

Protective Effects of Tocotrienol-rich fraction against Urban Air Pollution

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ABSTRACT

Aim: In the present study, we evaluated the protective effects of tocotrienol-rich fraction (TRF) on PM2.5-induced skin cell damages *in vitro*. Consumer trends are moving towards taking functional food and beverages over supplements to support wellness. To ensure that TRF could be successfully incorporated into functional food and beverages, we assessed if the use of a water-dispersible powder TRF (DVP 30-WD) in different consumer products led to perceived sensorial differences.

Materials & Methods: Human dermal fibroblast (NHDF) cells were treated with PM2.5 in the presence or absence of TRF. Oxidative stress level, inflammation status and skin barrier function were assessed after the treatments. A Triangle Taste Test was carried out in accordance with the ASTM Standard Test Method for Sensory Analysis—Triangle Test (ASTM E1885).

Result: TRF inhibited PM2.5-induced reactive oxygen species (ROS) generation in NHDFs. In addition, TRF had a significant anti-inflammatory effect, as evidenced by the significant attenuation of PM2.5-induced up-regulation of cyclooxygenase-2 (COX-2). TRF showed to restore the skin barrier function after the exposure of PM2.5, according to the increased level of filaggrin protein expression. In the Triangle taste test, a total of 10 panellists participated in the study. Samples (milk, yogurt, orange juice, carbonated soda and cultured milk drink) using a water-dispersible powder format of TRF (DVP 30-WD) were indistinguishable in terms of taste from the original drinks, indicating that DVP 30-WD is organoleptically acceptable.

Conclusion: TRF may potentially be used as an active ingredient in functional food and beverages against pollutant-induced skin damages.

METHODOLOGY

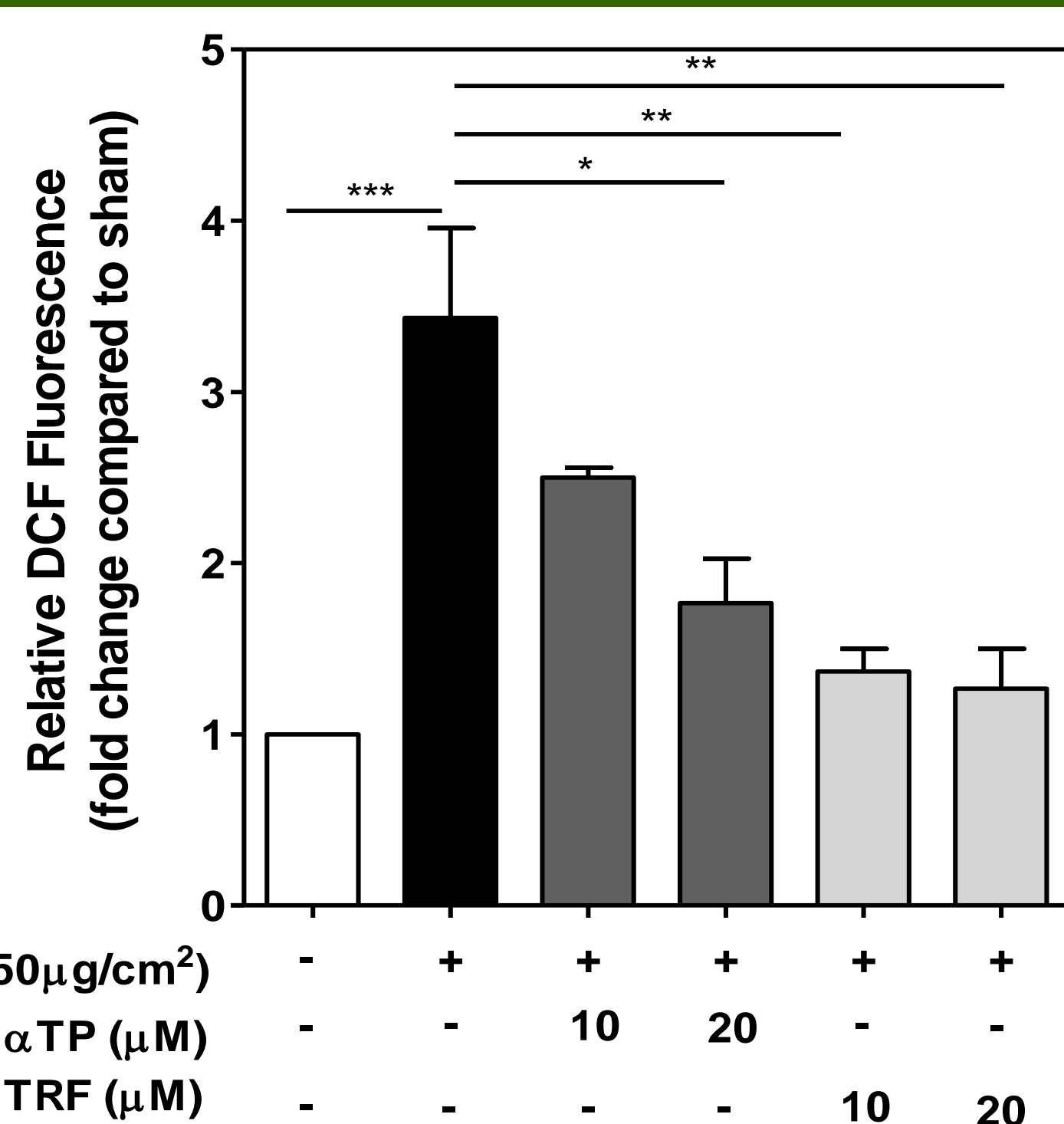
in vitro

The immortalized human dermal fibroblast cell (NHDF) cells was used to demonstrate the ability of TRF to alleviate air pollution via attenuation of oxidative stress, inflammation and impairment of skin barrier.

The Triangle Taste Test was done in accordance with the ASTM Standard Test Method for Sensory Analysis—Triangle Test (ASTM E1885). Assessors received a set of three samples and were informed that two of the samples were alike and that one was different (i.e. contains DVP 30-WD at GRAS-recommended levels). The assessors reported which samples they believed to be different. The number of correct responses were counted and the significance was determined by reference to a statistical table (corresponding to the number of assessors at $\alpha = 0.05$). In this triangle taste test, up to 10 panellists were used for each category of every day consumer products (e.g. milk, yoghurt, orange juice, carbonate drinks and cultured milk drinks).

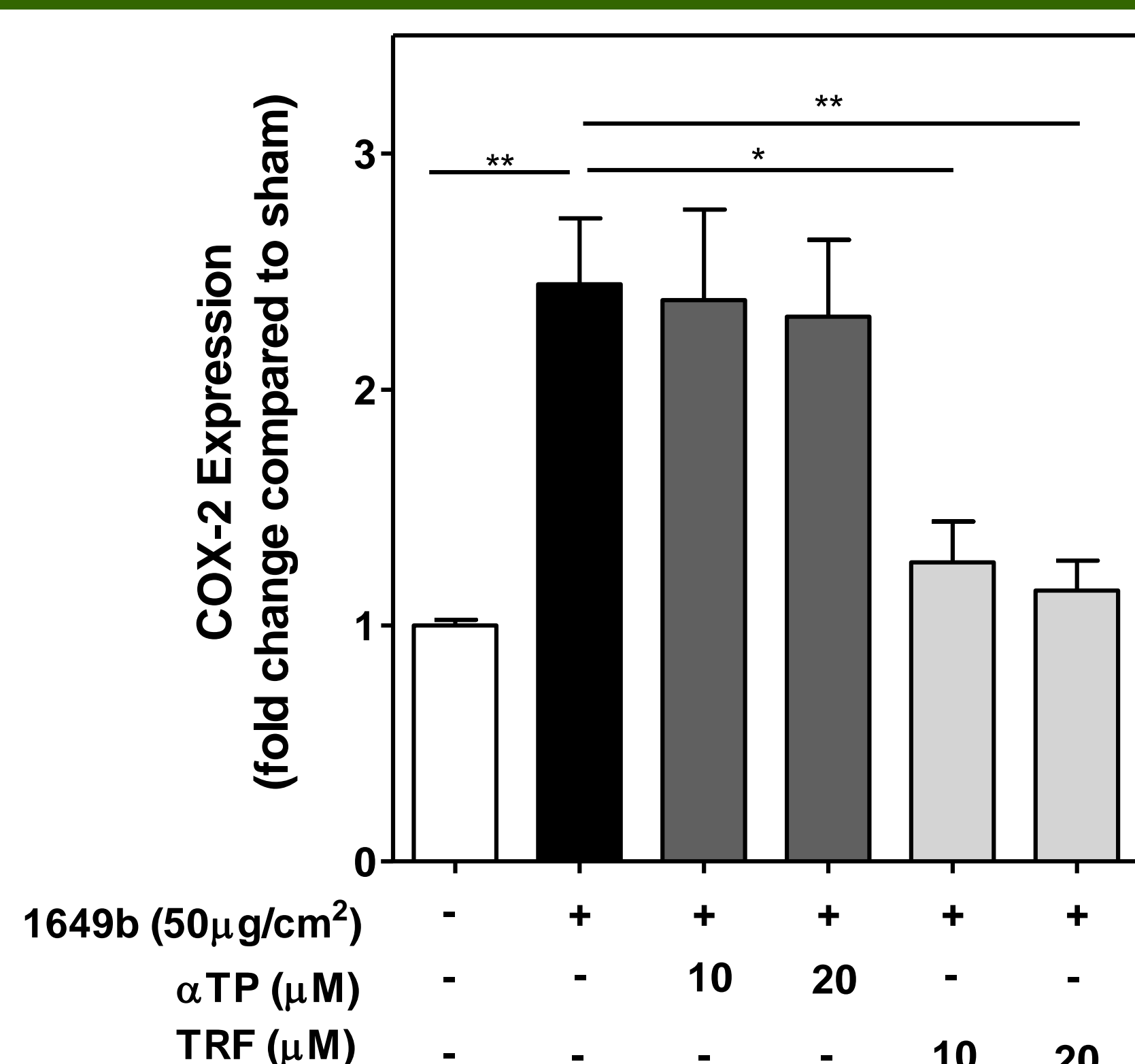
Triangle Taste Test

RESULT: Oxidative stress *in vitro*



When NHDFs cells were treated with either 20 µM TRF or αTP for 24 hours, a significant reduction in PM2.5-induced intracellular oxidative stress was observed due to their antioxidative biological activities. The data was evidenced by DCFHDA assay. However, when a lower concentration of treatment was used (10µM) the data showed that TRF was able to suppress the free radicals that generated by PM2.5 but not αTP.

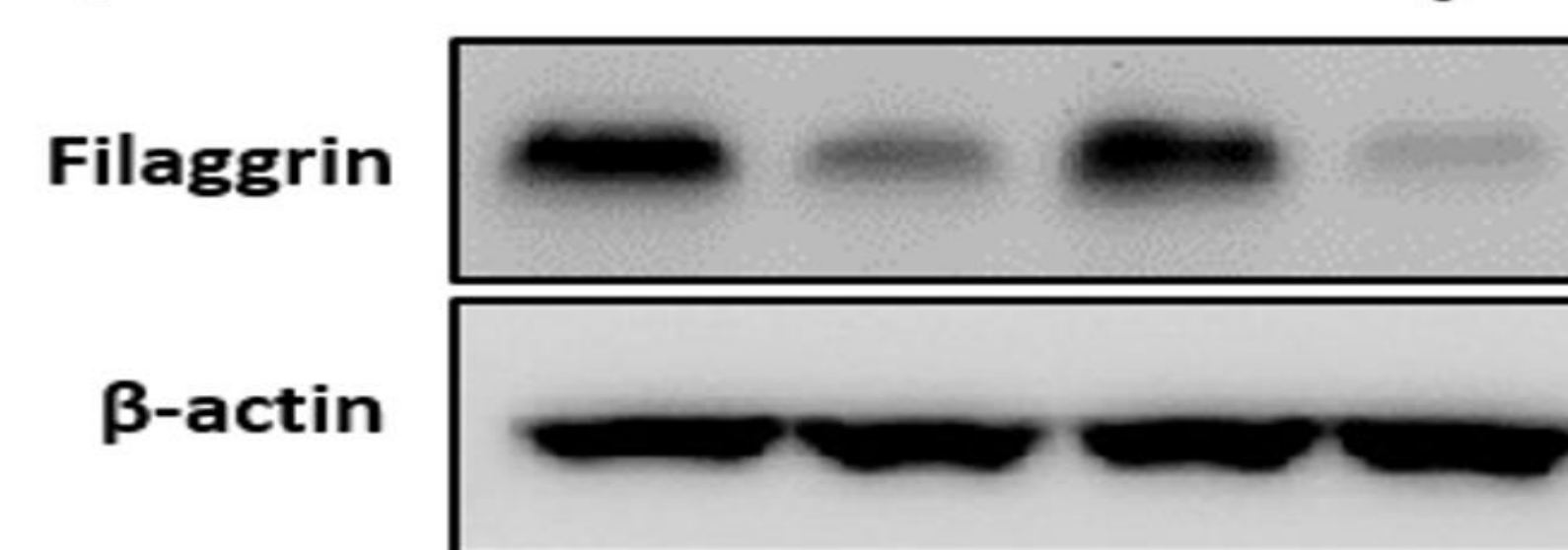
RESULT: Inflammation *in vitro*



When NHDFs cells were treated with either 10 µM or 20 µM TRF for 24 hours, only TRF showed a significant reduction in PM2.5-induced inflammation as evidenced by Cxclloxygenase-2 (COX-2) protein activity. Treatment with αTP in dose dependent did not show amelioration of inflammation.

RESULT: Skin barrier function *in vitro*

1649b (50µg/cm ²)	-	+	+	+
20µM TRF	-	-	+	-
20µM αTP	-	-	-	+



This western blotting analysis showed TRF was able to increase the protein expression of Filaggrin upon the PM2.5 stimulation but not for αTP which treated at the same concentration.

RESULT: Triangle Taste Test

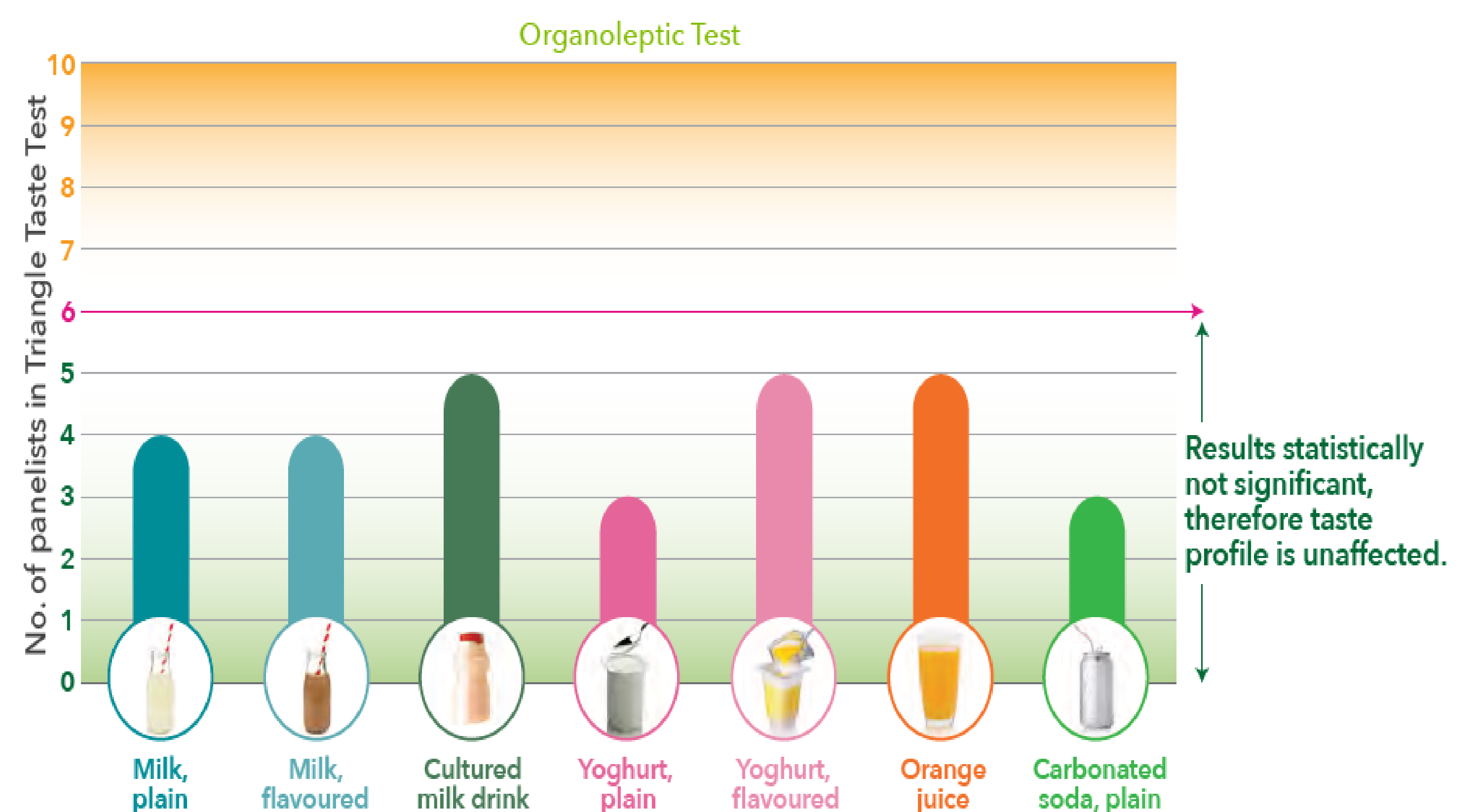


Figure 4: Triangle taste test results after the incorporation of DVP-30 WD into various beverages and yoghurt. The minimum significant number of correct judgements is above 6. DVP-30 WD is organoleptically acceptable.

Triangle taste test results showed that the number of correct judgements for each food or beverage category was below the minimum significant number required ($\alpha = 0.05$) to conclude that a perceptible difference exists between the samples. Therefore, the addition of DVP 30-WD at GRAS-recommended levels is organoleptically acceptable.

CONCLUSION

Tocotrienol-rich fraction

In vitro: Reduces oxidative stress and inflammation;
Protects skin barrier

Human study: Organoleptically acceptable