ORIGINAL SUBMISSION

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November 10, 2016

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, Maryland 20740

Subject: Notice of a GRAS Exclusion for Desugared Fermented Orange Juice

Dear Sir/Madam:

In accord with 21 C.F.R. part 170, subpart E, Sumol + Compal Marcas S.A. hereby submits the enclosed notice that the use of desugared fermented orange juice as an ingredient in carbonated and non-carbonated beverages is excluded from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because the notifier has determined that such use is generally recognized as safe (GRAS).

Sincerely,

(b) (6) [redacted]

Ricardo Carvajal

RC/sas
Enclosures

GRAS NOTICE FOR DESUGARED FERMENTED ORANGE JUICE SUBMITTED BY SUMOL + COMPAL MARCAS S.A.

Part 1 – Signed statements and certification

(1) Applicability of 21 C.F.R. part 170, subpart E

We submit this GRAS notice in accordance with 21 C.F.R. part 170, subpart E.

(2) Name and address of the notifier

Sumol + Compal Marcas S.A.
Rua Dr. António João Eusébio, 24
2790-179 Carnaxide
Portugal

(3) Name of the notified substance

Desugared Fermented Orange Juice

(4) Applicable conditions of use of the notified substance

(a) Foods in which the substance is to be used

The substance is to be used as an ingredient in carbonated and non-carbonated beverages, such as fruit drinks, fruit-flavored drinks, and drinks that contain fruit juice in part, and that contain less than 0.5% (v/v) ethanol, all of which are traditionally perceived by consumers to be "non-alcoholic."[1]

(b) Levels of use in such foods

The substance is intended to be used as an ingredient in carbonated and non-carbonated beverages. There is no limit on the level of use in such beverages, provided that the content of ethanol in the finished product is below 0.5% (v/v). For the purpose of estimating the daily intake of the

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[1] *cf.* FDA, CPG Sec. 510.400, Dealcoholized Wine and Malt Beverages ("Beverages such as soft drinks, fruit juices, and certain other flavored beverages which are traditionally perceived by consumers to be 'non-alcoholic' could actually contain traces of alcohol (less than 0.5 percent alcohol by volume) derived from the use of flavoring extracts or from natural fermentation. FDA also considers beverages containing such trace amounts of alcohol to be 'non-alcoholic.'").

substance, we assume that its intake will not exceed that of pure (100%) orange juice.

(c) Purpose for which the substance is used

The substance is intended to be used as a substitute for regular orange juice.

(d) Description of the population expected to consume the substance:

The population expected to consume the substance consists of members of the general population who consume at least one of the products described above.

(5) Basis for the GRAS determination

The statutory basis for our conclusion of GRAS status is through scientific procedures in accordance with section 170.30(a) and (b).

(6) Exclusion from premarket approval

The notified substance is not subject to the premarket approval requirements of the FDC Act based on our conclusion that the notified substance is GRAS under the conditions of its intended use.

(7) Availability of data and information

If FDA asks to see the data and information that are the basis for our conclusion of GRAS status either during or after FDA's evaluation of our notice, we will agree to make the data and information available to FDA. Further, upon FDA's request, we will allow the Agency to review and copy the data and information during customary business hours at the above address, and will provide FDA with a complete copy of the data and information either in an electronic format that is accessible for the Agency's evaluation or on paper.

(8) Applicability of FOIA exemptions

None of the data and information in Parts 2 through 7 of our GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. § 552.

(9) Certification

We certify that, to the best of our knowledge, our GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of the substance.

sumol+compal

(b) (6)

Name: Paulo Monteiro Marques
Title: Applied Research Director

2016.11.09
Date:

Please address correspondence to:

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Part 2 – Identity, method of manufacture, specifications, and physical or technical effect

(1) Identity of the notified substance

(a) Quantitative composition

The subject of this GRAS notice is a desugared fermented orange juice with a total sugars content of ≤ 2.0 g/L and an ethanol content of ≤ 1% (v/v).[2] All the other nutritionally relevant components of orange juice, such as vitamin C, carotenoids, flavonoids, etc. are present in the desugared fermented orange juice in similar concentrations as in regular orange juice.

(b) Characteristic properties

Desugared fermented orange juice has the appearance and sensory properties of orange juice. However, the sweetness-to-acid balance differs because of its low sugars content.

(c) Any known toxicants that could be in the source

There are no known toxicants in the orange juice used as a raw material in the production of desugared fermented orange juice. There are three potential undesirable by-products of fermentation: ethyl carbamate, biogenic amines, and methanol. As shown in Part 6(3) of this notice, these by-products are not present at pertinent levels in desugared fermented orange juice.

(2) Description of method of mannfacture

For the production of desugared fermented orange juice, regular orange juice or orange juice for manufacturing [3] is treated with viable cells of *Schizosaccharomyces pombe* strain PYCC 4197 (PYCC, 1989). The characteristics of this strain are shown in Annex 1.

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[2] The ethanol content of any beverages in which the substance is used as an ingredient will be less than 0.5% (v/v).

[3] 21 C.F.R. § 146.135. The term "orange juice for manufacturing" is the food prepared for further manufacturing use. *Id.* § 146.151. It differs from orange juice in that the oranges may deviate from the standards for maturity, among other differences. *Id.*

The production of desugared fermented orange juice comprises three steps, i.e., (1) the production of *Schizosaccharomyces pombe* PYCC 4197 biomass, (2) treatment of regular orange juice or orange juice for manufacturing[4] with this biomass at about 25°C for conversion to ethanol and CO_2 of the naturally present sugars [mainly sucrose (3.3–5 g/100 mL), fructose (2.3–3.6 g/100 mL) and glucose (1.7–3.4 g/100 mL)], and (3) removal of the biomass when the fermentation rate has dropped below a rate of -0.05°Bx/30 min due to exhaustion of the sugars present in the juice and thus available for fermentation. The fermented orange juice is then heat treated for pasteurization and dealcoholization.

The obtained desugared orange juice is then standardized by the addition of water to correct for the volume decrease due to loss of water during the pasteurization and dealcoholization step.

Using an ultra-clean filling system, the desugared fermented orange juice is then filled into aseptic bags that are fit for the storage of liquid foods.

For preserving the organoleptic quality of the desugared fermented orange juice, storage at 0–4 °C prior to its use in a beverage should not exceed 30 days.

(3) Specifications for food-grade material

Production of the desugared fermented orange juice is conducted in accord with current Good Manufacturing Practice (cGMP) requirements and a Hazard Analysis Critical Control Point (HACCP) system is established and applied.

The specifications of the desugared fermented orange juice comprise compositional and microbiological parameters. For purpose of specifications, limit values are applied for:

Sugars	≤ 2.0 g/L
Ethanol	≤ 1.0% v/v
Yeast and molds	< 100 CFU/mL
Bacteria	< 1000 CFU/mL

Additional quality criteria that are not part of the specifications but that are checked for performance purposes include:

Total yeast cells	≤ 10^5/mL
Appearance	characteristic of orange juice
Flavor	characteristic, free from off-flavors

4 As defined in 21 C.F.R. § 146.135 and § 146.151, respectively.

(4) Data and information bearing on physical or other technical effect

Our GRAS notice does not include data and other information bearing on physical or other technical effect because such data and other information are not necessary to demonstrate safety.

Part 3 – Dietary exposure

(1) Nutritional properties

Citrus juices, especially orange juice, are rich sources of vitamin C (Sanchéz-Moreno et al., 2003). Orange juice, for example, can provide 90–150% of the RDA of vitamin C with one serving size. Orange juice also is a good source of certain flavonoids, carotenoids, and folates. A comparison of their contents in orange juice and the corresponding desugared fermented orange juice is presented in Table 1. This data shows that the nutritional value of orange juice is not reduced during the production of desugared fermented orange juice, except for its sugars and thus energy content.

(2) Intended use

Desugared fermented orange juice is intended to be used as an ingredient in carbonated and non-carbonated beverages, such as fruit drinks, fruit-flavored drinks and drinks that contain fruit juice in part, and that contain less than 0.5% (v/v) ethanol, all of which are traditionally perceived by consumers to be "non-alcoholic."[5] Used in such beverages as a substitute for regular orange juice, the desugared fermented orange juice helps reduce the intake of sugar and thus energy (calories). Used as an ingredient in such drinks, desugared fermented orange juice provides all the nutrients (other than sugars) for which orange juice is a significant dietary source.

(3) Estimated Daily Intake (EDI)

Desugared fermented orange juice is intended to be used as an ingredient in carbonated and non-carbonated beverages. While the availability of this desugared fermented orange juice may, on the one hand, result in the formulation of new beverages which could not be made with regular orange juice, it is, on the other hand, highly unlikely that this new product would substitute for all orange juice consumed. Therefore, for the purpose of estimating the daily intake of desugared fermented orange juice, it is assumed that its intake will not exceed that of pure (100%) orange juice.

(a) EDI in adults

Based on National Health and Nutrition Examination Survey (NHANES) 2005–2010 data, the intake of water from fruit juice and fruit drinks (both regular and diet) was 74 and 90 mL/d among 20–50 year old consumers.

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[5] *See supra* note 1.

Data from adults 19+ years of age participating in the 2003-2006 NHANES reveal a population average per capita intake of 100% orange juice of 50.3 mL/d. Among consumers only, which represent 23.8% of the surveyed population, the intake was 210 mL/d on average and 259 mL/d for the 75th percentile consumer (O'Neil et al., 2012).

These figures give, therefore, an indication of the estimated daily intake of desugared fermented orange juice in this particularly health conscious segment of the population.[6]

Since 2003–2006, the consumption of orange juice has decreased continuously in the United States. By 2015, the consumption had decreased by almost 40% (Statista, 2016). This decrease has been attributed to a negative impact of the Nutrition Facts Panel on fruit juice (Kim et al., 2014).

On the one hand, the daily intake of 100% orange juice estimated from the NHANES 2003-2006 data may, therefore, be an overestimate of current intake. But on the other hand, the availability of a desugared fermented orange juice with a nutritional profile potentially perceived as more desirable due to its low sugars content may make some past consumers return to this new beverage which still provides all the nutritional values of 100% orange juice, except for its sugars content and the associated calories.

(b) EDI in children

The average consumption of 100% fruit juice (fruit of any kind) among children 2–18 years of age was 106 mL/d based on NHANES 2007–2010 data. Among consumers of 100% fruit juice only, the mean consumption was 313 mL/d (293 mL/d for 2–8 year olds and 331 mL/d for 9–18 year olds) (Nicklas et al., 2015).

The consumption of 100% fruit juice and fruit drinks[7] among 1–5 year old children (consumers and non-consumers of fruit juice) has also been examined based on NHANES 2001–2006 data (Fulgoni & Quan, 2012). The data demonstrate that in that age group the daily intake of 100% fruit juice and fruit drinks is 305 and 358 mL/d, respectively, i.e., not much different in absolute terms. If expressed in terms of mL/kg bw/d, it is obviously higher in the 1–5 year age group than in the 2–18 year age group.

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[6] The consumption of 100% orange juice was associated with generally better nutrition and improved biomarkers of health (O'Neil et al., 2012). People with a healthier lifestyle are likely to be the first and best acceptors of desugared fermented orange juice.

[7] According to the authors, most fruit drinks actually contain 10% or less fruit juice and a substantial amount of added sugars.

Data extracted from the 2005–2010 NHANES data show that, among children age 4–13 years (users and non-users), 194.8 mL/d fruit juice and fruit drinks (regular and low-calorie) are consumed. In the 4-8 year old subgroup, the intake is somewhat higher (214 mL) than in the 9–13 year old subgroup (176 mL) and there is a slight change away from 100% fruit juice to fruit drinks with increasing age (Drewnowski et al., 2013). Intake data for consumers only were not reported.

Part 4 – Self-limiting levels of use

There are no known self-limiting levels of use for desugared fermented orange juice.

Part 5 - Experience based on common use in food before 1958

Because the statutory basis for our conclusion of GRAS status is not through experience based on common use in food, our notice does not include evidence of a substantial history of consumption of the notified substance for food use by a significant number of consumers prior to January 1958.

Part 6 – Narrative

(1) *Schizosaccharomyces pombe*

Schizosaccharomyces pombe strain PYCC4197 is a wild type strain that is deposited at the Portugese Yeast Culture Collection at Faculdade de Ciências e Tecnologia, Lisbon, Portugal.

Schizosaccharomyces pombe was first described as a new species by Lindner (1893). It was isolated at that time from millet beer which was sent from East Africa to Germany.

Schizosaccharomyces pombe is a yeast species that has been and still is used in certain traditional brewing processes and that, more recently, is used as a model in molecular and cell biology. The genome of *Schizosaccharomyces pombe* has been fully sequenced and it was found to contain the smallest number of protein-coding genes then recorded for eukaryotic cells (Wood et al., 2002).

The life cycle, chromosome map, and main metabolic pathways of *Schizosaccharomyces pombe* have been examined (Gutz et al., 1974). Analysis of the genomic and phenotypic diversity of 161 natural isolates mostly from brewed beverages revealed moderate genetic diversity and suggested spreading from Europe to other countries by cultural uses (Jeffares et al., 2015).

Schizosaccharomyces pombe has been used both as a donor and acceptor organism for genetically modified microorganisms with technological beneficial use (FDA, 2003; Bourdichon et al., 2012). An *E. coli* derived gene encoding for a phytase was expressed in *Schizosaccharomyces pombe*. This phytase is intended for use in animal feed. Hence, its safety for this purpose was evaluated in standard genotoxicity studies as well as an acute and a subchronic (90-day) toxicity study in rats. The results of these and other toxicity studies demonstrated that both the refined and the unrefined phytase did not produce any adverse effects under the conditions of these tests. The unrefined test article included fermentation broth from which most, but not all, of the *Schizosaccharomyces pombe* cells have been removed (Ciofalo et al., 2003).

This enzyme was subsequently accepted for use in animal feed for different species by the European Food Safety Authority (EFSA, 2006 a,b, 2008a, 2012) and in the US by AAFCO (2012).

A *Saccharomyces cerevisiae* strain that harbors the *Schizosaccharomyces pombe* gene encoding for malate permease was the subject of GRAS Notice GRN 000120. In its answering letter, the FDA acknowledges that *Schizosaccharomyces pombe* is a yeast that was first isolated from African beer and that has frequently been found in sugar-containing products in tropical and subtropical regions as well as in grape must and cider in moderate climates (FDA, 2003).

In 2008, the European Food Safety Authority included *Schizosaccharomyces pombe* in the list of microorganisms with QPS, i.e., Qualified Presumption of Safety, status for the addition to food or feed (EFSA, 2008b).

A specific strain of *Schizosaccharomyces pombe* is offered as a starter culture for wine production by a Portuguese company under the trademark ProMalic® (Proenol, 2016). *Schizosaccharomyces pombe* has been found to be particularly useful for the deacidification of grape must with excessive acidity (Ethiraj et al., 1983; Snow & Gallander, 1979).

(2) Ethanol

Desugared fermented orange juice contains small residual amounts of ethanol. According to its specifications, the ethanol content is less than 1% (v/v) and less than 0.5% may be achieved.

Orange juices were found to naturally contain ethanol in widely varying concentration (mean: ~ 500 ppm; range: 150–900 ppm) (Lund et al., 1981). Results of more recent analyses are in keeping with these values (Moshonas & Shaw, 1994; Nisperos-Carriedo & Shaw, 1990; Farnworth et al., 2001; Hagenmaier, 2001). Thus, desugared fermented orange juice contains on average slightly higher concentrations of ethanol than regular orange juice. However, desugared fermented orange juice is intended to be used as an ingredient in formulated drinks and beverages. As long as such beverages contain less than 0.5% v/v ethanol, they are considered "non-alcoholic"[8] and as such qualify for use by the general population.

It is for this reason that the intended use of desugared fermented orange juice is limited to beverages that are traditionally perceived by consumers to be "non-alcoholic," i.e., contain less than 0.5% (v/v) ethanol.

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[8] *See supra* note 1.

(3) Undesirable by-products of fermentation

(a) Ethyl carbamate

Ethyl carbamate is a naturally occurring by-product in fermented foods and beverages. It is formed by the chemical reaction between urea and ethanol. Urea is a product of the microbial degradation of L-arginine and citrulline.

Differences in the L-arginine concentrations of the fruit juices determine the urea and thus the ethyl carbamate levels of the final fermented product to some extent. Orange juice contains about 44 mg/100 mL L-arginine (Souci et al., 1994). For grape juice, reported arginine concentrations vary over a wide range [from 28 ± 7 mg/100 mL up to 110 mg/100mL (Weits et al., 1971; Rapp & Reuther, 1971)]. However, for the present case more important than differences of L-arginine concentrations in the fruit juice is the fact that *Schizosaccharomyces pombe* contains urease, which prevents an accumulation of urea in fermented musts (Lubbers, 1993; Benito et al., 2016). Indeed, the urea content of white wines fermented with *Schizosaccharomyces pombe* alone was lower than in wines made with mixed fermentations and with *Saccharomyces cerevisiae* alone (Benito et al., 2012). Accordingly, the levels of urea in the desugared fermented orange juice and consequently the formation of ethyl carbamate is also expected to be low.

(b) Biogenic amines

The public health risks of biogenic amines, such as histamine and tyramine, have been evaluated by different authoritative bodies (e.g., EFSA, 2011; FAO & WHO, 2013). In wine, tyramine and histamine typically occur in concentrations that do not present a risk to human health. However, histamine concentrations of more than 10 mg/L have also been reported (Konakovsky et al., 2011; EFSA, 2011 at Tables 10, 11).

The tyrosine content of orange juice was reported to increase from 284 ± 46 mg/L to 425 ± 18 mg/L during fermentation for 9 days (Cerrillo et al., 2015) using *Pichia kluyveri* for starting the fermentation (Escudero-López et al., 2013). Whether the fermentation of orange juice with *Schizosaccharomyces pombe* leads to a similar increase of tyrosine is not known.

However, no indication could be found in the scientific literature of the presence of tyrosine decarboxylase or histidine decarboxylase in *Schizosaccharomyces pombe*. Therefore, there is no indication that tyramine and histamine is formed during the fermentation of orange juice with *Schizosaccharomyces pombe*. Direct evidence for this is provided by a comparative study on the use of different yeasts in red wine production in which the biogenic amines after fermentation were measured. For none of the analyzed amines there was a difference between fermentations with

Schizosaccharomyces pombe, *Saccharomyces cerevisiae*, and *Kluyveromyces thermotolerans* (Benito et al., 2015, 2016).

Recently, the formation of melatonin in orange juice during alcoholic fermentation with *Pichia kluyveri* was reported (Fernández-Pachón et al., 2014). The observed concentrations increased from 3.15 µg/L on day 0 (melatonin naturally present in orange) to 21.8 µg/L on day 15 of fermentation. These concentrations are well within the range of melatonin concentrations that have been found, using the essentially same analytical method, in *Schizosaccharomyces cerevisiae*, fermented red wines (Iriti & Vigentini, 2015; Rodriguez-Naranjo et al., 2011a, b, 2013; Gomez et al., 2012).

Whether *Schizosaccharomyces pombe* has the same capability of forming melatonin like *Saccharomyces cerevisiae* is not known.

(c) Methanol

Methanol is yet another potential undesirable by-product of alcoholic fermentations including the fermentation of orange juice (Oluwanisola & Adediran, 2007).

In a comparative study on the volatile compounds formed in the production of white wine with either *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe* insignificantly lower concentrations were found when *Schizosaccharomyces pombe* was applied (Benito et al., 2013, 2014). However, methanol due to its lower boiling point will be removed during the ethanol evaporation step even more completely than ethanol. Therefore, at the most, trace amounts of methanol might occur in desugared fermented orange juice.

(4) Animal studies

Standard toxicity tests in experimental animals have not been reported for fermented orange juice. However, this product has been administered in two recent nutritional studies in experimental animals.[9]

In the first study, the bioavailability of polyphenols present in fermented orange juice was examined in rats. Orange juice fermented with *Pichia kluyveri* at 20 °C for 10 days and then pasteurized at 80 °C for 30 seconds was fed to 10-week old rats housed in metabolic cages by gavage at a dose of 2 mL (single dose). Blood was collected at 2, 6, and 10 hours after dosing and urine was collected at four occasions, i.e., at 6, 12, 24, and 36 hours after dosing. The results demonstrate that the examined flavonoids occur in

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9 An early study on the vitamin C content of native and fermented orange juice has been reported by Lepkovsky et al. (1925).

fermented orange juice mainly in glycosylated form, i.e., as glucosides or rutinosides with ferulic acid-4'-O-glucoside and hesperetin-7-O-rutinoside being the two by far most prevalent compounds (accounting together for about 87% of all quantitated polyphenols). The urinary analyses indicate that half of the ingested ferulic acid is absorbed. In contrast, the absorption of hesperetin and naringenin is very small (not more than 5%). Adverse effects due to the administration of 2 mL (single dose) of fermented orange juice were not reported (Escudero-López et al., 2014).

In the second study consisting of two experiments, the same research group examined the effect of the ingestion of fermented orange juice on certain biochemical plasma parameters, such as blood lipids and other parameters considered to be cardiovascular risk biomarkers in mice. In the first experiment, four groups of eight-week old mice (8 mice/group) received water (control) or orange juice (10% in water) or fermented orange (10% in water) or ethanol (0.1% in water) for a period of 12 weeks. According to the authors, the applied dose of orange juice or fermented orange juice corresponds to the intake of 250 mL orange beverage per day in humans. In the second experiment, two groups of mice (18 mice/group) received twice this dose of fermented orange juice or water for 12 weeks. In neither experiment, body weights and weight gains were affected by the treatments. Adverse effects were not reported. According to the authors, the consumption of the fermented orange juice may protect against cardiovascular risk factors in healthy mice by improving the blood lipid profile and antioxidant status (Escudero-López et al., 2015).

(5) Nutritional and health related consequences

The consumption of fruit is generally associated with better health. However, the consumption of fruit falls short of national recommendations. In general, whole fruit contributes about 65% of all fruit servings while fruit juice contributes about 35% depeuding upon age (Drewnowski & Rehm, 2015). Thus, tbe consumption of 100% fruit juice provides a significant amount of vitamins and minerals as well as other bioactive compounds with the potential to positively affect human health (Hyson, 2015).

Fruit juice is underconsumed rather than overconsumed and adverse effects to human health, directly or indirectly, are not known.[10]

A comparative analysis of orange juice and desugared fermented orange juice has shown that ascorbic acid, carotenoids, flavonoids and folates are not affected by the fermentation of the orange juice with *Schizosaccharomyces pombe* (Table 1). In contrast, the content of total sugars and thus energy is decreased hy about 98 and 87%, respectively. Hence,

[10] For example, by displacing other foods.

the introduction in the food supply of desugared fermented orange juice under the intended conditions of use and at the estimated highest achievable levels is not associated with any risk to human health directly or indirectly but may indeed help to increase fruit consumption because it offers an acceptable alternative to consumers who are concerned about the sugars consumption that is inherently associated with the consumption of 100% fruit juice or even whole fruits.[11]

Among children in which the intake of energy with fruit juice or fruit drinks is not problematic from an energy intake point of view (O'Connor et al., 2006), a reduction in the frequency of sugar intake, particularly between meals, may still have a beneficial effect on dental health.

(6) Historical and present use of fermented orange juice

The term "orange wine" encompasses quite different beverages such as white wine (made from grapes) flavoured with macerated orange peel or wine with an orange color that it got from contact with grape skins.[12] However, this term also is applied to "fruit wine" made from orange juice rather than grape juice. This type of orange wine has a long history of human consumption, for example, in Southern Europe (Thiel et al., 1874) and on the French island of Martinique, where it was already known more than two hundred years ago.[13] However, the fermentation of orange juice was known at about that time not only on the Caraïbes but also in Portugal.[14] Typically, sucrose was added to the orange

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[11] It has been argued that fruit juice is nutritionally inferior to whole fruit because of a lower fiber content and a different satiating effect (Boulton et al., 2016). However, this discussion is more complex (Drewnowski & Rehm, 2015) and it is beyond the scope of this GRAS notice.

[12] *See* https://en.wikipedia.org/wiki/Orange_wine.

[13] For references see:

a) Vin d'orange de la Martinique en 1803 (http://books.google.fr/books?id=c5E5AAAAcAAJ&pg=RA1PA105&dq=vin+d%27oage&hl=fr&sa=X&ei=sTzBUJHMM2k0AWdmYH4DQ&redir_esc=y#v=onepage&q=vin%20d'orange&f=false).

b) Vin d'orange de Martinique en 1794 (http://books.google.fr/books?id=Z06M9M7DQ5QC&pg=PA505&dq=vin+d%27orange&hl=fr&sa=X&ei=wj3BUPcOHuPM0AWhw4DoDw&redir_esc=y#v=onepage&q=vin%20d'orange%f=false).

[14] Vin d'orange en 1780 par J.E. Bertrand (https://books.google.fr/books?id=cRZbAAAAQAAJ&pg=PA443&dq=vin+d%27orage&hl=fr&sa=X&ei=LzrBUPaHHMQk0AXVu4HIDQ&redir_esc=y#v=onepage&q=vin%20d'orange&f=false).

juice and baker's yeast (*Saccharomyces cerevisiae*) was used for starting the fermentation. From Portugal, the product reached France and other regions.

In recent times, the production of orange wine has attracted renewed interest in different countries including Spain (Bedoya et al., 2005), Brazil (Corazza et al., 2001), Colombia (Olivero et al., 2011), Mexico (Purcco Salvador, 2013), Honduras (Méndez V., 2006; Salinas Jiménez, 2007), and Argentina (Ferreyra et al., 2009). In all those studies, *Saccharomyces cerevisiae* was used for the fermentation.

The fermentation of orange juice with *Pichia kluyveri* was studied more recently. This yeast was isolated from the natural microbiota present in the orange fruit but it ferments only reducing sugars and therefore results in a fermented product which still contains significant concentrations of sugar and a low ethanol content (only 0.87%) (Escudero-Lopéz et al., 2013).

In a study conducted in Turkey, orange juice with added sugar was subjected to spontaneous fermentation. The alcohol and total sugar concentration in the final analyses reached 12.2 and 6%, respectively. While the flavor compounds were analysed in detail, no attempt was made to characterize the microbiology of this product (Selli et al., 2003).

Schizosaccharomyces pombe which has been found naturally occurring in fermenters used for the fermentation of sugar cane (El-Tabey Shehata, 1960; Lehtonen & Suomalainen, 1977 as cited in Maza Gomez, 2002) was examined for use in malo-alcoholic fermentation about 30 years ago (Maconi et al., 1984) but was proposed for the production of fruit wine only more recently (Joshi & Attri, 2005).

In December 2015, an orange wine produced with *Saccharomyces cerevisiae* was placed on the market in Spain under the Tarangino tradename (Martinez, 2015).[15] Earlier, in 2011, a sparkling orange wine named "Burnarj" hit the Spanish market.[16] In the US, Florida Orange Groves & Winery offers a semi-sweet orange wine.[17]

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[15] *See also* http://www.larazon.es/local/comunidad-valenciana/crean-un-vino-de-naranjas-valencianas-AN9807354#.Ttt1P3II0xaOt4SD (accessed August 10, 2015).

[16] https://www.alimarket.es/noticia/80244/vegasud-presenta-su-espumoso-de-naranja---burnarj- (accessed March 25, 2016).

[17] http://www.floridawine.com/orange-sunshine-sweet (accessed March, 25, 2016).

(7) Conclusions

In summary, the GRAS determination based on scientific procedures of the proposed use of desugared fermented orange juice as an ingredient of fruit drinks, fruit-flavored drinks, and drinks that contain fruit juice in part (all of which are perceived as non-alcoholic when their ethanol content is below 0.5% (v/v) ethanol), relies on:

(1) the safety of regular orange juice or orange juice for manufacturing which is used as the starting material for the production of desugared fermented orange juice;

(2) the qualified presumption of safety of *Schizosaccharomyces pombe* that is used for degrading the naturally present sugars in the manufacturing of desugared fermented orange juice, and

(3) the absence of undesirable by-products of fermentation at pertinent levels (ethyl carbamate, biogenic amines, methanol) from desugared fermented orange juice.

The safety of desugared fermented orange juice is further supported by the historical and current safe use of fermented orange juice produced with different fermenting microorganisms in different countries.

An independent panel of scientific experts, qualified by training and experience to evaluate the safety of food ingredients, concluded that under the conditions of intended use in foods, Sumol + Compal's desugared fermented orange juice is GRAS through scientific procedures. The panel's opinion is included at Annex 2.

Part 7 - List of supporting data and information

All of the data and information listed below are generally available.

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Table 1 **Energy and nutrient content of orange juice before and after fermentation with *Schizosaccharomyces pombe*[1,2]**

Content	Orange juice	Desugared fermented orange juice	Difference
Energy (kcal/100 mL)	40.2	5.3	-87%
Total Sugars (g/100 mL)	8.53	0.13	-98%
Total Vitamin C (mg/100 mL)	48.43	44.88	-7%
Total Carotenoids (µg/100 mL)	3.28	2.88	-12%
Total Flavonoids (mg/100 mL)	284	315	+11%
Folates (µg/100 mL)	20.3	19.8	-2%

Notes

(1) Values are means of the analyses of three batches run at pilot plant scale.

(2) Under the fermentative action of *Schizosaccharomyces pombe* ethanol is formed. Malate is degraded and glycerol as well as pyruvate are formed as minor by-products of this process (data not shown).

1

Annex 1

Characteristics of *Schizosaccharomyces pombe* PYCC 4197

Description and characteristics of *Schizosaccharomyces pombe* PYCC 4197
(copied from PYCC, 1989)

General information

Taxonomy

Taxon name:	Schizosaccharomyces pombe
Check of identity:	M.J. Nolasco, Aug. 1989

Collection details

PYCC strain status:	Open
Substrate of isolation:	unknown
Country of origin:	unknown
Deposited by:	R.J. Planta
Preservation:	Glass beads; 20% Glycerol; -150°C
Price per culture:	75

Growth conditions

Conditions for growth:	YM
Temperatures:	25-37

Physiology

Fermentation

D-Glucose (F1):	positive
D-Galactose (F2):	negative
Maltose (F3):	positive
Sucrose (F5):	positive
a,a-Trehalose (F6):	negative
Melibiose (F7):	negative
Lactose (F8):	negative
Cellobiose (F9):	negative
Melezitose (F10):	negative
Raffinose (F11):	positive
Inulin (F12):	negative

Assimilation-Growth

50% D-Glucose (O4):	growth
Starch formation (M1):	no
Urea hydrolysis (M3):	yes
Diazonium Blue B reaction (M4):	negative
Methanol (C41):	no growth
Ethanol (C42):	no growth

Yeasts physiological data:

C1 D-Glucose +
C2 D-Galactose -
C3 L-Sorbose -
C4 D-Glucosamine -
C5 D-Ribose -
C6 D-Xylose -
C7 L-Arabinose -
C8 D-Arabinose -
C9 L-Rhamnose -
C10 Sucrose +
C11 Maltose +
C12 a,a-Trehalose -
C13 Me a-D-Glucoside -
C14 Cellobiose -
C15 Salicin -
C16 Arbutin spliting -
C17 Melibiose -
C18 Lactose -
C19 Raffinose +
C20 Melezitose -
C21 Inulin d
C22 Starch -
C23 Glycerol -
C24 Erythritol -
C25 Ribitol -
C26 Xylitol -
C27 L-Arabinitol ?
C28 D-Glucitol -
C29 D-Mannitol -
C30 Galactitol -
C31 myo-Inositol -
C32 D-Glucono-1,5-lactone +
C33 2-Keto-D-Gluconate ?
C34 5-Keto-D-Gluconate ?
C35 D-Gluconate -
C36 D-Glucuronate -
C37 D-Galacturonate ?
C38 DL-Lactate -
C39 Succinate -
C40 Citrate -
C43 Propane 1,2 diol ?
C44 Butane 2,3 diol ?
C45 Quinic acid ?
C46 D-glucarate ?
C47 D-Galactonate ?
C48 Palatinose ?
C49 Levulinate ?
C50 L-Malic acid ?
C51 L-Tartaric acid -
P11 Phenol ?
C52 D-Tartaric acid ?
C53 meso-Tartaric acid ?
C54 Galactaric acid ?
C55 Uric acid ?
C56 Gentobiose ?
C57 Ethylene glycol ?
C58 Tween 40 ?
C59 Tween 60 ?
C60 Tween 80 ?
N1 Nitrate -
N2 Nitrite -
N3 Ethylamine -
N4 L-Lysine -
N5 Cadaverine -
N6 Creatine -
N7 Creatinine -
N8 Glucosamine ?
N9 Imidazole ?
N10 D-Tryptophan ?
N11 D-Proline ?
N12 Putrescine ?
V1 w/o vitamins -
V2 w/o myo-Inositol ?
V3 w/o Pantothenate ?
V4 w/o Biotin ?
V5 w/o Thiamin ?
V6 w/o Biotin & Thiamin ?
V7 w/o Pyridoxine ?
V8 w/o Pyridoxine & Thiamin ?
V9 w/o Niacin ?
V10 w/o PABA ?
O1 Cycloheximide 0.01% -
O2 Cycloheximide 0.1% -
O3 Acetic acid 1% ?
O6 10% NaCl -
O7 16% NaCl ?
O8 Growth at pH=3 ?
O9 Growth at pH=9.5 ?
O10 Fluconazole ?
P1 Protocatechuic acid ?
P2 Vanillic acid ?
P3 Ferulic acid ?
P4 Veractric acid ?
P5 p-Hidroxybenzoic acid ?
P6 m-Hidroxybenzoic acid ?
P7 Gallic acid ?
P8 Salicylic acid ?
P9 Gentisic acid ?
P10 Catechol ?

2

Annex 2

EXPERT PANEL OPINION

The Generally Recognized as Safe (GRAS) Status of Desugared Fermented Orange Juice for General Use in Non-Alcoholic Beverages

1. Introduction

The undersigned, an independent panel of experts qualified by their scientific training and national and international experience to evaluate the safety of food and food ingredients (the "Expert Panel"), was specially convened by Sumol+Compal Marcas S.A. (hereinafter "S+C") to evaluate the safety and Generally Recognized as Safe (GRAS) status of the use as an ingredient in non-alcoholic carbonated and non-carbonated beverages of desugared fermented orange juice. For purposes of the Expert Panel's evaluation, safe or safety means that there is a reasonable certainty of no harm under the intended conditions of use of the ingredient in foods, as stated in 21 CFR §170.3(i).

Albert Bär Ph.D., of Bioresco Ltd. performed a comprehensive search of the scientific literature through April 29, 2016 relating to the safety of *Schizosaccharomyces pombe*, the yeast species that is used by S+C for the fermentation of orange juice, as well as the safety and historical use of traditionally fermented orange juice. Dr. Bär summarized the results of the literature search and prepared a safety dossier, "Desugared Fermented Orange Juice" (May 16, 2016) for consideration by the Expert Panel.

The Expert Panel (Drs. Borzelleca, Nicolosi, and Pariza) individually and collectively critically evaluated the safety dossier and other available data and information that the members of the Expert Panel believed to be pertinent to the safety of the intended use of desugared fermented orange juice. The dossier included a summary of information and data, both favorable and unfavorable, compiled from the search of the scientific literature, a description of the production process of desugared fermented orange juice, its specifications and stability, intake estimates under its intended conditions of use, and a safety assessment.

Following its critical evaluation of this data and information, the Expert Panel jointly and unanimously concluded that S+C's desugared fermented orange juice, manufactured consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food-grade specifications, is safe for use as an ingredient in non-alcoholic carbonated and non-carbonated beverages. The Expert Panel further concluded unanimously that the use as an ingredient in non-alcoholic carbonated and non-carbonated beverages of S+C's desugared fermented orange juice is Generally Recognized as Safe (GRAS) based on scientific procedures. It is also the unanimous opinion of this Expert Panel that other qualified experts would concur with these conclusions.

Summarized below are the data, information, and interpretive analysis supporting the Expert Panel's conclusions.

2. Summary And Basis For This GRAS Determination

Desugared fermented orange juice is produced under current GMP standards from regular orange juice intended for direct human consumption or orange juice intended for further manufacturing.[1]

In a first step of the production process, a sufficient amount of washed, viable *Schizosaccharomyces pombe* (strain PYCC 4197) biomass is added to the orange juice. Fermentation is then conducted at a temperature of about 25°C until the fermentation rate has dropped below a rate of -0.05 °Bx/30 min due to exhaustion of the sugars present in the juice and thus available for fermentation. The biomass is removed and the desugared orange juice is heat treated for pasteurization and dealcoholization. Since this step is associated with a volume decrease, mainly due to the evaporation of ethanol, water is then added to reconstitute the original volume of orange juice. According to S+C, the orange juice used as starting material as well as all ingredients used for the production of the *Schizosaccharomyces pombe* biomass are of food-grade purity and all materials that come in contact with the biomass and the orange juice before, during and after production of desugared, fermented orange juice are fit for use in a food producing process.

The specifications of desugared, fermented orange juice provide for a low sugars content ($\leq$ 2 g/L), a low ethanol content ($\leq$ 1.0%) and a standard microbiological purity.[2]

The safety of *Schizosaccharomyces pombe* biomass as such and of its use in the fermentation of orange juice is evidenced by the following:

(a) Its history of safe use in traditional fermented beverages for human consumption (Lindner, 1893; Jeffares et al., 2015; El-Tabey Shehata, 1960);

(b) Its more recent and/or current proposed or actual use in the production of wine (Benito et al., 2012, 2013, 2014, 2015, 2016; Ethiraj et al., 1983; Snow & Gallander, 1979; Proenol, 2016);

(c) The results of toxicity studies of an unrefined phytase preparation obtained from genetically modified *Schizo-saccharomyces pombe* (Ciofalo et al. 2003);

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[1] As defined in 21 CFR §146.135 and §146.15, respectively.

[2] The Expert Panel notes that desugared fermented orange juice is intended to be used as an ingredient of non-alcoholic beverages only, and not for direct human consumption as such.

(d) Its Qualified Presumption of Safety (QPS) status (EFSA, 2008).

The safety of orange juice fermented with *Schizosaccharomyces pombe* (or other microorganisms used traditionally for the fermentation of fruit juices) has not been examined in standard toxicity tests. There are no data that suggest or indicate that fermented orange juice and fermented grape juice would have a different safety profile. Like wine, there is a historical use for fermented orange juice. Recently, the production of orange wine has attracted interest in different countries (including the U.S., where a semi-sweet orange wine has been placed on the market) without reports of adverse effects upon consumption. This history of safe use provides additional evidence of safety.

3. Nutritional Considerations

Desugared fermented orange juice may substitute in certain applications for regular orange juice. Comparative data on the composition of orange juice and desugared fermented orange juice in terms of their content of ascorbic acid, carotenoids, flavonoids and folates indicate that the fermentation process does not adversely affect the nutritional value of orange juice. Only the content of total sugars and thus calories is reduced by about 98 and 87%, respectively. In view of the contribution that orange juice typically makes to the total daily energy and sugars intake, this difference has no adverse nutritional implications.

4. Conclusion

We, the undersigned Expert Panel members, have individually and collectively critically evaluated published and unpublished data and information pertinent to the safety of the use as an ingredient of non-alcoholic carbonated and non-carbonated beverages of S+C's desugared fermented orange juice, produced consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food-grade specifications, and unanimously conclude that such use is safe.

We further unanimously conclude that the use as an ingredient of non-alcoholic carbonated and non-carbonated beverages of S+C's desugared fermented orange juice, produced consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food-grade specifications, is Generally Recognized as Safe (GRAS) based on scientific procedures.

It is our unanimous opinion that other qualified experts would concur with these conclusions.

(b) (6)

B[redacted] 21 July 2016

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4. Conclusion

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It is our unanimous opinion that other qualified experts would concur with these conclusions.

By:

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4. <u>Conclusion</u>

We, the undersigned Expert Panel members, have individually and collectively critically evaluated published and unpublished data and information pertinent to the safety of the use as an ingredient of non-alcoholic carbonated and non-carbonated beverages of S+C's desugared fermented orange juice, produced consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food-grade specifications, and unanimously conclude that such use is safe.

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