Annex 2

FORM FOR THE SUBMISSION OF SUBSTANCES TO BE EVALUATED BY JECFA

In completing this form, only brief information is required. The form may be retyped if more space is needed under any one heading provided that the general format is maintained.

Name of Substance(s):	Chymosin from *Camelus dromedarius* expressed in *Aspergillus niger*
Question(s) to be answered by JECFA *(Provide a brief justification of the request in case of re-evaluations)*	

1. Proposal for inclusion submitted by:
Danish Veterinary and Food Administration.

2. Name of substance; trade name(s); chemical name(s), IUPAC name, C.A.S number (as applicable):
Substance: Chymosin from dromedary camel (*Camelus dromedarius)* expressed in *Aspergillus niger (*formerly *Aspergillus niger var.awamori*).

Chemical name: Chymosin (rennin, aspartic proteinase);
CAS 9001-98-3, EC 3.4.23.4, IUBMB No: 3.4.23.4

3. Names and addresses of basic producers:
Chr-Hansen A/S
10-12 Bøge Alle
DK-2970 Hørsholm
Denmark

4. Identification of the manufacturer that will be providing data (Please indicate contact person):
Chr-Hansen A/S commits to provide data to support the proposal for inclusion of the chymosin in the list of substances to be evaluated by JECFA.

Contact of manufacturer:
Chr-Hansen A/S
10-12 Bøge Alle
DK-2970 Hørsholm
Denmark

Contact person:
Christina Westphal Christensen, Senior Regulatory Affairs Partner for food cultures & enzymes
dkchwe@chr-hansen.com
Mobile: +45 52 18 04 19

5. Justification for use:
The chymosin enzyme preparation is used as a processing aid during food manufacture to coagulate milk.
The chymosin catalyze the hydrolysis, at a very particular site in the amino acid chain, of κ-casein - the main protein in milk. This is the absolute first key step in all cheese-making, through which the liquid milk is coagulated (precipitated) and converted to a semi-solid form by the catalytic action of coagulants, such as chymosin. Therefore, the most important production process in which chymosin is used is the production of cheese.

Moreover, chymosin can be used in the production of fermented milk products, where it can be used to increase the viscosity of the preparation. Quarg (quark) is an example of fermented milk product in which coagulants, like chymosins, are used to increase the final viscosity of the product.

6. Food products and food categories within the GSFA in which the substance is used as a food additive or as an ingredient, including use level(s):
The enzyme preparation is not added to final foodstuffs as a food additive or as an ingredient, but it is used as a processing aid during food manufacturing. The chymosin is used in coagulation of milk during cheese-making and production of other fermented milk products.
The chymosin preparation is generally used following the Quantum Satis (QS) principle, i.e. at a level not higher than the necessary dosage to achieve the desired enzymatic reaction – according to Good Manufacturing Practice. The range of dosage recommended for the chymosin is comprised between 2 and 60 IMCU per kg milk, when expressed as International Milk Clotting Units, or between 0.004 and 0.13 mg of enzyme protein per kg milk.

7. Is the substance currently used in food that is legally traded in more than one country? (please identify the countries); or, has the substance been approved for use in food in one or more country? (please identify the country(ies))
The enzyme has been approved in: Denmark[1] (2009), France[2] (2010), Canada[3] (2010), Brazil[4] (2014), South Korea[5] (2018), Japan[6] (2019), Mexico[7] (2019).
Chr-Hansen A/S has also applied for inclusion of the enzyme in the upcoming European Union list of food enzymes, expected implemented in 2025[8].

8. Are you aware of any current impediments in international trade due to lack of a JECFA evaluation and/or Codex standard? If so, please provide details.
Not aware of any.

9. Are you aware of risk assessments, either on-going or completed within the last 10 years, at a national or regional level for this additive? If so, please provide the name, address and contact details of the organization having performed the risk assessment.
The enzyme has undergone a meticulous risk assessment for its use in dairy processing in the countries mentioned in section 7

10. Please provide details if this food additive is of particular relevance to the livelihood and food safety in developing countries
Not aware of any.

11. Please indicate the type of data that are available in the table below.
Ensure that the available data are directly relevant to the substance of interest in this request. In particular, for substances obtained from natural resources, characterization of the products in commerce and a relevant set of biochemical and toxicological data on such products are essential for JECFA to develop a specifications monograph and the related safety. Such data/information typically include: components of interest; all components of the final products; detailed manufacturing process; possible carryover of substances; etc.

	Data available? (Y / N)

[1] Veterinary and Food Administration
[2] AFSSA – French Food Safety Agency
[3] Health Canada
[4] ANVISA - Agência Nacional de Vigilância Sanitária
[5] MFDS -Ministry of Food and Drug Safety
[6] MHLW – Ministy of Health, Labour and Welfare
[7] COFEPRIS: Comisión Federal para la Protección contra Riesgos Sanitarios;
[8] Regulation (EC) No. 1332/2008

Toxicological data	
(i) Metabolic and pharmacokinetic studies (please specify)	***N***
(ii) Short-term toxicity, long-term toxicity/carcinogenicity, reproductive toxicity, and developmental toxicity studies in animals and genotoxicity studies (please specify)	***Y*** (please, see "Comments to the Toxicological data")
(iii) Epidemiological and/or clinical studies and special considerations (please specify)	***N***
(iv) Other data (please specify)	***N***
Comments to the Toxicological data: (ii) The following food toxicity program has been performed: • Test for mutagenic activity (Ames Test) performed in accordance with OECD Guideline 471 • *In Vitro* Mammalian Chromosomal Aberration Test in accordance with OECD Guideline 473 • Repeated dose 90-day oral toxicity study in Rodents performed in accordance with OECD Guideline 408	

	Data available? (Y / N)
Technological data	
(i) Specifications for the identity and purity of the listed substances (specifications applied during development and toxicological studies; proposed specifications for commerce)	***Y***
(ii) Technological and nutritional considerations relating to the manufacture and use of the listed substance	***Y***

	Data available? (Y / N)
Dietary exposure assessment data	
(i) Levels of the listed substance used in food or expected to be used in food based on technological function and the range of foods in which they are used	***Y***
(ii) Estimation of dietary exposures based on food consumption data for foods in which the substance may be used.	***Y***

	Data available? (Y / N)
Other information: *(please specify)*	***Y*** (data available as required by JECFA guidelines)

12. Specify earliest date when data can be made available to JECFA. (Data shall only be submitted in response to a JECFA call for data; do NOT include any data intended for JECFA to this form.)
September 2021