CASSIA GUM
Chemical and Technical Assessment (CTA)

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1. Summary

This Chemical and Technical Assessment summarizes data and information on cassia gum submitted to JECFA by Lubrizol[1] in a dossier dated 4 December 2008. Cassia gum is the purified flour from the endosperm of the seeds of *Cassia tora* and *Cassia obtusifolia* which belong to the *leguminosae* family. Seeds of *Cassia occidentalis* are a naturally occurring contaminant in the source material. Cassia gum is comprised of at least 75% high molecular weight (approximately 200,000-300,000) polysaccharide consisting primarily of a linear chain of 1,4-β-D-mannopyranose units with 1,6 linked α-D-galactopyranose units. The ratio of mannose to galactose is about 5:1. The composition of saccharides is: mannose (77.2-78.9 %), galactose (15.7-14.7 %) and glucose (7.1-6.3 %). Like most polysaccharides, the following formula applies: $(C_6H_{10}O_5)_n.H_2O$. Cassia gum is related to carob bean gum, tara gum and guar gum in terms of structure and chemical properties.

Manufacture of Cassia gum includes cleaning of the source material by which the content of *C. occidentalis* is reduced to no more than 0.05%, de-husking and de-germing by thermal mechanical treatment followed by milling and screening of the endosperm. The ground endosperm is further purified by extraction with isopropanol.

The intended use of Cassia gum is as thickener, emulsifier, foam stabilizer, moisture retention agent and/or texturizing agent in cheese, frozen dairy desserts and mixes, meat products and poultry products.

2. Description

Cassia gum[2] is primarily the ground purified endosperm of the seeds of *C. tora* and *C. obtusifolia*, (Fam. *Leguminosae*) containing less than 0.1% of *C. occidentalis*. The seeds are dehusked and de-germed by thermal mechanical treatment followed by milling and screening of the endosperm. The ground endosperm is further purified by extraction with isopropanol.

It consists mainly of high molecular weight (approximately 200,000-300,000) polysaccharides composed of galactomannans; the mannose:galactose ratio is about 5:1. Other galactomannans previously evaluated by JECFA are carob bean gum, guar gum and tara gum.

Semi-refined Cassia gum normally containing detectable amounts of anthraquinones has been accepted for use in pet food by several countries.

3. Method of manufacture

3.1. Manufacturing principle

The raw material, a mixture of seeds from *C. Tora and C. obtusifolia* is subject to different mechanical cleaning steps in order to reduce contents of *C. occidentalis* to no more than 0.05% and to remove other impurities, such as, farm waste, undeveloped seeds and stones.

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[1] Lubrizol Advanced Materials Europe BVBA, Chaussée de Wavre 1945, 1160 Brussels, Belgium

[2] The Trade name of the refined Cassia Gum manufactured by the sponsor is RheoRanger™ SR. Although the sponsor produces other grades of cassia gum under other names, RheoRanger™ SR is the only cassia product that the sponsor intends to market for use in human food. Diagum™ CS is the name for the sponsor's semi-refined Cassia gum, which is produced in the same way as the refined Cassia gum, except that the semi-refined Cassia gum does not go through an isopropanol extraction. During the development of the refined Cassia gum the trade name Diagum™ SR was used for refined Cassia gum.

Following the cleaning step the raw material is subject to a de-husking and splitting process i.e. a thermal and mechanical treatment removing husk and germ from the seeds resulting in splits. Finally the splits are ground to a uniform small particle size powder, extracted with isopropanol and dried.

3.2. Detailed description

The following information on manufacture has been submitted by the sponsor. *C. tora and C. obtusifolia* are harvested when the plants are ripe (e.g. November-January) in India. The seeds that are used in the production of cassia gum are generally picked in the wild. These seeds are often contaminated with seeds from *C. occidentalis.* Before starting the seed splitting process, the seeds are passed through grading machines to differentiate them on the basis of their size. By this step, underdeveloped seeds are removed and *C. occidentalis* seeds are reduced to no more than 0.05%. A composite sample of each fraction is given to the laboratory for inspection for the presence of *C. occidentalis*. Table 1, below, summarizes the specifications for *C. occidentalis* and other foreign matter applied to cassia seeds intended for use in the production of refined Cassia gum.

Table 1. Cassia gum seeds - seed quality control*

Component	Specification	Method
Dark seeds	<1%	By selection based on colour (raw material spec.)
C. occidentalis	< 0.1%	By selection based on colour and shape (raw material spec.)
C. occidentalis	< 0.05%	By selection size and shape-sieving (initial processing)

* These are raw material specifications. Subsequent processing ensures far lower levels in the splits. For example, *C. occidentalis* is < 0.001 % in the final splits.

Before splitting, the seeds are subject to several different mechanical cleaning steps to remove foreign matter and seeds other than *C. tora* and *C. obtusifolia*. Further purification of the material is achieved during the splitting process.

Before starting the splitting process, the seeds are passed through grading machines to differentiate them on the basis of their size. By this step, underdeveloped seeds are removed and *C. occidentalis* seeds are reduced to no more than 0.05%. A composite sample of each fraction is given to the laboratory for inspection for the presence of *C. occidentalis*.

3.2.1 Splitting the seeds

The seed consists of an outer husk, an endosperm (split) and the ovary or germ. Only the endosperm or split, which contains mainly polysaccharides, is used for the production of the cassia gum. Both husk and germ are removed in the de-husking and splitting process. The impact of the splitting procedure is that both husk and germ are loosened from the endosperm and made brittle by heating and can be removed in the subsequent purification procedure after pulverization. The split (endosperm), however, remains intact at these temperatures. Due to its much greater particle size, the split can be separated from husk and germ particles through a couple of physical cleaning steps. The production of cassia splits follows the well-known production procedure of the related guar gum splits which involves both roasting and mechanical processing.

The splitting procedure starts with roasting of the seeds. All seeds are heated for several minutes. During the roasting process the endosperm (split) remains intact and flexible, while husk and germ, which are more sensitive to heat, become brittle. Mechanical stress pulverizes husk and germ and the powder is separated from the intact split by sieving. Remaining traces of husk and germ on the split particles are finally removed through a series of physical cleaning steps. A composite sample is examined to ensure the material meets the specifications for splits intended for use in the production of refined Cassia gum. The content of material other than *C. tora* and *C. obtusifolia* has to be zero.

The concentration of anthraquinones in the seed originally is approximately 10,000 mg/kg. After splitting, polishing and brushing, the concentration of anthraquinones in the split can be reduced to below 250 mg/kg measured by a UV test method. This means the splitting-dehusking-degerming step removes the majority of the anthraquinones. By mechanical means the anthraquinone content cannot be further reduced in the splits.

3.2.2 Processing the splits

The reduction of the anthraquinone content from 250 mg/kg to below the level of detection of the HPLC analytical method (< 0.5 mg/kg) is done by a specially adapted extraction process with isopropanol.

After drying of the product in a dryer, the batches are transferred to a blender where they are packed after mixing into 20 kg bags.

The drying process drives off residual isopropyl alcohol and water allowing a suitably low specification for isopropyl alcohol of 0.075%.

The extraction step allows refined Cassia gum, intended for use in food, to achieve much lower impurity levels than semi-refined Cassia gum, and reduces the residual anthraquinones to less than the limit of 0.5 mg/kg.

4. Characterization

4.1. Natural sources of Cassia gum

Cassia gum[3] is the flour from the purified endosperm of the seeds of *C. tora* and *C. obtusifolia*, which belong to the *leguminosae* family. *C. tora* and *C. obtusifolia* closely resemble one another but have been classified by some botanists as distinct species. *C. tora* and *C. obtusifolia* grow wild in subtropical regions of the world, and *C. tora* is occasionally cultivated. *C. tora* and *C. obtusifolia* are annual ruderal plants that ripen after approximately 100 days.

4.2. Impact of admixtures of C. obtusifolia *with* C. tora

The raw seed material used for refined Cassia gum typically will contain some *C. obtusifolia* along with *C. tora*. Data collected from 1999-2003 show that *C. tora* content averaged from 52.7 – 81.5%; the remainder being *C. obtusifolia.* The *C. tora* and *C. obtusifolia* seeds grow in the same sites and are difficult to distinguish visually; they are therefore harvested together. Investigations have shown that the two are remarkably similar if not interchangeable (Crawford and Friedman, 1990a, Crawford et al, 1990b), but there are some detectable chemical differences in both amino acid and anthraquinone content (Upadhyaya and Singh, 1986). Koshioka and Takino (1978) and Upadhyaya and Singh (1986) suggest that C. *tora* is a phytochemically distinct species derived from *C. obtusifolia*. This is also the conclusion of Randell (1995), who discussed the taxonomy and evolution of the two species. However, regional differences in *C. obtusifolia* and *C. tora* cultivars may easily obscure the minor differences between these closely related species. The close similarity in seed chemistry, morphology and ecological requirements between the two plants is far more significant than the relatively minor differences between them.

4.3. Chemical Structure and Physical Properties of Cassia Gum

Cassia Gum is comprised of at least 75 % polysaccharide consisting primarily of a linear chain of 1,4-β-D-mannopyranose units with 1,6 linked α-D-galactopyranose units. The ratio of mannose to galactose is about 5:1. The following composition was revealed in the sugar analysis conducted using HPLC: mannose (77.2-78.9 %), galactose (15.7-14.7 %) and glucose (7.1-6.3 %).

[3] Cassia Gum, per se, does not have a CAS Registration Number. The CAS number for "galactomannan" is 11078-30-1

Like most polysaccharides, the following formula applies: $(C_6H_{10}O_5)_n \cdot H_2O$. The structure, ratio of mannose to galactose units, and molecular weight of cassia gum are outlined in Table 2 below. For comparison purposes, Table 2 also contains structural properties of several other galactomannans. The typical physical properties of cassia gum are shown in Table 3.

Table 2. Structural properties of Cassia gum and other gallactomannans

Substance	Structure	Mannose:Galactose	Molecular Weight
Cassia gum	1,4-β-D-mannopyranose units with 1,6-α-D-galactopyranose units attached to every fifth mannose.	5:1	200,000-300,000
Guar gum[4] (CAS No. 9000-30-0)	1,4-β-D-mannophyranose units with 1,6-α-D-galactopyranose units attached to every alternate mannose.	2:1	50,000-8,000,000
Locust (Carob) bean gum[5] (CAS No. 9000-40-2)	1,4-β-D-mannopyranose units with 1,6-α-D-galactopyranose units attached to every fourth or fifth mannose.	4:1	50,000-3,000,000
Tara gum[6] (CAS No. 39300-88-4)	1,4-β-D-mannopyranose units with side chains of 1,6-α-D-galactopyranbose attached to approximately every third unit.	Approximately 3:1	Not reported

Table 3. **Physical properties of food grade Cassia gum**

Appearance	Off-white fine power
Odour	Neutral
Taste	Neutral
Bulk density	0.6 kg/l
Particle size < 250 micron	≥ 99 %
Solubility	Insoluble in ethanol; disperses well in cold water and forms a colloidal solution
pH (1%)	5.5-8.0
1% Viscosity	≥ 260 mPas
Break Strength	1,200 – 1,800 g/cm^2

4.4 Possible impurities (including degradation products)

4.4.1 Seeds from C. occidentalis

[4] JECFA specifications for Guar gum published in FAO JECFA Monographs 5 (2008)

[5] JECFA specifications for Carob bean gum published in FAO JECFA Monographs 5 (2008)

[6] JECFA specifications for Tara gum published in FAO JECFA Monographs 1 (2006)

C. occidentalis, another species of Cassia that has been associated with muscle toxicity[7], generally does not grow in conjunction with *C. tora* or *C. obtusifolia*, but is an occasional impurity for which the collected seeds need to be inspected. *C. occidentalis* seeds are noticeably smaller and differently shaped (flat disks instead of the longish seeds of *C. tora* and *C. obtusifolia*) and can be easily recognized, both as seeds and later on as splits. The sponsor has a specification to limit the presence of *C. occidentalis* in the cassia seeds that are the raw material used to produce refined Cassia gum. The content of *C. occidentalis* in seeds for refined Cassia gum has to be less than 0.1%, and the content of darkened splits[8] and foreign matter is limited to a combined level of 1.0%[9]. The sponsor has developed a *Raw Material Specification Sheet* for seeds used in the manufacture of refined Cassia gum and a specific test standard for *C. Occidentalis* (Lubrizol, 2008[2]). This specification applies to seeds prior to any cleaning.

4.4.2 Anthraquinones

As indicated above, the primary impurities of concern in cassia gum are anthraquinones, which occur naturally in the seeds from which the cassia gum is produced.

4.4.2.1 Levels of anthraquinones in C. obtusifolia / C. Tora *seed*

Anthraquinones and related compounds have been known for years to occur in many *Cassia* species (Takahashi et al, 1978; Takido, 1958, 1960). Koshioka and Takino developed a quantitative method for measuring anthraquinones in cassia seeds based on optical absorbance at 500 nm (Koshioka and Takino, 1978). Using this method, Japanese and Formosan samples of *C. obtusifolia* and *C. tora* seeds were found to contain anthraquinones at levels ranging from 0.80% to 1.10%. The authors also measured free, bound, and total anthraquinones in 14 samples of powdered seeds of *C. obtusifolia* and *C. tora* used as crude drugs from Korea, China, Japan, Formosa and Vietnam. The results ranged from 0.01 to 0.04% for free anthraquinones, 1.01 to 1.29% for bound anthraquinones, and 1.04 to 1.31% for total anthraquinones.

It is well established that most of the anthraquinones in cassia are bound to sugars in the form of glycosides (with the aglycon occurring usually in a reduced form, e.g. anthrones, dianthrones, or oxanthrone). Using thin-layer chromatography, Crawford and Friedman, 1990a and Crawford et al, 1990b, showed that sickle-pod seeds contained 1-2% by weight of total anthraquinones. No alkaloids or saponins were present. The major portion of the anthraquinones exists as the di- or tri-glycosides. These include rhein, physcion, obtusifolin, emodin, questin, lesser amounts of others, and chrysophanic acid. Chrysophanic acid made up virtually 50% of the total anthraquinones in the seed samples (Crawford and Friedman, 1990a, Crawford et al, 1990b).

4.4.2.2 Levels of anthraquinones in refined Cassia gum

The bulk of the anthraquinone content in cassia seeds is located in the outer husk. In the refined Cassia gum production process, the endosperm is separated from the husk and the germ and purified in a series of steps culminating in an isopropanol/water extraction step. The de-husking that occurs early in the production process separates and discards the outer husks from the endosperm, resulting in a major reduction in the anthraquinone content. Both the concentration and number of different anthraquinones present are reduced. The isopropanol/water extraction at the end of the production process dramatically reduces the anthraquinone content of the gum. While the average anthraquinone content in whole seeds is at least 10,000 mg/kg, no individual anthraquinone is detectable in the refined Cassia gum at 0.5 mg/kg, based on HPLC analyses.

4.5 Analytical data for anthraquinones

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[7] Muscle toxicity has also been associated with components of *C. obtusifolia,* although *C. occidentalis* appears to be somewhat more toxic. Steps taken to ensure that refined cassia gum will not contain harmful levels of muscle toxins from *C. obtusifolia* or *C. tora* are discussed later in this section.

[8] The endosperm that remains when the husk and germ are removed from the seed is referred to as a "split".

[9] Each shipment of seeds is analyzed for compliance with the raw material specifications upon delivery.

Prior to using the HPLC method, the sponsor determined total anthraquinone levels in cassia gum via the optical colour analytical method of Koshioka and Takino (1978) for total anthraquinones. This method is based on the optical absorbance of the sample at a wavelength of 500 nm. Because the cassia complex mixture may include substances in addition to anthraquinones that absorb at 500 nm, the colorimetric procedure tends to exaggerate the actual anthraquinone content.

The maximum limit for anthraquinones in finished refined Cassia gum is established at 0.5 mg/kg. This level reflects recent improvements in purification techniques as well as analytical methodology. The primary reason the sponsor is able to achieve anthraquinone levels of 0.5 mg/kg is the utilization of its novel isopropanol/water extraction process at the completion of the process for making semi-refined cassia gum. A key factor in documenting the reductions in anthraquinone levels is the use of a new analytical procedure based on HPLC analysis conducted by Dr. W. Dekant at the University of Würzburg, Germany.

The new anthraquinone analytical method allows for the chromatographic separation and quantification of the individual anthraquinones present in the sample. During the development of refined Cassia gum, analyses were performed on the gum using the HPLC method, which has a detection limit of 0.5 mg/kg. The only anthraquinone detected in semi-refined cassia gum was physcion (1,8-dihydroxy-3-methyl-6-methoxyanthraquinone), at less than 10 mg/kg. Furthermore, in most HPLC analyses of semi-refined Cassia gum and all analyses of refined Cassia gum, no anthraquinone was detected at 0.5 mg/kg. The finding of physcion occurred in two HPLC analyses of a single sample of semi-refined cassia gum (before and after acid hydrolysis) performed by Dekant in July 2003 (5.20 mg/kg physcion without acid hydrolysis and 8.59 mg/kg after hydrolysis, Lubrizol 2008[1]). The sponsor thereafter incorporated an additional washing step into the preparation of samples of refined Cassia gum as well as into the process to be used for the commercial production of the gum. Dr. Dekant analyzed five samples of the additionally cleaned gum post-acid hydrolysis using the same procedure and analytical sensitivity as in the previous tests and found no anthraquinones at a detection limit of 0.5 mg/kg. Collectively, these analyses demonstrate that the current purification process effectively removes virtually all of the anthraquinone content initially present in cassia seeds. This is the basis for the current anthraquinone specification limit of 0.5 mg/kg.

When the HPLC method was used to analyse *C. obtusifolia* seeds, a range of anthraquinones (chrysophanic acid, emodin, questin, obtusifolin, etc.) are observed in amounts consistent with the data obtained by Crawford et al (Crawford and Friedman, 1990a, Crawford et al, 1990b). This confirms the sensitivity of the analytical method to the full range of anthraquinones that are potentially present based on detection in the *C. obtusifolia* seeds.

4.6 Rationale for proposed specifications

Cassia gum is related to carob bean gum, guar gum and tara gum in terms of structure and chemical properties, although in terms of functionality cassia gum is more comparable to locust bean gum than to guar gum. Cassia galactomannans can be differentiated from other galactomannans by electrophoresis. The molecular weight of cassia gum is estimated to be between 200,000 and 300,000.

4.7 Method of analysis of cassia gum in foods

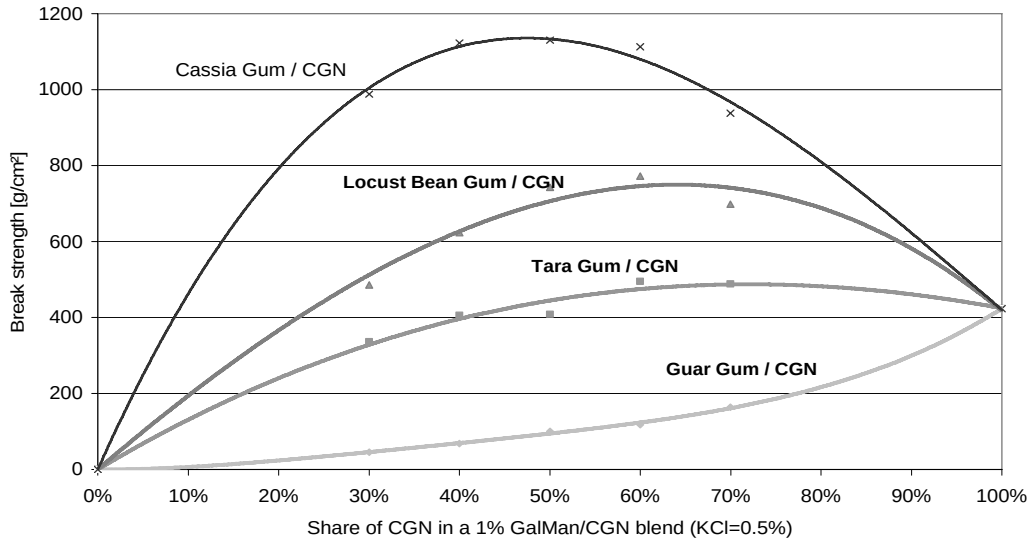
The methods used to analyze cassia gum are the same classical analytical methods used for the other gums such as guar gum or locust bean gum. There are several different AOAC methods (Association of Official Analytical Chemists) for the analysis of gums (galactomannans) in foods. These methods are based on analyzing the sugars formed from the hydrolyzed gums, or the precipitation of the gums in concentrated alcohol solution, or comparison of the IR spectrum of the separated and purified separated gum to known standard spectra. The specific method used depends on the particular food matrix and the ease of removing potentially interfering substances

5. Functional uses

Refined Cassia gum is a galactomannan that differs from related galactomannan gums, such as locust bean gum, tara gum and guar gum, in having fewer galactose molecules next to the long mannose

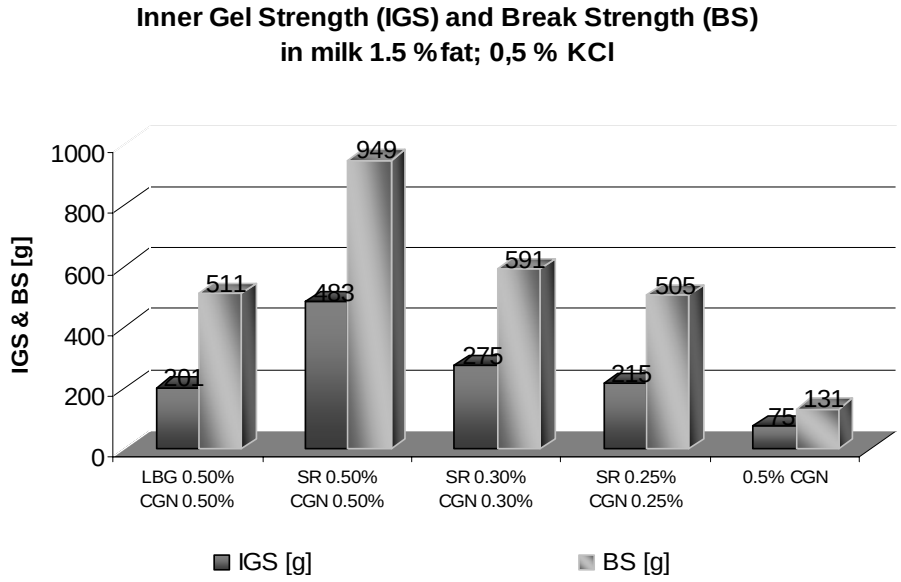
chain. This fact, however, has a significant effect on the synergy of food grade Cassia gum with anionic food gums like carrageenan or xanthan. A high number of galactose side chains prohibit the synergistic gelling effect with anionic polymers. As a result, a smaller amount of hydrocolloid blend containing cassia gum is needed in a food product to achieve the same effect as with carrageenan alone or blends of carrageenan with other related galactomannans. The break strength and gelling properties of pure carrageenan, for example, can be achieved with 2/3 less of a mixture of carrageenan and cassia gum. A comparison of the synergistic effect of the combination of carrageenan (identified as CGN) with regular cassia gum and with other regular food gums in the market is shown in Figure 1 below.

Figure 1. Break strengths contribution of different gums at increasing carrageenan levels



A comparison of gum combinations in aqueous solutions, specifically milk products, yields similar results. Cassia (SR)/carrageenan gels provide roughly double the gel and break strength of locust bean gum (LBG)/carrageenan gels in the same concentration. Carrageenan, alone, shows only roughly 1/3 of the gel performance compared to cassia/carrageenan gels (Figure 2).

Figure 2. Gelling properties of some combinations of gums with carrageenan



In summary, by replacing carrageenan and/or a galactomannan by cassia in a given food recipe, the amount of total hydrocolloid can be reduced significantly. This has a positive impact on the cost efficiency of producing food products.

The food applications for which cassia gum is intended to be used by the sponsor are listed in Table 5. Cassia gum (semi-refined cassia gum) has been used since the early 1990's as a thickening and gelling agent in pet foods (canned meat for cats and dogs).

Table 5. Use levels of Cassia gum in foods

Foods	Maximum Level	Technological Functions
Cheeses, except natural cheeses, including curd and whey cheeses, cream, natural, grating, processed, spread, dip and miscellaneous cheeses.	3.000 mg/kg	Emulsifying / stabilizing / thickening / gelling properties and water retention.
Frozen Dairy Desserts and mixes, including ice cream, ice milks, sherbets, and other frozen dairy desserts and specialties.	2.500 mg/kg	Emulsifying / stabilizing properties and controlling overrun.
Meat products, including all meat and meat containing dishes, salads, appetizers, frozen multi course meat meals and sandwich ingredients prepared by commercial processing or using commercially processed meats with home preparation.	3.500 mg/kg	Emulsifying / stabilizing / gelling properties and water retention.
Poultry products, including all poultry and poultry containing dishes, salads, appetizers, frozen multi course poultry meals, and sandwich ingredients prepared by commercial processed poultry with home preparation.	3.500 mg/kg	Emulsifying / stabilizing / gelling properties and water retention.

The applications include the use of cassia gum in the food categories above, as described in United States Code of Federal Regulation 21 C.F.R. 170, at levels ranging from 2,500 mg/kg to 3,500 mg/kg (0.25 % - 0.35 %).

6. Reactions and fate in foods

Based upon its chemical structure, cassia gum is stable during food processing and storage and it could only degrade, if at all, into sugars. To confirm the stability of the cassia gum of the sponsor, samples from six master batches, designated LL 42 to LL 47 (see Table 6), were tested in July 2003 and again in March 2005. These master batches were manufactured according to the procedure used for refined Cassia gum, including purification with isopropanol. The test results are reported below (the first number in each column is the 2003 result; the second number is the 2005 result).

Table 6. Stability data on batches of Cassia gum (2003 – 2005)

	LL42	LL43	LL44	LL45	LL46	LL47
Protein content [%]	5.7/5.4	5.7/5.5	5.7/5.6	5.6/5.6	5.5/5.4	5.7/5.5
Fat content [%]	0.3/0.4	0.3/0.3	0.3/0.3	0.3/0.4	0.3/0.3	0.3/0.3
Ash content [%]	0.17/0.2	0.16/0.2	0.21/0.2	0.24/0.3	0.19/0.2	0.20/0.2
pH value	8.02/8.14	7.41/7.88	7.55/7.66	7.67/8.11	7.75/7.61	7.47/8.03
LOD [%]*	3.5/11.0	4.7/11.0	4.9/9.9	7.95/10.2	7.54/10.9	3.48/10.7
Gel strength [g]	173/179	181/180	175/174	181/179	178/173	194/187
* "LOD [%]" is the percentage of the sample lost on drying.						

It is concluded that the product is stable without chemical modifications for at least 20 months. All changes in the values are within the limits of our specification. The only significant changes are observed in the LOD value. This is due to the fact that the product is hydroscopic until a final moisture content of 8-12% is achieved.

At the level recommended for its usage in the foodstuffs selected, Cassia gum has no interaction with the nutrients.

Except for the possible degradation to sugar, there are no particular degradation products of Cassia gum in the foodstuffs where it is used.

7. References

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