



Scientific Opinion on the substantiation of a health claim related to coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and N-methylpyridinium, and reduction of DNA damage by decreasing spontaneous DNA strand breaks

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SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and *N*-methylpyridinium, and reduction of DNA damage by decreasing spontaneous DNA strand breaks pursuant to Article 13(5) of Regulation (EC) No 1924/2006¹

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

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 Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver 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. The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. Coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and *N*-methylpyridinium (NMP), which is the subject of the health claim, is sufficiently characterised. Reduction of DNA damage by decreasing spontaneous DNA strand breaks is a beneficial physiological effect. In weighing the evidence, the Panel took into account that one human intervention study showed that daily consumption of coffee C21 (750 ml/day) for four weeks decreased spontaneous DNA strand breaks in habitual coffee drinkers after coffee withdrawal over the previous four weeks, but that no other human studies in which these results have been replicated were provided, and that no evidence was provided for a mechanism by which coffee (including coffee C21) could exert the claimed effect. The Panel concludes that a cause and effect relationship has not been established between the consumption of coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and NMP, and a reduction of DNA damage by decreasing spontaneous DNA strand breaks.

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KEY WORDS

coffee, C21, DNA damage, DNA strand break, health claims

¹ On request from the Competent Authority of Germany following an application by Tchibo GmbH, Question No EFSA-Q-2014-00624, adopted on 22 April 2015.

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SUMMARY

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 Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and *N*-methylpyridinium (NMP), and reduction of DNA damage by decreasing spontaneous DNA strand breaks.

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.

The food that is the subject of the health claim is coffee C21. Coffee C21 is a blend of roasted coffee arabica (*Coffea arabica* L.) standardised by its content of caffeoylquinic acids, trigonelline and NMP. Caffeoylquinic acids, trigonelline and NMP can be measured in ground and brewed coffee by established methods. The Panel considers that the food, coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and NMP, is sufficiently characterised.

The claimed effect proposed by the applicant is ‘reduction of the amount of spontaneous DNA strand breaks in white blood cells’. The target population proposed by the applicant is the general population. Spontaneous DNA strand breaks normally occur during the DNA repair process. DNA strand break, which may also be induced by genetic or environmental factors, is a type of DNA damage which can be measured by the comet assay (single-cell gel electrophoresis). The Panel considers that the reduction of DNA damage by decreasing spontaneous DNA strand breaks is a beneficial physiological effect.

The applicant identified six human intervention studies as being pertinent to the health claim.

These studies (except for one) were carried out with coffee types which did not comply with the specifications of coffee C21, which is standardised by its content of caffeoylquinic acids, trigonelline and NMP, were uncontrolled or did not report on spontaneous DNA strand breaks. The Panel considers that no conclusions can be drawn from these five studies for the scientific substantiation of the claim.

One placebo-controlled, randomised, single-blind, parallel study investigated the effect of consuming coffee C21 on spontaneous DNA strand breaks in healthy non-smoking males, who were accustomed to a daily coffee consumption level similar to that being investigated in this study. A total of 90 subjects were randomised (stratified by body mass index) in two groups. In the first four-week run-in period, all participants refrained from consuming coffee and instead consumed water. During the following four weeks participants consumed either coffee C21 (n=45; 45 g coffee/750 ml per day) or the same volume of water (n=45). The investigator was unaware of the treatment group allocation.

Difference in spontaneous DNA strand break changes in peripheral white blood cells between groups was the primary outcome of this study.

At baseline (i.e. after the run-in period), there was no difference in spontaneous DNA strand breaks between the groups. During the four-week intervention period spontaneous DNA strand breaks decreased in the intervention group and increased in the control group.

The Panel considers that this study shows that daily consumption of coffee C21 (750 ml/day) for four weeks decreases spontaneous DNA strand breaks in habitual coffee drinkers after coffee withdrawal over the previous four weeks.

The applicant provided two animal and two *in vitro* studies in support of a mechanism by which coffee C21 could exert the claimed effect. The Panel considers that these studies do not provide

evidence for a mechanism by which coffee C21 could reduce DNA damage by decreasing spontaneous DNA strand breaks.

In weighing the evidence, the Panel took into account that one human intervention study showed that daily consumption of coffee C21 (750 ml/day) for four weeks decreased spontaneous DNA strand breaks in habitual coffee drinkers after coffee withdrawal over the previous four weeks, but that no other human studies in which these results have been replicated were provided, and that no evidence was provided for a mechanism by which coffee (including coffee C21) could exert the claimed effect.

The Panel concludes that a cause and effect relationship has not been established between the consumption of coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and NMP, and a reduction of DNA damage by decreasing spontaneous DNA strand breaks.

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BACKGROUND

Regulation (EC) No 1924/2006⁴ 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).

STEPS TAKEN BY EFSA

- The application was received on 12/09/2014.
- The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.
- On 20/10/2014, during the validation process of the application, EFSA sent a request to the applicant to provide missing information.
- On 30/10/2014, EFSA received the missing information as submitted by the applicant.
- The scientific evaluation procedure started on 06/11/2014.
- On 26/11/2014, 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 clock was stopped on 05/12/2014 in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
- On 19/12/2014, EFSA received the applicant's reply and the clock was re-started.
- During its meeting on 22/04/2015, the NDA Panel, having evaluated the data submitted, adopted an opinion on the scientific substantiation of a health claim related to coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and *N*-methylpyridinium, and reduction of DNA damage by decreasing spontaneous DNA strand breaks.

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, a coffee standardised by its content of caffeoylquinic acids, trigonelline and *N*-methylpyridinium, and reduction of DNA damage by decreasing spontaneous DNA strand breaks.

⁴ Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

EFSA DISCLAIMER

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.

INFORMATION PROVIDED BY THE APPLICANT

Applicant's name and address

Tchibo GmbH, Überseering 18, D-22297 Hamburg, Germany.

Food/constituent as stated by the applicant

According to the applicant, the food for which the claim is made is coffee C21, a special blend of roasted pure arabica coffee (*Coffea arabica* L.) without any non-coffee ingredients, which is standardised by its content of caffeoylquinic acids (chlorogenic acids), *N*-methylpyridinium and trigonelline.

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.

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording 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

The target population proposed by the applicant is the general population.

The conditions of use proposed by the applicant are three large cups of coffee C21 (each 250 ml, prepared from two pads of 7.5 g ground coffee) per day.

ASSESSMENT

1. Characterisation of the food/constituent

The food that is the subject of the health claim is coffee C21.

Brewed coffee is a mixture of compounds, including coffee constituents, such as caffeine, caffeoylquinic acids and trigonelline, together with compounds formed during roasting, such as *N*-methylpyridinium (NMP), nicotinic acid, nicotinamide and melanoidins (Lang et al., 2008).

Coffee C21 is a blend of roasted coffee arabica (*Coffea arabica* L.). The roasting is accomplished with regular coffee manufacturing roasters which apply heat to dry beans. Roasted ground coffee (coffee C21) is standardised by its concentrations of chlorogenic acids (also called caffeoylquinic acids, polyphenols), trigonelline (alkaloid) and NMP (pyridine derivative). The concentrations of chlorogenic acids, trigonelline and the thermal degradation product NMP depend on the degree of roasting. The desired composition is obtained by blending different coffee roasts and by adjusting the roasting conditions (temperature and time). A decline of 6.6 % in caffeoylquinic acids was reported during 45 months of storage at room temperature.

The applicant indicated that ground coffee C21 contains 10.18 mg/g of caffeoylquinic acids, 3.82 mg/g of trigonelline and 1.10 mg/g of NMP. Upon a request by EFSA for clarification of the standardisation of coffee C21, the applicant indicated the minimum amount of caffeoylquinic acids, trigonelline and NMP in ground coffee C21 (9.16 mg/g, 3.44 mg/g and 0.99 mg/g, respectively).

According to the applicant, coffee C21 is prepared with a standard drip filter coffee machine, using a 20:1 (w/w) ratio of tap water to ground coffee, which results in a > 90 % extraction of caffeoylquinic acids, trigonelline and NMP.

Caffeoylquinic acids, trigonelline and NMP can be measured in ground and brewed coffee by established methods.

The Panel considers that the food, coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and NMP, which is the subject of the health claim, is sufficiently characterised.

2. Relevance of the claimed effect to human health

The claimed effect proposed by the applicant is ‘reduction of the amount of spontaneous DNA strand breaks in white blood cells’. The target population proposed by the applicant is the general population.

Spontaneous DNA strand breaks normally occur during the DNA repair process. DNA strand breaks may also be induced by genetic or environmental factors (e.g. mutagenic or pro-oxidant chemicals and radiation). Such DNA strand breaks alter DNA properties, may induce anomalies during DNA replication and translation, and require repair for the maintenance of cell functioning and survival.

A DNA strand break is a type of DNA damage which can be measured by the comet assay (single-cell gel electrophoresis) (EFSA NDA Panel, 2011).

The Panel considers that the reduction of DNA damage by decreasing spontaneous DNA strand breaks is a beneficial physiological effect.

3. Scientific substantiation of the claimed effect

The applicant performed a literature search in PubMed with the following terms: ‘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’, ‘methyl pyridinium and DNA damage and trial’ and ‘niacin and DNA damage and trial’.

The applicant identified six human intervention studies as being pertinent to the health claim.

These studies (except for one) were carried out with coffee types which did not comply with the specifications of coffee C21, which is standardised by its content of caffeoylquinic acids, trigonelline and NMP (Steinkellner et al., 2005; Bichler et al., 2007; Hoelzl et al., 2010; Misik et al., 2010), were uncontrolled (Steinkellner et al., 2005; Bichler et al., 2007; Bakuradze et al., 2011) or did not report on spontaneous DNA strand breaks (Steinkellner et al., 2005; Hoelzl et al., 2010). The Panel considers that no conclusions can be drawn from these five studies for the scientific substantiation of the claim.

The placebo-controlled, randomised, single-blind, parallel study by Bakuradze et al. (2015) investigated the effect of consuming coffee C21 on spontaneous DNA strand breaks in healthy non-smoking males (aged 19-50 years; body mass index (BMI) 19-32 kg/m²), who were accustomed to a daily coffee consumption level similar to that being investigated in this study (i.e. 45 g/750 ml). A total of 90 subjects were randomised (stratified by BMI) in two groups. In the first four-week run-in period, all participants refrained from consuming coffee and instead consumed water (750 ml). During the following four weeks participants consumed either coffee C21 (n=45; 45 g coffee/750 ml per day) or the same volume of water (n=45). Upon a request by EFSA for clarification of the caffeine consumption, the applicant indicated that the consumption of coffee C21 led to a daily ingestion of 531 mg of caffeine in the intervention group. The investigator was unaware of the treatment group

allocation. During the run-in and the intervention periods participants were instructed to consume their usual diet and to avoid consuming coffee and caffeine-containing products and foods rich in polyphenols. Participants recorded their food intake in the final seven days of each study period. At the end of the run-in and intervention periods blood samples were taken for determination of spontaneous DNA strand breaks through the comet assay. At those visits urine samples and body weight measurements were also taken.

Difference in spontaneous DNA strand break changes in peripheral white blood cells between groups was the primary outcome of this study. The sample size (target significance level $\alpha = 5\%$, at a power of 80%) was calculated based on a previous human intervention study, which also used the comet assay (Bakuradze et al., 2011).

Six participants withdrew from the study ($n=3$ in each group) owing to 'private' reasons. Compliance was assessed by monitoring trigonelline and NMP in urine. Upon a request by EFSA for clarification, the applicant indicated that these six participants were not included in the statistical analyses as they withdrew from the study in the run-in period (i.e. before the collection of baseline values).

Daily energy and macronutrient intake during the run-in and the intervention periods were not different between the groups. No changes in body weight were observed between the groups during the whole study period.

An analysis of covariance (ANCOVA) test, with baseline data as covariate, was used to assess the difference in spontaneous DNA strand break changes between groups. Additionally, the Wilcoxon rank sum test was used to cross-check the ANCOVA results.

The results of comet assays were presented as means, with standard deviations, of spontaneous DNA strand breaks, expressed as tail intensities (TI %). At baseline (i.e. after the run-in period), there was no difference in the mean of spontaneous DNA strand breaks between the groups. During the four-week intervention period the mean of spontaneous DNA strand breaks 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; ANCOVA, $p = 0.0002$; Wilcoxon rank sum test, $p = 0.0005$).

The Panel considers that this study shows that daily consumption of coffee C21 (750 ml/day) for four weeks decreases spontaneous DNA strand breaks in habitual coffee drinkers after coffee withdrawal over the previous four weeks.

Mechanism by which the food could exert the claimed effect

The applicant indicated that the effect of coffee C21 on the decrease of spontaneous DNA strand breaks was mainly exerted by NMP, the major alkylpyridinium compound in roasted coffee. According to the applicant, NMP would protect against the formation of DNA strand breaks by inducing the activity of enzymes involved in the cellular defence against oxidative damage and 'other stress' by inducing the nuclear translocation of the transcription factor nuclear-factor-E2-related factor 2 (Nrf2) and thereby regulating antioxidant response element (ARE)/electrophile response element (EpRE) pathways.

The applicant provided two animal (Paur et al., 2010; Vicente et al., 2014) and two *in vitro* studies (Bakuradze et al., 2010; Boettler et al., 2011) in support of a mechanism by which coffee C21 could exert the claimed effect.

These studies investigated the effect of different coffee brews/extracts (different from coffee C21) on cytosolic and nuclear concentrations of Nrf2, and on the expression and activity of ARE/EpRE-dependent enzymes. The results on the effect of NMP on the expression of ARE-dependent enzymes in the two *in vitro* studies were inconsistent. The Panel notes that none of the

studies provided addressed whether the proposed changes in cytosolic and nuclear concentrations of Nrf2, or the expression and activity of ARE/EpRE-dependent enzymes, would affect DNA strand breaks.

The Panel considers that the animal and *in vitro* studies do not provide evidence for a mechanism by which coffee C21 could reduce DNA damage by decreasing spontaneous DNA strand breaks.

Weighing the evidence

In weighing the evidence, the Panel took into account that one human intervention study showed that daily consumption of coffee C21 (750 ml/day) for four weeks decreased spontaneous DNA strand breaks in habitual coffee drinkers after coffee withdrawal over the previous four weeks, but that no other human studies in which these results have been replicated were provided, and that no evidence was provided for a mechanism by which coffee (including coffee C21) could exert the claimed effect.

The Panel concludes that a cause and effect relationship has not been established between the consumption of coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and NMP, and a reduction of DNA damage by decreasing spontaneous DNA strand breaks.

CONCLUSIONS

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

- The food, coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and NMP, which is the subject of the health claim, is sufficiently characterised.
- The claimed effect proposed by the applicant is ‘reduction of the amount of spontaneous DNA strand breaks in white blood cells’. The target population proposed by the applicant is the general population. A reduction of DNA damage by decreasing spontaneous DNA strand breaks is a beneficial physiological effect.
- A cause and effect relationship has not been established between the consumption of coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and NMP, and a reduction of DNA damage by decreasing spontaneous DNA strand breaks.

DOCUMENTATION PROVIDED TO EFSA

1. Health claim application on coffee C21 and reduction of DNA damage by decreasing spontaneous DNA strand breaks pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0428_DE). September 2014. Submitted by Tchibo GmbH.

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ABBREVIATIONS

ANCOVA	analysis of covariance
ARE	antioxidant response elements
BMI	body mass index
DNA	deoxyribonucleic acid
EpRE	electrophile response element
NMP	<i>N</i> -methylpyridinium
Nrf2	nuclear-factor-E2-related factor 2
TI %	tail intensity