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OPINION OF THE SCIENTIFIC PANEL

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Coffee C21 and protection of DNA from strand breaks: evaluation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006

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Abstract

Following an application from Tchibo GmbH submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Germany, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to Coffee C21 and protection of DNA from strand breaks. The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. The food proposed by the applicant as the subject of the health claim is Coffee C21. The Panel considers that Coffee C21, a coffee standardised by its concentration of caffeoylquinic acids (CQA), trigonelline and *N*-methylpyridinium (NMP), is sufficiently characterised in relation to the claimed effect. The Panel considers that the claimed effect, protection of DNA from strand breaks, is a beneficial physiological effect. Out of the two human intervention studies from which conclusion could be drawn, one study provides some evidence that daily consumption of Coffee C21 (750 mL/day) for 4 weeks decreases DNA strand breaks in habitual coffee drinkers after coffee withdrawal over the previous four weeks. However, the results of this study were not replicated in another study conducted under similar conditions in the same study centre. No studies performed in a different setting, from which conclusions could be drawn, were available. No evidence has been provided for a mechanism by which coffee (including Coffee C21) would reduce DNA damage in human cells by reducing DNA strand breaks. The Panel concludes that a cause and effect relationship has not been established between the consumption of Coffee C21 and protection of DNA from strand breaks.

Keywords: Coffee, C21, DNA damage, DNA strand break, health claim

Requestor: Competent Authority of Germany following an application by Tchibo GmbH

Question number: EFSA-Q-2019-00423

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Competing interests: A waiver was granted to an expert of the working group, Jean-Louis Bresson. Pursuant to Article 21(6) of the afore-mentioned Decision, the concerned expert was allowed to take part in the discussion and in the drafting phase of the scientific output.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1924/2006 harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of this Regulation lays down provisions for the addition of claims (other than those referring to the reduction of disease risk and to children's development and health), which are based on newly developed scientific evidence, or which include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

According to Article 18 of this Regulation, an application for inclusion in the Community list of permitted claims referred to in Article 13(3) shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

1.2. Interpretation of the Terms of Reference

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: Coffee C21 and protection of DNA from strand breaks.

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of Coffee C21, a positive assessment of its safety, nor a decision on whether Coffee C21 is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

2. Data and methodologies

2.1. Data

Information provided by the applicant

Food/constituent as stated by the applicant

According to the applicant, the food for which the health claim is made is 'Coffee C21, a blend of pure Arabica roast coffees (*Coffea arabica* L.) without any non-coffee ingredients. The roasting is accomplished with regular coffee manufacturing roasters by applying heat to the dry beans until the desired roast degree to provide the desirable composition is obtained. Coffee C21 (roasted) is defined by the following composition of key ingredients extractable by a standardised procedure mimicking usual household conditions: 10.18 mg/g of caffeoylquinic acids (CQA), 3.82 mg/g of trigonelline and 1.10 mg/g of N-methylpyridinium (NMP) (± 10%). Coffee C21 is consumed as a beverage'.

Health relationship as claimed by the applicant

According to the applicant, 'consumption of Coffee C21 leads to a reduction of the amount of spontaneous DNA strand breaks in white blood cells, which are measured by the comet assay'.

Mechanism by which the food/constituent could exert the claimed effect as proposed by the applicant

The applicant claims that 'intense roasting of coffee enhances the ability of coffee extracts to upregulate the nuclear factor erythroid 2-related factor 2 (Nrf2), a key positive regulator of gene expression of cell defence/cell repair genes. Dark roast coffee was found to be more efficient than light roast coffee in the activation of Nrf2'. Coffee C21 exerts its action by two pathways: a direct pathway – scavenging of free radicals by antioxidants in dark roast coffee, and an indirect pathway – activation of endogenous protective responses via modulation of gene expression'.

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wordings for the health claim: 'regular consumption of Coffee C21 contributes to the maintenance of DNA integrity in cells of the body'.

Specific conditions of use as proposed by the applicant

According to the applicant, the target population for the intended health claim is 'part of the general population, which drinks coffee'. The daily consumption of two to three large cups (in total 500–750 mL) over the day is recommended by the applicant to obtain the claimed effect.

Data provided by the applicant

The health claim application on Coffee C21 and protection of DNA from strand breaks pursuant to Article 13.5 of Regulation 1924/2006, was presented in a common and structured format as outlined in the Scientific and technical guidance for the preparation and presentation of applications for authorisation of health claims.

As outlined in the General guidance for stakeholders on health claim applications, it is the responsibility of the applicant to provide the totality of the available evidence.

2.2. Methodologies

The general approach of the NDA Panel for the evaluation of health claim applications is outlined in the EFSA General guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016).

The scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health are outlined in a specific EFSA guidance (EFSA NDA Panel, 2018).

The application does not contain data claimed as confidential or proprietary.

3. Assessment

In line with the General scientific guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016) in assessing each specific food/health relationship which forms the basis of a health claim the NDA Panel considers the following key questions:

i) the food/constituent is defined and characterised;
ii) the claimed effect is based on the essentiality of a nutrient; OR the claimed effect is defined and is a beneficial physiological effect for the target population, and can be measured *in vivo* in humans;
iii) a cause and effect relationship is established between the consumption of the food/constituent and the claimed effect (for the target group under the proposed conditions of use).

Each of these three questions needs to be assessed by the NDA Panel with a favourable outcome for a claim to be substantiated. In addition, an unfavourable outcome of the assessment of questions (i) and/or (ii) precludes the scientific assessment of question (iii).

3.1. Characterisation of the food/constituent

The food proposed by the applicant as the subject of the health claim is 'Coffee C21', a special blend of pure roast coffee beans – either ground or as intact beans – from different Arabica varieties (*Coffea arabica* L.), without any non-coffee ingredients. The beverage, coffee, is brewed from the ground coffee beans using hot water.

The roasting is carried out with regular industrial coffee roasters whereby heat is applied to dry beans. The process of roasting induces qualitative and quantitative changes in the chemical composition of the coffee beans.

Coffee C21 is standardised by the concentration of CQA, trigonelline and the thermal degradation product NMP.

CQA, trigonelline and NMP can be measured by established methods (Lang et al., 2013). The minimum amount of caffeoylquinic acids, trigonelline and NMP in ground Coffee C21 is 9.16 mg/g, 3.44 mg/g and 0.99 mg/g, respectively, as claimed by the applicant (EFSA NDA Panel, 2015). As the concentrations of CQA, trigonelline and NMP depend on the degree and the duration of roasting, the desired standardised composition of Coffee C21 is obtained by blending different Arabica roast coffees (i.e. prepared under different roasting conditions in terms of load, temperature and time). The applicant stipulates that the extraction efficiency from the ground coffee powder, when prepared with

a drip filter coffee machine and using a tap-water-to-coffee-powder ratio of 20:1, amounts to > 90% for CQA, trigonelline and NMP.

The Panel considers that the food, Coffee C21 – standardised by its content of CQA, trigonelline and NMP, which is the subject of the health claim, is sufficiently characterised in relation to the claimed effect. The Panel notes that different techniques of coffee brewing – but using the same ground coffee powder – may yield substantial differences in concentrations of CQA, trigonelline and NMP in the final coffee drink (Caprioli et al., 2015).

3.2. Relevance of the claimed effect to human health

The claimed effect proposed by the applicant is reduction of DNA damage by decreasing spontaneous DNA strand breaks. The proposed target population is 'part of general population, which drinks coffee'.

DNA strand breaks occur spontaneously during the DNA repair process but can also be induced by, e.g. environmental factors (such as mutagenic or pro-oxidant chemicals, radiation). Such DNA strand breaks alter DNA properties, may induce anomalies during DNA replication and translation and require repair for maintenance of cell functioning and survival. Direct measurements of DNA strand breaks can be obtained *in vivo* by using the traditional comet assay (single cell gel electrophoresis - SCGE) (EFSA NDA Panel, 2018).

The Panel considers that protection of DNA from strand breaks is a beneficial physiological effect.

3.3. Scientific substantiation of the claimed effect

A claim on Coffee C21 and reduction of DNA damage by decreasing spontaneous DNA strand breaks has already been assessed by the Panel with an unfavourable outcome (EFSA NDA Panel, 2015).

The applicant performed a literature search in PubMed using the following key words: 'Coffee AND DNA damage', 'Coffee AND DNA strand break(s)', 'Coffee AND Comet assay', 'Caffeine AND DNA damage AND trial', 'Chlorogenic acid AND DNA damage AND trial', 'Trigonelline AND DNA damage AND trial', 'Methylpyridinium AND DNA damage AND trial', 'Niacin AND DNA damage AND trial'. In addition, reference lists of relevant retrieved articles were searched manually.

Seven publications reporting on six human intervention studies were identified by the applicant as pertinent to the health claim (Misik et al., 2010; Bakuradze et al., 2015; Richling et al., unpublished study report, 2017; Shaposhnikov et al., 2018; Hochkogler et al., 2019; Pahlke et al., 2019; Schipp et al., 2019;). Two publications (Hochkogler et al., 2019; Pahlke et al., 2019) report on the same study.

The Panel notes that among the studies identified by the applicant, two studies (Misik et al., 2010; Shaposhnikov et al., 2018) assessed the effects of coffee types which did not comply with the specifications provided for Coffee C21. The Panel considers, therefore, that no conclusions can be drawn from these studies for the scientific substantiation of the claim.

Four placebo-controlled, randomised, single-blind, parallel studies (Bakuradze et al., 2015; Richling et al., 2017; Pahlke et al., 2019; Schipp et al., 2019) investigated the effect of Coffee C21 on DNA strand breaks using a similar design. The sample populations of the studies were healthy, non-smoking, habitual coffee drinkers accustomed to the required amount of coffee to be consumed during the study, comprising both males and females, except for the study by Bakuradze et al. (2015), in which only male subjects were recruited. Subjects were between 19 and 50 years of age and had a BMI between 19 and 32 kg/m^2. The studies had a parallel design and run-in periods of 4 weeks during which all subjects were asked to consume (warm) water in prespecified quantities and to avoid consuming coffee and caffeine-containing products and also foods rich in polyphenols, but otherwise maintained their usual diet. Compliance was assessed in all studies by measuring the NMP-to-creatinine ratio in spot urine samples. A concentration of > 0.2 nmol/μmol was taken as indicative of coffee consumption. In all studies coffee was prepared using a pad machine and the coffee powder complying with the specifications given in Section 3.1.

During the last 7 days of the run-in and the intervention periods, participants recorded their food intake. Blood samples were taken at the end of the run-in ('baseline') and at the end of the intervention period. The primary outcome in all studies was DNA strand breaks in peripheral white blood cells. DNA strand breaks were assessed by the comet assay (OECD, 2016) and the results expressed as tail intensities (TI%). Power calculations (80% power) were based on the studies by Bakuradze et al. (2011, 2015).

The study by Bakuradze et al. (2015) was already evaluated by the Panel in the previous assessment of the claim on Coffee C21 (EFSA NDA Panel, 2015). A total of 90 subjects were randomised (stratified by BMI and using a computer-generated randomisation list) into two groups (n = 45 per group) before the run-in period (750 mL/day water). The intervention group consumed 750 mL/day Coffee C21 without milk in three equal portions per day for 4 weeks; the control group drank equal volumes of water. Six subjects (three per group) dropped-out during the run-in period for private reasons. The statistical analyses were carried out in the population of completers (n = 42 per group).

At baseline, there was no statistically significant difference in the mean TI% between groups. During the 4-week intervention period, the mean ± SD TI% decreased in the intervention group and increased in the control group (from 0.32 ± 0.11 to 0.27 ± 0.09 TI% in the intervention group and from 0.31 ± 0.12 to 0.37 ± 0.15 TI% in the control group; i.e. mean difference at the end of the study ± SE −0.10 ± 0.03 TI%, analysis of covariance (ANCOVA) p = 0.0002).

Notwithstanding possible methodological limitations (e.g. randomisation before the run-in period; drop-outs during the run-in and statistical analysis on completers only), the Panel considers that this study provides some evidence that daily consumption of Coffee C21 (750 mL/day) for 4 weeks decreases DNA strand breaks in habitual coffee drinkers after coffee withdrawal over the previous 4 weeks.

In the study by Schipp et al. (2019), a total of 100 subjects were randomised before the run-in period (stratified by gender and BMI by using a computer-generated randomisation list) into two groups (n = 50 per group). The intervention group consumed 500 mL/day Coffee C21 without milk in four equal portions per day for 4 weeks; the control group drank equal volumes of warm water.

One subject in the intervention group dropped-out because of acute illness and one subject in the control group was excluded because of non-compliance with coffee restriction during the run-in period (NMP to creatinine ratio in urine samples > 0.2 nmol/μmol). In addition, for one subject in the intervention group the sample to be analysed with the comet assay was destroyed by accident prior to its evaluation. For the intention-to-treat (ITT) analysis, missing values were imputed by carrying forward the baseline observation. Results were presented for the ITT population (all randomised, n = 100) and the per protocol (PP) population (all compliant participants who finished the study with no missing end of trial outcomes, n = 97, n = 48 in the intervention and n = 49 in the control group).

At baseline, mean ± SD TI% were significantly higher in the intervention group versus the control group (1.08 ± 0.36 vs. 0.92 ± 0.34 TI%, p = 0.029, t-test). For the PP population (as reported in the unpublished study report provided to EFSA by the applicant), there was a statistically significant decrease in TI% in the intervention group and no significant changes in the control group resulting in mean ± SD TI% at the end of the study of 0.86 ± 0.28 vs. 0.92 ± 0.33 TI%, respectively. The difference between groups in absolute changes from baseline was statistically significant in the ITT (−0.22 TI%, no further details provided) and the PP population (mean difference ± SD −0.23 ± 0.49 TI%; p = 0.03, Wilcoxon Rank Sum Test (WRST)).

The Panel notes that the higher TI% in the intervention group compared with the control group at the end of the run-in period (i.e. baseline) has not been explained by the applicant. The Panel also notes that the statistical analysis presented by the applicant did not account for the imbalances that were observed at baseline, particularly since correlation analysis provided in the unpublished study report (provided to EFSA by the applicant) indicated that the magnitude of the reduction in TI% depended on the corresponding baseline value. It was observed that higher reductions in TI% were associated with higher baseline values. For this reason, subgroup analyses were presented in the unpublished study report in subjects with baseline TI% values above and below the overall baseline mean value. Based on the WRST analysis, a significant difference in TI% changes was observed between the intervention and control group in those individuals with baseline TI% below the overall mean, but not in those individuals with baseline TI% values above the mean. When EFSA proposed to take into account baseline values in the analysis, the applicant argued that based on a comparison of variances at baseline and at the end of the study in the intervention group, the impact of differences at baseline on the outcome was negligible in this study. The Panel considers that neither the presented information on the subgroup analyses nor on the comparison of variances allows to attribute differences in changes between groups to the actual intervention (Coffee C21) rather than to existing differences in TI% at baseline. Therefore, the Panel considers that no conclusions can be drawn from the study for the scientific substantiation of the claim.

Pahlke et al. (2019) randomised subjects after the run-in period (stratified by gender and BMI by using a computer-generated randomisation list) into two groups. The intervention group consumed 750 mL/day Coffee C21 without milk in three equal portions per day for 8 weeks; the control group drank equal volumes of warm water.

In the publication, it is reported that 96 subjects were randomised (n = 48 per group). In the unpublished study report, a number of 99 (n = 49 and 50) is reported in the text and 98 (n = 49 and 49) in the subject flow chart. This flow chart also indicates that two randomised subjects (one per group) dropped out during the run-in period. The Panel notes that the reporting is not consistent between the publication and the study report as well as between the text and the flow chart of the study report, both with respect to the number of subjects randomised and the timing of randomisation (before or after the run-in period).

One subject in the intervention group dropped out during the study. In the publication, it is reported that 10 subjects were excluded because of non-compliance, while in the corresponding unpublished study report (provided to EFSA by the applicant), the number was 9. The information provided in the study report is as follows. Seven subjects were excluded because of non-compliance to coffee restriction, i.e. four subjects during the run-in and three subjects in the control group during the intervention period. In the intervention group, two subjects were excluded because no NMP was detected in urine, which is indicative of no coffee consumption. The number of compliant subjects who finished the study was n = 42 in the intervention and n = 44 in the control group. The Panel notes that subjects who did not adhere to coffee restriction during the run-in phase were nonetheless randomised into the study but withdrawn thereafter from the analysis because of non-adherence to the protocol during the run-in period.

Among the set of samples analysed each day, one random sample was chosen and subjected to UV-B irradiation (as positive control) to verify the performance of the comet assay. For one of these random samples, the results for the positive control were not as required according to the protocol. As this indicated an issue with the performance of the comet assay, the whole sample set analysed on that day (n = 11, i.e. 5 in the intervention and 6 in the control group) was eliminated from the PP analysis. Finally, the PP population consisted of 37 participants in the intervention and 38 in the control group.

The results were presented for the ITT population (n = 96) and the PP population (all compliant participants who adhered to the protocol and had evaluable samples, n = 75; 78% of the ITT population). For ITT analysis, missing values were imputed by carrying forward the baseline observation.

At baseline in the PP population, there was no statistically significant difference in the mean TI% between the groups (mean ± SD: intervention vs. control: 0.53 ± 0.21 vs. 0.49 ± 0.22 TI%). At the end of the intervention period in the PP population, TI% was reduced on average by 0.15 TI% in the intervention and by 0.05 TI% in the control group (i.e. mean ± SD TI% at the end of the study: 0.38 ± 0.11 TI% and 0.44 ± 0.13 TI%, respectively). In the ITT population, TI% decreased by on average 0.02 TI% in the intervention group and increased by on average 0.10 TI% in the control group (i.e. 0.51 ± 0.21 and 0.48 ± 0.22 TI%, respectively, at baseline vs. 0.49 ± 0.35 and 0.58 ± 0.37 TI% at the end of the study).

The difference between groups in absolute changes from baseline was statistically significant both in the ITT (WRST, p = 0.047) and the PP (ANCOVA, p = 0.026) population. The choice of the statistical tests used was based on the Shapiro–Wilk test for normality. Following a request from EFSA to justify why different statistical tests were used for the ITT and the PP population considering that the assumption of normality relates to the distribution of the outcome variable in the population from which the sample is drawn and not to the distribution in the sample itself, the applicant argued that owing to the 'systematic selection process of excluding subjects with special behaviour' to obtain the PP population, the 'PP sample population and ITT sample population cannot in general be regarded as originating from and representing the same general population'. The Panel notes that, if the characteristics of the subjects included in and excluded from the PP analysis are not comparable, the principle of randomisation may not hold for the PP population. This would be supported by the observed differences between the ITT and the PP populations with respect to changes in TI% from baseline to the end of the study in the control group (i.e. increase by 0.10 TI% vs. decrease by 0.05 TI%, respectively), which suggests that subjects excluded from the PP analysis had different characteristics than subjects included in the PP analysis.

For the ITT analysis, baseline observations were carried forward to impute missing values and therefore changes from baseline will be zero for all imputed values. The Panel notes that if the same number of zero values are imputed in each group, these imputed values will have no impact on the result in statistical analyses based on differences in ranks (as is the case in the non-parametric WRST test), while they could have an impact on the results in analyses based on continuous variables (e.g. parametric ANCOVA).

The Panel considers that, owing to the important methodological limitations (i.e. number of subjects randomised unclear, exclusion of a substantial number of subjects from analysis, limitations of

the ITT analysis due to the method used to impute missing data and the subsequent approach towards statistical analysis, possible violation of the principle of randomisation in the PP population), no conclusions could be drawn from this study for the scientific substantiation of the claim.

In the study by Richling et al. (unpublished study report, 2017), that was conducted in the same study centre as the study by Bakuradze et al. (2015), a total of 99 subjects were randomised after the run-in phase (stratified by gender and BMI by using a computer-generated randomisation list) into two groups (n = 49 in the intervention and n = 50 in the control group). The intervention group consumed 750 mL/day Coffee C21 without milk in three equal portions per day for 4 weeks; the control group drank equal volumes of warm water.

Two subjects in the control group dropped out during the intervention. One subject in the intervention group was excluded from the PP analysis because of non-compliance to coffee abstention during the run-in period. Results were presented for the ITT population (all randomised, n = 99) and PP population (compliant participants who finished according to the protocol and who had evaluable samples, n = 96 (n = 48 per group)). For ITT analysis, missing values were imputed by carrying forward the baseline observation. The Panel notes that one subject who did not adhere to coffee restriction during the run-in phase was nonetheless randomised into the study but withdrawn thereafter from the analysis because of non-adherence to the protocol during the run-in period.

At baseline in the PP and ITT population, there was no statistically significant difference in the mean TI% between the groups (mean ± SD: 0.61 ± 0.12 and 0.62 ± 0.15 TI% in the intervention and the control group, respectively, both for the ITT and the PP population). At the end of the intervention period, there were no statistically significant differences in TI%, analysed by ANCOVA, between the intervention and the control group (i.e. mean ± SD TI% 0.63 ± 0.14 TI% and 0.67 ± 0.13 TI%, respectively), both in the ITT and the PP population.

The Panel considers that this study does not show an effect of daily consumption of Coffee C21 (750 mL/day) for 4 weeks on DNA strand breaks in habitual coffee drinkers after coffee withdrawal over the previous 4 weeks.

Overall, the Panel notes that out of the two studies from which conclusion could be drawn, one study (Bakuradze et al., 2015) provides some evidence that daily consumption of Coffee C21 (750 mL/day) for four weeks decreases DNA strand breaks in habitual coffee drinkers after coffee withdrawal over the previous four weeks. The statistically significant findings of this study were not replicated in another study (Richling et al., 2017) performed under similar conditions in the same study centre. The Panel also notes that no studies performed in a different setting, from which conclusions could be drawn, are available.

Studies on the mechanism of action as proposed by the applicant

The applicant claims that physiological effects of coffee consumption in humans may depend on the level of roasting because changes in composition occur during the roasting process.

The applicant proposes potential mechanistic pathways for dark roast coffee to exert an effect on DNA integrity, namely:

a) A direct pathway through scavenging of free radicals by antioxidants present in dark roast coffee and hence preventing oxidative damage to DNA strands.
b) Indirect pathways active through modulation of gene expression by coffee constituents leading to activation and upregulation of endogenous cellular DNA protective responses (mainly, but not exclusively, via activation of the nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant/electrophile response element (ARE/EpRE) system).

Regarding the direct pathway, the applicant noted that recent evidence from animal and human studies convincingly showed that plasma concentrations of coffee constituents with antioxidant capacity were far too low to explain efficient DNA protective radical scavenging. Therefore, this pathway was not considered relevant by the applicant in the context of this claim application.

In relation to the indirect pathway, the applicant cited the following studies that were conducted either with coffee as such or single coffee constituents: 3 human studies (Boettler et al., 2011a; Volz et al., 2012; Pahlke et al., 2019), 6 animal studies (Cavin et al., 2008; Paur et al., 2010; Balstad et al., 2011; Salomone et al., 2014; Vicente et al., 2014; Shi et al., 2018) and 11 *in vitro* studies (Bakuradze et al., 2010; Kalthoff et al., 2010; Paur et al., 2010; Boettler et al., 2011a,b; Volz et al., 2012; Sauer et al., 2013; Fratantonio et al., 2017; Jung et al., 2017; Priftis et al., 2018; Shen et al., 2018).

The applicant claimed – on the basis of human studies, animal studies and *in vitro* studies – that many phytochemicals in the human diet, including coffee constituents, can modulate the Kelch-like

ECH-associated protein 1 (Keap1)/Nrf2 system, leading to translocation of Nrf2 to the cell nucleus and upregulation of gene expression for antioxidant and other cell defensive pathways. The applicant further claimed – on the basis of the literature provided – that this is associated with better prevention or repair of DNA strand breaks (as determined by the comet assay), and that this is further confirmed by experimental Nrf2 knockdown studies in animals that showed abolishment of the DNA protective potential of phytochemicals.

As regards the many specific coffee constituents (and their potential alterations during processing of the coffee beans), the Panel notes that the literature on both the effects and the mechanisms of action of the different coffee constituents is incomplete, diverse and not consistent, and that inferences on the link with DNA integrity are in most instances reported in hypothetical and speculative terms. Indeed, none of the studies provided by the applicant unequivocally demonstrates that the proposed changes in cytosolic and nuclear concentrations of Nrf2, the expression and activity of ARE/EpRE-dependent enzymes, or any other cellular cascade triggered by coffee constituents, directly affects DNA strand breaks in humans and as such improves DNA integrity. In particular, the only human intervention study that investigated DNA strand breaks and Nrf2 translocation, which was provided by the applicant and is described above (Pahlke et al., 2019), did not allow conclusions to be drawn on the link between Nrf2 translocation and DNA strand breaks, owing to the methodological limitations of this study. The other human studies that were provided by the applicant (Boettler et al., 2011a; Volz et al., 2012), compared in one-arm sequential trials Nrf2-gene transcription levels in periods in which subjects refrained from coffee consumption and restricted the amounts of polyphenols in the diet with periods of coffee consumption, but did not investigate DNA strand breaks.

The Panel considers that the human, animal and *in vitro* studies submitted do not provide evidence for a mechanism by which coffee (including Coffee C21) could protect DNA from strand breaks.

Weighing of the evidence

In weighing the evidence, the Panel takes into account that out of the two human intervention studies, from which conclusion could be drawn, one study provides some evidence that daily consumption of Coffee C21 (750 mL/day) for 4 weeks decreases DNA strand breaks in habitual coffee drinkers after coffee withdrawal over the previous 4 weeks. However, the results of this study were not replicated in another study conducted under similar conditions in the same study centre. The Panel notes that no studies performed in a different setting, from which conclusions could be drawn, are available. The Panel also takes into account that no evidence has been provided for a mechanism by which coffee (including Coffee C21) would protect DNA from strand breaks.

The Panel concludes that a cause and effect relationship has not been established between the consumption of Coffee C21 standardised by its concentrations of CQA, trigonelline and NMP, and protection of DNA from strand breaks.

4. Conclusions

On the basis of the data presented, the Panel concludes that:

- The food/constituent, Coffee C21 standardised by its concentrations of CQA, trigonelline and NMP, which is the subject of the health claim, is sufficiently characterised in relation to the claimed effect.
- The claimed effect proposed by the applicant is reduction of DNA damage by decreasing spontaneous DNA strand breaks. The target population proposed by the applicant is 'part of general population which drinks coffee'. Protection of DNA from strand breaks is a beneficial physiological effect.
- A cause and effect relationship has not been established between the consumption of Coffee C21 and protection of DNA from strand breaks.

Documentation as provided to EFSA

Health claim application on Coffee C21 and protection of DNA from strand breaks pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0487_DE). Submitted by Tchibo GmbH, Überseering 18, D-22297 Hamburg, Germany.

Steps taken by EFSA

1) This application was received by EFSA on 1/7/2019.

2) The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence pursuant to Article 13(5) of Regulation (EC) No 1924/2006.
3) The scientific evaluation procedure started on 12/8/2019.
4) On 10/9/2019, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was suspended on 24/9/2019 and was restarted on 9/10/2019, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
5) On 29/10/2019, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was suspended on 25/11/2019 and was restarted on 5/12/2019, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
6) During its meeting on 25/2/2020, the NDA Panel, having evaluated the data, adopted an opinion on the scientific substantiation of a health claim related to Coffee C21 and reduction of DNA damage by decreasing spontaneous DNA strand breaks.

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Abbreviations

ANCOVA	analysis of covariance
ARE/EpRE	antioxidant/electrophile response element
BMI	body mass index
CQA	caffeoylquinic acids
ITT	intention-to-treat
Keap 1	Kelch-like ECH-associated protein 1
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
NMP	*N*-methylpyridinium
Nrf2	nuclear factor erythroid 2-related factor 2
OECD	Organisation for Economic Co-operation and Development
PP	Per Protocol
SCGE	single-cell gel electrophoresis

SD	Standard Deviation
TI	tail Intensity
UV	ultraviolet
WRST	Wilcoxon rank sum test