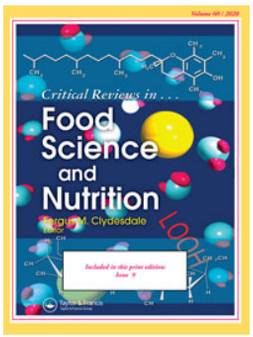

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REVIEW

Evidence of antiplatelet aggregation effects from the consumption of tomato products, according to EFSA health claim requirements

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ABSTRACT

The beneficial effect of fresh tomatoes or processed tomato products on platelet aggregation depends on the presence of bioactive compounds in these products, in sufficient quantities, to produce a relevant physiological effect, when consumed as part of a normal diet. This work is focused on reviewing the development on tomato products bioactive compounds, particularly with reference to its potential biological activity with beneficial effect on the prevention of platelet aggregation.

The most relevant studies found show that all bioactive compounds found in Water-soluble tomato concentrate are in tomato fruit and other tomato products, and there is enough evidence of their beneficial effects. According to the European Food Safety Authority requirements, further intervention studies (human clinical trials) using valid markers should be performed in order to demonstrate the beneficial effects of tomato products as consumer products (paste, puree, sauce or juice) on platelet aggregation. Our PubMed review results support the development of promising nutritional strategies involving tomatoes and tomato products to tackle cardiovascular disease as antiplatelet aggregation.

KEYWORDS

tomato; health claims; platelet and antiplatelet

Introduction

Cardiovascular disease (CVD) is considered a serious global public health problem. According to World Health Organization (WHO) statistics, at present, CVD is the leading cause of death in the world with 17.7 million people deaths every year. Even though in Europe CVD global mortality has fallen considerably over recent decades, it remains the major cause of premature death (WHO 2017). The WHO defines CVD as a group of disorders in the heart and blood vessels that includes coronary heart disease (heart attacks), cerebrovascular diseases (stroke), hypertension, peripheral vasculopathies, rheumatic heart disease, congenital heart disease and heart failure. These disorders originate from a chronic inflammatory vascular process that ends up producing endothelial dysfunction, arteriosclerosis. This systemic disease is stimulated by numerous factors, with high plasma concentrations of low-density lipoprotein cholesterol (LDL-c) being the main cause (Rafieian-Kopaei et al. 2014). Cholesterol, a necessary component for the proper functioning of the organisms, is transported by LDL to the tissues. LDL have high affinity for connective tissue proteins in the walls of the arteries, where they can be oxidized. Modified LDL act as cytotoxic agents for the endothelium and chemoattractant for monocytes, which become macrophages when they reach the intima layer (Pentikäinen et al. 2000). Activated macrophages recognize oxidized LDL-c and phagocyte them. These lipoproteins tend to accumulate in the cytoplasm, transforming into foam cells and originating the atheroma plaque. Thus, endothelial dysfunction allows increased platelet adhesion, which is the first step in platelet activation.

Platelets play an important role in CVD. Platelet hyperaggregability is associated with the risk factors of coronary artery disease (e.g. smoking, hypertension, and hypercholesterolemia) (Willoughby, Holmes, and Loscalzo 2002). In order to reduce cardiovascular (CV) risks, it is important to use strategies that covers the entire population, acting on risk factors such as an unhealthy diet, physical inactivity, smoking and excessive alcohol consumption. These risk factors can lead to hypertension, hyperglycemia, hyperlipidemia, and overweight or obesity, which are indications to increased risk of CVD. In fact, dietary risks are responsible for approximately half of the death and disability caused by CVD, with a cost of €102 billion/year in European Union (EU) (European Heart Network 2017). According to World Heart Federation (WHF), a diet high in fresh fruits and vegetables protects the heart, whereas a low fruit and vegetable intake accounts for about 20% of CVD worldwide. A 73% reduction in the risks of new major cardiac events has been shown by taking a diet low in saturated fats and with plenty

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of fresh fruits and vegetables in comparison with a typical diet of people living in the high-income countries (WHF 2017).

Although fruits and vegetables contain components that protect against heart diseases and stroke, it is important to note that the benefits of diet are not limited to its nutrient content; it also has to provide other protective factors contained especially in food of plant origin, so-called 'bioactive compounds'. Bioactive compounds are components of food that influence cellular activity and physiological mechanisms and have beneficial effects on health, and may have varied properties, structures and functions. The beneficial effects of a diet rich in fruits and vegetables cannot be attributed to a single compound or mixture of compounds, but to the synergistic effect of all of them, in order to obtain health beneficial effect which depends on the amount of bioactive compounds intake and its variety. Evidence shows that some fruits bioactive compounds are useful in reducing CVD risk factors (Alissa and Ferns 2017; Bonnefoy, Drai, and Kostka 2002; Liu et al. 2000; Torres-Urrutia et al. 2011).

Several studies suggest that bioactive compounds present in foods as tomato products have potential benefits against CVD (Cámara, Fernández-Ruiz, Cámara, et al. 2019). The content and bioavailability of bioactive compounds are highly variable in tomatoes depending on its varieties and type of use: fresh or processed as tomato paste, puree, juice, soup, sauce or ketchup (Fernández-Ruiz et al. 2004; García-Valverde et al. 2013; Hallmann et al. 2013; Mozos et al. 2018).

Consumption of tomato products attenuates postprandial oxidative stress induced by lipemia and associated inflammatory response, which demonstrates a protective role of tomato against CVD (Burton-Freeman et al. 2012). Hsu et al. (2008) reported that tomato paste exerts antioxidant and lipid-lowering effects in hamsters due to the increased activities of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase), the reduction of total and LDL cholesterol and the increase of HDL cholesterol respectively. Li et al. (2015) demonstrated the reduction of serum cholesterol and inflammatory adipokine levels in 25 healthy young females (20–30 years old) by daily consumption of 280 ml of tomato juice (containing 32.5 mg of lycopene) for 2 months. According to Xaplanteris et al. (2012), daily tomato paste consumption exerts a beneficial midterm but not short-term effect on endothelial function. On the other hand, Paran et al. (2009) studied the effect of tomato extract in 50 subjects with moderate hypertension treated with one or two antihypertensive agents (ACE inhibitors and calcium channel blockers). Results showed a reduction in both systolic and diastolic blood pressure after 6 weeks of tomato extract supplementation. Tomato oleoresin interferes inflammatory signaling in endothelial cells, imitating the inflammatory process reduction in the vessel walls and reducing blood pressure. Prevention of adhesion molecules overexpression through inhibition of NF-kB signaling is one of the main mechanisms of carotenoids to reduce leukocyte adhesiveness to endothelium (Armoza et al. 2013). As an example, lycopene is hypolipidemic and inhibits prothrombotic and pro-inflammatory factors (Mordente et al. 2011; Mozos et al. 2018; Müller et al. 2016).

The beneficial effects of fresh tomato and tomato products on platelet aggregation reduction depend on the presence of different bioactive compounds, such us phenolic compounds, carotenoids and others, in quantities enough to produce a relevant physiological effect when consumed as part of a normal diet (Cámara et al. 2013; Hsiao et al. 2005). This is the case of lycopene, a bioactive compound present in tomato and tomato products, that can exert CV beneficial effects acting as an antioxidative, anti-inflammatory, antiatherogenic, cardioprotective and antiplatelet agent (Jacques et al. 2013; Mozos et al. 2018). However, there is limited number of studies focused on the efficacy of lycopene supplementation in reducing some CVD risk factors (e.g. oxidative stress, inflammation, blood pressure, lipid metabolism …). Burton-Freeman and Sesso (2014) concluded that consumption of tomato-based products is more effective in the management of CVD risk factors (except for blood pressure) than lycopene supplementation. On the other hand, Pereira et al. (2017) reported that both fresh tomatoes and lycopene supplementation mitigated cardiac-remodeling and enhance diastolic dysfunction after a myocardial infarction in male Wistar rats. A systematic review and meta-analysis on tomato and lycopene supplementation as well as CV risk factors has been recently performed by Cheng et al. (2017). Authors concluded that the available evidence on the effects of tomato products and lycopene supplementation on CV risk factors supports the view that increasing the intake of these has positive effects on blood lipids, blood pressure and endothelial function.

Because tomatoes include several nutrients associated with theoretical or proven effects and are widely consumed all year-round, they may be considered a valuable component of a cardioprotective diet (Willcox, Catignani, and Lazarus 2003). In addition, the statement about tomatoes and health benefits could be used as a health claim in food marketing.

EU health claims scientific evidence requirements

Nutrition and health claims made on food are regulated in the EU under Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 with the aim of ensuring any claims made on the labeling, presentation or advertising of food in the EU are clear, accurate and based on scientific evidence. This is not only to protect consumers; it also promotes innovation and guarantees fair competition. This Regulation establishes a Community list of possible nutritional, health promotion and/or risk reduction indications as defined in Article 2. Health claims are those that affirm, suggest or imply that there is a relationship between a food category, a food or one of its constituents, and health. Disease Risk Reduction Statements are those health claims that state, suggest or imply that consumption of a food category, food or one of its constituents significantly reduces a risk factor for the occurrence of a human disease. A beneficial effect may relate

to maintenance or improvement of a function. This implies the reduction of a risk factor for the development of a human disease (not reduction of the risk of disease).

In Europe, EFSA is the body in charge of evaluating the health claim applications. In this sense, the characterization of food must include information about the botanical source, manufacturing process, chemical specifications, batch-to-batch reproducibility and stability. In addition, it is necessary to verify the biological activity of a compound to be able to declare that it is bioactive.

Regarding reduction of disease risk claims related to CVD (Cámara, Fernández-Ruiz, Domínguez, et al. 2018), evidence should provide that the consumption of the food/constituent (tomato-based product) also reduces (or beneficially affects) one or more risk factors for the disease. Several disease risk reduction claims related to CV and heart health have been evaluated by the Panel on Nutrition, Novel Foods and Food Allergens (NDA) with a favorable opinion (e.g. Limicol; Plant sterols; Plant stanol esters; Danacol®; Oat beta-glucan; Barley beta-glucan; Trans-free spreadable fats). The scientific substantiation of all these claims has been based on evidences for a sustained reduction in LDL-c concentrations with continuous consumption of the food/constituent, whereas evidence for a reduction in the risk of the disease directly (i.e. on disease outcomes) has not been provided (EFSA 2018).

Focusing on the reduction of platelet aggregation claim, EFSA recognizes that platelet hyperactivity and hypercoagulability states are more commonly observed in subjects with CV risk factors, as healthy subjects at very low risk of CV disease normally have non-activated circulating platelets. Thus, a reduction in platelet aggregation is a beneficial physiological effect.

Objective

With all previous information that suggests a positive effect of tomato and tomato products to prevent CVD due to different risk factors reduction, this review is aimed to review and analyze the scientific evidence on platelet aggregation prevention by the intake of tomato products and its potential health claims according to EFSA requirements.

Methodology

First, a review of the approved EFSA health claims on tomato products and platelet aggregation prevention has been performed using the public information published in EFSA Journal and web page.

Secondly, a scientific literature review was conducted. When searching for evidence-based information, systematic reviews, meta-analyses, and critically-appraised articles are considered the highest level of evidence. According to EFSA, human studies are essential to scientifically support health claims. In relation to the types of intervention studies necessary to explain the effect, they must be well-controlled studies (specifying either the control/placebo), using the markers considered valid by EFSA and with healthy individuals.

Search strategy. We conducted a literature search following EFSA's approach (EFSA 2017) on PubMed database [http://www.ncbi.nlm.Nih.gov/PubMed] last retrieve 19th November 2018.

Inclusion criteria. The inclusion criteria considered the English language, year of publication, human studies and the keywords 'tomato', 'platelet' and 'antiplatelet'.

Exclusion criteria. Articles related to other health benefits of tomato/tomato product consumption different from platelet aggregation as well as in vitro and animal studies were excluded from the selection.

Results

EU health claims approved on tomato products and platelet aggregation prevention

Among the claim applications submitted to EFSA, only related to the Water-soluble tomato concentrate (WSTC) properties is relevant to this review (EFSA 2009, 2010). Provexis Natural Products Limited submitted a health claim application to EFSA pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of United Kingdom; based on newly developed scientific evidence including a request for the protection of proprietary data. The food constituent that was the subject of the health claim was a lycopene-free and fat-free WSTC developed in two different forms named WSTC I (completely water-soluble sirup) and its low-sugar derivative, WSTC II, supplied in powder format.

WSTC I is an aqueous concentrate of tomato paste and consists of soluble solids concentrated from a commercially available tomato paste, being common tomato, *Solanum lycopersicon L.*, the starting material for the tomato paste. WSTC is produced in a 5-step process. Tomato paste is used as the starting material, for purposes of standardization and quality control. The first step involves the addition of purified water to tomato paste, followed by the second step, which utilizes physical means (centrifugal separation, membrane filtration) to concentrate the water-soluble tomato compounds of interest from the cold-break tomato paste, and removes unwanted suspended solids. The resulting straw-colored aqueous concentrate is then pH-adjusted by the addition of food-grade citric acid and concentrated by evaporation at low temperature. WSTC I comprises, on a dry weight basis, mainly carbohydrates (~80% w/w), with minor amounts of protein and free amino acids (~6 and ~3% w/w, respectively), and lesser amounts of flavonoids, simple phenolics (0.2% w/w), and low-molecular weight organic acids (0.7% w/w), including citric acid, malic acid, ascorbic acid, and dehydroascorbic acid, and their derivatives. Other constituents accounts for approximately 8.2% (w/w) of WSTC. No carotenoids or long-chain fatty acids are detectable. The WSTCs were standardized on the total quantity of 37 identified constituents, which altogether have been shown to inhibit platelet aggregation in vitro in different degrees. All constituents occur naturally in tomatoes, with a 3 g serving of WSTC I, providing levels of

Table 1. Summary of the found studies in PubMed by using 'tomato', 'platelet' and 'antiplatelet' as keywords for CV prevention.

Total number of studies	Studies in the last 10 years	Studies in the last 5 years
48	29	19
(27 humans and 18 in animals)	(12 humans and 11 in animals)	(8 humans and 6 in animals)
5 reviews	4 reviews	3 reviews
4 clinical trials	1 clinical trial	1 clinical trial

constituents that are comparable to levels derived from 2.5 tomatoes.

O'Kennedy, Raederstorff, and Duttaroy (2017) give detailed information about the production and composition of the WSTC. The authors literally expressed that: "3 g Fruitflow® 1 gives an equivalent dose of bioactives (approximately 65 mg) to 150 mg Fruitflow® 2. Of this quantity, 6–10% (up to 9 mg) are known nucleoside derivatives (F1), 13–15% (up to 10 mg) are known phenolic conjugates (F2), and 8–10% (up to 7 mg) are known flavonoid derivatives (F3), including a minimum of 2.4 mg quercetin derivatives per dose. This amount of bioactive compounds, which comprises a single daily dose, is equivalent to that found in about 3 average servings of tinned tomato soup. (…) Three representative components, one from each of the three broad fractions F1, F2 and F3 (…), were selected. These three compounds were adenosine, which represents a group of nucleosides/nucleotides found in F1, chlorogenic acid, which accounts for a group of phenolic derivatives found in F2, and rutin, which represents a group of flavonoid derivatives found in F3".

In 2009, EFSA Panel on Dietetic Products, Nutrition and Allergies gave permission to Provexis Natural Products Ltd to use a health claim stating that their WSTC "helps maintain normal platelet aggregation". The document was based on the results of 8 human and 7 animal studies (EFSA 2009). In these studies, in order to achieve the beneficial effect, 3 g WSTC I, or 150 mg WSTC II in up to 250 mL of either fruit juices, flavored drinks or yogurt drinks (unless heavily pasteurised) should be consumed daily; the target population were adults between 35 and 70 years of age. The health claim application was based largely on study reports, conducted with WSTC, that were (and some of them still are) unpublished by the applicants as the request was based on newly developed scientific evidence including a request for protection of proprietary data as it is mentioned in EFSA (2009) decision. The authorization was extended, in 2010, to additional conditions of use as powdered single-serve sachets, tablets, and capsules (EFSA 2010). The Panel considered that the bioavailability of potentially active compounds in WSTC, when administered as powder, tablets or capsules, would not be different from that observed in other food matrices for which the health claim was previously authorized (i.e. fruit juices, flavored drinks or yogurt drinks) as long as these are easily dissolved in water. One unpublished study addressed the acute effects of different forms of the tomato extract on platelet aggregation. The study was a double-blind placebo-controlled, randomized crossover design with three interventions (corresponding to 3 g WSTC I (sirup), WSTC II 150 mg (powder) produced at room temperature, and WSTC II 150 mg (powder) produced at 65 °C) and one control. The results showed that the three WSTC formulations reduced platelet aggregation. The Panel concluded, that, a cause and effect relationship had been established between the consumption of WSTC (i.e. WSTC I and II corresponding to the specifications provided by the applicant) and the reduction in platelet aggregation under the new conditions of use proposed by the applicant (i.e. consumed as powder, tablets or capsules). In order to achieve the claimed effect, 3 g WSTC I or 150 mg WSTC II as powder, tablets or easily dissolved capsules with at least 200 mL of liquid should be consumed daily. This WSTC is marketed under the name Fruitflow® (FF), being commercially available in different countries worldwide. FF is commercially produced by DSM Nutritional Products (Basel, Switzerland), in sirup and in powder formats. The sirup format was used in the clinical trial performed by O'Kennedy, Raederstorff, and Duttaroy (2017) with the EFSA-approved daily dose of 3 g, containing a minimum of 65 mg antiplatelet components. FF is encapsulated using red size 00 Vegecaps provided by Provexis plc in sealed, desiccated containers, and stable below 25 °C.

Recently, Uddin et al. (2018) carried out a randomized, double-blinded, placebo-controlled human intervention study which demonstrated that consumption of Fruitflow® lowers blood pressure in pre-hypertensive males due to its angiotensin converting enzyme-inhibitory effect. Results concluded that, compared to placebo, consuming a single dose of 150 mg Fruitflow® results in a significant reduction in 24-hour average blood pressure as well as average wake-period and sleep-period SBP.

Scientific evidence on platelet aggregation prevention by tomato and tomato products

Our literature search performed in PubMed using the keywords 'tomato', 'platelet' and 'antiplatelet' results in 48 studies, being the most relevant ones, 5 reviews and 4 clinical trials (Table 1). Neither meta-analysis nor systematic reviews, the highest level of evidence studies, were found.

According to the relevant reviews selected, the antiplatelet positive effects of tomato products can be attributed to different compounds: lycopene (Phang et al. 2011), flavonoids and vitamin E (Willcox, Catignani, and Lazarus 2003), as well as other compounds (McEwen 2014). The two more recent reviews were performed on WSTC (O'Kennedy, Raederstorff, and Duttaroy 2017; Osińska et al. 2017), being the positive effect attributed to the extract components mentioned previously in this review.

Focused also in lycopene and non-lycopene compounds in tomato/tomato products and tomato extract, Phang et al. (2011) provided information about the role that nutrient

and non-nutrient supplements play on platelet aggregation and risk of thrombosis. Authors concluded that compounds present in these products can modify platelet activation and/or hemostasis pathways by means of several mechanisms. Although, there is a need of further long-term randomized controlled trials as well as cohort and case-control studies.

Willcox, Catignani, and Lazarus (2003) consider that cardioprotective effects of tomatoes, like reduction of platelet aggregation and adhesion, are due to flavonoids and α-tocopherol, as a 70% reduction of platelet aggregation in healthy subjects was shown when tomato extract is incubated with blood. McEwen (2014) reviewed the influence of tomato juice and tomato extract on platelet function including a randomized, double-blinded, placebo-controlled crossover study with tomato extract supplementation carried out in 90 healthy subjects with normal platelet function. Changes from baseline hemostatic function were measured 3 hours after supplementation, with a result of significant reductions in platelet aggregation, induced by ADP and collagen.

The most recent reviews are the ones conducted by O'Kennedy, Raederstorff, and Duttaroy (2017) and Osińska et al. (2017) on WSTC. O'Kennedy, Raederstorff, and Duttaroy (2017) included the background to this product discovery and the biological and regulatory work necessary to obtain its approved health claim. Fruitflow® compounds show antiplatelet activity by (1) affecting ADP, collagen, thrombin, and TXA2-mediated signaling, (2) affecting integrin activation and fibrinogen binding and (3) down-regulating platelet PDI, which concludes that Fruitflow® can be beneficial in primary prevention of CVD for humans who are vulnerable to develop this disease. Osińska et al. (2017) reported that WSTC is able to inhibit platelet aggregation in response to ADP, collagen, arachidonic acid, and thrombin (in vitro studies). According to these authors, the mechanism is multidirectional: (1) blocking of the receptors for ADP, collagen and von Willebrand factor; (2) inhibition of the activation of the αIIbβ3 integrin and GPIIb/IIIa glycoprotein, and (3) inhibition of the expression of P-selectin on the platelet surface. Moreover, polyphenols present in these tomato extract can block protein disulfide isomerase (PDI) and have an influence in the aggregation, secretion and binding of fibrin. Tomato extract can increase the concentrations of cAMP and cGMP in platelet cytosol as well as inactivate tissue factor (TF), a protein that play an important role in the activation of the extrinsic pathway of the coagulation cascade. In vitro animal and human studies showed that tomato extract is able to inhibit the activation of inflammatory processes in the endothelium by (1) reducing the synthesis of inflammatory cytokines (TNF-α, IL-1β, IL-12) and the expression of cell adhesion molecules (ICAM-1, VCAM-1); and (2) increasing the production of anti-inflammatory interleukin 10 (Schwager et al. 2016). On the other hand, phenolic acids of this tomato extract can inhibit the expression of the nuclear factor-κB (NF-κB) (Navarrete, Alarcón, and Palomo 2015). Finally, some studies showed the hypotensive action of polyphenols present in tomato extract. The proposed mechanism is the inhibition of angiotensin-converting enzyme (ACE) (Biswas et al. 2014). Osińska et al. (2017) concluded that hypertensive humans with low or moderate CV risk, without previous CVD episodes and those in whom a good pressure control cannot be aimed, are prime candidates to benefit from the use of water-soluble tomato extract in primary prophylaxis. In addition, the action of this extract is reversible, being a safer option for these patients than the acetylsalicylic acid (ASA).

Related to the four relevant clinical trials, three of them studied the effects of antiplatelet components of WSTC on healthy humans (O'Kennedy, Crosbie, Van Lieshout, et al. 2006; O'Kennedy, Crosbie, Whelan, et al. 2006; O'Kennedy, Crosbie, et al. 2017) while one of them was focused on humans with type 2 diabetes and tomato juice consumption (Lazarus, Bowen, and Garg 2004). The studies conducted on WSTC concluded that tomato extract could play an important role in primary prevention of CVD as a functional food or dietary supplement by reducing platelet activation and possibly thrombotic events.

The benefits of using ASA in the primary prophylaxis of CVD may only slightly exceed the risk of serious bleeding. This warrants the search for alternative, safer preparations with antiaggregatory properties, which could be used in patients burdened with CV risk factors. Recently O'Kennedy, Crosbie, et al. (2017) conducted a randomized controlled trial comparing the effect of water-soluble tomato extract Fruitflow®, with 75 mg ASA in healthy subjects. Results showed that the effect of Fruitflow® on platelet aggregation suppression was approximately one-third that of daily intake of 75 mg ASA. Moreover, the reversible action of FF renders it less likely to overextend the time to form a primary hemostatic clot than ASA, an important safety consideration for primary prevention.

As we mentioned in methodology section, information provided by other studies that discuss the antiplatelet activity of compounds that occur in tomatoes, but outside the context of a tomato-based matrix, have been excluded from this review. Although they may support the bioactivity of specific compounds as adenosine and iosine (Fuentes, Pereira, et al. 2014), chlorogenic acid (Fuentes, Caballero, et al. 2014), rutin (Sheu et al. 2004), guanosine (Fuentes et al. 2013a), and other natural products (Fuentes and Palomo 2013; Rangel-Huerta et al. 2015).

Animal studies (ex vivo and in vivo) and in vitro studies can be also used as supportive evidence. This is the case of Rodríguez-Azúa et al. (2014), who showed the antiplatelet activity of tomato (fresh tomato hybrids, paste of different stages, and pomace) in vitro, ex vivo, and in vivo. In addition, bleeding time was shown to be prolonged after consuming 1.0 g tomato pomace/kg per day. Therefore, tomato and its industrial derivatives could play a remarkable role in CVD prevention. Also, the in vitro study performed by Yamamoto et al. (2003), using methodology based on platelet activation due to high shear stress like in coronary artery stenosis. Results showed an effect on platelets activity independent of coagulation, and dependent on tomato varieties: only one variety had thrombolytic activity; four varieties had

very strong inhibition; 6 showed a trend of inhibition, 9 had no effect. The responsible compounds had molecular weights over 10000 Da and were heat stable (tested in the most active variety). The aggregation was significantly inhibited at all stages of ripening (of the most active variety), but mostly at green, phase 1, phase 2 and pink phase rather than at mature and over-mature phase. Fuentes, Forero-Doria, et al. (2013) performed an in vivo study in mice using 200 mg tomato pomace extract/kg, and assessing thrombus formation at site of irradiation by helium-neon laser in mouse carotid artery, and the preventive effect of pomace against thrombus formation.

Once we have reviewed the scientific evidence on antiplatelet aggregation prevention by the intake of tomato products, now we are going to evaluate if these studies can be used as scientific evidence (included valid markers to prove a reduction of platelet aggregation) for a health claim application according to EFSA requirements previously mentioned.

In January 2018, EFSA published the last 'Guidance for the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health' (EFSA 2018). The guidance considers that CV health, in general, is not sufficiently defined for a scientific evaluation and should be accompanied by a specific claim, such us the reduction of platelet aggregation, and described the characteristics of the human intervention studies to be performed in order to sustain a specific claim (e.g. appropriate outcome variables and methods of measurement, suitable study group(s), appropriate duration of the study and suitable controls). Platelet hyperactivity and hypercoagulability states are more commonly observed in subjects with CV risk factors, while healthy subjects at very low risk of CV disease normally have non-activated circulating platelets. Thus, EFSA considers that a reduction in platelet aggregation is a beneficial physiological effect, as it has been mentioned before. In relation to the outcome variables and methods of measurement, the percentage of inhibition in platelet aggregation should be measured using light transmission aggregometry (LTA) according to well-accepted and standardized protocols (Michelson 2009; Paniccia, Priora, A.A. Liotta, and Abbate 2015) in subjects with platelet activation during sustained exposure to the food/constituent (at least 4 weeks). Other outcome variables, such as thromboxane A2 (TXA2), or plasma soluble P-selectin (P-sel), are not well-established markers of platelet aggregation but can be used as supportive evidence for the scientific substantiation of these claims.

Considering this information, we have analyzed if the relevant studies (Table 1: 5 reviews and 4 clinical trials) found in Pubmed search, meet the new EFSA requirements.

In all studies, the target population is well defined, and subjects are clearly categorized as healthy humans or subjects with CD risk factors. Related to the clinical trials, the time of exposure to tomato, or tomato products with the exception of WSTC, is lower than the minimum of 4 weeks required by EFSA, and the method for assessing the inhibition of platelet aggregation is not completely described without information about LTA technique. Thus, our analysis showed that only the clinical trial performed by O'Kennedy, Crosbie, et al. (2017) could meet all EFSA 2018 requirements for the substantiation of the specific claim "reduction of platelet aggregation", as the study was conducted in healthy humans with no medical history of serious disease, neither hemostatic disorder, and the inhibition in platelet aggregation was measured using LTA (light transmission aggregometry).

Conclusion

As it is reported, different epidemiological studies and interventional trials evaluate the association between tomato products intake and CVD and/or their risk factors progression. To date, the EFSA NDA Panel has evaluated a claim on maintenance of normal platelet aggregation (WSTC case), with a favorable opinion and the commercialization of Fruitflow®. After reviewing the most relevant studies found in our search, we consider that all bioactive compounds found in WSTC are in tomato fruit and other tomato products, and there is enough evidence of their beneficial effect, as WTSC is made from tomato concentrate, and the same bioactive content of a single dose of Fruitflow® is found in around 12 g of tomato paste (30% Natural Tomato Soluble Solids, NTSS) and 70 ml tomato juice (5% NTSS). Further intervention studies (human clinical trials) using EFSA valid markers should be performed in order to demonstrate the beneficial effect of tomato products as consumer products (paste, puree, sauce or juice) on platelet aggregation. Our PubMed review results support the development of promising nutritional strategies involving tomatoes and tomato products to tackle CVD as antiplatelet aggregation.

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