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POTASSIUM POLYASPARTATE

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1. Summary

This Chemical and Technical Assessment summarizes data and information on potassium polyaspartate, used as a wine stabilizer, submitted to JECFA by ESSECO Srl (dossier dated 26/11/2018). The Committee has not previously evaluated this additive.

Potassium polyaspartate is a low-molecular weight, highly polydisperse polyelectrolyte (Mn 1000 g/mol, Mw 5000 g/mol). The repeat units of D- or L-potassium aspartate are linked together by α and β peptide bonds at a ratio of 30:70, respectively. The polypeptide is produced by a two-phase process. In the first phase, the solid state thermal polymerization of L-aspartic acid leads to polysuccinimide. In the second phase the cyclic succinimide is hydrolysed with potassium hydroxide under controlled conditions that lead to opening of the ring. The product produced in this way is not the same as the potassium salt of high molecular weight polyaspartic acid produced by polycondensation of L-aspartic acid in the presence of catalysts.

Potassium polyaspartate produced in this way, is proposed for use as a stabilizer against tartrate crystal precipitation in red, rosé and white wines. Studies submitted verified that it has stabilizing properties similar to those of the metatartaric acid (MTA) and carboxymethylcellulose (CMC) and it does not have negative impacts on the sensory properties, such as colour, of the resultant wine. Depending on the level of instability of the wine to be treated a typical use level of 100 to 200 mg/litre and a maximum use level of 300 mg/litre is suggested.

Potassium polyaspartate has been approved and authorized for use as an additive for wine in the European Union (EU) in COMMISSION REGULATION (EU) 2017/1399 of 28 July 2017 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council and the Annex to Commission Regulation (EU) No 231/2012 as regards potassium polyaspartate and COMMISSION DELEGATED REGULATION (EU) 2017/1861 of 2 August 2017 amending Regulation (EC) No 606/2009 as regards certain oenological practices)¹.

FSANZ (Australia New Zealand Food Standard Code) has assessed an application from Enartis Pacific Pty Ltd. to amend the Code to permit the use of potassium polyaspartate as a food additive (stabilizer) in wine at a maximum permitted level of 100 mg/litre. FSANZ has prepared a draft food regulatory measure, where it concluded that there are no public health and safety concerns from the use of potassium polyaspartate as a food additive in wine at the proposed level. Pursuant to section 31 of the Food Standards Australia New Zealand Act 1991 (FSANZ Act), the calls for submissions to assist consideration of the draft food regulatory measure have been already concluded and the variation will be published in the Commonwealth of Australia Gazette².

¹<http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R1399&from=EN>

²<http://www.foodstandards.gov.au/code/applications/Documents/A1161%20CFS.pdf>

Moreover, a GRAS notification (GRN 000770) has been submitted on behalf of Enartis USA to the Food and Drug Administration (FDA). Based on the information provided, as well as other information available to FDA, there are no questions to the conclusion that potassium polyaspartate is GRAS under its intended conditions of use³.

Potassium polyaspartate also meets the OIV specification (OIV-OENO 572-2017).

2. Description

Potassium polyaspartate is a copolymer of D- and L-potassium aspartate repeat units linked together by α and β peptide bonds. It is produced in a two-phase process; a thermal polycondensation of L-aspartic acid to the polysuccinimide, followed by hydrolysis, using potassium hydroxide. Even though the starting material is L-aspartic acid, the polypeptide produced in this way does not have any optical activity and it is of low-molecular weight and high polydispersity (number average degree of polymerisation less than 10 and polydispersity index of 5). The ratio of α to β peptide bonds is 30 to 70 respectively.

The product is a light-brown, odourless powder containing more than 89% dry matter. It is very soluble in water, but only slightly soluble in organic solvents such as methanol, acetone, ethyl acetate and hexane.

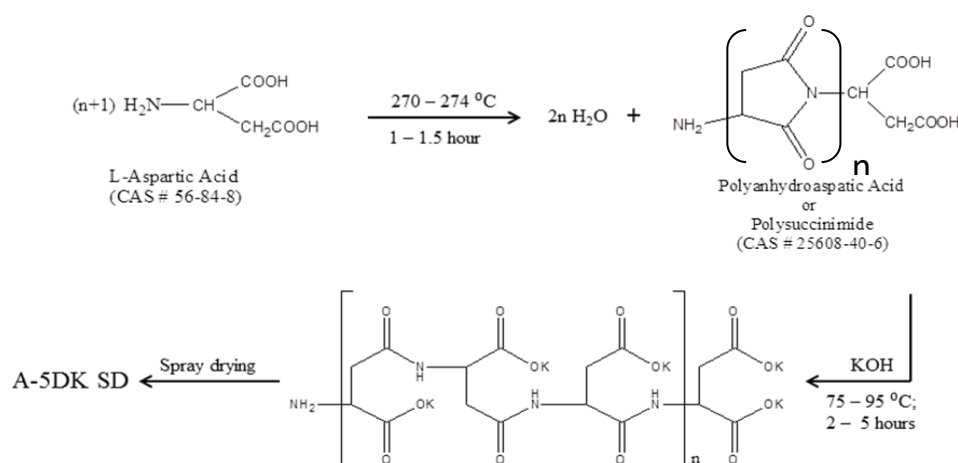
Potassium polyaspartate has stabilizing properties similar to those of metatartaric acid (MTA), carboxymethylcellulose (CMC) and yeast mannoprotein.

Poly L-aspartic acid of high molecular weight is produced synthetically using a different process using a catalyst (Thombre et al, 2005) - this product has not been evaluated under the present submission.

3. Manufacturing

3.1. Manufacturing principle

Potassium polyaspartate is produced from L-aspartic acid according to the reaction reported below.



The thermal process transforms the aspartic acid in polysuccinimide. The water-insoluble polysuccinimide is treated with potassium hydroxide under controlled conditions, which allow hydrolysis and opening of the rings. A 40% solution of potassium polyaspartate at pH 8.3 is obtained. The last step of the production of A-5D K/SD is a spray drying phase. The product is a light tan powder

³<https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm625987.pdf>

(92-95% dry matter). Almost complete racemization is reported during the thermal polycondensation and the hydrolytic opening of the ring may proceed at either carbonyl leading to a non-optically active polypeptide with both α and β peptide bonds.

4. Chemical characterization

4.1. Composition

Potassium polyaspartate, evaluated in this submission, is a polydisperse, low molecular weight polyelectrolyte (Mw approximately 5000, PDI close to 5). The repeat units, D or L potassium aspartate, are linked together via α or β peptide bonds. The intermediate polysuccinimide formed is water insoluble and not expected as impurity in the water soluble final product. Non-hydrolysed cyclic succinimide repeat units are not present in the polymeric chains, since the FTIR spectrum and the potassium content show hydrolysis and more than 90% substitution with K. Unreacted aspartic acid is the main impurity (<1%).

Data from ^1H and ^{13}C NMR found in the literature from commercial samples of polyaspartic acid (PAA) produced by thermal polymerisation (W. Joentgen et al, 2004) revealed that about 70% of the amide bonds are formed by the β -carboxyl groups. This percentage is in agreement with the findings for laboratory-synthesized PAA. Polarimetry analyses show that PAA is composed of a racemic mixture of D- and L-aspartic acid, although optically pure L-aspartic acid is used in the reaction. This result agrees with that of Kokufuta et al. (1978) and Low et al (1996) who report polarimetry measurements after complete hydrolysis with acid showed complete aspartic acid racemisation.

4.2 Identification

The FTIR spectrum shows the characteristic peaks of a polyamide. If subjected to acid hydrolysis, the polyamide is hydrolysed back to aspartic acid and can be measured by HPLC-FLD. The degree of succinimide hydrolysis and formation of the potassium salt is calculated, based on the potassium content of the product, to be greater than 90%.

GPC chromatographic analysis shows a weight average molecular weight of approximately 5000 g/mol and polydispersity index close to 5.

The additive is very soluble in water.

4.3 Analytical methods

The specifications monograph cites general tests included in the *FAO Combined Compendium of Specifications* (FAO JECFA Monographs 1, vol. 4, 2006) and a specific test for the determination of free aspartic acid and a method of assay. Free aspartic acid is determined by HPLC. Potassium polyaspartate is completely hydrolysed using acid, total aspartic acid is determined by HPLC and percent potassium polyaspartate is calculated by subtraction of the free aspartic acid content. A GPC method for the determination of the molecular weight, molecular weight distribution and polydispersity index is also provided.

4.4 Possible impurities

Possible impurities include the unreacted monomer (free aspartic acid). Even though only L-aspartic acid is originally used, the thermal polycondensation process results in racemisation and therefore both D and L aspartic acid should be considered as final impurities. Based on the data submitted on five non-consecutive batches of product, the aspartic acid does not exceed 1% on the dried basis.

Based on analytical data from four different batches and further information provided by the sponsor the levels of lead in the product are below 2 mg/kg.

4.5 Rationale for proposed specifications

Identification of the product is based on the characteristic polyamide peaks of its FTIR spectrum (Joentgen et al., 2004). The formation of the potassium salt is supported by the solubility characteristics, its pH and its content in potassium. The method of production leads to polydisperse low molecular weight product as verified by GPC. The specifications are consistent with the monograph for potassium polyaspartate in the OIV, RESOLUTION OIV-OENO 572-2017.

5. Functional uses

Potassium polyaspartate is evaluated as a new food additive to be used as a stabilizer against tartrate crystal precipitation (anti-scaling additive) in wine (red, rosé and white wine) at the suggested use level of 100-200 mg/l (normal use level) and at the maximum use level of 300 mg/l.

5.1. Technological function

Wine, in its normal state, is naturally supersaturated with significant concentrations of potassium bitartrate and calcium tartrate. Potassium bitartrate can form crystals and precipitate from the wine during storage resulting in undesired sediment in the wine. Although potassium bitartrate crystals in wine do not pose a health risk, their presence affects the aesthetics and consumer acceptability of the wine. Accordingly, before the delivery for domestic or international trade, white wine and sparkling wines in particular, need to be stabilized against tartrate salt precipitation, which is from a natural grape source. The concentration of tartaric acid itself in the wine, alcohol, colloids, calcium, and potassium, the pH value of the wine, the duration and temperature of storage, and the surface (roughness) of the storage container all impact on tartrate salt precipitation in wine (Pilone and Berg 1965). Tartaric acid predominantly precipitates as the potassium salt followed by calcium salt.

Various physical and chemical techniques can be applied during the winemaking process to prevent the formation of these deposits by reducing the concentration of potassium and tartaric acid in wines. Traditionally, part of the potassium bitartrate present in wines is removed by precipitation at low temperatures (cold stabilization) (Ribéreau-Gayon et al. 2006) but recently new techniques have been authorized such as electrodialysis and cation exchangers. Nonetheless, all these subtractive techniques have some disadvantages: cold stabilization is expensive and can sometimes be poorly selective with respect to phenols and polysaccharides; electrodialysis requires a high initial investment for the machinery and involves high operating costs and, unlike cold stabilization, is sometimes excessively selective towards phenols and polysaccharides; finally, the use of cation exchangers is not recommended when the instability is due to an excessive tartaric acid content. Thus, these physical methods are expensive and difficult for smaller wineries to adopt.

Alternative additives to stabilize wine to prevent the precipitation of tartrate into the relatively insoluble potassium bitartrate and calcium tartrate are metatartaric acid, carboxymethylcellulose and mannoproteins. Metatartaric acid is very effective for all wine types but is unstable over time de-esterifying into tartaric acid within few months or after a few days under heating, and can be used only for wines with short shelf life; cellulose gums are effective and stable in white wines but can cause colour instability when used in red wines; mannoproteins have a variable effect and therefore need preliminary tests to evaluate their effectiveness and to determine the optimal dose, which is specific to the wine being treated. Thus, among the crystallization inhibitors, only metatartaric acid is considered as a complete inhibitor of tartaric salt crystal nucleation and growth, until its hydrolysis occurs as the other additives merely slow crystal appearance and possibly their growth.

The aim of the European project STABIWINE (FP7, GA n. 314903, <http://www.stabiwine.eu>) was to identify new compounds for the tartaric and protein stabilization of wines. Potassium polyaspartate (KPA) added to wine even in very low dosage was found to inhibit the formation of tartrate crystals; the natural wine composition is not altered by subtraction of salts, and no refrigeration or other physical

process are required. The new additive is more efficient than metatartaric acid and carboxymethylcellulose and less expensive than yeast mannoproteins.

5.2. Food categories and use levels

The optimal dose of potassium polyaspartate depends on the level of instability of the wine to be treated. In most cases, an addition of 100 mg/L of potassium polyaspartate into wine, (providing a 100 mg/l concentration in the final product), is sufficient to obtain a complete inhibition of tartrate crystal formation during storage. In case of wines with very high levels of tartrate instability, the dose can increase up to 200 mg/l and very rarely to 300 mg/l. Therefore, a conservative approach has been used to calculate the consumer exposure by using 300 mg/l as maximum value.

Before addition to wine, the powder is first dissolved by agitation in a quantity of cold water or wine equivalent to approximately ten times the weight of polyaspartate. The low viscosity of this concentrated solution (20-30 centipoise) does not pose any handling problem. The concentrated solution is then incorporated into the wine through a pumping over operation or by dosage on-line, in order to uniformly disperse the additive in the whole mass of wine, avoiding aeration of the wine with consequent oxidative reactions. After addition, the wine continues its production process toward final filtration, bottling and packaging.

6. Reactions and fate in foods

Polyaspartates are an effective stabilizing agent, able to inhibit the formation of tartrate crystals while preserving the original wine composition. Potassium polyaspartate is to be preferred to the sodium salt for use in wine, as in the first case provision of cations to wine with the treatment is negligible (less than 5% of the natural potassium content). The addition of 100 mg/l potassium polyaspartate has been shown to inhibit the formation of tartrate crystals in both white and red wines, even in case of high instability (up to 450 $\Delta\mu\text{S}/\text{cm}$ measured with mini-contact test). Its stabilizing effect has longer persistence than metatartaric acid which in an acid environment is de-esterified to tartaric acid and progressively loses its stabilizing capability in few months. Potassium polyaspartate is chemically stable in wine conditions and its effect remains unchanged for long periods of time.

In contrast to carboxymethylcellulose (CMC), potassium polyaspartate is very easy to solubilize in water and in wine, and its addition to wine immediately before bottling does not affect its membrane filterability, even at 0.45 μm porosity. Due to its high negative specific charge at wine pH, potassium polyaspartate has been shown to interact with wine colloids like proteins and polyphenols, increasing of turbidity of instable wines. Therefore, if required, a remedial procedure of wine fining with bentonite following the addition of potassium polyaspartate is recommended.

From a sensory perspective, no variation in taste and aroma of wines following stabilization with potassium polyaspartate has been reported, even following the addition of high dosages at 300 mg/L (30 g/hL or 300 ppm). In some cases, a reduced sensitivity of potassium polyaspartate added wine to oxidation was observed, probably to be linked with a chelating effect of the polymer toward bivalent cations (Fe^{2+} , Cu^{2+}) which are known to catalyze the oxidation reaction of some wine components.

6.1 Summary of stability studies

The stability of the potassium polyaspartate (A-5D K/SD) in wine was assessed both directly and indirectly. Direct assessment was by measuring the concentration of aspartic acid unit (the potential degradation product of polyaspartate, that is also naturally present in wine) during the wine storage. Indirect assessment was by the evaluation of the effectiveness of the additive on the stability of various wine characteristics over time.

Potassium polyaspartate (A-5D K/SD) was added to different red and white wines, which were then bottled and analyzed during aging, after five and 12 months of storage. Only a small increase in the aspartic acid concentration was observed in all the wines after five and 12 months of bottle aging. After 5 months, the maximum increase of the aspartic acid in the wine corresponded to hydrolysis of 1.3% of the additive for the white wine and 6.3% for the red wine, while after 12 months the maximum increase of aspartic acid corresponded to 4.8% (white wine) and 7.5% (red wine) of the additive after addition of 100 mg/L potassium polyaspartate.

Similar results were obtained from two additional experiments with two wines (one white wine and one red wine), to which potassium polyaspartate (A-5D K/SD) was added, and monitored over time by assessing the tartaric stability and by quantifying the release of aspartic acid monomers. After addition of 100 mg/L of potassium polyaspartate (A-5D K/SD) to a Dolcetto variety red wine, the concentration of aspartic acid after 12 months of storage showed that the difference between the treated and the control wine is limited to 1.75% of the potassium polyaspartate (A-5D K/SD) added to the wine. This value is close to the impurity of the commercial preparation potassium polyaspartate (A-5D K/SD) in aspartic acid (about 1%), residual of the raw material used in the production process.

The tartaric stability was also monitored through analysis of tartaric acid content before and after cold storage, showing that the wine treated with potassium polyaspartate (A-5D K/SD) remained stable for 12 months of storage at room temperature, while the instability of control wine increased over time. The same experiment was repeated with white wine. Results obtained after 6 and 12 months of storage at room temperature confirmed that only the batch to which 100 mg/L of potassium polyaspartate (A-5D K/SD) was added remained stable. Data obtained by analysis of free aspartic acid show a non-significant analytical difference between the control and the wine to which PPA was added, suggesting that also in white wine the degradation of potassium polyaspartate (A-5D K/SD) into its monomer is negligible.

Assuming that hydrolysis is the main reaction occurring in wine during storage, aspartic acid is the only degradation product formed. The quantification of the aspartic acid after storage shows a non-significant increase compared to the control, thus it can be concluded that the potassium polyaspartate (A-5D K/SD) is stable in wine for at least 12 months. This conclusion is also supported by the maintenance of the stabilization effect over time as observed in the experiment where the tartaric stability was measured.

7. References

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