22/12/2015

Report

Study on Munroes Ltd Authentic Jamaican Jerk Marinades

Innovation Voucher Number:

IV-2015-1202

Dr. Joanna Przyborska
SHANNON APPLIED BIOTECHNOLOGY CENTRE,
INSTITUTE OF TECHNOLOGY, TRALEE

Contents

List of Tables

Introduction

AM Munroes Corporation Limited, Dublin is a company specialised in manufacture of condiments and seasoning. Munroes have developed original and gluten free marinades made from natural ingredients. Munroes required expertise in order to characterise its products (Munroes marinades). The Munroes Innovation Voucher (IV-2015-1202) completed at Shannon ABC involved:

1. Quantifying protein, fat, carbohydrate (Group 2 Nutritional Analysis), vitamin and mineral levels in Munroes marinades,
2. Completion of a desk based study to investigate nutritional and health claims relevant to Munroes marinades (EFSA guidelines),
3. Perform anti-oxidant testing on Munroes marinades,
4. Shelf life testing on Munroes marinades (Day 0, 1 month, 3 months, 6 months & 12 months).

1 Nutrition claims of Original Munroes Marinade and Gluten Free Munroes Marinades

Nutrition claims relate to all foods: foods for particular nutritional uses (PARNUTS), natural mineral waters and water intended for human consumption and food supplements. Nutrition claims (as stated by Regulation 1924/2006) defines a nutrition claim as: "*any claim which states, suggests or implies that a food has particular beneficial nutritional properties due to:*

a) The energy (calorific value) it
    i. provides:
    ii. provides at a reduced or increased rate; or
    iii. does not provide; and/or
b) The nutrients or other substances it
    i. contains;
    ii. contains in reduced or increased proportions; or
    iii. does not contain"

Nutrients is qualifying as "*protein, carbohydrate, fat, fibre, sodium, vitamins, minerals listed in the Annex to Directive 90/496/EEC, and substances which belong to or are components of one of those categories*".

Nutrition claims of Original and Gluten Free Munroes Marinades were established based on the data derived from Tests Certificates (see Appendices, section 5.1 and 5.2). Nutrition claims for Vitamins and Minerals were established based on data derived from vitamin and mineral analysis of Munroes Original marinades (see Appendices, section 5.3 and 5.4).

1.1 Original Munroes Marinade

Tables (Table 1 and Table 3) below contain permitted nutrition claims and their condition of use (as in Annex of Regulation 1924/2006).

Table 1 Permitted nutrition claims for Munroes Original Marinade

Nutrition Claim	**Condition of Use (regulated by Annex of Regulation 1924/2006)**	**Munroes marinade (Original)**
Low in fat	Product contains no more than 3g of fat per 100g for solids or 1.5g of fat per 100ml for liquids (1.8g of fat per 100ml for semi-skimmed milk)	Total Fat: 0.7g/100g
Low saturated fat	The sum of saturated fatty acids and trans-fatty acids in the product does not exceed 1.5g per 100g for solids or 0.75g per 100 ml for liquids and in either case, the sum of saturated fatty acids and trans-fatty acids must not provide more than 10% of energy.	a) Saturated fatty acids: 0.19g/100g b) % energy from the saturated fatty acids: [(0.19 x 9)/120]x100= 1.42% (*) (see conversion factor in Table 2)

$$(*)\ \%\ \text{energy from the nutrient} = \frac{\text{grams of nutrient per 100g of product} \times \text{conversion factor for nutrient}}{\text{total energy per 100g of product}} \times 100$$

Table 2 Conversion factors from Directive 90/496/EEC

Nutrient	Conversion factor	Amount of energy per 1g of nutrient
Protein	4 kcal/g , 17kJ/g	1g protein x 4kcal = 4 kcal per g protein
Fat	9 kcal/g , 37kJ/g	1g fat x 9 kcal = 9 kcal per g fat

Table 3 Nutrition claims for vitamins and minerals for Original Munroes Marinade

Nutrition claim (Vitamin/mineral)	RDA*	Claims that are a "Source of "	Claims that are "High"	Munroes marinade (Original)
Source of Vitamin B12	2.5µg	0.38µg	0.76µg	0.73µg/100g: 29% RDA
High in Manganese	2mg	0.30mg	0.60mg	1.77mg/100g: 88.5% RDA
Source of Copper	1mg	0.15mg	0.30mg	0.225mg/100g: 22.5% RDA

* Recommended Daily Allowance

1.2 Gluten Free Munroes Marinade

Table 4 contains permitted nutrition claims and their condition of use (as in Annex of Regulation 1924/2006).

Table 4 Permitted nutrition claims for Munroes Gluten Free Marinade

Nutrition Claim	Condition of Use (regulated by Annex of Regulation 1924/2006)	Munroes marinade (Gluten Free)
Low in fat	Product contains no more than 3g of fat per 100g for solids or 1.5g of fat per 100ml for liquids (1.8g of fat per 100ml for semi-skimmed milk)	Total Fat: 2.3g/100g
Low saturated fat	The sum of saturated fatty acids and trans-fatty acids in the product does not exceed 1.5g per 100g for solids or 0.75g per 100 ml for liquids and in either case, the sum of saturated fatty acids and trans-fatty acids must not provide more than 10% of energy.	a) Saturated fatty acids: 0.47g/100g b) % energy from the saturated fatty acids: [(0.49 x 9)/92]x100= 4.79% (*) (see conversion factor in Table 2)
Source of	At least 12% of the energy value of the	% energy from protein:

protein	food is provided by protein	[(3.73 x 4)/92]x100=16.2% (*) (see conversion factor in Table 2)
Reduced in sugar	The reduction in content is at least 30% compared to a similar product	Total sugars content: - Original sauce: 23.9g/100g - Gluten Free sauce: 12.2g/100g That gives 48% reduction in sugar content in Gluten Free sauce vs. Original sauce
Increased in protein	Product meets the conditions for the claim 'source of' (at least 12% of the energy value of the food is provided by protein) and the increase in content is at least 30% compared to a similar product	Protein content: - Original sauce: 2.23g/100g - Gluten Free sauce: 3.73g/100g That gives 40% increase in protein content in Gluten Free sauce vs. Original sauce. % energy from protein: [(3.73 x 4)/92]x100=16.2% (*) (see conversion factor in Table 2)
Increased in unsaturated	Product meets the conditions for the claim 'source of' (at least 12% of the energy value of the food is provided by unsaturated fatty acids) and the increase in content is at least 30% compared to a similar product	Unsaturated fatty acids content: - Original sauce: 0.48g/100g - Gluten Free sauce: 1.74g/100g That gives 72% increase in unsaturated fatty acids content in Gluten Free sauce vs. Original sauce % energy from unsaturated fatty acids: [(1.74 x 9)/92]x100=17% (*) (see conversion factor in Table 2)

$$(*)\ \%\ \text{energy from the nutrient} = \frac{\text{grams of nutrient per 100g of product} \times \text{conversion factor for nutrient}}{\text{total energy per 100g of product}} \times 100$$

Summary:

1) Nutrition claims of Original Munroes Marinade are:
    a. Low in Fat.
    b. Low in Saturated Fat.
    c. Source of Vitamin B12.
    d. High in Manganese.
    e. Source of Copper.

2) Nutrition claims of Gluten Free Munroes Marinade are:

    a. Low in Fat.

    b. Low in Saturated Fat.

    c. Source of Protein.

    d. Increased in Protein.

    e. Reduced in Sugar.

    f. Increased in Unsaturated Fat.

2 Shelf-life study

Shelf-life analysis of Munroes Original and Gluten Free marinades were conducted at Day 0, 1 month, 3 months, 6 months and 12 months. One to twelve month testing were carried out under accelerated (37°C) conditions. Aerobic, anaerobic bacterial levels and yeast and mould counts were monitored in each product at teach time point.

The Munroe's original and gluten-free formulae passed the aerobic plate count criteria i.e. was within acceptable levels for food consumption at Day 0 and 3 month accelerated

shelf life time points when placed into category 7 (<10^6 cfu/g). All samples were tested in triplicate using the standard procedure for aerobic, anaerobic and yeast & mould plate counts. Dilutions used for testing included 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} dilutions.

Table 1: Food Safety Authority of Ireland guidelines to microbiological testing of ready-to-eat foods.

Food category		Examples	ACC result (cfu/g)		
			Satisfactory	Borderline	Unsatisfactory
1	Ambient stable canned, bottled, cartoned and pouched foods immediately after removal from container	Canned products such as tuna, salmon, corned beef, soups, stews, desserts, fruit; UHT products	<10	Not applicable	See footnote[a]
2	Foods cooked immediately prior to sale or consumption	Takeaway food, burgers, kebabs, sausages, pizza, ready-meals (cook/chill and cook/freeze) after regeneration	<10^3	10^3 - <10^5	≥10^5
3	Cooked foods chilled but with minimum handling prior to sale or consumption; canned pasteurised foods requiring refrigeration	Whole pies, sausage rolls, samosas, flans, quiches, chicken portions; canned ham, pasteurised foods including fruit juice and soups; desserts	<10^4	10^4 - <10^7	≥10^7
4	Bakery and confectionery products without dairy cream, powdered foods	Cakes without dairy cream, soup powders, milk powder, powdered dairy products, other reconstituted powdered foods ready-to-eat after reconstitution or warming	<10^4	10^4 - <10^6	≥10^6
5	Cooked foods chilled but with some handling prior to sale or consumption	Sliced meats, cut pies, pâté, sandwiches without salad, hot smoked fish (mackerel, etc.), molluscs, crustaceans and other shellfish out of shell	<10^5	10^5 - <10^7	≥10^7
6	Non-fermented dairy products and dairy desserts, mayonnaise and mayonnaise-based dressings, cooked sauces	Most milk and butter, cream, ice-cream, fresh cheese (cream cheese, mascarpone, paneer), trifle with dairy cream, cakes with dairy cream, satay	<10^5	10^5 - <10^7	≥10^7
7	Food mixed with dressings, dips, pastes	Coleslaw, dips, taramasalata, humous	<10^6	10^6 - <10^7	≥10^7

Food category		Examples	ACC result (cfu/g)		
			Satisfactory	Borderline	Unsatisfactory
8	Extended shelf-life food products requiring refrigeration	MAP or vacuum packed products, e.g. meat, fish, fruit and vegetables	$<10^6$	10^6 - $<10^8$	$\geq 10^8$ See footnote[B]
9	Raw ready-to-eat meat and fish, cold smoked fish	Sushi, smoked salmon, gravalax	$<10^6$	10^6 - $<10^7$	See footnote[C]
10	Preserved food products-pickled, marinated or salted	Pickled or salted fish, cooked shellfish in vinegar, vegetables in vinegar or oil, herbs, spices	Not applicable	Not applicable	See footnote[C]
11	Dried foods	Fruits, berries, vine fruits, nuts, sunflower seeds, herbs, spices, dried fish	Not applicable	Not applicable	See footnote[C]
12	Fresh fruit and vegetables, products containing raw vegetables	Whole fruit, pre-prepared fruit salads, vegetable crudités, salads, sandwiches with salad, mixed commodity salads containing raw vegetables	Not applicable	Not applicable	See footnote[C]
13	Fermented, cured and dried meats, fermented vegetables, ripened cheeses	Continental sausages/ salamis, jerky, sauerkraut, olives, bean curd, cheddar, stilton, brie, fermented milk drinks and butter, yoghurt, etc	Not applicable	Not applicable	See footnote[C]

[A] Food category 1: These products are unsatisfactory if spore forming anaerobes are present but these require special tests for detection and enumeration. Spore forming aerobes are also usually absent in foods that have been cooked in their container but low levels may occur in canned fish products. Most Food Category 1 foods are sterile if sampled direct from the container, but if they undergo further preparation before consumption then they should be assessed as Food Category 5

[B] Determine the predominant micro-organisms. "Unsatisfactory" if the predominant organisms is $>10^6$ yeast, $>10^7$ Gram negative bacilli or *Bacillus* spp., or $>10^8$ lactic acid bacteria

[C] ACCs not routinely performed. For spoilage investigation, "Unsatisfactory" if the predominant organisms is $>10^6$ yeast, $>10^7$ Gram negative bacilli or *Bacillus* spp., or $>10^8$ lactic acid bacteria unless added as a processing aid

Results:

Summary of counts (detailed counts in excel file)

Day 0: Aerobic was satisfactory in original and gluten-free formulations. ($<10^6$ cfu/g) at both day 0 and 3, 6 and 12 month accelerated time points.

Anaerobic was satisfactory in original and gluten-free formulation (0 cfu/g) at day 0, 3, 6 and 12 month accelerated time point.

Mould and yeast counts were satisfactory in gluten-free and original formulations at day 0, 3, 6 and 12 month accelerated time point.

3 Desk study regarding reported health benefits associated with the natural ingredients of Munroes Marinades

The main aim of this portion of Munroes Innovation voucher was to characterise health benefits of 12 marinade ingredients (olive oil, onion, ginger, garlic, scotch bonnet peppers, pimento, black pepper, thyme, nutmeg, scallions, soy sauce and brown sugar). Scientific findings presented here are in relation to the effect of Munroes marinade components in association with human and animal health. Various studies were employed including *in vivo*/*in vitro* human and animal models in addition to *in vitro* assays.

EFSA (European Food Safety Authority) recommendations and current status in relation to health claims associated with food/food constituents were also included in this report.

Health claims (as stated by Regulation 1924/2006 on nutrition and health claims) are defined as: "*any claim that states, suggest or implies that a relationship exists between a food category, a food or one of its constituents and health*". The Commission website was used to check the current legal status of authorised, rejected and unauthorised health claims.

There are four types of health claims: Article 14 health claims (reduction of disease risk claims and claims referring to children's development and health), Article 13(5) health claims (health claims other than disease risk reduction and children's development and health, based on newly develop scientific evidence), Article 13 health claims (health claims other than disease risk reduction and children's development and health).

3.1 Authorised EU health claims for vitamins and minerals

Vitamin and mineral analysis of Munroes Original marinade (see Appendices, section 5.3 and 5.4) allowed to determine the nutrition claims. These, in turn, allowed to establish their health claims:

- **Vitamin 12 (biotin)**

The Table 5 contains authorised health claims (claim type Art.13 (1)) for **vitamin B12** (biotin) and their condition of use. The claim may be used only for food which is at least a source of biotin as referred to in the claim SOURCE OF [NAME OF VITAMIN/S] AND/OR

[NAME OF MINERAL/S] as listed in the Annex to Regulation (EC) No 1924/2006. Commission Regulation (EU) 432/2012 of 16/05/2012

Table 5 Authorised EU health claims for vitamin B12 (biotin)

Claim	Health relationship	EFSA opinion reference
Biotin contributes to normal energy-yielding metabolism	Energy-yielding metabolism	2009;7(9):1209
Biotin contributes to normal functioning of the nervous system	Function of the nervous system	2009;7(9):1209
Biotin contributes to normal macronutrient metabolism	Contribution to normal Macronutrient metabolism	2009;7(9):1209, 2010;8(10):1728
Biotin contributes to normal psychological function	Contribution to normal psychological functions	2010;8(10):1728
Biotin contributes to the maintenance of normal hair	Maintenance of normal hair	2009;7(9):1209, 2010;8(10):1728
Biotin contributes to the maintenance of normal mucous membranes	Maintenance of normal skin and mucous membranes	2009;7(9):1209
Biotin contributes to the maintenance of normal skin	Maintenance of normal skin and mucous membranes	2009;7(9):1209, 2010;8(10):1728

- **Manganese**

The Table 6 contains authorised health claims (claim type Art.13 (1)) for **manganese** and their condition of use. The claim may be used only for food which is at least a source of manganese as referred to in the claim SOURCE OF [NAME OF VITAMIN/S] AND/OR [NAME OF MINERAL/S] as listed in the Annex to Regulation (EC) No 1924/2006. Commission Regulation (EU) 432/2012 of 16/05/2012

Table 6 Authorised EU health claims for manganese

Claim	Health relationship	EFSA opinion reference

Manganese contributes to normal energy-yielding metabolism	Contribution to normal energy-yielding metabolism	2009;7(9):1217, 2010;8(10):1808
Manganese contributes to the maintenance of normal bones	Maintenance of bones	2009;7(9):1217
Manganese contributes to the normal formation of connective tissue	Contribution to normal formation of connective tissue	2010;8(10):1808
Manganese contributes to the protection of cells from oxidative stress	Metabolism of fatty acids	2009;7(9):1217

- **Copper**

The Table 7 contains authorised health claims (claim type Art.13 (1)) for **copper** and their condition of use. The claim may be used only for food which is at least a source of copper as referred to in the claim SOURCE OF [NAME OF VITAMIN/S] AND/OR [NAME OF MINERAL/S] as listed in the Annex to Regulation (EC) No 1924/2006. Commission Regulation (EU) 432/2012 of 16/05/2012

Table 7 Authorised EU health claims for copper

Claim	Health relationship	EFSA opinion reference
Copper contributes to maintenance of normal connective tissues	Maintenance of connective tissues	2009;7(9):1211
Copper contributes to normal energy-yielding metabolism	Contribution to normal energy-yielding metabolism	2009;7(9):1211, 2011;9(4):2079
Copper contributes to normal functioning of the nervous system	Maintenance of the normal function of the nervous system	2009;7(9):1211
Copper contributes to normal iron transport in the body	Iron transport	2009;7(9):1211
Copper contributes to normal skin pigmentation	Maintenance of skin and hair pigmentation	2009;7(9):1211

Copper contributes to the normal function of the immune system	Maintenance of the normal Function of the immune system	2009;7(9):1211, 2011;9(4):2079
Copper contributes to the protection of cells from oxidative stress	Protection of DNA, proteins and lipids from oxidative damage	2009;7(9):1211

3.2 Olive Oil (oil of *Olea europea*)

Major components of olive oil are glycerols (98%) of the total oil weight. Minor components (2%) include more than 230 chemical compounds; e.g.aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile compounds and antioxidants. Phenolic and carotenes are among of constituents with antioxidant properties of olive oil (Servili and Montedoro 2002). Oleic, palmitic and linoleic acids are main fraction of glycerols and their quantity are as follows 55-83, 7.5-20 and 3.5-21%, respectively (Silva *et al.* 2015).

3.2.1 Authorised EU health claims

EFSA recommendation contains an authorised health claim (Entry ID 1333, 1638, 1639, 1696, 2865) that 'olive oil polyphenols contribute to the protection of blood lipids from oxidative stress on the basis of human studies showing significantly reduced levels of oxidised low density lipoprotein (LDL) in plasma after virgin olive oil consumption. The health claims pursue to Article 13(1) of Regulation (EC) No 1924/2006. The claim may be used only for oil which contains at least 5 mg of hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol) per 20 g of olive oil. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 20 g of olive oil (EFSA 2011).

Other claims proposed were rejected:

- maintenance of normal blood pressure (ID 3781)
- maintenance high density lipoprotein (HDL) cholesterol concentration (ID 1639)
- anti-inflammatory properties (ID 1882)
- contributes to the upper respiratory tract health (ID 3468)

- can help to maintain a normal function of gastrointestinal tract (ID 3779)
- contributes to body defences against external agents (ID 3467)

3.2.2 Non-authorised EU health claims

EFSA recommendation contains an non-authorised health claim (Entry ID 1316, 1332, 4244) that ‘olive oil contribute to the maintenance of normal blood LDL-cholesterol concentrations, maintenance of normal (fasting) blood concentration of triglycerides, maintenance of normal blood HDL-cholesterol concentrations and maintenance of normal blood glucose concentrations’. The claims pursue to Article 13(1) of Regulation (EC) No 1924/2006. (EFSA 2011). The Panel considered that references provided in relation to these claims were narrative reviews. No original data was available for scientific substantiation of the claimed effects.

3.2.3 Relevance of olive oil to health

There are number of studies showing the potential therapeutic effect of olive oil/olive oil constituent(s). Health claims of olive oil are:

3.2.3.1 Cardioprotective activity

Urinary proteomics was applied to assess cardiovascular health improvements of olive oil consumption in humans. Proteomics biomarkers allow to diagnose of disease on early presymptomatic stage. In a randomized, parallel, controlled, double-blind study 69 participants received 20ml of low or high phenolics (18 or 286mg of caffeic acid equivalents per kg, respectively) for 6 weeks. Urinary proteomic biomarkers of coronary artery disease (CAD), chronic kidney disease (CKD) and diabetes were investigated. Urinary proteomic biomarkers were measured at baseline and 3 and 6 week alongside blood lipids, the antioxidant capacity, and glycation markers. The consumption of both olive oils improved the proteomic CAD score at endpoint compared with baseline (mean improvement: -0.3 for low-phenolic OO and -0.2 for high-phenolic OO; $P < 0.01$) but not CKD or diabetes proteomic biomarkers. However, there was no difference between groups for changes in proteomic

biomarkers or any secondary outcomes including plasma triacylglycerols, oxidized LDL, and LDL- cholesterol (Silva *et al.* 2015).

3.2.3.2 Hypotensive effect

In vivo, human model

- Effect of mono-unsaturated fatty acids (MUFA), one of the olive oil constituent, on blood pressure of hypertensive women was studied. Sixteen hypertensive women ate a diet rich with olive oil for 4-week. After that time the following parameters were checked: plasma lipids, erythrocyte membrane and blood pressure. There was a significant increase in high-density lipoprotein (HDL) cholesterol concentration after the olive oil diet, moreover, a significant decrease in plasma HDL2 cholesterol concentration and an increase in plasma HDL3 cholesterol concentration was observed. The membrane free-cholesterol concentration increased significantly and the phospholipid concentration decreased significantly in erythrocytes after the olive oil diet, though MUFA diet produced a significant decrease in the concentration of membrane esterified cholesterol. Ratio of cholesterol to phospholipids was raised significantly in the erythrocyte membrane of hypertensive women after the dietary olive oil. Reduction in systolic and diastolic pressure was observed in significant manner. The study showed a beneficial effects of dietary olive oil (rich in MUFA) on the plasma lipids and lipoprotein profile, lipid composition of erythrocyte membrane, and blood pressure in hypertensive women (Ruiz-Gutierrez *et al.* 1996).
- The effect of olive oil supplemented diet on 24-hour ambulatory blood pressure (BP), blood glucose and lipids was evaluated by PREDIMED (The PREvención con DIeta MEDiterránea) trial. In a one year, randomized, parallel-design, controlled trial 235 participants (56.5% women; mean age=66.5 years) at high cardiovascular risk (85.4% with hypertension) were assessed. Adjusted changes from baseline in mean systolic BP were -2.3 (95% confidence interval [CI], -4.0 to -0.5) mm Hg with olive oil and 1.7 (95% CI, -0.1 to 3.5) mm Hg in the control group-low fat diet (P<0.001). Respective changes in mean diastolic BP were -1.2 (95% CI, -2.2 to -0.2) and 0.7 (95% CI, -0.4 to 1.7) mm Hg (P=0.017). Mean changes from baseline in fasting blood glucose were -6.1 and 3.5 mg/dL (P=0.016) in the diet with olive oil and control diet,

respectively; those of total cholesterol were -11.3, and -4.4 mg/dL (P=0.043), respectively. The trial showed that supplemented with olive oil diet reduced 24-hour ambulatory BP, total cholesterol, and fasting glucose in hypertensive patients (Domenech *et al.* 2014).

- Polyphenol-rich olive oil and its effect on blood pressure (BP) and endothelial function was studied. In a double-blind, randomized, crossover dietary intervention study 24 women with high-normal BP or stage 1 essential hypertension received two diets (polyphenol-rich olive oil (~30 mg/day) and polyphenol-free olive oil) for 2 months with a 4-week washout between diets. Baseline values consisted of 4-months period before introduction of diets. The following parameters were measured: systolic and diastolic BP, serum or plasma biomarkers of endothelial function, oxidative stress, and inflammation, and ischemia-induced hyperemia in the forearm. In polyphenols-rich olive oil diet a significant ($P < 0.01$) decrease in systolic and diastolic BP was observed: -7.91 mm and -6.65 mm Hg, respectively when compared to baseline values. Similar results was observed for serum asymmetric dimethylarginine (ADMA) (-0.09 ± 0.01 µmol/l, $P < 0.01$), oxidized low-density lipoprotein (ox-LDL) (-28.2 ± 28.5 µg/l, $P < 0.01$), and plasma C-reactive protein (CRP) (-1.9 ± 1.3 mg/l, $P < 0.001$). A significant increase in plasma nitrites/nitrates (+4.7 ± 6.6 µmol/l, $P < 0.001$) and hyperemic area after ischemia (+345 ± 386 perfusion units (PU)/sec, $P < 0.001$) were observed after polyphenols-rich olive oil diet (Moreno-Luna *et al.* 2012).
- Anti-hypertensive effects of monounsaturated fatty acids (MUFA) of extra virgin olive oil and polyunsaturated fatty acids (PUFA) of sunflower oil were studied. In a double-blind, randomized, crossover study 23 hypertensive patients received MUFA or PUFA diet for 6 months. Patients were crossed over to the other diet. At the end of MUFA diet resting BP was significantly lower ($P = 0.05$ for systolic BP; $P = 0.01$ for diastolic BP) when compared to PUFA diet, moreover patients on MUFA diets received significantly reduced daily dosage of anti-hypertensive drug in comparison to those on PUFA diet (-48% vs - 4%, P<.005). In case of 8 patients on MUFA diet no anti-hypertensive drug was needed whereas those on PUFA diet needed additional anti-hypertensive therapy (Ferrara *et al.* 2000).

In vivo, animal model

- Olive oil (OO) was studied on its effect on decrease on blood pressure. Chronic administration of virgin olive oil VOO (rich in oleic acid-70-80%) over 14 days (2g/kg) significantly reduced the blood pressure (BP; -26 ± 4.0 mm Hg, $P < 0.001$) in treated rats when compared with rats that received vehicle alone (water used was control). Similarly, acute (2 h) exposure to VOO (2g/kg) reduced systolic BP (-20 ± 0.3 mm Hg, $P < 0.001$) when compared to the control group. (Terés *et al.* 2008).

3.2.3.3 Effect on plasma lipids

In vivo, human model

- In a randomized, crossover, controlled trial olive oil was assessed for its effect on plasma lipids concentration and lipid oxidative damage and compared with phenolic content. Six research centres from 5 European countries was participating in this study where 200 healthy male volunteers were randomly assigned to 3 sequences of daily administration of 25 mL of 3 olive oils for 3 weeks. Olive oils contained: low (2.7 mg/kg of olive oil), medium (164 mg/kg), or high (366 mg/kg) phenolic content. A linear increase in high-density lipoprotein (HDL) cholesterol levels was observed for low-, medium-, and high-polyphenol olive oil: mean change, 0.025 mmol/L (95% CI, 0.003 to 0.05 mmol/L), 0.032 mmol/L (CI, 0.005 to 0.05 mmol/L), and 0.045 mmol/L (CI, 0.02 to 0.06 mmol/L), respectively. Total cholesterol-HDL cholesterol ratio decreased linearly with the phenolic content of the olive oil. Triglyceride levels decreased by an average of 0.05 mmol/L for all olive oils. Oxidative stress markers decreased linearly with increasing phenolic content. Mean changes for oxidized low-density lipoprotein (LDL) levels were 1.21 U/L (CI, -0.8 to 3.6 U/L), -1.48 U/L (-3.6 to 0.6 U/L), and -3.21 U/L (-5.1 to -0.8 U/L) for the low-, medium-, and high-polyphenol olive oil, respectively. Olive oil and its phenolic content can provide benefits for plasma lipid levels and oxidative damage (Covas *et al.* 2006).
- Olive oil and its effect on postprandial lipid metabolism was studied by Demmelmair et al. In a randomised crossover study 20 volunteers consumed muffins containing olive, rape-seed or hydrogenated oil. On the seventh day of each diet week subjects consumed a test meal with 0.5 g of the corresponding oil and 0.5 mg ^{13}C labelled linoleic acid per kg body weight after a 12 h fast. Blood was sampled before the meal

and in hourly intervals thereafter. Breath was collected at baseline and in half hourly intervals. After 8 hours sampling was stopped. Fasted plasma phospholipid fatty acids showed significantly higher oleic acid after olive oil (12.3±0.3 %), significantly higher α-linolenic acid after rapeseed oil (0.32±0.03 %) and significantly higher palmitic acid after hydrogenated oil (30.3±0.3%), although stool fat indicated that hydrogenated oil was not adequately absorbed. Fasted plasma triacylglycerol and cholesterol (total, LDL, VLDL and HDL) were not different between the diets. Postprandial increase of triacylglycerol was similar with olive and rapeseed oil, but lower after hydrogenated oil reflecting its poor absorption. Cumulative recovery of ^{13}C after 8 h was 10.7±0.6 % after olive oil, 11.6±0.6 % after hydrogenated oil and 13.1±0.8 % after rapeseed oil, indicating significantly greater linoleic oxidation after rapeseed oil. This study demonstrates beneficial effects of olive oil and rapeseed oil on postprandial lipid metabolism (cholesterol and LDL-cholesterol) (Demmelmair *et al.*).

3.2.3.4 Anti-inflammatory activities

In vivo, animal model

- COX-like inhibitory activity of olive oil component - oleocanthal was presented by Beauchamp et al. (2005). Daily consumption of 50g of extra virgin oil containing up to 200µg per ml of oleocanthal corresponding to effect of 10% ibuprofen (non-steroidal anti-inflammatory drug) dosage recommended for adult (Beauchamp *et al.* 2005).
- Anti-inflammatory effect of olive oil was tested in acute and chronic inflammation model in rats. The effect was compared to other oils (sunflower, palm and fish). For 8 weeks rats received diets enriched with six different type of oils: 2% corn oil (basal diet, BD), 15% high oleic sunflower oil (HOSO), 15% virgin olive oil (VOO), 15% virgin olive oil supplemented with 600 ppm polyphenols from this oil (PSVOO), 15% palm olein (POL), and 15% fish oil (FO). After that time carrageenan was injected. Rats treated with BD, PSVOO and FO diets for 3 weeks received Freund's adjuvant-complete (CFA) to induce arthritis. Indomethacin (1mg/kg, COX inhibitor) was also used in this experimental group. Carrageenan caused local oedema at 1 to 5 hour after the injection in group BD. Inflammation index significantly diminished in rats fed

VOO, at 5 h after injection, and it was markedly reduced in the PSVOO group both at 3 and 5 h after injection compared to the BD group. No significant changes in oedema formation were observed in the other diet groups, including the FO group. In case where CFA and indomethacin were used, both groups (PSVOO and FO) were able to prevent to some extent the development of the inflammation. PSVOO was even more effective than FO compared to BD in this model of chronic inflammation. Anti-inflammatory effect of indomethacin in PSVOO group was stronger than in other group (BD, FO). This study shows that virgin olive oil with a higher content of polyphenolic compounds has protective effects in both models of inflammation (Martinez-Dominguez *et al.* 2001)

- Hydroxytyrosol-supplemented refined olive oil was tested in an animal model of rheumatoid arthritis. Rheumatoid arthritis was induced by Freund's adjuvant-complete (CFA) collagen type II in rats on days 1 and 21. Hydroxytyrosol-supplemented refined olive oils (0.5 and 5mg/kg) were administrated by gavage from day 23 until day 35. The treatment at 5-mg/kg dose significantly decreased paw edema ($P<0.01$), histological damage, cyclooxygenase-2 and inducible nitric oxide synthase expression, and markedly reduced the degree of bone resorption, soft tissue swelling and osteophyte formation, improving articular function in treated animals. Acute inflammation, induced by carrageenan, was also evaluated for hydroxytyrosol-supplemented refined olive oils at 0.5 and 5 mg/kg. Both doses significantly reduced paw edema ($P<.001$). The study suggested that the supplementation of refined olive oil with hydroxytyrosol may be advantageous in treatment of rheumatoid arthritis in both chronic and acute inflammation (Silva *et al.* 2015).

3.3 Onion (*Allium cepa*)

3.3.1 Non-authorized EU health claims

EFSA Journal 2010;8 (2):1489 with Scientific Opinion contains the substantiation of health claims related to various food(s)/food constituents with antioxidant activity, antioxidant content and antioxidant properties (ID 1988). That claim pursues to Article 13(1) of Regulation (EC) No 1924/2006. Entry ID refers to *Allium cepa* (common name-onion) that has antioxidant properties. Proposed wording: "Specific antioxidant for smokers". Conditions

of use: bulb/leaf: 0.5-1g/day. It's been assumed that food/food constituent(s), here: onion, has ability to work as a scavenger of free radicals. Provided in vitro studies did not exert a beneficial physiological effect in humans. Therefore, the Panel considered that there is no relationship between consumption of onion and its beneficial physiological effect as antioxidant activity, antioxidant content and antioxidant properties (EFSA 2010).

3.3.2 Relevance of onion to health

There are number of studies showing the potential therapeutic effect of onion/onion constituent(s). Health claims of onion are:

3.3.2.1 Hepatoprotective/antioxidant effect

In vivo, animal model

- The effect of oral administration of onion on hepatic and renal function was study in vivo in animal model. Rats were divided into groups where treated groups were given varied doses of onion extract (0.5ml and 1.0 ml/100g body weight/day). Control group received placebo. Different hepatic and renal biomarkers were screened (glutathione (GSH), glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA), hepatic aspartate transaminase (AST), alkaline phosphatase (ALP), renal Na+/K+-ATPase activity). After 6 weeks of treatment both doses of onion indicated significant ($p<0.05$) increase in hepatic and renal levels of GSH, GST, SOD and CAT whereas MDA level was decreased. Both doses of onion also significantly ($p<0.05$) enhanced renal Na+/K+-ATPase activity. Only a high dose of onion caused significant ($p<0.05$) increase in hepatic aspartate transaminase (AST), alkaline phosphatase (ALP), and decrease in plasma AST activities. That results show that onion at both doses indicates antioxidant enhancement effect on hepatic and renal functions (Suru and Ugwu 2015).
- Hepatoprotective effect of onion extract was also study by other researchers. Aqueous extract of onion (made from fresh bulb) was tested in vivo in animal model. Rats were treated cadmium (Cd) to induce oxidative damage. Rats were divided into groups: Cd-treated group $3CdSO_4{*}8H_2O$, as Cd; 1.5 mg/kg body weight per day/orally, extract

treated group (0.5 and 1.0 ml/100 g body weight per day/orally for a week and then co-treated with Cd (1.5 mg/kg body weight per day/orally for three weeks). Control group received water only. Liver biomarkers were screened (GSH, GST, SOD, CAT, AST, ASP, ALP). Cadmium caused a marked ($p < 0.001$) increase in the levels of lipid peroxidation and GST, whereas GSH, SOD and CAT levels were decreased in the liver. A decrease in hepatic activities of ALT, AST and ALP was observed and a concomitant increase in the plasma activities of ALT and AST. Onion extracts significantly attenuated these adverse effects of Cd in a dose dependant-manner. This study shows protective effect of onion aqueous extract on Cd-induced oxidative damage in rat liver. This enhancing antioxidant effect is sufficient for preventing and protection liver against toxic agents. (Obioha *et al.* 2009)

3.3.2.2 Antioxidant and hypoglycaemic/hypolipidaemic properties

In vivo, animal model

- Anti-hyperglycaemic and antioxidant properties of onion were presented by El-Demerdash et al. (2005) in animal model. Rats were divided into groups: A-control, which received water as placebo. In B-group diabetes was induced by single dose of alloxan to rats in concentration of 120mg/kg body weight (BW). Group B was then subdivided into other groups: B1-was kept as diabetic group, B2-recived orally 1ml of onion juice (aqueous extract) per 100g BW (equivalent 0.4g/100g BW) for four weeks. In alloxan-induced rats the level of plasma glucose increased significantly ($p < 0.05$) (by 199%) in comparison to control group. Treatment of alloxan-diabetic rats with the juice onion reduced their plasma glucose levels by 70% compared with the diabetic group. In alloxan-diabetic rats the activities of plasma AST,ALT, LDH, AlP and Acp were significantly ($p < 0.05$) increased by 49, 60, 37, 51 and 58%, respectively, relative to their normal levels. In contrast, the activities of AST, ALT, LDH, AlP and AcP were significantly ($p < 0.05$) decreased in the liver tissue of alloxan-diabetic rats by 47%, 38%, 41%, 35% and 36%, respectively and increased in testes by 38%, 32%, 35%, 31% and 33%, respectively compared to the control values. Brain LDH activity was significantly ($p < 0.05$) increased by 58% in alloxan-diabetic rats. The study showed that the levels of free radicals were significantly ($p < 0.05$)

increased in plasma, liver, testes, brain and kidney by 28%, 16%, 22%, 38% and 22%, respectively in alloxan-diabetic rats as compared to control values. After treatment of alloxan-diabetic rats with onion the level of free radicals was significantly ($p < 0.05$) decreased in plasma and tissues as compared with the mean value of diabetic group. The activity of GST was significantly ($p < 0.05$) increased in liver, testes and kidney of both diabetic and, onion -treated diabetic rats compared with the control values (El-Demerdash *et al.* 2005).

- Potency of onion (*Allium cepa*) with respect to its hypoglycaemic and hypolipidaemic effects on the diabetic situation was presented by Campos et al. (2003). Study was performed using animal. Rats were divided into four groups. A normal control (group A), and a non-diabetic group (group B) were treated daily with 1 ml onion solution (0.4 g onion/rat). Groups C and D were made diabetic by an intraperitoneal injection of streptozotocin (STZ) (60 mg/kg body weight) in citrate buffer (pH 6.3). Groups C and D were the STZ diabetic control and STZ diabetic rats with onion intake, respectively. Onion increased the fasting serum high-density lipoprotein (HDL) levels, and demonstrated alleviation of hyperglycaemia in STZ diabetic rats. The hypoglycaemic and hypolipidaemic actions of onion (*Allium cepa*) were associated with antioxidant activity, since onion decreased SOD activities while no increased lipid hydroperoxide and lipoperoxide concentrations were observed in diabetic rats treated with onion (Campos *et al.* 2003).

3.3.2.3 Anticancer properties

In vivo, human model

- In meta-analysis presented by Zhou et al. (2013) nine studies were included in relation to intake of Allium vegetables (onions, garlic, shallots, leeks, chives) intake and prostate cancer risk. All included studies were published between 1997-2001 (3 cohort case and 6-case-controlled). It has been found a significantly decreased risk of prostate cancer for intake of allium vegetables (OR = 0.82, 95% CI 0.70, 0.97). This was statistically heterogeneity among studies (p=0.012 for heterogeneity; I^2 =59.2%. (Zhou *et al.* 2013)

- Allium vegetables (onions, garlic, shallots, leeks, chives) and their effect on gastric cancer risk was presented in another meta-analysis study. Twenty-one studies

(published from 1972 to 2010), which included 543,220 subjects, were analysed. It has been found that Allium vegetables consumption was associated with a reduced gastric cancer risk. In onion studies (13 studies performed: 5 hospital-based, 7 population-based case control and 1 cohort studies) the result showed that increased consumption of onions was associated with reduced gastric cancer (OR, 0.52; 95% CI, 0.36–0.68). In subgroup analysis by study design hospital-based case-control studies (OR, 0.53; 95% CI, 0.23– 0.82), population-based case-control studies (OR,0.52; 95% CI, 0.29 – 0.76), and cohort studies (OR, 0.50;95% CI, 0.16 – 0.84) gave similar results. Meta-analysis shows that consumption of large quantity of Allium vegetables (onions, garlic, shallots, leeks, chives) reduced the risk for gastric cancer (odds ratio, 0.54; 95% confidence interval, 0.43-0.65). Specific analyses for onion, garlic, leek, Chinese chive, scallion, garlic stalk, and Welsh onion yielded similar results, except for onion leaf. The estimated summary odds ratio for an increment of 20 g/day of Allium vegetables consumed (approximately the average weight of 1 garlic bulb) was 0.91 (95% confidence interval, 0.88-0.94), based on case-control studies from the dose-response meta-analysis (Zhou *et al.* 2011)

3.3.2.4 Anti-platelet activity

In vivo, human model

- Anti-platelet activity of onion soup was studied. Two varieties of soup were tested: low; (8.2mg/L) and high (114.8mg/L) in quercetin). Two types of onions were used to prepare soups (near-isogenic high- and low-quercetin onions). In a randomized, double-blind, cross-over study, 6 volunteers (age 34 (SD 7) years; weight 68·5 (SD 3·6) kg; BMI 23·1 (SD 2·0) kg/m2) were participated in the experiment. Blood samples were taken before and after either low- or high quercetin onion soup (600ml) ingestion. Blood samples were taken at 0,1 and 3 h were also used to examine the effect of soup ingestion on platelet function and signalling. Platelets were prepared from blood samples (450µl) using with collagen (50µl; 0.5, 1, 2 and 3µg/ ml final concentration). Soup ingestion contained high quercetin concentration inhibited platelet aggregation at 1 and 3h after ingestion and this effect was greater 3h after consumption of high-quercetin soup, when platelets were stimulated with 0.5, 1 and 2 µg/ml collagen. Collagen-stimulated platelet aggregation was inhibited by ingestion

of the high-quercetin soup. The inhibitory effect was dependent on the concentration of collagen used to stimulate platelet aggregation. The inhibition of platelet aggregation by collagen after ingestion of the high-quercetin soup was also time-dependent, more potent effects being seen 3 h after ingestion of the soup compared with 1 h (Hubbard *et al.* 2006)

In vivo, animal model

- Anti-platelet effect of onion was studied in streptozotocin (STZ) induced diabetic rats. Thromboxane B2 (TXB_2) level in serum of diabetic rats was elevated compared to that in normal. Four day of onion treatment (0.5g/ml/kg/day) significantly reduced TXB_2 level in diabetic rats. In normal rats TXB_2 level was unaltered after treatment with onion. The study also revealed that onion has an effect on TXB_2 formation, platelet aggregation and arachidonic acid (AA)-release in platelets from diabetic and normal rats. Onion showed a significant inhibitory effect on collagen- or AA-induced TXB_2 formation with greater potency in diabetic platelets than in normal. Similarly, more potent inhibitory effects of onion in diabetes were observed in collagen- or AA-induced platelet aggregation and collagen-induced AA release response. Onion may be considered as a powerful antithrombotic agent in treatment of diabetes. (Jung *et al.* 2002)

In vitro

- Anti-platelet action of aqueous extract of onion (0.1–1.0 g/ml) was tested in animal model using rat platelet-rich plasma. Aggregation was induced by various agents (collagen, thrombin and arachidonic acid (AA)). IC 50 values of onion for collagen (6 µg/ml)-, thrombin (0.4 U/ml) - and AA (30 µM)-induced aggregations were 0.17 ± 0.01, 0.23 ± 0.03 and 0.34 ± 0.02 g/ml, respectively. In addition, effect of onion on TXB_2 formation was examined in rat washed platelet. IC50 value of onion for collagen (6 µg/ml)-induced TXB2 formation was 0.12 ± 0.01 g/ml. Aqueous extract of onion significantly inhibits the collagen-induced AA release what may suggest that onion possess antithrombotic effect (Moon *et al.* 2000).
- Aqueous extract of boiled and raw onions and they effect on the collagen-induced platelet aggregation was studied. In in vitro model, rabbit and human platelet-rich plasma were used. The concentration of onion required for 50% inhibition was

estimated as 90mg/ml plasma, moreover antiplatelet action of onion was dose-dependent. Boiled onion extract had reduced inhibitory activity (Ali *et al.* 1999).

3.4 Ginger (*Zingiberis officinale*)

3.4.1 Non-authorized EU health claims

EFSA Journal 2010;8 (2):1493 with Scientific Opinion contains the substantiation of health claims related to various food(s) /food constituents claiming maintenance of joints (entry ID 2649). That claim pursues to Article 13(1) of Regulation (EC) No 1924/2006. Entry ID refers to extract of *Zingiberis officinale* (common name-ginger) that helps to maintain mobility of joints. Proposed wording: "helps to maintain mobility of joints and to avoid morning stiffness". Conditions of use: powder: 5.0-0.1g/day; aqueous extract 2.5-0.05g/day or Food supplement with 1.8g of ginger root in the daily dose. There are number of in vitro and animal studies showing the effect of food/food constituent(s) on human chondrocytes/cartilage explants and chondrocyte cell lines but no human in vivo studies were done to prove an effect of food/food constituent(s), here: ginger roots, on the maintenance of normal joints in humans. Therefore, the Panel considered that there is no relationship between consumption of ginger and maintenance of normal joints (EFSA 2010).

3.4.2 Relevance of ginger to health

There are number of studies showing the potential therapeutic effect of ginger/ginger constituent(s). Health claims of ginger are:

3.4.2.1 Anti-emetic properties

In vivo, human model

- The effect of ginger in nausea and vomiting of pregnancy was studied in a single-blind, randomized, placebo-controlled trial. Pregnant women (67) were included in the study and they received 250mg of ginger capsules 4 times a day. Control group

received placebo at the same prescribed form. Ages of women were 24.1 +/- 4.8 and 23.3 +/- 5 years, respectively. The mean gestational age was 13 +/- 3 weeks, and the mean parity was 1.6 +/- 0.8. There was a higher rate of improvement in ginger group than the placebo users (85% versus 56%; $p < 0.01$). Vomiting occurrences (daily measurement) among ginger users was also significantly lower than among the women who received the placebo (50% versus 9%; $p < 0.05$). It can be suggested that 1000mg of ginger daily may decreasing effect on nausea and vomiting women during pregnancy (Ozgoli *et al.* 2009)

- The effectiveness of ginger in nausea and vomiting was tested in women after elective caesarean section. Double-blinded and placebo-controlled trial was included in this study. Women (n=116) who underwent elective caesarean section received spinal-epidural anaesthesia with preoperative antiemetic treatment additionally they were given 2 ginger capsules (each contained 1000mg of dry powdered ginger). One capsule was given a half-hour before induction of anaesthesia and the second two hours after surgery. Placebo group (n=123) received no ginger. Occurrences of nausea and vomiting were monitored both intraoperative and postoperative. Postoperative pain and pruritus were also assessed. The incidence of postoperative nausea in ginger and placebo groups were 52% and 61%, respectively (p=0.149). Occurrence of intraoperative nausea was less in the ginger group compared to placebo (mean difference was -0.396, 95% CI -0.738, -0.054) and the result was statistically significant (p=0.023). The incidence of intraoperative vomiting was 27.35% in the ginger group and 36.59% in the placebo group, and the difference was not statistically significant (p=0.126). The number of episodes of vomiting during surgery was less in the ginger group compared to placebo: (mean difference -0.158, 95% CI -0.626, 0.311) although statistically insignificant (p=0.505). Furthermore, postoperatively, there was no statistical difference in the incidence of nausea and vomiting assessed at 0, 2, 2 ½ and 24h after surgery. There were also no differences in postoperative pain or pruritus. Ginger in dry powdered form reduces the occurrence of intraoperative nausea but has no effect on incidence of nausea, vomiting, or pain during and after an elective caesarean section when combined spinal epidural anaesthesia is applied (Kalava *et al.* 2013).

- Chittumma et al. (2007) were compared the effectiveness of ginger and vitamin B6 in treatment of nausea and vomiting during pregnancy. In a randomized and double-blind controlled trial 126 pregnant women (gestational age of < or = 16), who had nausea and vomiting, were received either 650mg of ginger or 25mg of vitamin B6 (three times a day for 4 days). Rhode's score was used to assess episodes of nausea, duration and number of vomits. This was recorded 24 hours before treatment (as baseline) and then each subsequent day of treatment. Ginger and vitamin B6 significantly reduced nausea and vomiting scores from 8.7 +/- 2.2 to 5.4 +/- 2.0 and 8.3 +/- 2.5 to 5.7 +/- 2.3 respectively, ($p < 0.05$). The mean score change after treatment with ginger was greater than with vitamin B6 (3.3 +/- 1.5 versus 2.6 +/- 1.3), ($p < 0.05$). There were some minor side effects in both groups 25.4% and 23.8% ($p = 0.795$) respectively, such as sedation, heartburn, arrhythmia. Ginger is more effective than vitamin B6 in treatment of nausea and vomiting in pregnancy, however some minor side effects of ginger treatment are observed (Chittumma *et al.* 2007).

- Ginger and its therapeutic effect on treatment of nausea and vomiting in pregnancy was also presented by another researchers. In the randomized, placebo-controlled trial 70 pregnant women (before or at 17 weeks gestation) were participated. They received 1000mg of ginger (or placebo) during 4 days. Visual analog scale was used for assessing the occurrence of nausea. Number of vomiting was also recorded 24h before and 4 days after treatment. Seven days later at follow-up check Linkert scale was also used to assess the severity of their symptoms. The visual analog scores of post therapy minus baseline nausea decreased significantly in the ginger group (2.1 +/- 1.9) compared with the placebo group (0.9 +/- 2.2, P =0.014). The number of vomiting episodes also decreased significantly in the ginger group (1.4 +/- 1.3) compared with the placebo group (0.3 +/- 1.1, P <0.001). Likert scales showed that 28 of 32 in the ginger group had improvement in nausea symptoms compared with 10 of 35 in the placebo group (P <.001). No adverse effect of ginger on pregnancy outcome was detected. Ginger is effective for nausea and vomiting during pregnancy (Vutyavanich *et al.* 2001).

3.4.2.2 Antioxidant activity

In vitro

- Antioxidant activity of CO_2 extract (871 mg/g dry extract) of ginger was studied. Different methods were employed in this study. DPPH assay was performed to determine the scavenger activity of ginger extract. The ginger extract showed a significant effect in inhibiting DPPH, reaching up to 90.1% at concentration 20 μg/ml and its IC_{50} was 0.64 μg/ml compared with the IC_{50} of synthetic antioxidant BHT of 7.02 μg/ml. IC_{50} values were with statistical significance $p \leqslant 0.01$ and had high regression coefficients of $R^2 = 0.985$ and $R^2 = 0.976$, respectively. Antioxidant activity of extract was also screened using linoleic acid model. The experiment for inhibiting the peroxidation of the linoleic acid by ginger extract were conducted at two temperatures: 37 and 80 °C. An intensive formation of conjugated dienes was observed on day 4 of incubation of the linoleic acid at 37 °C, and the maximum was reached on day 10. Three different concentrations of ginger extract (0.01. 0.02 and 0.05%) were used. The highest antioxidant activity was found at 0.02%, and it was the same as that of the inhibition of the synthetic antioxidant BHT, i.e. 62.9%. The concentrations of the ginger extract 0.05% and 0.01% showed a weaker effect in inhibiting lipid peroxidation. At 80 °C, a peak in conjugated dienes formation was observed at 5 h of storage, although the level of conjugated dienes was lower than that at 37 °C. Most efficient was again the 0.02% concentration, whose inhibition after 5 h was higher than that of BHT: 50.5% and 44.7%, respectively. The comparison of the antioxidant activity of the ginger extract for the two temperatures showed a greater value at a lower temperature reaching 71.6% as compared with 69.3% of BHT after 8 d of storage.
- Ability of extract for lipid peroxidation was also performed by TBARS. The antioxidant activity of 0.05% ginger extract was higher than that of the control – 67.7% and 61.3%, respectively, after 10 d at 37 °C and 36.4% and 31.8%, respectively, at 80 °C after 4 h. The free radical scavenger properties of ginger extract was also studied by deoxyribose assay. Ferric-EDTA was incubated with H_2O_2 and ascorbic acid at pH 7.4. Hydroxyl radicals were formed in free solution and were detected by their ability to degrade 2-deoxy-d-ribose into fragments that on heating with TBA at low pH form a pink chromogen. Addition of ginger extract to the

reaction mixture removed the hydroxyl radicals from the sugar and prevented it from degradation. The effect from inhibiting the hydroxyl radicals was greater at 37 °C rather than at 80 °C – IC_{50} at 37 °C was 1.90 µg/ml (R^2 = 0.967), while at 80 °C a higher concentration of ginger extract was needed – IC_{50} = 2.78 µg/ml (R^2 = 0.975). The quercetine used as a control had IC_{50} of 4.61 µg/ml (R^2 = 0.834) at 37 °C and 7.41 µg/ml (R^2 = 0.984) at 80 °C (Stoilova *et al.* 2007)

3.4.2.3 Anti-atherogenic properties

In vivo, animal model

- Ginger extract and its effect on plasma lipids was investigated. In ex vivo study standardized ginger extract was assessed in terms of its effect on development of atherosclerosis in apolipoprotein E-deficient (E(0)) mice. Plasma cholesterol level and LDL peroxidation and aggregation was subjected to this study. E(0) mice (n = 60; 6 weeks old) were divided into three groups of 20 and fed for 10 weeks via their drinking water with the following: group A) placebo (control group), 1.1% alcohol and water (11 mL of alcohol in 1 L of water); group B) 25 µg of ginger extract/day in 1.1% alcohol and water and group C) 250 µg of ginger extract/day in 1.1% alcohol and water. Aortic atherosclerotic lesion areas were reduced 44% (P<0.01) in mice that consumed 250 µg of ginger extract/day. Consumption of 250 µg of ginger extract/day resulted in reductions (P<0.01) in plasma triglycerides and cholesterol (by 27 and 29%, respectively), in VLDL (by 36 and 53%, respectively) and in LDL (by 58 and 33%, respectively). These results were associated with a 76% reduction in cellular cholesterol biosynthesis rate in peritoneal macrophages derived from the E(0) mice that consumed the high dose of ginger extract for 10 weeks (P<0.01). Furthermore, peritoneal macrophages harvested from E(0) mice after consumption of 25 or 250 µg of ginger extract/day had a lower (P<0.01) capacity to oxidize LDL (by 45 and by 60%, respectively), and to take up and degrade oxidized LDL (by 43 and 47%, respectively). Consumption of 250 µg of ginger extract/day also reduced (P<0.01) the basal level of LDL-associated lipid peroxides by 62%. In parallel, a 33% inhibition (P<0.01) in LDL aggregation (induced by vortexing) was obtained in mice fed ginger extract. Consumption of ginger extract by E(0) mice significantly attenuates the development of atherosclerotic lesions. This antiatherogenic effect is associated with

a significant reduction in plasma and LDL cholesterol levels and a significant reduction in the LDL basal oxidative state, as well as their susceptibility to oxidation and aggregation (Fuhrman *et al.* 2000).

3.4.2.4 Anticancer properties

In vivo, animal model

- Anti-cancer and cytoprotective activity of ginger was studied in vivo animal model. Wistar albino rats (150-200g) were divided into groups. Group A received 1ml of necrotizing agents for induction of cells death (80% ethanol, 0.6M HC1, 0.2M NaOH and 25% NaCl) 30 min after administration of ginger extract (96% ethanolic extract) in dose of 500 mg/kg. Ginger extract (96% ethanol extract) in dose of 500 mg/kg orally exhibited highly significant cytoprotection against 80% ethanol, 0.6M HC1, 0.2M NaOH and 25% NaCl induced gastric lesions. Ulcer Index (mean+S.E.) was as follow: For 80% Ethanol 7.62+0.18 in control and 0.75+0.25 in ginger group, for 0.6%M HCl 7.12±0.29 in control and 1.50+1.89 in ginger group, for 0.2M NaOH 6.87+0.29 in control and 1.12+1.24 in ginger group and 25% NaCl 5.87+0.35 in control and 1.37±0.18 in ginger group. In group B ginger extract was given 30 min before induction of ulcer (by ulcerogenic drug: indomethacin, aspirin and reserpine). In this group is has been observed that the extract significantly ($p \leq 0.05$) prevented the occurrence of gastric ulcers induced by non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin, aspirin but also by reserpine (non-significant occurrence). These observations suggest cytoprotective and anti-ulcerogenic effect of the ginger. (al-Yahya *et al.* 1989)
- Anti-ulcerogenic activity of ginger was also studied by Zaman et al. (2014). Ginger root extract in powdered form was made in aqueous solution. Albino rats (150-250g) were randomly divided into 4 groups. Group I-distilled water -2ml/kg body weight, group II- omeprazole (anti-ulcerative) 10mg/kg body weight, group III-test rats-ginger 200mg/kg body weight, groups IV-test rats-ginger 400mg/kg body weight. Half-on hour later rats were treated with indomethacin (25mg/kg body weight) to induce gastric damage. Gastric damage caused by indomethacin gave a total severity score of

22.33±2.25. Using of omeprazole (10mg/kg), ginger (200mg/kg) and ginger (400mg/kg) along with indomethacin gave a mean total severity score to 4±2.28, 10.6±3.26 and 6.2±3.35, respectively. The percentage of inhibition of gastric ulcers was 40.91%, 57.58% and 65.91% by ginger 200mg/kg, ginger 400mg/kg and omeprazole, respectively. The study clearly shows that ginger significantly (n=6, $P \leq 0.001$, for each group) inhibits gastric damage induced by indomethacin, it can be considered then as a gastro-protective agent (Zaman *et al.* 2014).

- Effect of ginger on induced duodenal ulcer was studied by Minaiyan et al. (2006). Different concentrations of hydroalcoholic extracts were tested: 100, 350 and 700mg/kg. Wistar rats were divided into groups where doses of 100, 350, 700 mg/kg of ginger extracts, ranitidine (50 mg/kg)-reference drug, sucralfate (500 mg/kg) and 5 ml/kg of vehicle were administered orally. After ulcer induction, the number, scoring, area and finally ulcer index were assessed for each duodenum. Administration of extract in dose of 350 mg/kg and ranitidine resulted in significant reduction in mucosal damage for the entire ulcer factors which were assessed. Larger doses of extract given (350 and 700 mg/kg) were effective to reduce the ulcer area and index but the lowest dose of extract (100 mg/kg) was not effective (Minaiyan *et al.* 2006).

3.4.2.5 Antiplatelet, anti-inflammatory and cholesterol lowering effect

In vivo, animal study

- An aqueous extract of raw ginger was examined for its efficacy on serum cholesterol and triglyceride levels as well as platelet thromboxane-B_2 (TXB_2) and prostaglandin-E_2 (PGE_2) production. Extract of ginger (50mg/kg and 500mg/kg) was administered daily for a period of 4 weeks orally to rats. Control (normal saline) was also employed in the study. A low dose of ginger (50 mg/kg) administered orally did not produce any significant reduction in the serum thromboxane-B_2 levels when compared to saline-treated animals but caused a significant changes in the serum PGE_2 at this dose. High doses of ginger (500 mg/kg) were more effective in lowering serum PGE_2. TXB_2 levels were significantly lower (by 50%) in rats who received 500 mg/kg ginger. A significant reduction in serum cholesterol was observed when a higher dose of ginger (500 mg/kg) was administered. A significant reduction in cholesterol level was

observed when high dose of ginger (500mg/kg) was given to rats, however no significant changes in triglyceride level was observed in the serum of rats receiving ginger extract. These results suggest that ginger could be used as an cholesterol-lowering, antithrombotic and anti-inflammatory agent (Thomson *et al.* 2002).

In vivo, human model

- The effects of ginger consumption on platelet thromboxane production was studied. Women were involved in this study. They consumed 5 g raw ginger daily for a period of 7 days. Thromboxane B_2 (TXB_2) determination was estimated before and after consumption in serum obtained after blood clotting. TXB_2 (pmol/ml serum) in ginger group was 782 ± 482 before consumption and 498 ± 164 (Mean ± SD, N = 7), after consumption. This study shows that ginger may be used as antithrombotic agent (Srivastava 1989).
- Synergistic effect on anti-platelet aggregation of ginger was presented by Young et al. (2006). Ginger and nifedipine (anti-platelet drug) was administrated in patients suffered from hypertension and in healthy volunteers. The study showed that 1g of ginger with 10mg nifedipine resulted efficient inhibition of platelet aggregation (Young *et al.* 2006).

3.5 Garlic (*Allium sativum*)

3.5.1 Non-authorised EU health claims

EFSA Journal 2010;8 (10):1799 with Scientific Opinion contains the substantiation of health claims related to various food(s) /food constituents with decreasing potentially pathogenic gastro-intestinal microorganisms (entry ID 2362). That claim pursues to Article 13(1) of Regulation (EC) No 1924/2006. The claimed effects are: “body’s defence system against microorganism” and “immune health”. Main entry health claims is related to Garlic bulb (*Allium sativum*), including condition of use (2-5g fresh garlic or 0.5-1g dried powder/day; containing: Alliin: 6-10mg, allicin: 3-5mg or any extract equivalent with these). Proposed wording: Garlic can contribute to the maintenance of the microbial balance in the gut, and to

the defence against harmful microorganisms (bacteria, fungi). The Panel stated that in vitro studies can be used as supportive evidence but there are not sufficient to predict the effect of consumption of food(s) /food constituents (here: garlic bulb) on decreasing harmful gastro intestinal bacteria or fungi. Moreover no human studies has been done to prove the effect of garlic bulb on claimed effect. The Panel concludes that there is no relationship between consumption of food(s) /food constituents (here: garlic bulb) and decreasing potentially pathogenic gastro-intestinal microorganism (EFSA 2010)

3.5.2 Relevance of garlic to health

There are number of studies showing the potential therapeutic effect of garlic/garlic constituent(s). Health claims of garlic:

3.5.2.1 Reducing cholesterol in plasma

In vivo, human model

- Garlic powder supplements (enteric-coated) with 9.6mg allicin-realising is shown to have hypocholesterilemic effect. After 12 weeks of supplementation and low fat combined diet (in a double-blind randomised, placebo controlled trial) it has been a significant reduction in total cholesterol (TC); -4.2%) and LDL cholesterol (-6.6%) in garlic group (n=22) when compares to placebo group, n=24 (2.0% and 3.7% for total cholesterol and LDL cholesterol, respectively) (Kannar *et al.* 2001)
- Effect of consumption of raw garlic on lipid and sugar profile was investigated by Mahmoodi et al. in 2011. Eighty five volunteers were divided into three group where: 1 group (fasting blood sugar; FBS) with blood sugar more than 126mg/dl (30 people), group 2 with cholesterol more than 245mg/dl (30 people) and group 3 with FBS more than 126 mg/dl and cholesterol more than 245mg/dl (25 people). Volunteers were given normal diet, no medication for sugar and lipid level reduction additionally there were supplied with raw garlic (10g/day divided into two meals). Garlic consumption were stopped 6 weeks before samples were collected. In the second group a significant decrease in FBS level (($P< 0.01$), TC ($P < 0.001$) and TG ($P < 0.01$).

When garlic consumption was stopped for 42 days is has been found that FBS and TG level was elevated and HDL level decreased. In the third group level of FBS (P <0.01) and also total cholesterol (P < 0.001) were significantly decreased whereas HDL-C level significantly increased after 42 days consumption of garlic. In all group any significant changes in hormonal profile (insulin, TSH, T3, T4) was not found. Garlic has been found to has more effect on lipid profile than glucose level (Mahmoodi *et al.* 2011).

- The cholesterol lowering effect was also observed in another randomised, placebo-controlled human study (meta-analysis study). Twenty-nine trials were included in the analysis and it was found that garlic significantly reduces total cholesterol and TAG level. Study also shown no significant reduction of LDL and HDL (Reinhart *et al.* 2009).
- The cholesterol lowering effect of garlic was also mentioned by Tapsell in 2006 (Tapsell *et al.* 2006). There is level III-3 evidence (National Health and Medical Research Council [NHMRC] levels of evidence) that consuming a half to one clove of garlic (or equivalent) daily may have a cholesterol-lowering effect of up to 9%. Reductions in blood pressure (an approximate 5.5% decrease in systolic blood pressure) was also observed in vivo studies after consuming 7.2g of aged garlic extract (Tapsell *et al.* 2006).
- Garlic powder tablets (900mg/day) was tested in a randomized, double-blind, placebo controlled trial in 42 healthy adults (mean age 52±12 years). Total serum cholesterol (TC) level in those group was greater than or equal to 220 mg/dL. After 12 weeks total serum cholesterol (TC) level 262 ±34 mg/dL was reduced to 247 ± 40 mg/dL ($p < 0.01$) in garlic group, and for placebo were 276 ± 34 mg/dL before and 274 ± 29 mg/dL after placebo treatment. LDL-C (low density lipoprotein cholesterol) was reduced by 11% and 3% in garlic and placebo group, respectively. It has been observed then significantly greater reduction in serum TC and LDL-C than placebo (Jain *et al.* 1993).

3.5.2.2 Preventing thrombosis

In vitro, human model

- Effect of garlic oil on platelet aggregation was studied in vitro by Bordia in 1978. Blood was collected from six healthy adults and aggregation agents were used (ADP, epinephrine, collagen) for inducing of aggregation. Garlic oil shown inhibitory effect on platelet aggregation induced by aggregation agents and this effect was dose related. Also oral administration of garlic oil (0.5mg/day) for 5 days had inhibitory effect on formation of thrombus (Bordia 1978).

- Influence of processed garlic on aggregation inhibition was also studied in vitro model. Study was performed using human blood platelets. Treatment of garlic with high temperature (200 degree C or in boiling water) for 3 min did not affect of anti-aggregation activity (IVAA) of garlic as compared to raw garlic. Same effect was observed for microwaved garlic. (Cavagnaro *et al.* 2007).

In vivo, human model

- The effect of consumption of fresh garlic bulb on platelet thromboxane was studied. After 26 weeks of garlic consumption (1 cloves, approx. 3g a day) there was 20% reduction of cholesterol and 80% reduction of thromboxane in serum (Ali and Thomson 1995).

- There is level III-1 evidence that 7.2 g of aged garlic extract has been associated with anticlotting (in-vivo studies) (Tapsell *et al.* 2006).

- In double-blind, placebo-controlled trial by Kiesewetter et al. in 1991 has been shown that administration of 800 mg of garlic powder over a period of four weeks causes complete solubilisation of thrombocyte aggregation. Aggregation disappeared and microcirculation of the skin increases by 47.6% (from 0.63 +/- 0.13 to 0.93 +/- 0.22 mm/s) and plasma viscosity decreases by 3.2% (from 1.25 +/- 0.34 to 1.21 +/- 0.43 mPas) and diastolic blood pressure by 9.5% (from 74 +/- 9 to 67 +/- 5 mmHg; (Kiesewetter *et al.* 1991).

- In another double-blind, placebo controlled trial the effect of dried garlic powder (Sapec) on fibrinolysis and platelet aggregation has been studied. The garlic powder (900mg) was consumed by 12 healthy volunteer. After 14 days of garlic powder consumption t-PA (tissue plasminogen activator) was significantly higher, platelet aggregation values were also significantly lower after 14 days treatment. Moreover, platelet aggregation, induced by different agents (ADP and collagen), and beta-TG

(beta-thromboglobulin) realising (after collagen stimulation), were significantly inhibited 2 and 4h after garlic powder consumption (Legnani *et al.* 1993).

3.5.2.3 Anticancer activity

In vivo, human model

- Aged garlic extract (AGE) was tested for colorectal adenomas in a double-blinded randomized study. High-AGE (2.4ml/day) and low-AGE (0.16ml/day) doses were administrated to 51 patients who were diagnosed with colorectal adenomas. Number and size of adenomas were screened using colonoscopy at 0, 6 and 12 months of intake of AGE extracts. In 37 patients chosen as efficacy evaluated subjects, 47.4% (9/19) in the high-AGE and 66.7% (12/18) in the low-AGE group had at least one new adenoma for the first and second interval (0 to 12 months after intake), and its relative risk was 0.71. The decrease rate of at least one adenoma was 50.0% (7/14) in the high-AGE group for the second interval (6 to 12 months after intake), whereas there was no decrease in subjects in the low-AGE group (p=0.02). The decrease rate of at least one adenoma was 50.0% (7/14) in the high-AGE group for the second interval (6 to 12 months after intake), whereas there was no decrease in subjects in the low-AGE group (p=0.02); (Tanaka *et al.* 2004)

In vitro studies using human cell line:

Organosulfur compounds isolated from garlic with anticancer properties:

- Organosulphur compounds in a garlic bulb 2.3% (Suleria *et al.* 2015). Main sulphur compound in garlic are **allicin** and **alliin**.
- Garlic sulphurous are:

a) Alliin (0.8% in a garlic cloves). Dehydrated garlic contains 2-2.5mg/g alliin in a powder.
b) Allicin content in fresh garlic 3.7mg/g (Amagase *et al.* 2001).
c) Ajoene.

d) DADS (metabolite of allicin).
e) DAS.
f) DATS.
g) DAMS.
h) SAC.

- **Ajoene** has been studied to show induction effect on apoptosis in leukemic human cell line as well as in blood cells of a leukemic patient. Ajoene in quantity of 20μM activates specific caspases in a time-dependent manner in leukaemia human cells (Dirsch *et al.* 2002)
- **Ajoene** has been found to have an effect on expression of apoptosis-related proteins: bcl-2 and caspase-3 induced by drugs (cytarabine and fludarabine) used in treatment of acute myeloid leukaemia (AML). Treated of KG1-resistant leukaemia cells with 40μM of ajoene showed reduction in the bcl-1- expression from 239.5 +/- 1.5 in control cultures to only 22.0 +/- 4.0 in ajoene-treated cultures. Ajoene had also enhancing effect on inhibitory fashion of two chemotherapeutic drugs: cytarabine and fludarabine (Ahmed *et al.* 2001)
- **Ajoene** and its inducing effect on apoptosis of human leukemic cells was also studied by Dirsch et al. in 1998. It's been suggested that ajoene may stimulate peroxide production and activate of nuclear factor κB .(Dirsch *et al.* 1998)
- The oil-soluble **diallyl sulfides (DAS)** include: **monosulfides (DAMS), disulfides (DADS)** and **trisulfides (DATS)** isolated form garlic are shown to have ant proliferative effect on human cells (HEK 293T) There were significant differences between the IC50 values of DAMS, DADS and DATS. RT-PCR was performed and the expression of COX-2 was compared with that of b actin. DATS inhibited COX-2 gene expression significantly stronger than DAMS and DADS. The data are suggestive of antineoplastic effect of DAS, mediated by controlling COX-2 expression (Elango *et al.* 2004)
- **Diallyl trisulfide (DATS)** and its anticancer properties was also presented by other researchers. DATS inhibit carcinogenesis of BaP (benzo(a)pyrene)induced breast cells in vitro. The DATS in quantity of 6 and 60 μM inhibits concurrently treated with BaP(1μM) human cell (MCF-10A cells) by an average of 71.1% and 120.8%, respectively, at 6. h. The 60 μM DATS pre-treatment decreased BaP-induced G2/M

cell cycle transition by 127%, and reduced the increase in cells in the S-phase by 42%; whereas 60 μM DATS CoTx induced a 177% increase in cells in G1. DATS effectively inhibited (P< 0.001) BaP-induced peroxide formation by at least 54%, which may have prevented the formation of BaP-induced DNA strand breaks. (Nkrumah-Elie *et al.* 2012)

- **Allicin** inhibits the proliferation of cancer cells. The influence of allicin on human tumor cells was studied. Treated with allicin and untreated SiHa cells were screened by MTT assay (expressed as percentage over control) after 24, 48 and 72 hours. Most of the cancer cells were killed with 100mM concentration of allicin. The research show that allicin decreases viability of SiHa cells in a time and concentration dependent manner (Oommen *et al.* 2004)
- **Water soluble S-allys cysteine (SAC)** is garlic compound with anticancer properties. SAC significantly reduce formation of liver carcinogen N-nitrosomorpholine (NMOR) in vitro. Addition (20, 40, and 80 mM) of SAC decreased NMOR formation by 16%, 27%, and 43%, respectively ($p < 0.05$). Both SAC and DADS reduced the mutagenicity of NMOR in *Salmonella typhimurium* TA100 ($p < 0.05$). SAC at 70 μmol/plate reduced the number of histidine revertants per plate by 51% ($p < 0.05$), whereas DADS at 0.12 μmol/plate reduced mutant colony number by 76% ($p < 0.05$) (Dion *et al.* 1997)

3.6 Scotch Bonnet Peppers and Pimento (*Capsicum chinense*, *Capsicum annum*)

These peppers belong to variety of chili peppers. Pimiento (pimento, cherry pepper) is a large, red chili pepper (*Capsicum annum*). Scotch Bonnet pepper (*Capsicum chinense*), is hotter than pimento. Most Scotch Bonnets have a heat rating of 100,000–350,000 Scoville units. Pimento has 100-500 Scoville unit, what means is very mild. The Scoville rating indicates how "spicy" a pepper is, which depends on its respective capsaicin content. The major and naturally occurring constituents are capsaicinoids ranges typically from 0.1 mg/g in chili pepper to 2.5 mg/g in red pepper and 60 mg/g in oleoresin red pepper (O'Neill *et al.* 2012).

3.6.1 Non-authorised EU health claims

EFSA Journal 2010;8 (2):1489 with Scientific Opinion contains the substantiation of health claims related to various food(s) /food constituents and protection of DNA, proteins and lipids from oxidative damage (entry ID 2043). That claim pursues to Article 13(1) of Regulation (EC) No 1924/2006. Entry ID refers to Capsicum extract- with capsaicin with reduction of oxidative stress properties. Proposed wording: "As an antioxidant helps reduce damage to the body tissue". Conditions of use: Animal study showing that capsaicin resists oxidative stress and depletion of intracellular thiols. Animal study (1-3mg/kg body weight for up to three days) shows capsaicin to be a potent antioxidant. The claimed effects refers to the protection of the body cells and molecules (DNA, proteins, lipids) from oxidative damage, including UV-induced oxidative damage. However in vitro and animal studies showed the free radical scavenger properties of different food constituents, no human trials has been done to prove the effect of food(s)/food constituent(s), on protection of cells and tissues from oxidative damage. Provided scientific evidences are not sufficient to establish relation between consumption of the food/food constituents and protection of body cells and tissue form oxidative damage (EFSA 2010).

3.6.2 Relevance of peppers to health

There are number of studies showing the potential therapeutic effect of peppers/peppers constituent(s). Health claims peppers are:

3.6.2.1 Prevention hypertension

In vivo, *in vitro* animal model

- Beneficial effect of capsaicin in reduction of high blood pressure was presented by Yang et al. (2010). Capsaicin showed stimulatory action on (TRPV1) channels in vitro and vivo animal model. Endothelial transient receptor potential vanilloid 1 (TRPV 1) is present in endothelial cells (ECs) of arteries. It is a non-selective Ca2+

channel. In the study TRPV 1 was isolated from the arteries of wild-type (WT) mice and *TRPV 1* $^{-/-}$ mice. Expression of TRPV 1 mRNA and protein was confirmed by RT-PRC and immunoblotting only in ECs of wild-mice. It has been shown that acute capsaicin stimulates an increase of free calcium in the cytosol in cultured ECs (in vitro) what in turn relaxes mesenteric arteries and thus reduces arterial pressure in hypertension. Regulation of TRPV 1 channel by capsaicin was also observed in vivo study. After 6 months treatment of animal (mice) with capsaicin (0.01%/day) there was a significantly increase in TRPV1 protein expression in mesenteric arteries of wild-type mice and the level of TRPV1 protein was higher in mesenteric arteries from TRPV1 transgenic mice. Chronic activation of TRPV1 by 6 month consumption of dietary capsaicin (0.01%) in WT mice moderately increased acetylcholine-induced endothelium-dependent relaxation (pD_2, 6.88 ± 0.16 in control and 7.52 ± 0.17 in capsaicin, $p < 0.05$. Furthermore, the maximum relaxation to acetylcholine was significantly enhanced in TRPV1-tg mice. This effect was not present in capsaicin-treated *TRPV1*$^{-/-}$ mice. Capsaicin was also administered intragastrically (15mg/kg body weight) in Wistar rats. During first hour capsaicin concentration in a plasma reached the peak of 10ng/ml and then declined rapidly. Maximum bioavailability of capsaicin taken orally was 0.106%. Capsaicin was rapidly metabolized in a liver. After 3 weeks of treatment (intragastrically) with capsaicin where was no changes in endothelium relaxation and blood pressure in MAs. Reduction in systolic blood pressure (SBP) was only observed after long capsaicin treatment. At fifth month arterial pressure reached significant reduction. The average 24 h blood pressure was significantly lower in capsaicin-treated compared with untreated rats (SBP, 181 ± 3 mmHg versus 198 ± 5 mmHg; and diastolic BP, 127 ± 2 mmHg versus 139 ± 3 mmHg, $p < 0.01$, $n = 7–8$). Chronic capsaicin treatment improved endothelium-dependent relaxation of SHR mesenteric arteries (pD_2, 6.97 ± 0.05 in control and 7.41 ± 0.04 in capsaicin, $p < 0.05$). This difference was eliminated by L-NAME (pD_2, 6.13 ± 0.29 in control and 6.31 ± 0.20 in capsaicin, $p > 0.05$). Endothelium-independent relaxation by nitroglycerin was comparable in both groups (pD_2, 7.16 ± 0.15 in control and 7.21 ± 0.14 in capsaicin, $p > 0.05$. Long term capsaicin treatment can reduce arterial pressure (Yang *et al.* 2010).

- Capsacin can be beneficial in preventing salt-induced increases of blood pressure. In vivo animal study Li and Wang (2003) presented effect of capsaicin in regulation of

blood pressure caused by high sodium diet. Rats (Wistar) were divided into groups: A-control plus normal sodium diet (0.5%, CON-NS), B-control plus high sodium diet (4%, CON-HS) and C-capsaicin pre-treatment plus high sodium diet (CAP-HS). After 4 weeks of treatment mean arterial pressure (MAP) and its response to bolus injection of calcitonin gene-related peptide (CGRP) and its antagonist, CGRP (8-37), were measured by carotid arterial catheterization. Baseline MAP was increased in CAP-HS compared with CON-HS and CON-NS rats, and it was higher in CON-HS than in CON-NS rats. MAP response to exogenous CGRP was enhanced in CAP-HS and CON-HS than in CON-NS rats, but MAP response to CGRP (8-37) was increased only in CON-HS rats. Plasma CGRP levels were not different among three groups, but CGRP content in dorsal root ganglia (DRG) was decreased in CAP-HS compared with CON-HS and CON-NS rats. Calcitonin receptor-like receptor (CRLR) expression in mesenteric resistance arteries was upregulated in CAP-HS and CON-HS compared with CON-NS rats (Li and Wang 2003).

3.6.2.2 Management of glucose and lipid metabolism in women with gestational diabetes mellitus (GDM)

In vivo, human model

- The effect of capsaicin supplementation was study in women suffer from GDM. In a randomized, double-blind, placebo controlled trial 44 women with 22-23 gestational weeks were divided into groups: A-capsaicin group (5mg/day) and B-placebo group (0 mg/day capsaicin). Glucose and insulin concentration were measured 2-h postprandially. Other metabolic parameters (fasting serum lipids, liver, kidney functions, calcitonin gene-related peptide (CGRP) were measured at 0 and 4 weeks of trial. In a capsaicin group there was significant ($P \leq 0.05$) decrease in 2-h PG and 2-h INS concentrations and 2-h postprandial HOMA-IR (2-h HOMA-IR) levels. The fasting serum total cholesterol and triglycerides concentrations also significantly decreased when compared to placebo group. The fasting serum apolipoprotein B and CGRP concentrations significantly increased in the capsaicin group ($P < 0.05$). The changes in the 2-h PG and 2-h INS concentrations and in the 2-h HOMA-IR were

negatively correlated with the change in the serum CGRP concentration ($P < 0.05$). Furthermore, the incidence of large-for-gestational-age (LGA) newborns was significantly lower in the capsaicin group than in the placebo group ($P = 0.022$).(Yuan *et al.*)

3.6.2.3 Weight management

In vivo, human model

- The effect of red pepper consumption on feeding behaviour and energy intake in humans was presented by Yoshioka et al. (1999). Two studies were involved in this case. In Study 1 they measured the influence of addition of red peppers to meals (consisted of high fat-HF and high carbohydrate-HC) and it's subsequently micronutrients intake and energy. Thirteen Japanese women were used as volunteers. After standardized dinner they ate previous day, they received breakfast (1883kJ) consisted of one of the four types: 1-HF, 2-HF and 10g red pepper, 3-HC and 4-HC and red pepper. Macronutrients intakes and energy as libitum were measured at lunch time. Study 2 involved impact of addition of a red-pepper appetizer on energy and macronutrients intake. Ten Caucasian men were taken to this experiment. After standardized breakfast they received appetizer (644kJ) consisted one of the following types: 1-mixed diet and appetizer, 2-mixed diet and 6g red-pepper appetizer. Study 1 showed that the macronutrient composition of breakfast did not affect the weight of ingested food, or energy and macronutrient intakes at lunch-time. However, protein and fat intake significantly decrease (protein by 20 % and 6 % for HF and HC conditions respectively, $P \leq 0.05$ and fat by 17 % and 11 % for HF and HC conditions respectively, $P \leq 0.05$) at lunch-time when the addition of red pepper to breakfast was applied. There was also decrease in energy intake (11 % and 4 % for HF and HC conditions respectively) when red pepper was ingested but this effect did not reach statistical significance. Prospective food consumption was also taken into account. The addition of red pepper to meals significantly decreased the prospective food consumption, red pepper decreased desire to eat immediately after the breakfast and before the lunch. Hunger was significantly lower in meals contained red pepper. The HC breakfast significantly reduced the desire to eat and hunger after breakfast. The addition of red pepper to the HC breakfast also significantly decreased the desire to

eat and hunger before lunch. Differences in diet composition at breakfast time did not affect energy and macronutrient intakes at lunch-time. However, the addition of red pepper to the breakfast significantly decreased protein and fat intakes at lunch-time. In Study 2 it has been established that red-pepper added to appetizer significantly decreased cumulative (lunch + mid-afternoon snack) carbohydrate intake (18 %, $P \leq 0.05$) and energy intake (11 %, $P \leq 0.05$) at lunch and snack. These results showed that the consumption of red pepper decreases appetite and subsequent protein and fat intakes in Japanese females and energy intake in Caucasian males (Yoshioka *et al.* 1999).

- Ludy and Mattes (2011) studied the effect of hedonically acceptable red pepper (RP) doses ingested in single meal on thermogenesis and appetite. Twenty-five volunteers (men and women), aged 23.0 ± 0.5 years, BMI 22.6 ± 0.3 kg/m were involved. Thirteen were spicy food users and twelve were non-users. In a randomized, crossover trial they consumed a standardized quantity of RP (1 g); their preferred quantity (regular spicy food users 1.8 ± 0.3 g/meal, non-users 0.3 ± 0.1 g/meal); or no RP. Energy expenditure and body temperature were grater after RP ingestion (1g) in comparison to no-RP users (Ludy and Mattes 2011).

- The effect of red pepper consumption on appetite and energy expenditure was studied by Westerterp-Plantenga (2005). Volunteers (24: 12 men and 12 women, age: 35+/-10 y; BMI: 25.0+/-2.4 kg/m2; range 20-30) received 30 min before each meal 0.9g red pepper (0.25% capsaicin; 80,000 Scoville Thermal Units) or placebo in either tomato juice or in two capsules that were swallowed with tomato juice. Study was performed for 2 consecutive days, four times. Visual Analogue Scale was used to determined hunger and satiety. Daly energy intake in the placebo was 11.5+/-1.0 MJ/d for the men and 9.4+/-0.8 MJ/d for the women. After capsaicin capsules, energy intake was 10.4+/-0.6 and 8.3+/-0.5 MJ/d ($P<0.01$); after capsaicin in tomato juice, it was 9.9+/-0.7 and 7.9+/-0.5 MJ/d, respectively (compared to placebo: $P<0.001$; compared to capsaicin in capsules: $P<0.05$). Energy % from carbohydrate/protein/fat (C/P/F): changed from 46+/-3/15+/-1/39+/-2 to 52+/-4/15+/-1/33+/-2 en% ($P<0.01$) in the men, and from 48+/-4/14+/-2/38+/-3 to 42+/-4/14+/-2/32+/-3 en% ($P<0.01$) in the women, in both capsaicin conditions. Increase in satiety was also observed (from 689 to 757 mmh in the men and from 712 to 806 mmh in the women, both ($P<0.01$)). The study showed that oral and gastrointestinal administration of capsaicin effects on

satiety, energy and fat intake. Oral exposure to capsaicin in short term gave stronger reduction in energy and increase in satiety which was related to perceiving of spiciness (Westerterp-Plantenga *et al.* 2005).

- Meta-analysis that investigate effect of capsaicin on energy intake was described by Whiting et al. (2014). Authors were assessed 74 clinical trials from the database. They chose and investigate 10 trials that made a research on capsaicin influence on weight management. Chosen studies were randomized, placebo-controlled trials where participants consumed capsaicin. The meta-analysis showed that capsaicin consumption caused statically significant reduction in ad libitum energy intake of 309.9kJ (74kcal) per meal (under the random effects model, with 95% confidence interval of 481.5-138.3kJ (115.0-33.0kcal) and $p \leq 0.0001$, with high heterogeneity $I2$=75.7%) what could have beneficial effect on weight loss (Whiting *et al.* 2014).

3.6.2.4 Effects of regular chili consumption on some indicators of metabolic and vascular function

In vivo, human model

- Effect of chili peppers on metabolic and vascular function was studied in a randomized cross-over dietary human trial. Volunteers (36; 22 women and 14 men), aged 46±12 (mean±SD) years; BMI 26.4±4.8 kg/m^2) were consumed chili blend (30 g/day; 55% cayenne chili) with their normal diet (chili diet).Control group was on chili free diet. Study was performed for 4 weeks each. Parameters such as: plasma glucose, serum lipids and lipoproteins, insulin, basal metabolic rate, blood pressure, heart rate, augmentation index (AIx; an indicator of arterial stiffness), and subendocardial-viability ratio (SEVR; a test of myocardial perfusion) were measured at the end of each diet. In men who were on chili diet significantly lower resting heart rate (P=0.02) and higher SEVR (P=0.05) was observed compared to control group. Also Aix of chili group was lower ($P \leq 0.001$) than the control group (Ahuja *et al.* 2006).

3.6.2.5 Effect of consumption of chili on postprandial hyperinsulinemia

In vivo, human model

- In a randomized, crossover, intervention study, 36 participants (age 46 +/- 12 years) with a BMI of 26.3 +/- 4.6 were placed on a chilli supplemented diet (30 g/d; 55% cayenne chilli) for 4 weeks. The postprandial effects of a bland meal after a bland diet (BAB), a chili meal after a bland diet (CAB), and a chili meal after a chili-containing diet (CAC) were evaluated. Serum insulin, C-peptide, and glucose concentrations and energy expenditure (EE) were measured at fasting and up to 120 min postprandially. Significant heterogeneity was observed between the meals for the maximum increase in insulin and the incremental area under the curve (iAUC) for insulin (P = 0.0002); the highest concentrations were with the BAB meal and the lowest with the CAC meal. When separated at the median BMI (26.3), the subjects with a BMI > or = 26.3 also showed heterogeneity in C-peptide, iAUC C-peptide, and net AUC EE (P < 0.02 for all); the highest values occurred after the BAB meal and the lowest after the CAC meal. Conversely, the C-peptide/insulin quotient (an indicator of hepatic insulin clearance) was highest after the CAC meal (P = 0.002); (Ahuja *et al.* 2006)

3.7 Black pepper (*Pepper nigrum*)

3.7.1 Non-authorized EU health claims

EFSA Journal 2011;9(6):2246 with Scientific Opinion contains the substantiation of health claims related to various food(s) /food constituents that: “Increase in satiety leading to a reduction in energy intake (entry ID 1884)”. That claims pursues to Article 13(1) of Regulation (EC) No 1924/2006. The claimed effects are “satiety/weight management/promotion of CCK release and soy foods”, “alginate forms a gel in the stomach and promotes an immediate feeling of satiety. It may also trap a portion of HCA. Piperine increases the bioavailability of the un-trapped HCA and enhances satiety”, “satiety”, and “weight management/satiety”. Main entry health claim (ID 1884) is related to sodium alginate,

HCA and piperine. Proposed wording: Helps manage appetite and hunger. Other alternative wording: contributes to reduce the appetite/Can help in the management of weight control/Promotes the feeling of fullness and satiety/Helps to feel full sooner/Helps to stay full longer/Assist weight management/Helps to reduce appetite and inhibits conversion of carbohydrates to fats/helps maintain a healthy level of appetite.

Number of nutrients/other substances that are essential to claimed effect: 3. Names of nutrient/other substances and Quantity in Average daily serving: 2.20 grams sodium alginate, 1500.00 milligrams HCA, 10 milligrams piperine. Weight of average daily food serving: 150 millilitre(s). Daily amount to be consumed to produce claimed effect: 300 millilitre(s).

The Panel conclude that no human studies were provided in relation to food(s)/food constituent(s), here; sodium alginate, HCA and piperine, and their claim effect (increase in satiety leading to a reduction in energy intake). Provided animal studies were not sufficient to predict the occurrence of an effect of the consumption of the food(s)/food constituent(s) on an increase in satiety leading to a reduction in energy intake in humans (EFSA 2011).

3.7.2 Relevance of black pepper to health

Piperine is major constituent of black pepper (Pepper nigrum L.). Its concentration is 50-90kg piperine per kg black pepper (Thiel *et al.* 2014).

There are number of studies showing the potential therapeutic effect of black pepper/ black pepper constituents. Health claims of black pepper are:

3.7.2.1 Antioxidant activity

In vivo, animal model

- Antioxidant effect of black pepper (*Pepper nigrum* L.) was studied in in vivo model using male Wistar rats. Different antioxidant profiles (tissue lipid peroxidation, enzymatic and non-enzymatic antioxidants) were determined in animals fed high-fat diet. Rats (95-115g) were divided into groups: group A-rats fed standard pellet diet, group B-high-fat diet (20% coconut oil, 2% cholesterol and 0.125% bile salts), group C-high-fat diet plus black pepper (0.25 g or 0.5 g/kg body weight) and group D-high-

fat diet plus piperine (0.02 g/kg body weight). Experiment was carried out for a period of 10 weeks. In groups where black pepper and piperine was applied, the level of antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and reduced glutathione (GSH)) in the liver, heart, kidney, intestine and aorta was maintained and was to near those of control rats. A level of thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD) was lowered. In rats on high-fat diet levels of TBARS, CD were elevated, whereas, SOD, CAT, GPx, GST and GSH were lowered. The data from the experiment indicates that black pepper and piperine can reduce high-fat diet induced oxidative stress to the tissue (Vijayakumar *et al.* 2004).

- Another experiment showing effect of black pepper constituent (piperine) on reducing inflammation and oxidative stress in vivo animal model was presented by Diwan et al. in 2013. Rats were divided into groups: group A-rats fed with high carbohydrate, high fat (HCHF; carbohydrate, 52%; fat, 24%; 25% fructose in drinking water) or corn starch (CS). Groups B and C were treatment groups (Group B-CS + piperine and group C-HCHF + piperine; 375 mg/kg food; approximately 30 mg/kg/day). Experiment was carried out for a period 16 weeks but treatment groups were supplemented with piperine for the last 8 weeks of this study. Non-treated rats (Group A) developed hypertension, high oxidative stress and inflammation-induced cardiac changes. Other abnormalities were also detected (reduced responsiveness of aortic rings, obesity, liver fibrosis, fat deposition, increased plasma liver enzymes. In treated group all abnormalities were reduced. This may suggest that piperine reduces symptoms of human metabolic syndrome in HCHF-fed rats (Diwan *et al.* 2013).

In vitro

- Raw extracts (non-hydrolysed and hydrolysed) of black pepper and white pepper were tested in terms theirs antioxidant activity. DPPH scavenging properties of black pepper was determined. Raw extract of non-hydrolysed black pepper had a significantly higher ($P \leq .001$) DPPH radical scavenging activity at 8 mg/mL than its white variety (white pepper). Non-hydrolysed extract of black pepper (at 1, 2, 4 and 8mg/mL) also indicated a significant ($P \leq .001$) scavenging activity on superoxide radical when compared to non-hydrolysed extract of white pepper. Both hydrolyse

and non-hydrolysed black pepper raw extracts shown also hydroxyl radical scavenging activity, moreover extracts were significantly ($P \leq .001$) more effective than white pepper extracts (hydrolysed and non-hydrolysed form). Reactive oxygen species (superoxide and hydroxyl) scavenging activities of white pepper extracts were able to scavenge in a concentration-dependant manner (Agbor *et al.* 2006).

- Crude extracts of black pepper (water and ethanolic) were evaluated by other researchers in terms their antioxidant activities. A few different methods were applied (ferric thiocyanate method, reducing power, DPPH assay, metal chelating, and active oxygen species such as H_2O_2, O_2, and OH quenching assay. DPPH assay showed abilities of water extract of black pepper (WEBP) and ethanolic extract of black pepper (EEBP) to scavenge of DPPH. Standards (BHA, BHT and α-tocopherol) were also used in the assay. The scavenging effect of WEBP and EEBP and standards on the DPPH radical decreased in the order of BHA- α-tocopherol –BHT-WEB-EEBP and were 79, 78, 76, 55, and 48% at the concentration of 75 µg/ml, respectively. Other radical scavenging properties of black pepper extracts was assayed by determination of inhibition of superoxide formation. Standards (BHA, BHT, and α-tocopherol) were also applied. The percentage inhibition of superoxide generation by 75 µg/ml concentration of WEBP and EEBP was found as 64.2 and 22.6% and lower than that same doses of BHA, BHT, and α-tocopherol (69.6, 82.2, and 75.4%), respectively. Superoxide radical scavenging activity of those samples followed the order: BHT-α-tocopherol-BHA-WEBP-EEBP. The thiocyanate method showed that both WEBP and EEBP exhibited effective and powerful antioxidant activity. WEBP and EEBP showed higher antioxidant activities than that of α-tocopherol at the same concentration (75 µg/ml). The percentage inhibition of peroxidation of WEBP and EEBP was found 95.5% and 93.3%, respectively, and greater than that of same concentration of α-tocopherol (70.4%). On the other hand, percentage inhibition of 75 µg/ml concentration of BHA and BHT was found 92.1% and 95.0%. Reductive ability of both extracts was also determined. WEBP and EEBP showed higher activities than control and these differences were statistically very significant ($P \leq 0.01$). EEBP exhibited stronger reducing power than WEBP, however this difference between the extracts was not found as significant, statistically ($P \leq 0.05$). Reducing power of WEBP, EEBP and standards followed the order: BHA- BHT- α-tocopherol- EEBP-WEBP. Metal chelating activity of extract was also study. The percentage of metal

chelating capacity of 75 µg/ml concentration of WEBP and EEBP, α-tocopherol, BHA, and BHT were found as 84, 83, 75, 69, and 66%, respectively. The scavenging ability of WEBP and EEBP on H_2O_2 was determined and compared to BHA, BHT and α-tocopherol as standards. Seventy-five micrograms per millilitre of WEBP and EEBP exhibited 83 and 63% scavenging activity on H_2O_2, respectively. However, BHA, BHT, and α-tocopherol showed 88, 97, and 93% H_2O_2 scavenging activity at the same concentration. These results showed that WEBP and EEBP had effective H_2O_2 scavenging activity. The H_2O_2 scavenging effects 75 µg/ml concentration of WEBP and EEBP and standards decreased in the order of BHT-α-tocopherol-BHA-WEBP-EEBP.). Polyphenols content (by Folin-Ciocalteu) in black pepper extract was determined as 54.3 and 42.8 µg Gallic acid equivalent in 1 mg WEBP and EEBP (Gulcin 2005).

3.7.2.2 Anti-platelet and anti-inflammatory activity

In vitro

- Piperine isolated from black pepper (*Pepper nigrum*) indicated anti-platelet activity. In the experiment it is shown that piperine indicates dose-dependent inhibitory activity on washed rabbit thrombocyte aggregation induced by collagen, arachidonic acid (AA) and platelet-activating factor (PAF) (Park *et al.* 2007)
- Son et al. (2014) confirmed previous results. Researchers presented that piperine inhibited collagen- and AA-induced platelet aggregation (in RAW264.7 cells) in a concentration-dependent manner, with IC_{50} values of 158.0 and 134.2 µM, respectively. Piperine showed also a mild inhibitory effect on platelet aggregation induced by TXA_2 receptor agonist U46619 (IC_{50} > 300.0 µM). Measurement of inflammatory mediators such as PGs in piperine treated cells was also study. LPS-stimulated (1 µg/mL LPS for 12 h) RAW264.7 cells under piperine treatment indicated inhibition of generation of PGE_2 and PGD_2. Piperine significantly inhibited the generation of both PGE_2 and PGD_2 in a concentration-dependent manner, with IC_{50} values of 7.7 and 10.1 µM, respectively. Piperine also indicated inhibitory effect on prostaglandin production in LPS stimulated RAW264.7 Cells. Piperine in concentration 10 to 100 µM significantly inhibits prostaglandin generation. Piperine

also inhibits another enzyme responsible for inflammation. Treatment of RAW264.7 cells with piperine significantly inhibited activities of TXA_2 Synthase and COX-2 in a concentration-dependant manner (Son *et al.* 2014).

- The anti-inflammatory effect of piperine was studied. Cells (fibroblast-like synoviocytes-FLS) derived from patients suffer from rheumatoid arthritis. FLS cells were treated with IL1β to stimulate production of IL6 and PGE_2 (inflammatory mediators). Addition of piperine to treated with IL1β cells greatly inhibited generation of inflammatory mediators (IL6 and PGE_2) in a dose dependant manner. Piperine also inhibited both the protein and mRNA expression levels of IL6 and COX-2. Inhibitory effect of piperine on the expression of the extracellular matrix degradation enzymes (MMPs) was also found. Piperine blocks production of MMP13 at protein and mRNA levels (Bang *et al.* 2009).

In vivo, animal model

- Anti-inflammatory effect of piperine was tested *in vivo* (animal model). Paws of rat were treated with carrageenan to induce arthritis. Piperine (100mg/kg) was administrated orally for 8 days. After this time it has been observed a significant reduction in paw volume compared to the vehicle-treated arthritic group. Rats treated with piperine could tolerate higher pressure on the affected paw. Piperine (100mg/kg) showed almost the same efficacy as prednisolone (10 mg/kg), which was used as a positive control. Piperine also provided a mild anti-edema effect at 20 mg/kg, although it was not statistically significant. Histological evaluation of ankle joints (H&E staining) treated with piperine (100mg/ml) shown smaller areas of lymphocyte infiltration into the joints compared to the corn oil treated group. This was evaluated by three different pathologist (five specimens were screened). That indicates that piperine significantly reduces inflammatory response in induced by carrageenan organs (Bang *et al.* 2009).
- Anti-inflammatory and analgesic activity of pure piperine and hexane and ethanolic extract of black pepper (*Piper nigrum* L.) was demonstrated by Tasleem et al. in 2014. The analgesic activity was determined by tail immersion method, analgesia-meter (paw pressure), hot plate and acetic acid induced writhing test. Anti-inflammatory activity was evaluated by carrageenan-induced paw inflammation in rats. Piperine at a dose of 5 mg/kg and ethanol extract at a dose of 15 mg/kg after 120

min and hexane extract at a dose of 10 mg/kg after 60 min exhibited significant (P<0.05) analgesic activity by tail immersion method, in comparison to ethanol extract at a dose of 10 mg/kg using analgesia-meter in rats. In hotplate method, piperine produced significant (P<0.05) analgesic activity at lower doses (5 and 10 mg/kg) after 120 min. A similar analgesic activity was noted with hexane extract at 15 mg/kg. In writhing test, ethanol extract significantly (P<0.05) stopped the number of writhes at a dose of 15 mg/kg, while piperine at a dose of 10 mg/kg completely terminated the writhes in mice. In the evaluation of anti-inflammatory effect using plethysmometer, piperine at doses of 10 and 15 mg/kg started producing anti-inflammatory effect after 30 min, which lasted till 60 min, whereas hexane and ethanol extracts also produced a similar activity at a slightly low dose (10 mg/kg) but lasted for 120 min (Tasleem *et al.* 2014).

3.7.2.3 Thyrogenic activity, modulation of apolipoproteins, lipids and hormonal levels

In vivo, animal model

- The effect of piperine on thyroid hormones and apolipoproteins in high-fat-diet (HFD) and anti-thyroid drug-induced hyperlipidemic rats was studied. Rats (male Wistar) were divided into two groups; A-control diet and B-high-fat diet (HFD) and then subdivided into four subgroups of ten animals each. Rats were treated for 10 weeks: 1% carboxymethyl cellulose; 10 mg carbimazole (CM)/kg body weight; 10 mg CM + 40 mg piperine/kg body weight, and 10 mg CM + 2 mg atorvastatin /ATV//kg body weight. Lipid profiles, hormone levels, and apolipoprotein levels were determined in all groups. In group where HFD and/or CM were applied the levels of total cholesterol, VLDL, LDL, triglycerides, free fatty acids, and phospholipids were significantly elevated moreover HDL levels were significantly reduced. CM treatment significantly reduced apo A-I levels and T3, T4 and testosterone levels while plasma apo B, thyroid stimulating hormone (TSH) and insulin levels were significantly elevated. If piperine and HFD were administrated there was reduction in plasma lipids and lipoproteins levels, except for HDL, which was significantly elevated. Levels of apo A-I, T3, T4, testosterone were improved and apo B, TSH, and insulin back to near normal levels after piperine application (Vijayakumar and Nalini 2006).

- Effect of piperine on thyroid hormone and glucose level in mice was investigated by Panda and Kar in 2003. Piperine (2.5 mg/kg/day) was administered orally for 15 days. It has been observed that thyroid hormones (T4 and T3) as well as glucose levels in serum were lowered whereas activity of hepatic 5'D enzyme and glucose-6-phospatase (G-6-Pase) was decreased. The decrease in T3, T3 and G-6-Pase concentrations were comparable to that of a standard antithyroid drug, Proylthiouracil (PTU) (Panda and Kar 2003).

3.7.2.4 Hepatoprotective activity

In vivo, animal model

- Hepatotoxicity was induced in mice with a single dose acetaminophen (900 mg/kg BW/ i.p.). After 30 min, piperine (25 mg/kg BW/ i.p.) and standard drug silymarin (25 mg/kg BW/ i.p.) were given to mice. In treated animal the level of different liver enzymes (aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP)) were decreased in comparison to control (untreated mice). Inflammatory agent such as tumour necrosis factor-alpha (TNF-α) and antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-s-transferase and glutathione) were also elevated in liver of treated mice. As demonstrated in the study piperine shows promising hepatoprotective effect as comparable to standard drug silymarin (Sabina *et al.* 2010).

3.7.2.5 Enhancement of bioavailability of supplement nutrients

BioPerine (BioPerine 2015)

It is a supplement (98% piperine), that significantly enhances the bioavailability of various supplement nutrients through increased absorption. It's been patented in U.S. and clinically tested in U.S. Clinical studies shown that BioPerine (5mg per dose) increases dramatically gastrointestinal absorption of some nutrients (Beta Carotene, Vitamin C, Selenium, Coenzyme Q10) as compared to control group receiving the nutrient alone. Selenium levels increased by 30%, beta-carotene increased by 60%, and the vitamin B_6 increase was slightly higher than beta-carotene.

3.8 Thyme (*Thymus vulgaris*)

3.8.1 Non-authorized EU health claims

EFSA Journal 2010;8 (2):1489 with Scientific Opinion contains the substantiation of health claims related to various food(s) /food constituents and protection of DNA, proteins and lipids from oxidative damage (entry ID 2151). That claim pursues to Article 13(1) of Regulation (EC) No 1924/2006. Entry ID refers to *Thymus vulgaris* (common name-thyme) with antioxidant properties. Proposed wording: "contains naturally occurring antioxidants, antioxidant help protect from free radicals, protects cells and tissues from oxidation and contributes to the total capacity of the body". Conditions of use: flower/leaf/ equivalent of 10g of leaf/the equivalent of 3-6g herb per day. The claimed effects refers to the protection of the body cells and molecules (DNA, proteins, and lipids) form oxidative damage, including UV-induced oxidative damage. However in vitro and animal studies showed the free radical scavenger properties of different food constituents, no human trials has been done to prove the effect of food(s)/food constituent(s), here: thyme, on protection of cells and tissues from oxidative damage. Provided scientific evidences are not sufficient to establish relation between consumption of the food/foos constituents and protection of body cells and tissue form oxidative damage (EFSA 2010).

3.8.2 Relevance of thyme to health

There are number of studies showing the potential therapeutic effect of thyme/thyme constituent(s). Health claims of thyme are:

3.8.2.1 Antimicrobial properties

In vitro

a) Antifungal properties

- Essential oils of thyme indicates antifungal properties. Thyme essential oils (TEO) inhibits growth of *Aspergillus flavus* (suspension 10^6) with minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) at 250µg/ml. TEO also showed to have and inhibitory effect on production of aflatoxins. Complete inhibition of both B1 and B2 aflatoxins (AFB1 and AFB2) was observed at 150µg/ml of TEO (Kohiyama *et al.* 2015).
- Inhibitory properties of thyme oils (different chemotypes) on growth of *Candida albicans* were also studied. Presence of thyme oil in the culture medium (thyme chemotype) indicated the most efficient inhibitory effect on growth of *C. albicans* giving MIC 80%=0.016µl/ml and Kaff=296µl/ml. *Thymus vulgaris* thymol chemotype also indicated decreasing effect on MIC 80% of well-known antifungal agent- amphotericin B. Decrease by 48% of MIC 80% was observed with concentration of thyme oil of 0.2µl/ml (Giordani *et al.* 2004).

b) Antibacterial properties

- Antibacterial activity of essential oils of thyme against 14 clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA) and 5 standard bacterial strains was presented by Tohidpour in 2010. Disc diffusion and agar dilution method were employed in the study. Extract (essential oil of *Thymus vulgaris*- EO) was obtained from air-dried aerial parts of *Thymus vulgaris* by hydrodistillation (4h) using Clevenger apparatus. Dried oils were investigated. EO with had inhibitory effect on all bacteria tested (14 clinical MRSA and *Bacillus cereus* ATCC 9634, *E. coli* ATCC 3428, *Klebsiella pneumonia* ATCC 13883, MRSA standard strain ATCC 33592 and *S. aureus* ATCC 25922. All the bacteria were sensitive to EO but in variable degrees with MIC values ranging 0.1-4% (v/v). The most sensitive bacteria was *E.coli* ATCC 3428 with MIC=9.25µg/ml, while for *K. pneumonia* ATCC 13883 was relatively high (MIC=55.5µg/ml). MIC for clinical MRSA isolates ranged from 18.5 to 37.5µg/ml. Inhibition zones of EO were also compared to standard antibiotics and in some cases zones were similar (Tohidpour *et al.* 2010).
- Antibacterial activity of thyme essential oils against 120 bacterial strains (*Staphylococcus, Enterococcus, Escherichia* and *Pseudomonas* genera) was showed.

Bacteria were isolated from patients suffered from oral cavity, respiratory, genitourinary tracts and as well as from hospital environment. Using agar diffusion method is has been found that thyme oil exhibited extremely strong activity against testes strains, especially *Staphylococcus* strains were most sensitive (Sienkiewicz *et al.* 2011).

- Chloroformic extract of thyme (made from dried powdered leaves) was also screened for inhibitory effect on isolated clinical and standard bacterial strains (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*). Agar well diffusion method was employed in the study (Abu-Darwish *et al.* 2012).
- Essential oil of thyme (CT thymol chemotype) indicated significant antibacterial activity against *Streptococcus pyogenes*. This strain plays role in the pathogenesis of tonsillitis. Disc diffusion method was used to determined inhibition zones. CT thymol was very effective in inhibition of *S. pyogenes* (+++) and *indicated MBC (minimal bactericidal concentration)* of 0.87 % (Sfeir *et al.* 2013)
- Anti-*Helicobacter pylori* activity of essential oils of thyme (*Thymus vulgaris*) was presented by Esmaeili in 2012. The thyme extract (essential oils) was prepared as described in (Tohidpour *et al.* 2010). Thyme oil was tested in different concentration in an agar dilution method. The MIC for thyme oil against *H. pylori* ATCC 700392 was determined as 10.8µg/ml (Esmaeili *et al.* 2012).

3.8.2.2 Antioxidant properties

In vivo, animal model

- Study on rats (*in vivo*) shows antioxidant activity of thyme oil. In the experiment rats (n=40) were fed with 42.5mg/kg body weight/day of thyme oil for 28 months, effectively the lifetime of a rat. Control group (n=40) was also applied and received normal diet (pellet). Rats were also different age in both groups (7-28 months). Activities of antioxidant enzymes: superoxide dismutase (SOD) glutathione peroxidases (GSHPx) and total antioxidant status (TAS) were measured in heart, liver and kidney in both group. SOD activity was found to be significantly higher in liver and heart of old rats fed thyme oil in comparison to their age-matched controls. In thyme treated rats GSHPx activity increased significantly with age and was higher in

old rats (by 67%) in comparison with young control. Oil thyme treated rats exhibited significantly higher liver, kidney and heart TAS than the respective age-matched controls. Antioxidant capacities (SOD, GSHPx and TAS activites) remained higer during life span of rats in rats whose diet contained thyme oils. (Youdim and Deans 1999).

In vitro, human cells

- Antioxidant activity of thyme extract (dry extract of 40% ethanolic extraction) was studied by Kozics in 2013. Activity of antioxidant enzyme (glutathione peroxidase; GPx) of human hepatocellular carcinoma cells (HepG2) cells was determined after thyme extract treatment. After 24h, 1 and 0.5mg/ml of thyme extract induced significantly GPx activity compared to control cells (no treatment).
- Thyme extract also exhibited cytotoxic effect on HepG2 cells. Different concentrations of thyme extract (0-100mg/ml) were used. MTT assay was employed in the study. After 24h, IC_{50} (inhibitory concentration that causes 50% cell death) for thyme extract was 4.3mg/ml. Thyme extract also shows reduction in DNA damage of HepG2 cells induced by H_2O_2 (300µM). H_2O_2 induces DNA single breaks in HepG2 cells, as determined using SCGE assay. Concentration of 1 and 0.5 mg/ml of thyme extract significantly reduces DNA strand breaks (Kozics *et al.* 2013).

In vitro antioxidant assays

- Antioxidant properties of different thyme preparation were determined in various assays (RP-reducing power, RSA-free radical scavenging activity, CBI-beta carotene bleaching inhibition and LPI- lipid peroxidation inhibition. Hydroalcoholic extract (80% methanol) was lyophilized. Infusion extract was; 1g of dried sample, thymus aerial parts in 200ml boiling water, 5min. Decoction extract was: 1g dried sample in 200ml water, heated and boiled for 5 min. Infusion and decoction mixtures were frozen and lyophilized. All lyophilized thyme extract were re-dissolved in 80% methanol, to obtain stock solution of 20mg/ml. Decoction preparation showed the highest RSA and RP (EC_{50} values 112 and 101 µg/mL, respectively), while infusion

revealed the highest LPI (7 µg/mL). Concerning CBI, it was the hydroalcoholic extract that showed the highest value (32 µg/mL; (Martins *et al.* 2015)

3.9 Nutmeg (seeds of *Myristica fragrance*)

3.9.1 Relevance of nutmeg to health

There are number of studies showing the potential therapeutic effect of nutmeg/nutmeg constituent(s). Health claims of nutmeg are:

3.9.1.1 Hepatoprotective/antioxidant properties

In vivo, animal model

- Aqueous extract of nutmeg was examined for its hepatoprotective activity in vivo in animal model. Rats were divided into group: control group-animal received standard pellet diet and saline; ISO (isoproterenol) group received ISO at dose of 85mg/kg body weight (BW), twice daily for a period of 2 days; nutmeg extract group received aqueous nutmeg extract orally (100mg/kg BW) for a period of 30 days and then ISO at dose mentioned above. ISO was given to rat to induce hepatotoxicity. There was a significant decrease in the concentration of DPPH radical and inhibition the formation of lipid peroxides induced by Fe^3 +/ADP/ascorbate system. Nutmeg extract did not alter the activities the enzymes: LDH, CK, AST, ALT, ALP, and GGT in the liver and heart when compared to control. In ISO administered rats, a significant ($P < 0.05$) elevation in the activities of these marker enzymes was observed by 64.74%, 68.45%, 88.55%, 91.60%, 232%, and 46%, respectively, when compared to control. The activities of marker enzymes were significantly ($P < 0.05$) reduced by 35.19%, 24.87%, 32.80%, 39.42%, 56.64%, and 21.62% for LDH, CK, AST, ALT, ALP, and GGT, respectively in pre-treated group compared to ISO administered group. ISO administration alone showed a significant ($P < 0.05$) decrease in the activities of LDH, CK, AST, ALT, ALP, and GGT by 44.03%, 38.90%, 43.54%, 30.24%, 55.3%, and 47.9%, respectively when compared to control. Pre-treatment of rats with nutmeg extract significantly ($P < 0.05$) increased the level of these marker enzymes by 56%,

44.44%, 55.2%, 21.87%, 52.38%, and 66.52%, respectively for LDH, CK, AST, ALT, ALP, and GGT compared to ISO administered rats. The study also showed the level of antioxidant enzymes (GSH, GPX, GST, SOD and CAT) in the heart tissue. Treatment with nutmeg extract alone increased the activities of antioxidant enzymes by 8.51%, 6.34%, 8.29%, and 6.73%, respectively for GPX, GST, SOD, and CAT along with GSH (13.71%). The pronounced oxidative stress effect of ISO is significantly ($P < 0.05$) attenuated by pre-treatment of animals with nutmeg extract by 29.57%, 53.62%, 27.61%, 93.50%, and 53.77%, respectively for GPX, GST, SOD, CAT, and GSH. These results showed that nutmeg exacta possess heptoprotective and antioxidant activity in ISO-induced damage in rats (Kareem *et al.* 2013)

- Hepatoprotective activity of nutmeg constituent was presented by Morita et al. (2003) in lipopolysaccharide (LPS) and GalN- induced damage model. Oral administration of myristicin, one of the essential oil of nutmeg, was found to possess extraordinary potent hepatoprotective activity. Myristicin markedly suppressed LPS/D-GalN-induced enhancement of serum TNF-alpha concentrations and hepatic DNA fragmentation in mice (Morita *et al.* 2003).

3.9.1.2 Anti-inflammatory effect

In vivo animal model

- Water extract of nutmeg was screened for its protective effect on a dextran sulphate sodium (DSS)-induced colitis in mice. Mice received 5% DSS for 7 days in a drinking water. Water extract of nutmeg was administrated orally (100, 300 and 1000mg/kg) for 7 days or distilled water was given to mice as control for the same period of time. Body weight was measured at day 0 to 7. After 7 days of the experiment colon was isolated and screened for its length. Clinical scores were also measured. Body weight in the control group continuously decreased. Nutmeg extract treatment did not show any effects on body weight. Control group showed colon shortening compared with the normal group. The nutmeg-treated groups exhibited less shortened and oedematous colons with less damage to the cecum than in the control group. Water extract of nutmeg treatment significantly inhibited the colon shortening in a dose-

dependent manner. The clinical score of the control group began to decrease rapidly from day 3. Nutmeg-treated groups had better clinical scores from day 3 to 5 compared to the control group. Nutmeg-treated groups showed relatively intact surface epithelia and cryptal glands, whereas the control group showed disruptions of the cryptal glands and infiltration of inflammatory cells. Water extract of nutmeg showed dose-dependent protective effects against histologically evaluated damage. Nutmeg extract also inhibits the activities of pro-inflammatory cytokines (IFN-c, TNF-a, IL-1b, and IL-6) in the colon mucosa. Oral administration of nutmeg water extract showed dose-dependent protective effects against colon shortening, clinical symptoms and histological observed damage in DSS-induced colitis model. Nutmeg treatment improved the histological score that reflects cryptal damages, and the severity and extent of inflammation (Kim *et al.* 2013).

- Anti-inflammatory activity of some organic fractions of nutmeg seed (organic solvents; n-hexane and methanol) were evaluated in carrageenan-induced oedema in rats and acetic acid-induced vascular permeability in mice. The methanol extract was extracted with ether, and then the ether soluble fraction was extracted with n-hexane. The n-hexane soluble fraction was fractionated by silica gel column chromatography (Fr-l-Fr-V), and the active principle was isolated from Fr-II by thin layer chromatography (Fr-VI-Fr-VII). All fractions and indomethacin (as reference drug) were administered orally. The methanol extract (1.5 g/kg), ether fraction (0.9 g/kg), n-hexane fraction (0.5 g/kg), Fr-II (0.19 g/kg) and Fr-VI (0.17 g/kg) showed a lasting anti-inflammatory activity, and the potencies of these fractions were approximately the same as that of indomethacin (10 mg/kg). The anti-inflammatory action of nutmeg extracts was due to myristicin, belonged to Fr-VI fraction (Ozaki *et al.* 1989).

- Evaluation of the effect of the ethanolic extract of nutmeg (Myristica fragrance) on some haematological indices was presented by Bamidele et al. (2011) using animal model. Twenty four rats (140 to 160 g) were randomly divided into four groups (six animals per group). Group I- as control, received 10 ml/kg normal saline (orally), Groups II, III and IV received 50% ethanolic seed extract of *M. fragrans* (orally) at doses of 100, 250 and 500 mg/kg, respectively. In all groups, the blood samples were obtained by cardiac puncture for analysis of haematological indices after 14 days of treatment. Normal saline has no effect on haematological indices. There were decreases in the values of RBC counts in Groups II and III compared with the control

group but not significant. There was only significant decrease in the value of RBC counts in Group IV compared with the control group at P≤0.05. The mean total WBC count in control group was 6.51±1.30 × 103 cells/mm^3 while those of Groups II, III and IV were 6.65±0.02, 6.72±0.07 and 7.16±0.07 × 103, cells/mm^3 respectively. There were significant increases in total WBC count in Groups III and IV compared with control group. The increases observed in the test groups were dose dependent. The mean PCV in control group was 38.19±0.27% while those of Groups II, III and IV were 38.11±0.33, 37.98±0.29 and 36.00±0.21% respectively. The mean PCV of the Group IV was significantly decreased compared to the control group (P≤0.05) while the Groups II and III were not significantly different. Also, the mean haemoglobin concentration (HbC) in Group II (12.32±0.76 g/dl) and Group III (11.86±0.43 g/dl) were statistically insignificant compared with control group (12.34±0.25 g/dl) while that of Group IV (11.44±0.10 g/dl) was significantly decreased. The mean platelet of Group II (143.17±16.17 × 103 cells/mm^3), Group III (103.33±2.14 × 103 cells/mm^3) and Group IV (88.33±2.30 × 103 cells/mm^3) were significantly different compared with that of control group (338.00±7.33 × 103 cells/mm^3). This study confirmed the anti-inflammatory properties of nutmeg seeds (Bamidele *et al.* 2011).

3.9.1.3 Antimicrobial effect

In vitro

- The ethyl acetate and ethanol extracts of nutmeg (*Myristica fragrance* seeds) was evaluated for its antibacterial activity against three Gram-positive cariogenic bacteria (*Streptococcus mutans* ATCC 25175, *Streptococcus mitis* ATCC 6249, and *Streptococcus salivarius* ATCC 13419) and three Gram-negative periodontopathic bacteria (*Aggregatibacter actinomycetemcomitans* ATCC 29522, *Porphyromonas gingivalis* ATCC 33277, and *Fusobacterium nucleatum* ATCC 25586). Serial microdilution method was used in the study. Ethyl acetate extract of nutmeg had the highest significant inhibitory effects against Gram-positive and Gram-negative bacteria with mean MIC value ranging from 0.625 to 1.25 ± 0.00 (SD) mg/mL; P = 0.017) and highest bactericidal effects at mean MBC value ranging from

0.625 mg/mL to 20 ± 0.00 (SD) mg/ml. Ethanol extracts of nutmeg exhibited good antibacterial activity against both groups of test pathogens compared to its ethyl acetate extracts. All of the extracts of *Myristica fragrans* seeds did not show any antibacterial activities against *Fusobacterium nucleatum* ATCC 25586. This study showed that nutmeg extracts can be used against oral pathogens (Shafiei *et al.* 2012).

- Water, acetone and ethanol extract of nutmeg seeds were tested for antibacterial activity against two Gram-positive bacteria (*Bacillus subtilis*, and *Staphylococcus aureus*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). Activities of extracts were compared with each other and between crust and bulb and with selected antibiotic (ciprofloxacin) used as positive control. Ethanol and acetone extracts exhibited antibacterial activity against gram positive bacterial species only, the diameter of inhibition zone reached 25mm against *S. aureus* by crust extract. Crust extract showed antibacterial activity more than the pulp extract. (Ibrahim *et al.* 2013).
- Dichloromethane and methanol extracts of nutmeg seeds (*Myristica fragrans*) was studied for their antibacterial activity against *Helicobacter pylori*. *H. pylori* is an infectious bacteria causing chronic gastritis, gastroduodenal ulcer and low grade gastric mucosa associated lymphoid tissue lymphoma. In evaluation of ant-helicobacter pylori activity of nutmeg extracts the agar dilution method was employed and 39 strains of *H. pylori* (38 clinical isolates and a standard strain) were used in the study. The final concentrations of the extracts tested were 100, 50, 25, 12.5, 6.25 and 3.125 mg/mL for each sample. The dichloromethane extract has an inhibitory effect on 4 out of the 39 *H. pylori* isolates tested MIC value of 6.25 mg/mL and MBC values of 6.25 mg/mL- 12.5 mg/mL *H. pylori* BAA 037 was the only strain susceptible to methanol extract of *M. fragrans* with MIC and MBC values of 25 mg/mL and 100 mg/mL respectively. Methanol extract of nutmeg seeds was also tested in vivo using animal model (rats). The effect of 500 mg/kg and 250 mg/kg body weight of the methanol extract of nutmeg on *H. pylori*-induced gastritis and colonization was investigated in rats. Bacterial density score of the gastric mucosa reduced from 5.0 ± 7.07 x 108 to 1.6 ± 1.4 x 104 and 3.45 ± 1.4 x 104 CFU/mL (mean ± SD, $p < 0.05$) after treatment with nutmeg extract (500 mg/kg body weight) and Ofloxacin (400 mg/kg), respectively (Oyedemi *et al.* 2014).

- Antibacterial effect of nutmeg extract (ethanol extract) against food-borne pathogen (*E.coli* O157) was studied. *E. coli* belongs to enterohemorrhagic strain causing haemorrhagic colitis and haemolytic uremic syndrome. Nutmeg extract at 0.01% and 0.1% reduced the population of pathogenic *E. coli* O 157 strains (14 serotypes of this strains was used). The antimicrobial activity of nutmeg was also examined with other pathogenic strains: enterotoxigenic, enteroinvasive and enteropathogenic *E. coli*. During 24 h incubation in the nutmeg ethanol extract (0.1%) the reduction in the viability of population of enteropathogenic *E.coli* (strain O111) was observed. Other strains did not show any sensitivity for nutmeg extract (Takikawa *et al.* 2002).

3.9.1.4 Hypolipidemic effect

In vivo, animal model

- Ethanolic extract of nutmeg (*Myristica fragrans*) was tested for its effect on induced-hyperlipidaemia. In an animal model, in 12 rabbits hyperlipidaemia was induced and then in 6 rabbits a dose of 500 mg/kg of the extract was administered orally daily for a period of 60 days . Rest of the animal (6 rabbits) was treated as a controls. The levels of lipoprotein lipids were significantly lower in the experimental group after 60 days. Lipids profiles of the control group in comparison to the nutmeg-treated group were; total cholesterol 573 +/- 61 vs. 209 +/- 27 mg/dl, low density lipoprotein (LDL) cholesterol 493 +/- 57 vs. 131 +/- 25 mg/dl, and triglycerides 108 +/- 14 vs. 67 +/- 9 mg/dl $P < 0.001$). High density lipoprotein (HDL) cholesterol levels were not significantly different (59 +/- 7 vs. 65 +/- 4 mg/dl, P = n.s.). Total cholesterol:HDL ratio and LDL:HDL ratio were significantly lower in the experimental group. There were significantly lower levels of total cholesterol in heart (3.7 +/- 0.5 vs. 2.2 +/- 0.5 mg/100 g) and liver (11.9 +/- 1 vs. 1.5 +/- 0.4 mg/100 g) (Ram *et al.* 1996).
- Hypolipidemic effect of nutmeg seeds extract (*Myristica fragrance*) was studied by Sharma et al. (1995) in an animal model. Ethanol extract of nutmeg seeds was used in this study. Rabbits were divided into 5 groups: Group 1-control group (given water only); Group 2- 200mg/day atherogenic diet+400mg cholesterol/kg/BW for 60 days; Group 3- atherogenic diet (200mg/day) + 400mg cholesterol for 60 days, from 61-120 days nutmeg extract (500mg/kg/BW/day). From 61 day onward normal diet was

given; Group 4- atherogenc diet (200mg/day) +cholesterol (400mg) + nutmeg extract (500mg/kg/BW/day) from day 1-120. Following parameters of serum were checked during experimental study (0, 30, 60, 90 and 120 days): total cholesterol (TC), triglyceride, phospholipids, HDL-cholesterol, LDL-cholesterol and VLDL. After sacrificed of animals same parameters were examined from isolated organs. TC, LDL and VLDL level was elevated in high fat and cholesterol diet but treatment with nutmeg seeds extract (group 3) TC, LDL and VLDL were lowered by 69, 1, 76, 3 and 56, 6% respectively. Ratio of cholesterol to phospholipids is usually high when atherosclerosis is diagnosed. In group 4 this ratio significantly decreased (from 1.74 to 1.13) after nutmeg extract treatment. Lipids parameters in organs also were improved after nutmeg treatment. TC, triglyceride and phospholipids contents of liver, heart and aorta were significantly reduced to near normal levels. In nutmeg extract-treated group 3 regression of aortic plaque was observed by 70.9-76.5%. In group 4 the aortic plaque was not even induced (Sharma *et al.* 1995).

3.10 Scallions (Welsh onion, *Allium fistulosum*)

3.10.1 Relevance of scallions to health

3.10.1.1 Anti-inflammatory/antioxidant properties

In vivo (*ex vivo*) animal model

- Aqueous extract of Welsh onion (scallions) was screened for its anti-inflammatory activity in in vivo (ex vivo) animal model. Twenty five mice were randomly divided into 5 groups (n = 5 in each group): Group 1; carrageenan (Carr) group- 50µl of 1% carrageenan was injected into hind paws. Group 2; Indomethacin (Indo) group- positive control (10mg/kg) was injected intraperitoneally 90 min before Carr injection. Indomethacin belongs to anti-inflammatory drug. Groups 3-5 welsh onion extract (WOE)-treated groups: WOE was administered orally at a dose of 0.25, 0.5, and 1 g/kg for 2 h before the injection of Carr. In the study following parameters were checked: paw volume, antioxidant enzymes profiles (CAT, SOD, GPX). Other anti-inflammatory markers such as nitrate and TNF-α were also determined.

In Indo group (Group 2) the oedema volume (caused by acute inflammation) was reduced by 9-46% after 1-5h of Indomethacin treatment respectively, in comparison to the group with carrageenan alone (Group 1). In Groups 3-5 the range of 0.25–1 g/kg of WOE showed a concentration dependent inhibition of oedema development after 5 h of carrageenan treatment. WOD at 1g/kg reduced oedema volume by 29.6% of that observed in the Group 1 after 5 h treatment. WOE treatment (0.25–1 g/kg) reduced lipid oxidation by 20–49%, of that observed in the Group 1. Carrageenan decreased the activities of CAT, SOD and GPX in the paw oedema by 43%, 74%, and 50%, respectively, in comparison to control group. In the range of 0.25–1 g/kg, WOE could increase the activities of CAT to 122–145%, SOD to 168–319%, and GPX to 121–176% of that observed in the group with carrageenan alone. Indomethacin also increased the activities of CAT (153%), SOD (334%), and GPX (179%) in comparison to the Group 1. Welsh onion aqueous extract in the same concentration range (0.25-1g/kg) had an decreasing effect on nitrate and TNF-α level in the serum of treated mice (by 17-53% and 24-51%, respectively of that observed in Group 1-Carr group). The result showed that welsh onion may be a source of anti-inflammatory compounds (Wang *et al.* 2013).

In vitro

- Scavenging properties of welsh onion was study by Wang et al. (2006). Aqueous extract of green leaves of welsh onion (WOE) possess antioxidant effect on reactive oxygen and nitrogen species. Concentration ranging from 0.05 to 1 mg/ml had an inhibitory activity on xanthine oxidase (1.0-97%). Further study also showed that this concentration of WOE contained 0.0007-0.0151mg/ml of flavonoids which had concentration-dependent activity on xanthine oxidase inhibition. NADH-PMS-NBT system was used for screening of potential superoxide scavenging activity of WOE. Trolox was used as positive control. WOE and trolox in concentration of 0.05-1.0mg/ml indicated scavenging activities against superoxide of 5.0–80.0% and 14.0–87.0%, respectively. WOE and trolox (as positive control) also exhibited hydroxyl radical scavenging properties. TBARS system was used in this case. In the range 0.1–0.5 mg/ml of WOE, TBARS production was decreased by increasing concentration of WOE. The scavenging activity of WOE against hydroxyl radical, in the range 0.05–0.2 mg/ml, was inferior to trolox.

Other antioxidant assays were also employed in the study.

Ferrozine assay showed the chelating activity of WOE. One mg/ml of WOE exhibited 80% chelating effects on ferrous ions. The chelating effect of EDTA, used as positive chelator showed 100% chelating (plateau) at 0.1–1 mg/ml. At 0.01–1 mg/ml, WOE indicated also other antioxidant activity- reduction of nitrix oxide generation; additionally this effect was in a concentration dependent manner.

The Fe^{3+}–ascorbate–H_2O_2 system was used to show the effect of WOE on oxidative damage of albumin induced by $^{\bullet}OH$. WOE showed a concentration-dependent reduction of albumin oxidation, induced by the Fe^{3+}–ascorbate–H_2O_2 system, which resulted in formation of a carbonyl group. The effects of trolox at 0.01 mg/ml reached a plateau of 70% inhibition. WOE, in the range 0.5–2 mg/ml, showed a concentration-dependent inhibition of protein oxidation induced by HOCl (Wang *et al.* 2006).

- Aqueous extract of Welsh onion green leaves (WOE) was investigated for its effect on the expression of scavenger receptors: class BI (SR-BI) and ATP-binding cassette A1 (ABCA1) receptors presented in macrophage RAW 264.7 cells. WOE in concentration of 0–0.5 mg/ml increased the protein expression of ABCA1 and SR-BI by a dose- and time-dependent manner in macrophages. Lipopolysaccharide (LPS) at 200 ng/ml decreased ABCA1 and SR-BI protein expression. WOE (0–1.0 mg/m) blocked LPS-decreased ABCA1 and SR-BI protein expression. Quercetin and kaempferol, was found to be two major flavonoids presented in WOE. Their quantity of 0–3.4 μg/ml also showed an inductive effect on the ABCA1 and SR-BI protein and a protective effect under LPS stimulation. Used in the study RT-PCR technique showed that WOE (0–1.0 mg/ml) increased the ABCA1 and SR-BI mRNA levels after 12 h treatment. LPS also obviously decreased ABCA1 and SR-BI mRNA in RAW 264.7 cells. These LPS resulted mRNA inhibitions were also markedly prevented by the addition of WOE, quercetin and kaempferol, respectively. These results suggest that WOE and its two major flavonoids, quercetin and kaempferol; have potential effect on increasing HDL receptor expression in macrophages (Duh *et al.* 2008).

3.10.1.2 Antiplatelet activity

In vivo, animal model

- Anti-thrombotic effect of welsh onion was study by Chen et al. (2000). In an in vivo study rats were fed raw or boiled Welsh onion juice (2g/kg/day) for 4 weeks. Control rats were on normal diet. Before and after feeding, their systolic blood pressure was measured by a tail-cuff method. Two days after the treatment period, tail bleeding time, platelet function (including platelet aggregation and adhesion), plasma levels of prostaglandins, and platelet cyclic nucleotide levels were determined. Administration of raw juice lowered systolic blood pressure ($P < 0.05$), while the boiled juice did not have the hypotensive effect. This treatment also prolonged the tail bleeding time (337 ± 10 s, $n = 6$; $P < 0.01$), while the boiled juice did not affect the bleeding time, compared to the control (308 ± 12 s vs. 279 ± 8 s). Raw juice feeding significantly shifted the ADP-induced platelet aggregation curve to the right ($P < 0.01$). When the results of platelet adhesion were plotted as percentage attached platelets vs. the local shear stress, the higher the shear stress, the less the attached platelets. Raw juice feeding suppressed platelet adhesion ($P< 0.01$). Chronic feeding of raw Welsh onion juice elevated cAMP level ($P < 0.01$), but not cGMP level, in platelets. Moreover, this treatment completely suppressed 2 μmol/L of ADP-stimulated thromboxane release from platelets without altering their basal thromboxane level. In contrast, the boiled juice did not have any effect. Chronic feeding of raw Welsh onion juice increased plasma 6-keto-$PGF_{1\alpha}$ levels ($P < 0.01$), but did not significantly affect NO metabolites ($P = 0.11$). In addition, feeding rats boiled Welsh onion juice had no effect on either PGI_2 or NO metabolites in plasma. Giving raw Welsh onion juice to rats for 28 days prolonged the bleeding time, diminished platelet adhesion and ADP-evoked platelet aggregation, elevated the concentration of cAMP in platelets, reduced ADP-stimulated thromboxane release in PRP, increased the plasma level of 6-keto-$PGF_{1\alpha}$, and lowered systolic blood pressure. Therefore, chronic oral administration of Welsh onion juice had antiplatelet effects, and these effects were mainly mediated by the PGI_2-cAMP pathway (Chen *et al.* 2000).

3.10.1.3 Hypertension treatment

In vivo, animal model

- Effect of green part of Welsh onion on lowering of blood pressure was presented in the study by Yamamoto et al. (2005). In an animal model, rats were fed a high-fat high-sucrose diet (HFS) with or without 5% Welsh onion for 4 weeks. Control diet was also applied. In HFS group systolic blood pressure was elevated and TBARS in plasma were increased. In HFS plus Welsh onion group lower blood pressure was observed. Rats in this group also had a significantly higher level of nitric oxide (NO) metabolites in both the urine and plasma, significantly lower activity of NADH/NADPH oxidase in the aorta, and suppressed angiotensin II production. TBARS parameter in the plasma was lowered in rats fed with Welsh onion. This study showed ant oxidative effect of green Welsh onion. This was evidenced by suppressing the angiotensin II production and then the NADH/NADPH oxidase activity, increasing the NO availability in the aorta, and consequently lowering the blood pressure in the rats fed with the HFS diet (Yamamoto *et al.* 2005).

3.10.1.4 Hyperlipidemia treatment

In vivo, animal model

- Two types of Welsh onions (green-leafy and white-sheath) were studied for their potential effect on hyperlipidemia in rats fed on diets high in fat and sucrose. Rats (n=28) were divided into four groups. Group 1-control, received control diet (CON). Group 2-received high-fat high-sucrose (HFS) diet. Group 3 received HFS and 5.0% of powdered green Welsh onion (GWO) diet. Group 4 received HFS and 5.0% of powdered white Welsh onion (WWO) diet. The experiment was carried out for 4 weeks. After that period the following parameters were checked: plasma total cholesterol, HDL-cholesterol, triacylglycerol and lipid profile of the liver. Total cholesterol in plasma of HFS rats was significantly higher than in CON group. For GWO rats total plasma cholesterol was significantly lower than in HFS group. Total lipids, triacylglycerol and total cholesterol on the liver of HFS rats were significantly higher than in CON group. The same values were significantly lower for GWO rats (but not for GWO rats) than in HFS group. The study also showed that two main flavonoids: kaempferol and quercetin are present in green and white Welsh onions. Kaempferol constituted 118±12 and 12±10 mg/kg of fresh weight of green and white

onion, respectively. Quercetin is present in green and white onion in quantity of 7±1 and 4±1 mg/kg of fresh weight, respectively. Green Welsh onion, as presented in the study, showed a significant lowering effect on plasma cholesterol and total lipids, triacylglycerol and cholesterol in the liver of rats fed with the diets high in fat and sucrose. Kaempferol may be considered as the main component responsible for lower lipid deposition (Yamamoto and Yasuoka 2010).

3.10.1.5 Hyperglycemia treatment

In vivo, animal model

- Welsh onion hot water extract was tested for its effect on fasting and postprandial hyperglycemia. Rats were treated with streptozotocin (STZ; 60 mg/kg) in citrate buffer at pH 4.5 to induce diabetes. Eighteen rats (n=18) were divided into three groups: Group 1-rats received soluble starch (1 g/kg); Group 2- rats received starch with an extract of the Welsh onion fibrous root (500 mg/kg); Group 3- rats received acarbose (50 mg/kg- anti-diabetic drug). Blood samples were collected from the tail tip after 30, 60, 120, 180, and 240 min and plasma glucose was measured. Glucose levels were expressed as increments from baseline, and areas under the response curves (AUC) were calculated using the trapezoidal rule. In groups 2 and 3 significant decrease incremental plasma glucose levels at 30, 60, and 120 min ($P < 0.01$).) were observed. Incremental plasma glucose levels in the acarbose group were significantly lower than those of the control group at 180 min ($P < 0.05$). Incremental plasma glucose levels in the Welsh onion group were not significantly different from the acarbose group. The AUCs for glucose responses in the Welsh onion (5,165 ± 894 mg·min/dL) and acarbose groups (3,752 ± 681 mg·min/dL) were significantly decreased, compared to the control group (11,147 ± 1,651 mg·min/dL, $P < 0.01$). There were no significant differences between the AUCs of the Welsh onion group and those of the acarbose group.
- The effect of chronic consumption of Welsh onion on hyperglycaemia was also presented in this study. Diabetes mice were fed an AIN-93G diet or a diet containing

either Welsh onion fibrous root extract at 0.5% or acarbose at 0.05% for 7 weeks. Fasting plasma glucose and blood glycated haemoglobin were measured. Welsh onion or acarbose diet did not significantly influence body weight, food intake, or feed efficiency ratio in diabetes mice. It was observed significant reduction in plasma glucose (326 ± 34 mg/dL) in Welsh onion diet compared to the control group (480 ± 44 mg/dL, $P < 0.01$). Plasma glucose was significantly reduced in the acarbose group (279 ± 29 mg/dL) as compared to the control group ($P < 0.01$). There were no significant differences between the plasma glucose levels of the Welsh onion and acarbose groups. Consumption of the fibrous root extract of the Welsh onion or acarbose significantly decreased blood HbA_{1C} (6.0 ± 0.5% and 5.3 ± 0.4%, respectively) compared to the control group (7.8 ± 0.6%, $P < 0.01$). There was no significant difference between blood HbA_{1C} levels in the Welsh onion and acarbose groups. The study presents Welsh onion as a potential food component that can be used as an effective hypoglycemic agent. The average intakes of fibrous root extract and acarbose were calculated as 512 and 51 mg/kg/day, based on food intake and final body weight, respectively (Kang *et al.* 2010).

3.11 Soy sauce

Soy sauce is a fermented food derived from soybean or/and wheat. Soy sauce contains salt (approximately 15–20%), water (approximately 50–70%), peptides, isoflavones, free sugar, and organic acids derived from the soybeans during fermentation. Soy sauce contains several antioxidants such as melanoidins (formed during fermentation), phenolic compounds and free amino acids (Moon *et al.* 2002).

3.11.1 Relevance of soy sauce to health

There are number of studies showing the potential therapeutic effect of soy sauce/soy sauce constituent(s). Health claims of soy sauce are:

3.11.1.1 Antioxidant activity

In vivo, human model

- Antioxidant activity of soy sauce was study in vitro and in vivo. In randomized, observed-blind, placebo-controlled and crossover trial dark soy sauce (DSS) was given to healthy human volunteers in volume of 30ml mixed with 200g plain boiled rice. Placebo was 200g of boiled rice with colourant (caramel). Blood and urine samples were collected pre-meal and at 1, 2, 3, and 4 h time points after eating. Vascular measurements—blood pressure, augmentation index, and heart rates were also taken at baseline, then at 1, 2, 3, and 4 h after the meal and just prior to blood sampling. The antioxidant activity of dark soy sauce, rice, and food colorant (caramel) was measured in vitro by the ABTS assay. Trolox was used as a standard. The antioxidant activity of the food was expressed as Trolox equivalent antioxidant capacity (TEAC). In this study high Trolox equivalent antioxidant capacity (TEAC) of the dark soy sauce was confirmed and was 122 ± 16 mM, $n = 3$ by the ABTS activity. The rice or caramel colorant had no significant antioxidant activity. Augmentation index (AIx) is an indication of blood flow efficiency in the vascular system. An increase in AIx denotes vasoconstriction while a decrease denotes a reduction in flow resistance (vasodilatation). In this trial no significant difference between placebo and soy sauce meal was observed for AIx, heart rate and systolic blood pressure at any time point. In the study one of the biomarker of lipid peroxidation was checked. The level of F_2-isoprostanes in urine and plasma was measured. Plasma total F_2-isoprostanes (combined measurement of free and esterified forms) were decreased at 3 h after the placebo meal and at 1–4 h after the DSS meal when compared with baseline. When DSS and placebo were compared, there was a maximal decrease in total F_2-isoprostanes 3 h after consuming DSS at which point the total F_2-isoprostanes concentration was significantly lower than after placebo (0.272 ng/ml vs. 0.310 ng/ml, $p = 0.02$). The DSS meal had also greater impact than placebo on lowering free F_2-isoprostanes, with levels differing significantly at 4 h (0.066 ng/ml vs. 0.078 ng/ml, $p = 0.04$). The placebo meal lowered the esterified F_2-isoprostanes at the 3 h time point ($p = 0.02$) while the DSS meal tended to reduce esterified F_2-isoprostanes throughout with statistically significant reductions from baseline at 3 h ($p = 0.01$) and 4 h ($p = 0.04$), and a borderline difference ($p = 0.07$) from placebo values at 3 h. This study shows antioxidant potential of dark soy sauce in vitro and in vivo models (Lee *et al.* 2006).

In vivo, animal model

- Dark soy sauce (DSS) was tested for its antioxidant activity in acrylamide (ACR)-induced toxicity in rats. DSS was administrated orally (0.5ml/kg/BW/day) for 2 weeks before and after ACR exposure (0.2mg/L). The body weight gain, brain weights and the gait abnormalities of ACR-treated rats were improved significantly ($p \leq 0.05$) when DSS was applied. DSS significantly improved the axonal degeneration, the ratio of myelinated nerves < 3 micron in diameter, degree of central chromatolysis of the ganglion neurons in peripheral nerves, and numbers of SYP (+) aberrant dots per mm cortex in the cerebellar molecular layer of ACR-treated rats no matter before, after, or during ACR-exposure ($p < 0.05$). DSS significantly decreased the malondialdehyde level and increased the superoxide dismutase activity in brain of ACR-treated rats when DSS treated during ACR-exposure ($p < 0.05$). This study showed that dark soy sauce may play protective role against ACR-developed neurotoxicity through an anti-oxidative action. And it is worthy to note that DSS treatment at the same time as ACR exposure plays a more effective protective role than before or after ACR exposure (Xichun and Min'ai 2009).

In vitro

- Antioxidant activity of melanoidins, the brown colour products in soy sauce, was studied by Moon et al. (2002). These are end products of Maillard reaction process which occurs between carbohydrates and amino acids. In the study the authors presented antioxidant effect of commercially available soy sauces. 29 different soy sauces were screened. Sauces were collected from: China (6), Taiwan (1), Thailand (3), Singapore (7), Malaysia (3), Indonesia (2), and The Philippines (7). Their contents of total nitrogen and 3-deoxy-glucosone (3-DG), which is an important intermediate product of the Maillard reaction products, were compared. Ferric reduction/Antioxidant power (FRAP) and Trolox Equivalent Antioxidant Capacity (TEAC) assays were employed in measurement of antioxidant activities of say sauces. Most soy sauces suppressed the lipid peroxidation effectively and showed strong free radical scavenging ability although big differences existed between samples. Low antioxidant activity indicates white and light coloured sauce, however they have a high nitrogen content (0.4811–0.283). Dark coloured and sweet sauces containing low nitrogen (0.253–0.295) showed higher effect. The FRAP value of soy sauces was positively correlated with brown color intensity (r^2=0.9634) and 3-DG content (r^2=0.9704), while not correlated with nitrogen content. In

summary, the Maillard reaction products in soy sauces play a more important role on their antioxidative effect than their nitrogen compounds (Moon *et al.* 2002).

- Antioxidant property of soy sauce was study by measurement its DPPH radical-scavenging activity. In the study performed by Aoshima and Ooshima (2009) total concentration of polyphenols content is soy sauce (expressed as mM gallic acid) was estimated as 30.3mM ± 0.2 (n=3). Soy sauce indicated high level of DPPH radical-scavenging activity (87.7% ± 0.1 (n=3)) (Aoshima and Ooshima 2009).
- Antioxidant activity of dark soy sauce constituent was study by Wang et al. (2007). 3-Hydroxy-2-methyl-4*H*-pyran-4-one (maltol) was identified in ethyl acetate extract of soy sauce in millimolar concentration. Its antioxidant action was confirmed by ABTS assay. Other, even more powerful, antioxidant components of dark soy sauce were melanoidins. Their structural characteristics was carried out by nuclear magnetic resonance (NMR) and electrospray-ionization time-of-flight mass spectrometry (ESI-TOF-MS) analysis (Wang *et al.* 2007).

3.11.1.2 Anti-allergic and hypoallergenic activity

In vivo, human model

- Shoyu polysaccharides (SPS) are components of soybean cell wall. They are resistant to enzymic hydrolysis, means that they are also present in soy sauce. Their quantity in soy sauce is estimated as 1%. Anti-allergic properties of SPS was studied in vivo by Kobayashi (2005).

  Oral supplementation of SPS was tested in patients suffered from allergic rhinitis. Two separate trials were carried out on patients with perennial allergic rhinitis (PAR) and seasonal allergic rhinitis (SAR). Patients with PAR: In a 4-week randomized, double-blind, placebo-controlled parallel group study (67), patients with PAR were treated with 600 mg of SPS (n=11) or a placebo (n=10) each day. After 4 weeks of treatment with SPS, symptom scores for runny nose, sore throat, and eye itching significantly decreased from the baseline within the group ($p \leq 0.05$) but no changes in these scores were observed after 4 weeks treatment in the placebo group (67). The total symptom score, calculated from the sum of individual scores, showed a significant difference between the two groups after 4 weeks of treatment ($p \leq 0.05$).

Patients with SAR: In an 8-week randomized, double-blind, placebo-controlled parallel group study (68), patients with SAR due to Japanese cedar pollen were treated with 600 mg of SPS (n=25) or a placebo (n=26) each day. After 4 weeks of treatment with SPS, scores of symptoms such as sneezing, nasal stuffiness, and hindrance of daily life were significantly different ($p \leq 0.05$) from those in the placebo-treated groups (68). The total symptom score, calculated from the sum of individual scores, showed a significant difference ($p \leq 0.05$) between the two groups after 4 to 8 weeks.

In these studies anti-allergic activity of shoyu polysaccharides (SPS) was confirmed Quantity of SPS (600mg/day) used in the studies corresponded to 60 ml of soy sauce per day (Kobayashi 2005).

- Hypoallergenic property of soy sauce was presented by Kobayashi et al. (2004). Wheat and soy beans are two main raw materials of soy sauce. Sera of patients suffer from wheat allergy was used for screening of allergenicity of 10 soy sauces. Inhibition ELISA and direct ELISA assays showed no wheat allergen detected in soy sauces, what suggested that during fermentation (brewing process) of soy sauce wheat allergens were completely degraded by microbial enzymes. In this case soy sauce may be considered as hypoallergenic food (Kobayashi *et al.* 2004).

3.12 Brown sugar

Brown sugar is made from sugarcane. Brown sugar contains sugar crystals and molasses. Originally, brown sugar was made from not fully refined sugar. Intensity of brown colour depended on the molasses content (from golden to brown). Nowadays, brown sugar is made by adding molasses to partially or full refined sucrose (3.5% molasses gives light brown sugar, whereas 6.5% molasses added to sugar gives dark brown colour) (Goldstein *et al.* 2015).

3.12.1 Relevance of brown sugar to health

3.12.1.1 Antioxidant activity

In vitro

- Antioxidant activity of 14 available cane and palm sugars was study by Sia et al. (2010). Cyclic voltammetry method was used for evaluation antioxidants in sugars. Sugars were dissolved in a phosphate buffer up to 50% w/v. Vitamin C was also assessed for its antioxidant activity using same method. Anodic current peaks (I_{pa}) of Vitamin C and sugars were compared. Assuming that the I_{pa} reflects antioxidant activity, comparing these values with vitamin C gives a rank order of antioxidant activity of the five sugars: *gula anau* > *gula merah* > China rock honey sugar > soft brown sugar > SIS raw sugar (Sia *et al.* 2010).
- Antioxidant content of natural sweeteners was determined and compared to refined sugars. The ferric-reducing ability of plasma (FRAP) assay was used to estimate total antioxidant capacity. Substantial differences in total antioxidant content of different sweeteners were found. Refined sugar, corn syrup, and agave nectar contained minimal antioxidant activity (<0.01 mmol FRAP/100 g); raw cane sugar had a higher FRAP (0.1 mmol/100 g). Dark and blackstrap molasses had the highest FRAP (4.6 to 4.9 mmol/100 g), while maple syrup, brown sugar, and honey showed intermediate antioxidant capacity (0.2 to 0.7 mmol FRAP/100 g) (Phillips *et al.* 2009).
- Electron spin resonance (ESR) spectroscopy was used to determine the anthocyanin content in refined sugar, plantation white sugar, soft brown sugar and raw sugar. Spectra of sugars were compared to standard anthocyanin. The ESR spectra of red and violet anthocyanins was predominantly $g \approx 2.0055$, which corresponded to an unpaired electron located in the pyrylium ring. Signals for Fe (III) and Mn (II), which naturally occur in plants, were found in raw sugar, soft brown sugar and standard anthocyanin. Signals for refined sugar and plantation white sugar were not present due to the refining process. ESR results were also correlated with the colour of the sugars (as determined by International Commission for Uniform Methods of Sugar Analysis and inductively coupled plasma optical emission spectroscopy) (Thamaphat *et al.* 2015).

4 References

Abu-Darwish, M. S., Al-Ramamneh, E. A., Kyslychenko, V. S. and Karpiuk, U. V. (2012). "The antimicrobial activity of essential oils and extracts of some medicinal plants grown in Ash-shoubak region - South of Jordan." *Pakistan journal of pharmaceutical sciences* 25(1): 239-246.

Agbor, G. A., Vinson, J. A., Oben, J. E. and Ngogang, J. Y. (2006). "Comparative analysis of the in vitro antioxidant activity of white and black pepper." *Nutrition Research* 26(12): 659-663.

Ahmed, N., Laverick, L., Sammons, J., Zhang, H., Maslin, D. J. and Hassan, H. T. (2001). "Ajoene, a garlic-derived natural compound, enhances chemotherapy-induced apoptosis in human myeloid leukaemia CD34-positive resistant cells." *Anticancer research* 21(5): 3519-3523.

Ahuja, K. D., Robertson, I. K., Geraghty, D. P. and Ball, M. J. (2006). "Effects of chili consumption on postprandial glucose, insulin, and energy metabolism." *The American Journal of Clinical Nutrition* 84(1): 63-69.

Ahuja, K. D. K., Robertson, I. K., Geraghty, D. P. and Ball, M. J. (2006). "The effect of 4-week chilli supplementation on metabolic and arterial function in humans." *European Journal of Clinical Nutrition* 61(3): 326-333.

al-Yahya, M. A., Rafatullah, S., Mossa, J. S., Ageel, A. M., Parmar, N. S. and Tariq, M. (1989). "Gastroprotective activity of ginger zingiber officinale rosc., in albino rats." *The American journal of Chinese medicine* 17(1-2): 51-56.

Ali, M., Bordia, T. and Mustafa, T. (1999). "Effect of raw versus boiled aqueous extract of garlic and onion on platelet aggregation." *Prostaglandins Leukotrienes and Essential Fatty Acids* 60(1): 43-47.

Ali, M. and Thomson, M. (1995). "Consumption of a garlic clove a day could be beneficial in preventing thrombosis." *Prostaglandins, Leukotrienes and Essential Fatty Acids* 53(3): 211-212.

Amagase, H., Petesch, B. L., Matsuura, H., Kasuga, S. and Itakura, Y. (2001). "Intake of garlic and its bioactive components." *Journal of Nutrition* 131(3s): 955s-962s.

Aoshima, H. and Ooshima, S. (2009). "Anti-hydrogen peroxide activity of fish and soy sauce." *Food Chemistry* 112(2): 339-343.

Bamidele, O., Akinnuga, A. M., Alagbonsi, I. A., Ojo, O. A., Olorunfemi, J. O. and Akuyoma, M. A. (2011). "Effects of ethanolic extract of Myristica fragrans Houtt. (nutmeg) on some heamatological indices in albino rats." *International Journal of Medicine and Medical Science* 3(6): 215-218.

Bang, J. S., Oh, D. H., Choi, H. M., Sur, B.-J., Lim, S.-J., Kim, J. Y., Yang, H.-I., Yoo, M. C., Hahm, D.-H. and Kim, K. S. (2009). "Anti-inflammatory and antiarthritic effects of

piperine in human interleukin 1β-stimulated fibroblast-like synoviocytes and in rat arthritis models." *Arthritis Research & Therapy* 11(2): R49-R49.

Beauchamp, G. K., Keast, R. S., Morel, D., Lin, J., Pika, J., Han, Q., Lee, C. H., Smith, A. B. and Breslin, P. A. (2005). "Phytochemistry: ibuprofen-like activity in extra-virgin olive oil." *Nature* 437(7055): 45-46.

BioPerine (2015, July 2015). Retrieved July 2015, 2015, from http://www.bioperine.com.

Bordia, A. (1978). "Effect of garlic on human platelet aggregation in vitro." *Atherosclerosis* 30(4): 355-360.

Campos, K. E., Diniz, Y. S., Cataneo, A. C., Faine, L. A., Alves, M. J. and Novelli, E. L. (2003). "Hypoglycaemic and antioxidant effects of onion, Allium cepa: dietary onion addition, antioxidant activity and hypoglycaemic effects on diabetic rats." *International journal of food sciences and nutrition* 54(3): 241-246.

Cavagnaro, P. F., Camargo, A., Galmarini, C. R. and Simon, P. W. (2007). "Effect of cooking on garlic (Allium sativum L.) antiplatelet activity and thiosulfinates content." *Journal of agricultural and food chemistry* 55(4): 1280-1288.

Chen, J. H., Chen, H. I., Tsai, S. J. and Jen, C. J. (2000). "Chronic consumption of raw but not boiled Welsh onion juice inhibits rat platelet function." *Journal of Nutrition* 130(1): 34-37.

Chittumma, P., Kaewkiattikun, K. and Wiriyasiriwach, B. (2007). "Comparison of the effectiveness of ginger and vitamin B6 for treatment of nausea and vomiting in early pregnancy: a randomized double-blind controlled trial." *Journal of the Medical Association of Thailand* 90(1): 15-20.

Covas, M. I., Nyyssonen, K., Poulsen, H. E., Kaikkonen, J., Zunft, H. J., Kiesewetter, H., Gaddi, A., de la Torre, R., Mursu, J., Baumler, H., Nascetti, S., Salonen, J. T., Fito, M., Virtanen, J. and Marrugat, J. (2006). "The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial." *Annals of internal medicine* 145(5): 333-341.

Demmelmair, H., Broekaert, I., Münch, K., Trak-Fellermeier, M. and Koletzko, B. "Comparison of the effects of rapeseed oil, olive oil and hydrogenated plant oil on postprandial lipids and fatty acid oxidation " *Division Metabolic Diseases and Nutritional Medicine* Dr. von Hauner Children's Hospital, Ludwig-Maximilians University, Munich, Germany

Dion, M. E., Agler, M. and Milner, J. A. (1997). "S-allyl cysteine inhibits nitrosomorpholine formation and bioactivation." *Nutrition and Cancer* 28(1): 1-6.

Dirsch, V. M., Antlsperger, D. S., Hentze, H. and Vollmar, A. M. (2002). "Ajoene, an experimental anti-leukemic drug: mechanism of cell death." *Leukemia* 16(1): 74-83.

Dirsch, V. M., Gerbes, A. L. and Vollmar, A. M. (1998). "Ajoene, a compound of garlic, induces apoptosis in human promyeloleukemic cells, accompanied by generation of reactive oxygen species and activation of nuclear factor kappaB." *Molecular pharmacology* 53(3): 402-407.

Diwan, V., Poudyal, H. and Brown, L. (2013). "Piperine attenuates cardiovascular, liver and metabolic changes in high carbohydrate, high fat-fed rats." *Cell biochemistry and biophysics* 67(2): 297-304.

Domenech, M., Roman, P., Lapetra, J., Garcia de la Corte, F. J., Sala-Vila, A., de la Torre, R., Corella, D., Salas-Salvado, J., Ruiz-Gutierrez, V., Lamuela-Raventos, R. M., Toledo, E., Estruch, R., Coca, A. and Ros, E. (2014). "Mediterranean diet reduces 24-hour ambulatory blood pressure, blood glucose, and lipids: one-year randomized, clinical trial." *Hypertension* 64(1): 69-76.

Duh, P.-D., Hsiao, W.-C. and Wang, B.-S. (2008). "An aqueous extract of Welsh onion green leaves increase ABCA1 and SR-BI expression in macrophage RAW 264.7 cells." *Food Chemistry* 107(3): 1029-1038.

EFSA (2010). "Scientific Opinion on the substantiation of health claims related to various food(s)/food constituent(s) and protection of cells from premature aging, antioxidant activity, antioxidant content and antioxidant properties, and protection of DNA, proteins and lipids from oxidative damage pursuant to Article 13(1) of Regulation (EC) No 1924/20061." *EFSA Journal* 8(2): 1489.

EFSA (2010). "Scientific Opinion on the substantiation of health claims related to various food(s)/food constituent(s) claiming maintenance of joints (ID 1799, 1973, 2022, 2178, 2202, 2254, 2255, 2311, 2394, 2417, 2418, 2458, 2649, 2794, 2798, 3119, 3144, 3274, 3283, 3318, 3339, 3495, 3511, 3523, 3555, 3624, 3699, 3748, 3770, 3835, 3884, 3892, 3904, 3943, 3978, 4012, 4020, 4056, 4137, 4175), maintenance of bone (ID 1764, 1907, 2418, 4012, 4020, 4056, 4175) and maintenance of muscles (ID 2254, 2311) pursuant to Article 13(1) of Regulation (EC) No 1924/2006." *EFSA Journal* 8(2): 1493.

EFSA (2010). "Scientific Opinion on the substantiation of health claims related to various food(s)/food constituents(s) and "immune function/immune system" (ID 433, 605, 645, 791, 1384, 1451, 1624, 1743, 1776, 1838, 1851, 1854, 1891,1895, 3074, 3191,3211, 3218, 3336, 3341, 3450, 3514, 3658, 3756, 3802,3805, 3841, 3935, 3979), "contribution to body defences against external agents" (ID 2344, 3208, 3213, 3270, 3278, 3285, 3294, 3309, 3387, 3424,3428, 3536, 3537, 3539, 3575, 3577, 3579, 3582, 3603, 3618, 3755, 3776,3777, 3778, 3801, 3804, 3840, 4367, 4472), reduction of inflammation (ID 1327, 1391) and decreasing potentially pathogenic gastro-intestinal microorganisms (ID 2362, 2730, 4064) pursuant to Article 13(1) of Regulation (EC) No 1924/2006." *EFSA Journal* 8(10): 1799.

EFSA (2011). "Scientific Opinion on the substantiation of health claims related to olive oil and maintenance of normal blood LDL-cholesterol concentrations (ID 1316, 1332), maintenance of normal (fasting) blood concentrations of triglycerides (ID 1316, 1332), maintenance of normal blood HDL-cholesterol concentrations (ID 1316, 1332) and maintenance of normal blood glucose concentrations (ID 4244) pursuant to Article 13(1) of Regulation (EC) No 1924/2006." *EFSA Journal* 9(4): 2044.

EFSA (2011). "Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL-cholesterol concentrations (ID 1639), maintenance of normal blood pressure (ID 3781), "anti-inflammatory properties" (ID 1882), "contributes to the upper respiratory tract health" (ID 3468), "can help to maintain a normal

function of gastrointestinal tract" (3779), and "contributes to body defences against external agents" (ID 3467)pursuant to Article 13(1) of Regulation (EC) No 1924/2006." *EFSA Journal* 9(4): 2033.

EFSA (2011). "Scientific Opinion Part I on the substantiation of health claims related to various food(s)/food constituent(s) not supported by pertinent human data (ID 411, 559, 1174, 1184, 1197, 1380, 1409, 1656, 1667, 1670, 1763, 1767, 1806, 1884, 1908, 1997, 2141, 2159, 2243, 2244, 2325, 2331, 2333, 2336, 2652, 2717, 2727, 2752, 2788, 2861, 2870, 2885, 2894, 3077, 3101, 3516, 3595, 3726, 4252, 4288, 4290, 4406, 4509, 4709) pursuant to Article 13(1) of Regulation (EC) No 1924/2006." *EFSA Journal* 9(6): 2246.

El-Demerdash, F. M., Yousef, M. I. and El-Naga, N. I. (2005). "Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats." *Food and Chemical Toxicology* 43(1): 57-63.

Elango, E. M., Asita, H., Nidhi, G., Seema, P., Banerji, A. and Kuriakose, M. A. (2004). "Inhibition of cyclooxygenase-2 by diallyl sulfides (DAS) in HEK 293T cells." *Journal of applied genetics* 45(4): 469-471.

Esmaeili, D., Mobarez, A. M. and Tohidpour, A. (2012). "Anti-Helicobacter Pylori Activities of Shoya Powder and Essential Oils of Thymus Vulgaris and Eucalyptus Globulus." *The Open Microbiology Journal* 6: 65-69.

Ferrara, L. A., Raimondi, A. S., d'Episcopo, L., Guida, L., Dello Russo, A. and Marotta, T. (2000). "Olive oil and reduced need for antihypertensive medications." *Archives of internal medicine* 160(6): 837-842.

Fuhrman, B., Rosenblat, M., Hayek, T., Coleman, R. and Aviram, M. (2000). "Ginger extract consumption reduces plasma cholesterol, inhibits LDL oxidation and attenuates development of atherosclerosis in atherosclerotic, apolipoprotein E-deficient mice." *Journal of Nutrition* 130(5): 1124-1131.

Giordani, R., Regli, P., Kaloustian, J., Mikail, C., Abou, L. and Portugal, H. (2004). "Antifungal effect of various essential oils against Candida albicans. Potentiation of antifungal action of amphotericin B by essential oil from Thymus vulgaris." *Phytotherapy Research* 18(12): 990-995.

Goldstein, D., Mintz, S., Krondl, M., Rath, E., Mason, L., Quinzio, G. and Heinzelmann, U. (2015). The Oxford Companion to Sugar and Sweets, Oxford University Press.

Gulcin, I. (2005). "The antioxidant and radical scavenging activities of black pepper (Piper nigrum) seeds." *International journal of food sciences and nutrition* 56(7): 491-499.

Hubbard, G. P., Wolffram, S., de Vos, R., Bovy, A., Gibbins, J. M. and Lovegrove, J. A. (2006). "Ingestion of onion soup high in quercetin inhibits platelet aggregation and essential components of the collagen-stimulated platelet activation pathway in man: a pilot study." *British Journal of Nutrition* 96(3): 482-488.

Ibrahim, K. M., Naem, R. K. and Abd-Sahib, A. S. (2013). "Antibacterial Activity of Nutmeg (Myristica fragrans) Seed Extracts Against Some Pathogenic Bacteria." *Journal of Al-Nahrain University* 16(2): 188-192.

Jain, A. K., Vargas, R., Gotzkowsky, S. and McMahon, F. G. (1993). "Can garlic reduce levels of serum lipids? a controlled clinical study." *The American Journal of Medicine* 94(6): 632-635.

Jung, Y. S., Kim, M. H., Lee, S. H., Baik, E. J., Park, S. W. and Moon, C. H. (2002). "Antithrombotic effect of onion in streptozotocin-induced diabetic rat." *Prostaglandins, Leukotrienes and Essential Fatty Acids* 66(4): 453-458.

Kalava, A., Darji, S. J., Kalstein, A., Yarmush, J. M., SchianodiCola, J. and Weinberg, J. (2013). "Efficacy of ginger on intraoperative and postoperative nausea and vomiting in elective cesarean section patients." *European Journal of Obstetrics & Gynecology and Reproductive Biology* 169(2): 184-188.

Kang, M.-J., Kim, J.-H., Choi, H.-N., Kim, M.-J., Han, J.-H., Lee, J.-H. and Kim, J.-I. (2010). "Hypoglycemic effects of Welsh onion in an animal model of diabetes mellitus." *Nutrition Research and Practice* 4(6): 486-491.

Kannar, D., Wattanapenpaiboon, N., Savige, G. S. and Wahlqvist, M. L. (2001). "Hypocholesterolemic Effect of an Enteric-Coated Garlic Supplement." *Journal of the American College of Nutrition* 20(3): 225-231.

Kareem, M. A., Gadhamsetty, S. K., Shaik, A. H., Prasad, E. M. and Kodidhela, L. D. (2013). "Protective effect of nutmeg aqueous extract against experimentally-induced hepatotoxicity and oxidative stress in rats." *Journal of Ayurveda and Integrative Medicine* 4(4): 216-223.

Kiesewetter, H., Jung, F., Pindur, G., Jung, E. M., Mrowietz, C. and Wenzel, E. (1991). "Effect of garlic on thrombocyte aggregation, microcirculation, and other risk factors." *International Journal of Clinical Pharmacology, Therapy and Toxicology* 29(4): 151-155.

Kim, H., Bu, Y., Lee, B. J., Bae, J., Park, S., Kim, J., Lee, K., Cha, J. M., Ryu, B., Ko, S. J., Han, G., Min, B. and Park, J. W. (2013). "Myristica fragrans seed extract protects against dextran sulfate sodium-induced colitis in mice." *Journal of medicinal food* 16(10): 953-956.

Kobayashi, M. (2005). "Immunological functions of soy sauce: hypoallergenicity and antiallergic activity of soy sauce." *Journal of Bioscience and Bioengineering* 100(2): 144-151.

Kobayashi, M., Hashimoto, Y., Taniuchi, S. and Tanabe, S. (2004). "Degradation of wheat allergen in Japanese soy sauce." *International journal of molecular medicine* 13(6): 821-827.

Kohiyama, C. Y., Yamamoto Ribeiro, M. M., Mossini, S. A. G., Bando, E., Bomfim, N. d. S., Nerilo, S. B., Rocha, G. H. O., Grespan, R., Mikcha, J. M. G. and Machinski Jr, M. (2015). "Antifungal properties and inhibitory effects upon aflatoxin production of Thymus vulgaris L. by Aspergillus flavus Link." *Food Chemistry* 173(0): 1006-1010.

Kozics, K., Klusová, V., Srančíková, A., Mučaji, P., Slameňová, D., Hunáková, Ľ., Kusznierewicz, B. and Horváthová, E. (2013). "Effects of Salvia officinalis and Thymus vulgaris on oxidant-induced DNA damage and antioxidant status in HepG2 cells." *Food Chemistry* 141(3): 2198-2206.

Lee, C. Y., Isaac, H. B., Wang, H., Huang, S. H., Long, L. H., Jenner, A. M., Kelly, R. P. and Halliwell, B. (2006). "Cautions in the use of biomarkers of oxidative damage; the vascular and antioxidant effects of dark soy sauce in humans." *Biochemical and Biophysical Research Communications* 344(3): 906-911.

Legnani, C., Frascaro, M., Guazzaloca, G., Ludovici, S., Cesarano, G. and Coccheri, S. (1993). "Effects of a dried garlic preparation on fibrinolysis and platelet aggregation in healthy subjects." *Arzneimittel-Forschung* 43(2): 119-122.

Li, J. and Wang, D. H. (2003). "High-salt-induced increase in blood pressure: role of capsaicin-sensitive sensory nerves." *Journal of hypertension* 21(3): 577-582.

Ludy, M. J. and Mattes, R. D. (2011). "The effects of hedonically acceptable red pepper doses on thermogenesis and appetite." *Physiology & Behavior* 102(3-4): 251-258.

Mahmoodi, M., Hosseini Zijoud, S. M., Hassanshahi, G. H., Toghroli, M. A., Khaksari, M., Hajizadeh, M. R. and Mirzajani, E. (2011). "The effects of consumption of raw garlic on serum lipid level, blood sugar and a number of effective hormones on lipid and sugar metabolism in hyperglycemic and/or hyperlipidemic individuals ; Benefit of raw garlic consumption." *Advances in Biological Chemistry* 1(2): 29-33.

Martinez-Dominguez, E., de la Puerta, R. and Ruiz-Gutierrez, V. (2001). "Protective effects upon experimental inflammation models of a polyphenol-supplemented virgin olive oil diet." *Inflammation Research* 50(2): 102-106.

Martins, N., Barros, L., Santos-Buelga, C., Silva, S., Henriques, M. and Ferreira, I. C. F. R. (2015). "Decoction, infusion and hydroalcoholic extract of cultivated thyme: Antioxidant and antibacterial activities, and phenolic characterisation." *Food Chemistry* 167(0): 131-137.

Minaiyan, M., Ghannadi, A. and Karimzadeh, A. (2006). "Anti-ulcerogenic effect of ginger (rhizome of Zingiber officinale Roscoe) on cystemine induced duodenal ulcer in rats." *DARU Journal of Pharmaceutical Sciences* 14(2): 97-101.

Moon, C. H., Jung, Y. S., Kim, M. H., Lee, S. H., Baik, E. J. and Park, S. W. (2000). "Mechanism for antiplatelet effect of onion: AA release inhibition, thromboxane A2synthase inhibition and TXA2/PGH2receptor blockade." *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)* 62(5): 277-283.

Moon, G., Lee, M., Lee, Y. and Trakoontivakorn, G. (2002). "Main component of soy sauce representing antioxidative activity." *International Congress Series* 1245: 509-510.

Moreno-Luna, R., Munoz-Hernandez, R., Miranda, M. L., Costa, A. F., Jimenez-Jimenez, L., Vallejo-Vaz, A. J., Muriana, F. J., Villar, J. and Stiefel, P. (2012). "Olive oil polyphenols decrease blood pressure and improve endothelial function in young women with mild hypertension." *American journal of hypertension* 25(12): 1299-1304.

Morita, T., Jinno, K., Kawagishi, H., Arimoto, Y., Suganuma, H., Inakuma, T. and Sugiyama, K. (2003). "Hepatoprotective effect of myristicin from nutmeg (Myristica fragrans) on lipopolysaccharide/d-galactosamine-induced liver injury." *Journal of agricultural and food chemistry* 51(6): 1560-1565.

Nkrumah-Elie, Y. M., Reuben, J. S., Hudson, A., Taka, E., Badisa, R., Ardley, T., Israel, B., Sadrud-Din, S. Y., Oriaku, E. and Darling-Reed, S. F. (2012). "Diallyl trisulfide as an inhibitor of benzo(a)pyrene-induced precancerous carcinogenesis in MCF-10A cells." *Food and Chemical Toxicology* 50(7): 2524-2530.

O'Neill, J., Brock, C., Olesen, A. E., Andresen, T., Nilsson, M. and Dickenson, A. H. (2012). "Unravelling the Mystery of Capsaicin: A Tool to Understand and Treat Pain." *Pharmacological Reviews* 64(4): 939-971.

Obioha, U. E., Suru, S. M., Ola-Mudathir, K. F. and Faremi, T. Y. (2009). "Hepatoprotective potentials of onion and garlic extracts on cadmium-induced oxidative damage in rats." *Biological trace element research* 129(1-3): 143-156.

Oommen, S., Anto, R. J., Srinivas, G. and Karunagaran, D. (2004). "Allicin (from garlic) induces caspase-mediated apoptosis in cancer cells." *European journal of pharmacology* 485(1-3): 97-103.

Oyedemi, T. O., Lawal, T. O. and Adeniyi, B. A. (2014). "Effect of Myristica fragrans Houtt. Seed (Nutmeg) on Helicobacter pyloriinduced gastritis in albino rats: in vitro and in vivo studies." *International Journal of Biological and Chemical Sciences* 8(4).

Ozaki, Y., Soedigdo, S., Wattimena, Y. R. and Suganda, A. G. (1989). "Antiinflammatory effect of mace, aril of Myristica fragrans Houtt., and its active principles." *Japanese journal of pharmacology* 49(2): 155-163.

Ozgoli, G., Goli, M. and Simbar, M. (2009). "Effects of ginger capsules on pregnancy, nausea, and vomiting." *Journal of Alternative and Complementary Medicine* 15(3): 243-246.

Panda, S. and Kar, A. (2003). "Piperine lowers the serum concentrations of thyroid hormones, glucose and hepatic 5'D activity in adult male mice." *Hormone and Metabolic Research* 35(9): 523-526.

Park, B. S., Son, D. J., Park, Y. H., Kim, T. W. and Lee, S. E. (2007). "Antiplatelet effects of acidamides isolated from the fruits of Piper longum L." *Phytomedicine : international journal of phytotherapy and phytopharmacology* 14(12): 853-855.

Phillips, K. M., Carlsen, M. H. and Blomhoff, R. (2009). "Total Antioxidant Content of Alternatives to Refined Sugar." *Journal of the American Dietetic Association* 109(1): 64-71.

Ram, A., Lauria, P., Gupta, R. and Sharma, V. N. (1996). "Hypolipidaemic effect of Myristica fragrans fruit extract in rabbits." *Journal of ethnopharmacology* 55(1): 49-53.

Reinhart, K. M., Talati, R., White, C. M. and Coleman, C. I. (2009). "The impact of garlic on lipid parameters: a systematic review and meta-analysis." *Nutrition Research Reviews* 22(01): 39-48.

Ruiz-Gutierrez, V., Muriana, F. J., Guerrero, A., Cert, A. M. and Villar, J. (1996). "Plasma lipids, erythrocyte membrane lipids and blood pressure of hypertensive women after ingestion of dietary oleic acid from two different sources." *Journal of hypertension* 14(12): 1483-1490.

Sabina, E. P., Souriyan, A. D. H., Jackline, D. and Rasool, M. K. (2010). "Piperine, an active ingredient of black pepper attenuates acetaminophen–induced hepatotoxicity in mice." *Asian Pacific Journal of Tropical Medicine* 3(12): 971-976.

Servili, M. and Montedoro, G. (2002). "Contribution of phenolic compounds to virgin olive oil quality." *European Journal of Lipid Science and Technology* 104(9-10): 602-613.

Sfeir, J., Lefrançois, C., Baudoux, D., Derbré, S. and Licznar, P. (2013). "In Vitro Antibacterial Activity of Essential Oils against Streptococcus pyogenes." *Evidence-Based Complementary and Alternative Medicine* 2013: 9.

Shafiei, Z., Shuhairi, N. N., Md Fazly Shah Yap, N., Harry Sibungkil, C. A. and Latip, J. (2012). "Antibacterial Activity of Myristica fragrans against Oral Pathogens." *Evidence-Based Complementary and Alternative Medicine* 2012: 825362.

Sharma, A., Mathur, R. and Dixit, V. P. (1995). "Prevention of hypercholesterolemia and atherosclerosis in rabbits after supplementation of Myristica fragrans seed extract." *Indian journal of physiology and pharmacology* 39(4): 407-410.

Sia, J., Yee, H.-B., Santos, J. H. and Abdurrahman, M. K.-A. (2010). "Cyclic voltammetric analysis of antioxidant activity in cane sugars and palm sugars from Southeast Asia." *Food Chemistry* 118(3): 840-846.

Sienkiewicz, M., Lysakowska, M., Ciecwierz, J., Denys, P. and Kowalczyk, E. (2011). "Medicinal chemistry." *Medicinal chemistry (Shariqah (United Arab Emirates))* 7(6): 674-689.

Silva, S., Bronze, M. R., Figueira, M. E., Siwy, J., Mischak, H., Combet, E. and Mullen, W. (2015). "Impact of a 6-wk olive oil supplementation in healthy adults on urinary proteomic biomarkers of coronary artery disease, chronic kidney disease, and diabetes (types 1 and 2): a randomized, parallel, controlled, double-blind study." *The American Journal of Clinical Nutrition* 101(1): 44-54.

Silva, S., Combet, E., Figueira, M. E., Koeck, T., Mullen, W. and Bronze, M. R. (2015). "New perspectives on bioactivity of olive oil: evidence from animal models, human interventions and the use of urinary proteomic biomarkers." *The Proceedings of the Nutrition Society* 74(3): 268-281.

Silva, S., Sepodes, B., Rocha, J., Direito, R., Fernandes, A., Brites, D., Freitas, M., Fernandes, E., Bronze, M. R. and Figueira, M. E. (2015). "Protective effects of hydroxytyrosol-supplemented refined olive oil in animal models of acute inflammation and rheumatoid arthritis." *The Journal of Nutritional Biochemistry* 26(4): 360-368.

Son, D. J., Akiba, S., Hong, J. T., Yun, Y. P., Hwang, S. Y., Park, Y. H. and Lee, S. E. (2014). "Piperine Inhibits the Activities of Platelet Cytosolic Phospholipase A(2) and Thromboxane A(2) Synthase without Affecting Cyclooxygenase-1 Activity: Different Mechanisms of Action Are Involved in the Inhibition of Platelet Aggregation and Macrophage Inflammatory Response." *Nutrients* 6(8): 3336-3352.

Srivastava, K. C. (1989). "Effect of onion and ginger consumption on platelet thromboxane production in humans." *Prostaglandins, Leukotrienes and Essential Fatty Acids* 35(3): 183-185.

Stoilova, I., Krastanov, A., Stoyanova, A., Denev, P. and Gargova, S. (2007). "Antioxidant activity of a ginger extract (Zingiber officinale)." *Food Chemistry* 102(3): 764-770.

Suleria, H. A. R., Butt, M. S., Khalid, N., Sultan, S., Raza, A., Aleem, M. and Abbas, M. (2015). "Garlic (Allium sativum): diet based therapy of 21st century–a review." *Asian Pacific Journal of Tropical Disease* 5(4): 271-278.

Suru, S. M. and Ugwu, C. E. (2015). "Comparative assessment of onion and garlic extracts on endogenous hepatic and renal antioxidant status in rat." *Journal of basic and clinical physiology and pharmacology* 26(4): 347-354.

Takikawa, A., Abe, K., Yamamoto, M., Ishimaru, S., Yasui, M., Okubo, Y. and Yokoigawa, K. (2002). "Antimicrobial activity of Nutmeg against Escherichia coli O157." *Journal of Bioscience and Bioengineering* 94(4): 315-320.

Tanaka, S., Haruma, K., Kunihiro, M., Nagata, S., Kitadai, Y., Manabe, N., Sumii, M., Yoshihara, M., Kajiyama, G. and Chayama, K. (2004). "Effects of aged garlic extract (AGE) on colorectal adenomas: a double-blinded study." *Hiroshima journal of medical sciences* 53(3-4): 39-45.

Tapsell, L. C., Hemphill, I., Cobiac, L., Patch, C. S., Sullivan, D. R., Fenech, M., Roodenrys, S., Keogh, J. B., Clifton, P. M., Williams, P. G., Fazio, V. A. and Inge, K. E. (2006). "Health benefits of herbs and spices: the past, the present, the future." *Medical Journal of Australia* 185(4 Suppl): S4-24.

Tasleem, F., Azhar, I., Ali, S. N., Perveen, S. and Mahmood, Z. A. (2014). "Analgesic and anti-inflammatory activities of Piper nigrum L." *Asian Pacific Journal of Tropical Medicine* 7, Supplement 1(0): S461-S468.

Terés, S., Barceló-Coblijn, G., Benet, M., Álvarez, R., Bressani, R., Halver, J. E. and Escribá, P. V. (2008). "Oleic acid content is responsible for the reduction in blood pressure induced by olive oil." *Proceedings of the National Academy of Sciences of the United States of America* 105(37): 13811-13816.

Thamaphat, K., Goodman, B. A., Limsuwan, P. and Smith, S. M. (2015). "Rapid screening for anthocyanins in cane sugars using ESR spectroscopy." *Food Chemisty* 171: 123-127.

Thiel, A., Buskens, C., Woehrle, T., Etheve, S., Schoenmakers, A., Fehr, M. and Beilstein, P. (2014). "Black pepper constituent piperine: Genotoxicity studies in vitro and in vivo." *Food and Chemical Toxicology* 66(0): 350-357.

Thomson, M., Al-Qattan, K. K., Al-Sawan, S. M., Alnaqeeb, M. A., Khan, I. and Ali, M. (2002). "The use of ginger (Zingiber officinale Rosc.) as a potential anti-inflammatory and antithrombotic agent." *Prostaglandins, Leukotrienes and Essential Fatty Acids* 67(6): 475-478.

Tohidpour, A., Sattari, M., Omidbaigi, R., Yadegar, A. and Nazemi, J. (2010). "Antibacterial effect of essential oils from two medicinal plants against Methicillin-resistant Staphylococcus aureus (MRSA)." *Phytomedicine : international journal of phytotherapy and phytopharmacology* 17(2): 142-145.

Vijayakumar, R. S. and Nalini, N. (2006). "Piperine, an active principle from Piper nigrum, modulates hormonal and apo lipoprotein profiles in hyperlipidemic rats." *Journal of basic and clinical physiology and pharmacology* 17(2): 71-86.

Vijayakumar, R. S., Surya, D. and Nalini, N. (2004). "Antioxidant efficacy of black pepper (Piper nigrum L.) and piperine in rats with high fat diet induced oxidative stress." *Redox Report* 9(2): 105-110.

Vutyavanich, T., Kraisarin, T. and Ruangsri, R. (2001). "Ginger for nausea and vomiting in pregnancy: randomized, double-masked, placebo-controlled trial." *Obstetrics & Gynecology* 97(4): 577-582.

Wang, B.-S., Lin, S.-S., Hsiao, W.-C., Fan, J.-J., Fuh, L.-F. and Duh, P.-D. (2006). "Protective effects of an aqueous extract of Welsh onion green leaves on oxidative damage of reactive oxygen and nitrogen species." *Food Chemistry* 98(1): 149-157.

Wang, B. S., Huang, G. J., Lu, Y. H. and Chang, L. W. (2013). "Anti-inflammatory effects of an aqueous extract of Welsh onion green leaves in mice." *Food Chemistry* 138(2-3): 751-756.

Wang, H., Jenner, A. M., Lee, C. Y., Shui, G., Tang, S. Y., Whiteman, M., Wenk, M. R. and Halliwell, B. (2007). "The identification of antioxidants in dark soy sauce." *Free radical research* 41(4): 479-488.

Westerterp-Plantenga, M. S., Smeets, A. and Lejeune, M. P. (2005). "Sensory and gastrointestinal satiety effects of capsaicin on food intake." *International Journal of Obesity* 29(6): 682-688.

Whiting, S., Derbyshire, E. J. and Tiwari, B. (2014). "Could capsaicinoids help to support weight management? A systematic review and meta-analysis of energy intake data." *Appetite* 73(0): 183-188.

Xichun, Z. and Min'ai, Z. (2009). "Protective role of dark soy sauce against acrylamide-induced neurotoxicity in rats by antioxidative activity." *Toxicology mechanisms and methods* 19(5): 369-374.

Yamamoto, Y., Aoyama, S., Hamaguchi, N. and Rhi, G. S. (2005). "Antioxidative and antihypertensive effects of Welsh onion on rats fed with a high-fat high-sucrose diet." *Bioscience, biotechnology, and biochemistry* 69(7): 1311-1317.

Yamamoto, Y. and Yasuoka, A. (2010). "Welsh onion attenuates hyperlipidemia in rats fed on high-fat high-sucrose diet." *Bioscience, biotechnology, and biochemistry* 74(2): 402-404.

Yang, D., Luo, Z., Ma, S., Wong, W. T., Ma, L., Zhong, J., He, H., Zhao, Z., Cao, T., Yan, Z., Liu, D., Arendshorst, W. J., Huang, Y., Tepel, M. and Zhu, Z. (2010). "Activation of TRPV1 by dietary capsaicin improves endothelium-dependent vasorelaxation and prevents hypertension." *Cell metabolism* 12(2): 130-141.

Yoshioka, M., St-Pierre, S., Drapeau, V., Dionne, I., Doucet, E., Suzuki, M. and Tremblay, A. (1999). "Effects of red pepper on appetite and energy intake." *British Journal of Nutrition* 82(2): 115-123.

Young, H. Y., Liao, J. C., Chang, Y. S., Luo, Y. L., Lu, M. C. and Peng, W. H. (2006). "Synergistic effect of ginger and nifedipine on human platelet aggregation: a study in hypertensive patients and normal volunteers." *The American journal of Chinese medicine* 34(4): 545-551.

Yuan, L.-J., Qin, Y., Wang, L., Zeng, Y., Chang, H., Wang, J., Wang, B., Wan, J., Chen, S.-H., Zhang, Q.-Y., Zhu, J.-D., Zhou, Y. and Mi, M.-T. "Capsaicin-containing chili improved postprandial hyperglycemia, hyperinsulinemia, and fasting lipid disorders in women with gestational diabetes mellitus and lowered the incidence of large-for-gestational-age newborns." *Clinical Nutrition*(0).

Zaman, S. U., Mirje, M. M. and Ramabhimaiah, S. (2014). "Evaluation of the anti-ulcerogenic effect of Zingiber officinale (Ginger) root in rats." *International Journal of Current Microbiology and Applied Sciences* 3(1): 347-354.

Zhou, X. F., Ding, Z. S. and Liu, N. B. (2013). "Allium vegetables and risk of prostate cancer: evidence from 132,192 subjects." *Asian Pacific Journal of Cancer Prevention* 14(7): 4131-4134.

Zhou, Y., Zhuang, W., Hu, W., Liu, G. J., Wu, T. X. and Wu, X. T. (2011). "Consumption of large amounts of Allium vegetables reduces risk for gastric cancer in a meta-analysis." *Gastroenterology* 141(1): 80-89.

5 Appendices

5.1 Group 2 Nutritional Analysis of Original Munroes Marinades

Food & Pharmaceutical

TEST CERTIFICATE

Medcalfe Way Bridge Street Chatteris Cambridgeshire PE16 6QZ England
Telephone: 01354 695858

Mr Thomas O'Sullivan
Advance Micro Services & Environmental Laboratories Ltd
Carrigeen Industrial Estate
Clonmel, Tipperary
Ireland
Fax: 052 61 78133

Certificate Number: TCHC221105-1 Final
Date Reported: 27/07/2015
Date Analysis Started: 21/07/2015
Order Number: INST-044170715

Lab Ref.	Sample Details	Method Number	Test	Result	Units	Flag
CHC320259	Desc: 2058400. Sample 1 - Original. Order No: INST-044170715 Date Received: 20/07/2015 Date Tested: 20/07/2015	AM/C/1015	Moisture (Loss on Drying)	66.9	g / 100g	
		AM/C/224	Protein (Nx6.25)	2.23	g / 100g	
		AM/C/1015	Total Fat (NMR)	0.7	g / 100g	
		AM/C/803	Ash	2.6	g / 100g	
		AM/C/901	Total Carbohydrate (by difference)	27.6	g / 100g	
		AM/C/309	Total Dietary Fibre (AOAC)	2.6	g / 100g	
		AM/C/901	Available Carbohydrate (by difference)	25.0	g / 100g	
		AM/C/901	Energy	120	kcal / 100g	
		AM/C/901	Energy	509	kJ / 100g	
		AM/C/403	Total Sugars (expressed as Glucose)	23.9	g / 100g	
		AM/C/1002	Sodium (ICP-OES)	741	mg / 100g	
		AM/C/107	Saturated Fatty Acids (in sample)	0.19	g / 100g	
		AM/C/107	Monounsaturated Fatty Acids (in sample)	0.35	g / 100g	
		AM/C/107	Polyunsaturated Fatty Acids (in sample)	0.13	g / 100g	

The results for saturated, monounsaturated and polyunsaturated fatty acids in the sample use a 0.956 conversion factor for non fatty acid material in the fat.

The values above for the total monounsaturated fatty acids and total polyunsaturated fatty acids are inclusive of both cis and trans components.

Karl Larder
Section Head - Nutritional Chemistry
For and on Behalf of ALS Food and Pharmaceutical

Disclaimers:

The testing results in this certificate relate only to the samples described above.
Unless otherwise stated, all results are expressed on an as received basis.
Opinions and interpretations expressed herein are outside the scope of UKAS accreditation.
Chemistry Samples will be retained for a period of 30 calendar days from the date reported unless otherwise agreed in writing with the Laboratory.

ilac-MRA UKAS TESTING 1282

5.2 Group 2 Nutritional Analysis of Gluten Free Munroes Marinades

Food & Pharmaceutical

TEST CERTIFICATE

Medcalfe Way Bridge Street Chatteris Cambridgeshire PE16 6QZ England
Telephone: 01354 695858

Mr Thomas O'Sullivan
Advance Micro Services & Environmental
Laboratories Ltd
Carrigeen Industrial Estate
Clonmel, Tipperary
Ireland
Fax: 052 61 78133

Certificate Number: TCHC221106-1 Final
Date Reported: 27/07/2015
Date Analysis Started: 21/07/2015
Order Number: INST-044170715

Lab Ref.	Sample Details	Method Number	Test	Result	Units	Flag
CHC320260	Desc: 2058401. Sample 2 - Gluten Free. Order No: INST-044170715 Date Received: 20/07/2015 Date Tested: 20/07/2015	AM/C/1015	Moisture (Loss on Drying)	75.4	g / 100g	
		AM/C/224	Protein (Nx6.25)	3.73	g / 100g	
		AM/C/1015	Total Fat (NMR)	2.3	g / 100g	
		AM/C/803	Ash	3.1	g / 100g	
		AM/C/901	Total Carbohydrate (by difference)	15.5	g / 100g	
		AM/C/309	Total Dietary Fibre (AOAC)	2.8	g / 100g	
		AM/C/901	Available Carbohydrate (by difference)	12.7	g / 100g	
		AM/C/901	Energy	92	kcal / 100g	
		AM/C/901	Energy	386	kJ / 100g	
		AM/C/403	Total Sugars (expressed as Glucose)	12.2	g / 100g	
		AM/C/1002	Sodium (ICP-OES)	707	mg / 100g	
		AM/C/107	Saturated Fatty Acids (in sample)	0.47	g / 100g	
		AM/C/107	Monounsaturated Fatty Acids (in sample)	1.42	g / 100g	
		AM/C/107	Polyunsaturated Fatty Acids (in sample)	0.32	g / 100g	

The results for saturated, monounsaturated and polyunsaturated fatty acids in the sample use a 0.956 conversion factor for non fatty acid material in the fat.

The values above for the total monounsaturated fatty acids and total polyunsaturated fatty acids are inclusive of both cis and trans components.

Karl Larder

Section Head - Nutritional Chemistry

For and on Behalf of ALS Food and Pharmaceutical

Disclaimers:

The testing results in this certificate relate only to the samples described above.
Unless otherwise stated, all results are expressed on an as received basis.
Opinions and interpretations expressed herein are outside the scope of UKAS accreditation.
Chemistry Samples will be retained for a period of 30 calendar days from the date reported unless otherwise agreed in writing with the Laboratory.

ilac-MRA UKAS TESTING 1282

5.3 Vitamin Analysis of Original Munroes Marinades

Food & Pharmaceutical

TEST CERTIFICATE

Medcalfe Way Bridge Street Chatteris Cambridgeshire PE16 6QZ England
Telephone: 01354 695858

Mr Thomas O'Sullivan
Advance Micro Services & Environmental Laboratories Ltd
Carrigeen Industrial Estate
Clonmel, Tipperary
Ireland
Fax: 052 61 78133

Certificate Number: TCHC223268-1 Final
Date Reported: 14/08/2015
Date Analysis Started: 04/08/2015
Order Number: INST-196230715

Lab Ref.	Sample Details	Method Number	Test	Result	Units	Flag
CHC323203	Desc: 2063460. Sample. Original. Order No: INST-196230715 Date Received: 28/07/2015 Date Tested: 28/07/2015	AM/V/754	Vitamin B12 (as cyanocobalamin)	0.73	µg / 100g	
		AM/V/755	Free Folic Acid	8.9	µg / 100g	
		AM/V/710	Vitamin C (as Ascorbic Acid)	0.69	mg / 100g	
		AM/V/702	Vitamin A (Retinol)	<60.0	µg / 100g	
		AM/V/702	Vitamin E (as DL a-tocopherol acetate)	0.33	mg / 100g	

Gemma A. Parr
Section Head Vitamins and Additives
For and on Behalf of ALS Food and Pharmaceutical

Disclaimers:

The testing results in this certificate relate only to the samples described above.
Unless otherwise stated, all results are expressed on an as received basis.
Opinions and interpretations expressed herein are outside the scope of UKAS accreditation.
Chemistry Samples will be retained for a period of 30 calendar days from the date reported unless otherwise agreed in writing with the Laboratory.

ilac-MRA UKAS TESTING 1282

5.4 Mineral Analysis of Original Munroes Marinades

southern scientific services ltd.

OUR REF: RP 2015 | SHANNON ABC | 001 |REV 01 – 24.09.15| PAGE 01 | 01

ANALYSIS REPORT

CUSTOMER:	**SHANNON APPLIED BIOTECHNOLOGY CENTRE**	**SAMPLE TYPE:**	
ADDRESS:	IT Tralee, Clash, Tralee, County Kerry	**CONDITION OF SAMPLE ON RECEIPT:**	Satisfactory
		DATE SAMPLED:	-
REPORT TO:	**JOANNE PRYBORSHE**	**DATE RECEIVED:**	20 July 2015
SAMPLED BY:	-	**DATE ANALYSED:**	20 July – 17 August 2015 & 17 September 2015
SAMPLING PT:	-	**DATE REPORTED:**	17 August 2015 & 23 September 2015
ORDER NO:		**WORK NO.:**	**33204 C**

TABLE OF RESULTS

Method:	Parameter:	Unit	C15-Jul 406 Sample Original
	Chemical Analysis:		
SCP 038	Iron	mg/kg AR	10.6
SCP 038	Zinc	mg/kg AR	3.89
SCP 038	Selenium	mg/kg AR	1.01
SCP 038	Manganese	mg/kg AR	17.7
SCP 038	Copper	mg/kg AR	2.25
SCP 038	Iodine	mg/kg AR	<0.5
SCP 053	Sulphur	mg/kg AR	522
SCP 053	Phosphorus	mg/kg AR	539
SCP 053	Calcium	mg/kg AR	187
SCP 053	Sodium	mg/kg AR	7,973
SCP 053	Potassium	mg/kg AR	2704
SCP 053	Magnesium	mg/kg AR	169

Jennifer Keane
Jennifer Keane
Chemistry Laboratory Manager

- **The results relate only to the items tested.**
- **The analysis report shall not be reproduced except in full without written approval of the laboratory.**

(registered office)
4 park business centre | farranfore | county kerry | ireland | telephone +353 66 976 3588 | fax +353 66 976 3589
dunrine | killarney | county kerry | ireland | telephone +353 64 66 33922 | fax +353 64 66 39022

web site www.southernscientificireland.com | e-mail info@southernscientificireland.com

directors: K. Murphy, M. Murphy & C. Murphy
registered in ireland no 323196 | vat reg no IE 6343196 M