Despite all the above, consumers want to go beyond strict compliance with the law. With the increase in purchasing power and life expectancy in global terms, there is a growing interest in health. The consumer wants to be informed of everything that affects their diet, and to be able to decide about what they consume. Therefore, in the face of the slightest uncertainty, it requires further investigation until all doubts are dissipated, and it is willing to assume a higher price if necessary, if all guarantees are given.

This implies, consequently, a greater interest of society for nutritional and biomedical research aimed at greater knowledge of the role of food in human health, as well as increasing pressure from consumer associations and media to clarify all those issues related to food security.

Regarding the case of the presence of 4MeI in caramel colors E150c and E150 d, the revision of maximum limits recommended by the Food Chemical Codex (FCC) has been particularly relevant after the implementation of the Proposal List of chemical substances 65 from California (Prop. 65). On August 30, 2018, Prop. 65 entered into force for all purposes, so that the companies thereafter must clearly identify, in their labeling, the chemicals included in the list if they exceed the levels considered acceptable. For its part, the FCC addressed this review after numerous requests from different organizations to review the maximum level of 4-MeI in caramel color due to the inclusion of 4-MeI in Prop. 65, as a chemical risk. And it has been recently, on December 1, 2018, when the Food Chemical Codex has established a new maximum limit of 4-MeI of 125 mg / kg, for this type of products.

During this process, it is important to highlight that the international association of caramel colors producing companies (ITCA) and European companies (EUTECA) actively participated with the FCC to help identify the new level. And in view of this situation, SECNA NATURAL INGREDIENTS GROUP, decided during 2018 to initiate a strategic research line aimed at drastically reducing the presence of 4-MeI in all its references of caramel color that it made.

Currently, a few manufacturers of caramel color, among which is SECNA NATURAL INGREDIENTS, have adhered to the commitment to reduce levels of 4-MeI in color base below the 125 mg / kg recommended by the FCC, in line with proposition 65. And as of today, all our references meet that criterion.

6. Identification and quantification of 4-Methylimidazole

A matter of capital importance in relation to the presence control of any potentially dangerous chemical substance in a food, is the correct identification and quantification, free of any doubt and using a generally recognized and accepted methodologies. At present, there are several procedures for the separation and / or determination of 4-MEI and its derivatives in caramel colors. These procedures are based primarily on high performance liquid chromatography coupled to an ultraviolet (UV) detector or a mass spectrometer (MS), although the use of MS spectrometry is replacing UV. In any case, non-MS based methods, according to our experience, are not enough for the analysis of 4-MEI in complex food matrices with low levels of this compound, due to the low reliability of its detection limit. These methods, however, can be applied successfully in simple matrices that contain a high concentration of 4-MEI, but are not advisable in complex matrices such as caramel color. The most selective methods are based on the MS capable of reaching a much lower detection and / or quantification limit, in addition to offering greater sensitivity and selectivity when confirming the presence of this molecule. In any case, in the literature we can find many other techniques for the quantification of this compound that can give good results, such as two-dimensional liquid chromatography, gas chromatography coupled to different detectors (including MS), thin layer chromatography, and even nuclear magnetic resonance for the distinction between imidazole isomers, among others.

Figure 6. SECNA GROUP chromatography laboratory equipment.

Regarding sample preparation, many studies have focused on methods of extraction and enrichment of imidazole compounds, using complex strategies such as the use of celite columns, cation exchange adsorbents, liquid-liquid or solid-liquid extractions, extractions with ionic pairs, extractions in solid phase and even extractions with supercritical fluids. Most of these procedures require a lot of time and are very expensive and complex. For this reason, in many occasions, simpler but at the same time reliable methods give better results with less effort. In this sense, there are simple methods that only require a preparatory work which includes a simple dissolution, dilution, filtration and direct injection of the sample. These techniques if combined with the use of the most advanced technologies already mentioned as liquid chromatography coupled to the MS gives very reliable and accurate results.

Figure No. 7. SECNA GROUP Chemical Analysis Laboratory

Finally, another relevant aspect that can provide some advantages in the quantification of 4-MEI is the use of internal standards. The internal standard is usually used when the concentration of the sample or the instrumental response can be variable. The use of internal standards eliminates problems of instrumental fluctuation and interference, minimizing errors derived from the equipment, since both the component of interest and the internal standard undergo the same variations, which facilitates obtaining reliable and consistent results. As an internal

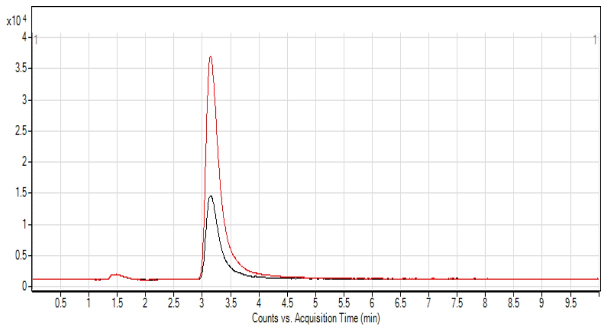

Figure nº 8. Caramel color chromatograms of MO-7 reference and with reduced 4-MeI.

standard in the determination of 4-MEI, the same deuterated compound is used, which gives it almost identical physicochemical properties. With the use of this methodology, analytical recovery percentages close to 100% can be achieved. As a result of all of the above, during our project, we used a high performance liquid chromatography method coupled to a mass spectrometer. The equipment used was an Agilent 6420 Triple Quad LC/MS Chromatograph. For the preparation of samples, work was always carried out by dissolution, filtration, dilution and direct injection, using as internal standard 4-MeI deuterated, making both the characterizations of the control samples and all the caramels developed both at pilot scale and during the scaled up. Analytical determinations were performed in triplicate demonstrating the method adequate reliability. Figure 8 shows the chromatograms of a sample of MO-7 caramel color in the 4-MeI reduction process.

7. The project to reduce the presence of 4-MeI in caramel color. The new product range.

Figura nº 9. Caramel colors reactor

In order to comply with the new and demanding requirements proposed by the FCC, the General Managment of SECNA NATURAL INGREDIENTS GROUP decided throughout 2018 to open a new line of strategic research whose main objective has been to replace the references of type dyes c and d, for products with the same technological characteristics, but with levels below 125 mg/kg. This implied an important investment in human and material resources, and an effort of our organization as a whole that to date we can affirm that has culminated successfully. During the project, we began by studying the different existing technological alternatives to develop the new caramel color formulations. For this, an extensive search of scientific-technical information was carried out that allowed us to identify different ways. These options were contrasted with the experience of more than 50 years of work in the development and manufacture of caramel colors of our company. From these reflections, a proposal of potential solutions emerged, prioritized by its technical and economic feasibility. Essentially, the Project was carried out in three phases. First, the proposals for process conditions and formulation in our pilot reactor were tested. Raw materials, intermediate products and final prototypes were analyzed following the strictest quality controls. All the usual physicochemical parameters were analyzed, and other issues that could affect the composition of the final product in any way were also considered. Of course, special care was taken in the quantitative determination of 4-MeI levels.

Once the results obtained at pilot scale were satisfactory, and after the complete characterization of the prototypes, we were able to begin the second phase of industrial development. During this phase, the new formulations and operating conditions were tested. Due to the large volume of industrial reactors necessary for processing, the readjustment of the operation required numerous tests, until the desired result was achieved, with a process time, efficiency and acceptable cost. Similarly, after the completion of each of the industrial adaptation tests, a complete physicochemical analysis of the manufactured product was performed. Finally, the third and final phase was the preparation of technical documentation by our quality assurance team. The new products had to pass all the usual quality controls of our company in order to properly document their production and performance (ISO22000: 2005 and FSSC22000 accreditation).

Table nº 4. Example of an analytical characterization technical sheet of a caramel color.

Parameter	version	version	Limits
Color EBC	35938	37570	32000-38000
@ 610 nm 0,1%	0,146	0,1544	0,120-0,180
IX	4,90	4,87	-
Be	32,7	32,7	32,0-34,0
Dry mass (%)	56,9	54,5	-
pH	2,8	2,8	2,4-3,4
Viscosity 30ºC (cps)	50	30	<1000
Haze (minutes)	>80	>80	>80
Gel (minutes)	>120	>120	>120
Color DEAE (%)	99	98	>50
4 Metil- imidazol / Color Base (mg/kg)	235,5	23,8	<125
Ratio @280/@560	34,66	36,00	<50
Color intensity / Dry mass	0,257	0,283	0,1-0,6

Figures 6, 7 and 8 show images of our laboratory and caramel color reactors

8. Conclusions.

The caramel color or also known as E150 is one of the dyes most used in human food. This product complies with all the guarantees required by the most demanding food safety agencies such as the FDA and the EFSA, and its safety is backed by its extensive history of consumption, with more than 160 years of use in food worldwide. Caramel dye is not a synthetic dye, in fact, it is a very suitable alternative to the use of many of them.

Regarding the production of caramel coloring, the 95/45/EC directive is the norm that governs the production and quality of these in Europe. All the technical sheets of SECNA NATURAL INGREDIENTS GROUP include detailed information that guarantees compliance with current regulations, in addition to making available to our customers the corresponding Halal, Kosher, NON-GMO certificates, etc. in specific products when required. Regarding the mandatory rule that refers to the levels of 4-MEI in caramel color, the reference document is "REGULATION (EU) No 231 / 2012of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council "in which the 4MeI levels must be <250 mg / kg.

During 2018 and part of 2019 SECNA NATURAL INGREDIENTS GROUP has developed an R&D project aimed at reducing the levels of 4-MeI in all its references, without affecting the technical characteristics of its products or their applicability. In this project, an in-depth analysis of the available scientific information has been carried out, as well as numerous tests in our laboratory and pilot facilities. At this point, the studies and tests for its industrial production were finally approached, having successfully completed this process throughout the month of April 2019.

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