

Ad hoc meeting with industry representatives – Joint dossiers on food enzymes produced from animal and plant sources

Triacylglycerol lipase: data gaps and proposals

EFSA FIP Unit – food enzyme team





EFSA-Q-2015-00131 Triacylglycerol lipase from pregastric tissues of cattle, goat and sheep

Enzyme activities and source tissues







Source material

Available data

- Pregastric tissues
 - "gullet"
- the oro-pharyngeal tissues (De Caro et al., 1995)
- Esophagus, pharyngeal end (Ramsey, 1961, 1962)

Species

- Cattle (Bos primigenius (Bos taurus))
- Goat (Capra aegagrus *hircus*)
- Sheep (Ovis aries)

Enzyme activities

pregastric lipase

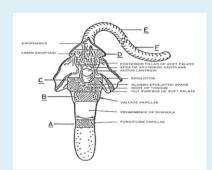
pregastric esterase

Product specific data

Description of the source material used for production



EFSA-Q-2015-00131 Triacylglycerol lipase from pregastric tissues of cattle, goat and sheep





Source material

Available data

Lipase from **pregastric tissue** of ruminants is secreted from the tongue region and washed into the stomach with the saliva and can be obtained from the stomach in accordance with the JECFA specification.

The area richest in the triacylglycerol lipase is referred to as "**the gullet**", the region which is bordered anteriorly by the vallate papillae of the tongue and posteriorly by the pharyngeal end of the esophagus.

- Medical Definition of gullet: the tube leading from the mouth to the stomach; esophagus
- Beef gullet e.g. used as snack for dogs

De Caro et al. (1995): The digestion of dietary triacylglycerols is initiated in the stomach by a lipase which is synthesized and secreted either by:

- the von Ebner glands of the posterior area of the tongue (lingual lipase in rodents);
- or the oro-pharyngeal tissues (pregastric lipase in ruminants);
- or the fundic gastric mucosa (gastric lipase in omnivores).

Timmermans et al. (1996): **Calf pregastric esterase** is secreted by the von Ebner's glands on the dorsal posterior region of the tongue and by glands located in the pharynx wall.

Product specific data

 Already available information, including the source of the enzyme, its composition and properties



Flavouring

production?

EFSA-Q-2015-00131 Triacylglycerol lipase from pregastric tissues of cattle, goat and sheep

Enzyme activities and source material

pregastric lipase and pregastric esterase two separate enzymes

one enzyme which can have dual activity depending on the hydrophobicity of the substrate

synonyms for one enzyme, triacylglycerol lipase (Regulatory chapter 5 in the International Dairy Federation Bulletin, the database Brenda, the JECFA specification)

Technical dossier



EFSA-Q-2015-00131 Triacylglycerol lipase from pregastric tissues of cattle, goat and sheep

History of previous consumption of the tissue

Source material

Available data

- Gullet from ox has been used for human consumption, at least in the 18th century, as an ingredient to make bouillon (in a Danish book about diet and eating habits, Boyhus, 2009).
- Gullet is to be considered equal to the items which may be used in meat products (section VI, Annex III of Reg. (EC) No 853/2004).
- Gullet can be grouped under point 1.11. of Annex I defining "Offal" as fresh meat other than that of the carcase, including viscera and blood. "Meat" is defined as "edible parts of e.g. domestic ungulates, which means domestic bovine (including Bubalus and Bison species), porcine, ovine and caprine animals, and domestic solipeds."
- Gullets are obtained from animals that are slaughtered and approved for human consumption in or outside of EU and are handled in accordance with specific procedures to avoid contamination and to preserve a good hygienic standard.

- History of previous consumption of the tissue, in particular on whether there is a documented history of use with absence of human health adverse effects
- Quantity of consumption in the EU or elsewhere
- By-products originating from the source organism and residues of any substances and materials used in the production process (EFSA, 2009)





EFSA-Q-2015-00131 Triacylglycerol lipase from pregastric tissues of cattle, goat and sheep

Source material







Methods used to ensure the absence of any risk of infectivity

Available data

Gullet from ruminants is not considered as specified risk material, is free from tonsils in accordance with the rules specified in Annex V of Reg. (EC) No 999/2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies.

Only gullet from animals considered free of any evidence of disease is being used for manufacturing of triacylglycerol lipase. Human health problems associated with other infective agents originating from gullet have not been reported.

- Documented compliance with meat inspection requirements and in accordance with good hygienic practice.
- Data on the absence of infectious agents in the source tissue and methods to ensure the absence of any risk of infectivity.



EFSA-Q-2015-00131 Triacylglycerol lipase from pregastric tissues of cattle, goat and sheep

Characteristics of the food enzyme

Properties of the food enzyme

Available data

Timmermans et al. (1996): Calf pregastric esterase has an • Amino acid sequence, apparent molecular mass of 50 kDa, as determined by SDS-PAGE analysis and about 46 kDa, as determined by gel titration.

The food enzyme exhibits activity at pH 4-8, and <10-65°C. No enzyme activity is left at above 65°C.

The optimal pH and temperature conditions for the food enzyme activity:

- pH 5.5-6.5
- temperature 30-50°C

Lai et al. (1997): Optimum pH conditions for pregastric lipase is pH 5.6-6.5 for goats and pH 5.5-6.2 for kids; the optimum temperature is 43-60°C. Optima for kid lipase extended slightly below pH 5.5 and higher than 60°C.

- molecular weight literature or experimental data
- Missing data about termostability



EFSA-Q-2015-00131 Triacylglycerol lipase from pregastric tissues of cattle, goat and sheep

Manufacturing process, raw materials

Available data

Description of the manufacturing • process is intended to cover all productions of triacylglycerol lipase including those from suppliers who • An updated list of all raw materials that have not contributed to this dossier.

- Detailed description of the concrete manufacturing process actually used.
- are actually used in the course of the food enzyme production.
- Clear indication at which step of the manufacturing each process raw material is used, as well as information on their functions.



EFSA-Q-2015-00131 Triacylglycerol lipase from pregastric tissues of cattle, goat and sheep

Chemical composition, properties and specifications

Food enzyme

Available data

- The chemical composition, impurities of at least
 3 commercial batches of several manufacturers
- *E. coli* determination is missing in the 2/3 batches from 1 applicant.
- *E. coli* was determined in 0.1 g sample of 1 batch, *Salmonella* sp. and *Listeria monocytogenes* were detected in 125 g sample in 1 batch, and in 25 g sample in other 2 batches.
- Description of the method for determination of total coliforms, E. coli, Salmonella and heavy metals – e-mails between 1 applicant and laboratories were only provided.

Product specific data

The chemical composition and impurities of at least 3 commercial batches of food enzyme, detailed description of the methods of determination and detection limits, units in which activity is expressed



EFSA-Q-2015-00131 Triacylglycerol lipase from pregastric tissues of cattle, goat and sheep

Toxicological data Available data **Product specific data** The food enzyme is obtained from • Any existing toxicological pregastric tissues of animals (cattle, studies (EFSA, 2009) goat and sheep) slaughtered for human consumption. Consequently, no toxicological data is included in the dossier, supported by the safe history of use.

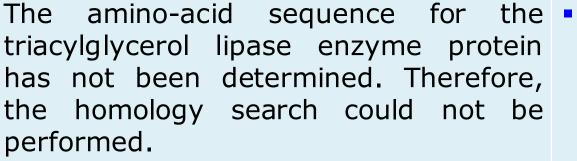


EFSA-Q-2015-00131 Triacylglycerol lipase from pregastric tissues of cattle, goat and sheep

Allergenicity



Available data



Product specific data

 Allergenicity assessment – a comprehensive literature search for possible adverse reactions, allergy after consumption of source material, published in the last 10 years







EFSA-Q-2015-00131 Triacylglycerol lipase from pregastric tissues of cattle, goat and sheep

Intended uses and exposure

Available data

- production and modified diary production), flavouring be used' production (typical flavour development in different cheese types)
- Budget Method, calculation of a Theoretical Maximum Daily Intake (TMDI)

Product specific data

 dairy processing (cheese Intended uses of the food enzyme aligned with the enzyme 'EC working document describing the food ingredient processes in which food enzymes are intended to

- Specify the intended uses
- Flowcharts for each intended food process and indication at which step(s) the food enzyme is added and yield factor
- Use levels to be expressed as mg TOS/kg raw material

The estimation of dietary exposure, if deemed necessary, will be carried by EFSA.





EFSA-Q-2015-00131 Triacylglycerol lipase from pregastric tissues of cattle, goat and sheep

Documented history of use of the enzyme and its reaction and fate in food

Available data

Product specific data

Triacylglycerol lipase powder has been used for cheese • Reaction and fate in food making at least since 1950 (Harboe, 1994).

Each type of pregastric lipase, depending upon the animal species (calf, kid, lamb), gives rise to its own characteristic flavour profile due to the fatty acid and/or regio- and stereoselectivity of the corresponding enzyme (De Caro et al., 1995). Production of flavour compounds from fat during cheese ripening by action of lipases and esterases was described by e.g. Wolf et al. (2009).

Triacylglycerol lipase is denatured by pasteurization in the production of enzyme modified dairy ingredients (EMD) and removed during the production of flavourings.

After the separation from cheese, the whey is commonly pasteurized and residual lipase activity is denatured.

- Intended and unintended reaction products resulting either from enzymatic chemical reactions of the food enzyme with food constituents or from the degradation of the food enzyme during storage and processing of the foodstuff



Stay connected





Subscribe to

efsa.europa.eu/en/news/newsletters efsa.europa.eu/en/rss



Receive job alerts

careers.efsa.europa.eu – job alerts



Follow us on Twitter

@efsa_eu

@plants_efsa

@methods_efsa

@animals_efsa



Follow us Linked in

Linkedin.com/company/efsa



Contact us

efsa.europa.eu/en/contact/askefsa