Consiglio Nazionale delle Ricerche

PUBBLICAZIONE AI SENSI DELL'ART. 19 DEL D.LGS. N. 33 DEL 14 MARZO 2013,
MODIFICATO DALL'ART. 18 DEL D.LGS N. 97 DEL 25 MAGGIO 2016
INTEGRATO DALL'ART.1 C. 145 DELLA LEGGE 27 DICEMBRE 2019 N. 160

BANDO N. 368.32 RIC
AREA STRATEGICA PRODUZIONI ALIMENTARI E ALIMENTAZIONE

CONCORSO PUBBLICO, PER TITOLI ED ESAMI, PER L'ASSUNZIONE CON CONTRATTO DI LAVORO A TEMPO PIENO E INDETERMINATO DI N. 5 UNITÀ DI PERSONALE PROFILO RICERCATORE - III LIVELLO PROFESSIONALE - PRESSO STRUTTURE DEL CONSIGLIO NAZIONALE DELLE RICERCHE

TRACCE DELLE PROVE D'ESAME ESTRATTE A SORTE

PER LA PRIMA PROVA VIENE ESTRATTA LA SERIE DI TRACCE CONTRADDISTINTE CON LA LETTERA "C"

PER LA SECONDA PROVA VIENE ESTRATTA LA SERIE DI TRACCE CONTRADDISTINTE CON LA LETTERA "D"

Consiglio Nazionale delle Ricerche

BANDO N. 368.32 RIC - Area strategica Produzioni alimentari e Alimentazione
Concorso pubblico per titoli ed esami per l'assunzione con contratto di lavoro a tempo pieno e indeterminato di n.5 unità di personale con profilo di Ricercatore – III livello professionale - presso strutture del Consiglio Nazionale delle Ricerche

PROVA SCRITTA A CARATTERE GENERALE DEL 27 MAGGIO 2019 – ORE 14.00

Il candidato, dopo aver scelto un elaborato tra i tre allegati, ciascuno estratto da un articolo pubblicato su rivista, dovrà definire un adeguato titolo e il riassunto in inglese (minimo 200, massimo 300 parole) attendendosi strettamente al contenuto dell'estratto.

1. Introduction

Type 2 diabetes mellitus (T2DM) is one of the most prevalent chronic diseases around the world, with an increase in its prevalence due to the increase in its risk factors such as obesity and physical inactivity. According to the National Health and Nutrition Survey, in 2012, in Mexico there were 6.4 million people with T2DM, which means an increase in prevalence from 7% in 2006 to 9.2% in 2012. T2DM is characterized by impaired pancreatic β-cell function that causes impaired insulin secretion and insulin resistance (IR) mainly in liver, muscle, and adipose tissue.

It is well recognized that the actively secreted products of adipose tissue known as adipokines can modulate different functions; adipokines play a pivotal role in the regulation of whole-body metabolism, as well as in inflammatory and immune responses, and are considered a link between obesity and the development of T2DM. Adiponectin regulates glucose and lipid metabolism through the reduction of fat storage (lipogenesis) and the promotion of fat utilization (fatty acid oxidation). Leptin is a cytokine-like molecule secreted by adipose tissue which regulates adipose tissue mass and body weight by inhibiting food intake and stimulating energy expenditure, thus maintaining energy homeostasis. Leptin correlates directly with adipose tissue mass. Obesity and T2DM are associated with increased plasma leptin levels, which fail to correct hyperglycemia in these patients because of the presence of leptin resistance, and these elevated plasma leptin levels are associated with IR, independent of obesity and insulin sensitivity.

In humans, resistin is mainly secreted by macrophages and monocytes and by organs such as spleen and bone marrow [11]. Since its discovery, resistin has been related to obesity and IR in many animal experiments, but the application of these findings to human studies has been difficult to determine. However, studies in humans have shown that serum resistin levels are higher in obese patients with T2DM compared with non-diabetic obese and that mRNA levels of resistin are higher in female patients with T2DM compared to healthy women. Accumulating evidence suggests that *n*-3 PUFAs from fish oil may counteract the adipokine dysregulation that occurs in obesity and its related diseases like T2DM, but it is not well established if the consumption of *n*-3 PUFAs affects circulating adiponectin, resistin, and leptin in humans; the results are inconclusive. For these reasons, we undertook a pilot study with the aim of investigating the effect of a six-month supplementation trial with *n*-3 PUFAs on adiponectin, resistin, leptin, and the lipid profile in adults with T2DM from Toluca, Mexico.

2. Materials and Methods

This study was a randomized, single-blind and placebo-controlled pilot study conducted in eight Urban Public Health Centers (UPHC). 54 patients with T2DM received 520 mg of DHA + EPA-enriched fish-oil (FOG) or a placebo (PG) daily. The supplementation period started after basal evaluation. Patients took two softgels per day of the assigned supplement for 24 weeks. Each 1.4 g *n*-3 PUFAs softgel contained a combination of 160 mg of eicosapentaenoic acid (EPA) with 100 mg of docosahexaenoic acid (DHA) from fish oil, so the total daily oral dose was 520 mg of *n*-3 PUFAs (320 mg of EPA and 200 mg of DHA, 2 g of total fat, 1.2 mg of vitamin E, gelatin and glycerine) during the six months of intervention.

HOMA-IR = (fasting insulin (μU/mL)) × (fasting glucose (mg/dL))/405

3. Results

Age, gender distribution, and diabetes duration were similar between groups without significant differences. In anthropometric measurements, only the FOG showed a significant decrease in waist circumference after supplementation ($p = 0.001$). At baseline, there was only a significant difference between groups in serum resistin levels ($p = 0.006$). Glucose serum levels only showed a significant decrease in FOG, and there were significant reductions in glycosylated hemoglobin, leptin, and leptin/adiponectin ratio in both groups (FOG and PG, respectively). Adiponectin did not show significant changes. Contrary to expected, resistin showed a significant increase in both groups. With respect to IR, there were significant increases in insulin serum levels and in HOMA-IR. The FOG showed a significant increase in total omega-3 fatty acids, particularly in EPA and DHA; *n*-6 to *n*-3 ratio decreased from 16:1 to 10:1.

4. Discussion

In a crossover model in which 16 T2DM patients were assigned to one of two consecutive 3.5-week periods of diabetic diets (foods rich in *n*-6 or *n*-3 PUFAs), the authors found a slight but significant reduction in body weight and BMI in both dietary periods. This suggests that *n*-3 PUFAs from food are more effective in controlling body weight than *n*-3 PUFAs from supplements. In our study, supplementation with *n*-3 PUFAs for 24 weeks only helped to

a significant positive effect of *n*-3 PUFA supplementation on glycemic control (glucose and glycosylated hemoglobin), but more studies are needed to test this hypothesis because most of the beneficial effects have been shown in epidemiologic studies based on a habitual fish diet consumed for years. As expected, there were significant reductions in leptin serum levels and hence in leptin/adiponectin ratio, which has been demonstrated to be related to obesity and T2DM. Contrary to expectation, adiponectin did not increase significantly, while resistin increased significantly. As the changes in adipokine profile were similar in both groups, we cannot affirm that higher doses of *n*-3 PUFAs would have led to a decrease in leptin and leptin/adiponectin ratio because there was no lead tendency toward lower leptin concentrations in subjects with the *n*-3 PUFA supplement than in subjects with the placebo. Leptin correlates directly with adipose tissue mass; however, we did not observe a significant reduction in body weight, BMI, and body fat, despite the reduction in serum leptin levels. When we used a bivariate correlation analysis, we found that serum leptin levels showed a positive correlation with BMI and body fat percentage. Leptin/adiponectin ratio showed a significant reduction in both groups related to the significant decrease in leptin levels. Leptin/adiponectin ratio was a useful measure of IR in non-diabetic white adults, but we did not observe a positive correlation between HOMA-IR and leptin serum levels; despite the reduction in this ratio, there was no reduction in HOMA-IR. However, these findings suggest the use of the leptin/adiponectin ratio as a useful tool to detect IR. On the other hand, resistin has been linked to obesity and IR since its discovery, but our results are not consistent with these findings because we found a weak negative correlation between resistin levels and HOMA-IR. Our data show an overall improvement in the lipid profile through the atherogenic index in the *n*-3 PUFA supplemented group, with an overall deterioration in the placebo group. Derosa et al. found an increase in HDL-cholesterol and a decrease in triacylglycerides after 18 months of supplementation with *n*-3 PUFA, in patients with impaired glucose metabolism.

With respect to diet analysis, our results are in line with those of a previous study of this group that showed that a Mexican population with T2DM had a very low intake of *n*-3 PUFAs and a high consumption of lipids, particularly saturated fatty acids. It is well known that the quality of dietary fat is a key determinant of IR and that saturated and trans fatty acids decrease insulin secretion and worsen insulin sensitivity; a factor which could explain the higher increase in HOMA-IR in FOG because of their higher intake of saturated fatty acids. Because of the very low intake of *n*-3 PUFAs in both our groups, we may not have been able to observe significant changes in adiponectin levels. *In vitro* human adipocyte studies found that EPA and DHA (100 μM) treatment for 48 h, increases adiponectin secretion, and that only EPA led to higher cellular adiponectin being introduced into the adipocytes, suggesting that the regulation of adiponectin by *n*-3 PUFAs is dose- and time-dependent. However, another study of supplementation with *n*-3 PUFAs, showed that for *n*-3 PUFAs, the duration of treatment is not associated with the effects observed and that the dosage could be more important, which in this case would suggest that in our study the *n*-3 PUFA dose we used can be considered low.

Although we found a beneficial effect of EPA + DHA supplementation on waist circumference, glucose, glycosylated hemoglobin, leptin, and leptin/adiponectin ratio in this population, these beneficial effects may not have been due to the supplement alone because we observed similar results in some of these parameters between patients who took the *n*-3 PUFA supplement and those that took the placebo. Similarly, there were significant increases in resistin, serum insulin, and HOMA-IR in both groups. It is possible that some of the beneficial effects observed in both groups were due to metformin and that the combined use of *n*-3 PUFA and metformin gave rise to better outcome measures. However, the significant increase in monounsaturated fatty acids and protein in the placebo group may also have contributed to the decrease in glycosylated hemoglobin, leptin, and leptin/adiponectin ratio and the increase in resistin, insulin, and HOMA-IR in this group. Interestingly, Schwingshack et al., 2001, and McAllan et al., 2014, have reported that macronutrient quality and composition, i.e. monounsaturated fat and protein can affect some of the parameters we have measured (leptin and glycosylated hemoglobin) and in the same direction we observed for the placebo group.

SERIE E – TRACCIA 2

1. Introduction

Green tea as the second most traditional style of tea products represents 20-22% of world tea production which is popularly consumed in Japan and China. The green tea from specific origins is strongly influencing the consumer's purchasing decision and costing much higher price compared to the other green teas from nongeographic regions. The famous teas of China are usually protected Geographical Indication (GI) products, such as Dongting Biluochun, Xihu longjing, etc. which is named according to their geographic origins. Green teas produced in specific geographical regions have found favorable acceptance among customers, who are willing to pay more for a product perceived as genuine, typical, and of higher quality. However, some unscrupulous producers fraudulently label their product from GI areas to take advantage of the higher price. In this respect, it is important to develop methods enabling the recognition of the geographic origin of green teas in order to protect the benefits of consumers and producers. Among the various analytical tools, metal element concentrations determined by inductively coupled plasma mass spectrometry (ICP-MS) is widely used and considered to be an effective and reliable tool to identify the geographic origin of teas. For example, twelve trace element concentrations of tea samples combined with chemometric procedures have been reported to distinguish African teas from Asian teas. Herrador et al. applied eight metal element concentrations to determine teas as green or black and oolong. Similarly, twenty-five elements were analyzed to classify teas from Asian, African, China, Indian and SriLankan. Pilgrim et al. achieved identification of the provinces of tea from different growing regions of Asia (China, India, Sri Lanka and Taiwan) by a combination of stable isotope (C, N & H) and twenty trace metal elements. However, most studies have focused on classifying different categories of tea (green, black, oolong) and teas from various countries. It is still a great challenge to identify the same category of tea from close regions. Moreover, trace metals other than rare earth elements were used for classification the origins of teas during the former studies. Herein, we take Dongting Biluochun (DTBLC) as an example of green teas to identify its geographic origin based on 37 metal elements including trace metals and rare earth elements (Rees). The method of determining the geographic origin of DTBLC was also used for other green teas from close regions. DTBLC as one of the top ten famous teas in China is well known for its floral aroma, fruity taste, delicate appearance, showy white hairs and especially limited yields. Authentic DTBLC had been granted the status of imperial tea since the Qing dynasty. The East (Dongting) Mountain and West (Dongting) Mountain areas around the Tai Lake in Jiangsu Province of China are the main production regions of DTBLC. The Tai Lake provides a unique growing location for tea due to the humid air, acidic soil and climate and results in the DTBLC with a characteristic high quality and high price. However, the Biluochun (BLC) teas from other regions are processed by similar procedures, which make them difficult to distinguish by their appearances. In order to protect the interests of consumers and legitimate producers, it is important to develop a reliable and accurate method for geographical tracing of authentic DTBLC. Herein, we developed a facile and reliable method to distinguish the geographical origin of DTBLC.

2. Materials and Methods (omissis)

3. Results and discussion

A large number of samples were employed in the analysis to ensure that there would be adequate data to create an exact model of the BLC profile from each region. 56 BLC teas were chosen as representative samples from 3 different regions (East Mountain, West Mountain and Non-GI regions). The 37 elements concentrations (Li, Be, Cr, Co, Ni, Ga, As, Sc, Se, Mo, Cd, Sb, Cs, Ba, Pb, Mn, Cu, Zn, Sr, Fe, Al, Mg, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu) of 56 BLC samples from different regions are analyzed by ICP-MS. As compared by the ANOVA test, there is no significant difference in the mean concentration of Ni, Se, Sb, Sr, Mg, Y, Er, Yb and Lu among the East Mountain, West Mountain and non-GI samples which are rejected for further statistical analysis. 28 elements in BLC samples were significantly different among the regions. The Duncan's multiple comparison was performed when there were statistically significant differences among the element concentration in ANOVA. The results indicate that the concentrations of metal elements from DTBLC region are mostly lower than BLC from non-GI region. Non-GI samples have the higher content of Li, Be, Cr, Ga, As, Cd, Cs, Pb, Cu, La, Ce, Nd, Pr, Sm, Eu, Gd, Tb and Tm compared with DTBLC samples. DTBLC (including East Mountain and West Mountain) has a higher content of Zn compared to Non- GI samples. Furthermore, East Mountain samples could be clearly separated from West Mountain and Non-GI samples based on the highest content of Sc and Fe and the lowest content of Al. Meanwhile, West Mountain sample could be distinguished from East Mountain and Non-GI samples based on the highest content of Ba. The contents of Mn and Co could be used to discriminate the East Mountain, West Mountain and Non-GI samples. The large standard deviations of some elements reflected the great variability among samples from the same region. The significant difference in element concentrations of BLC samples makes it possible to distinguish them from different regions and provides reliable results for further statistical analysis.

In order to evaluate the difference of DTBLC and non-GI BLC, 28 elements with significant differences was processed by PCA. The first principal component (PC1) describes the maximum possible variation and the second PC accounts for the second most and so on. The first five factors explained 76.5% of the total variability. The content of Li, As, Ce, Nb, Pb and Cu had the highest weight on the first PC (explaining 26.5% of the variability). Fig. 2 shows the 3D scatter plot of the scores of PC1 versus PC2 versus PC3, together explaining 64.4% of the total variability. It is shown that BLC samples from different regions cluster into three groups, East Mountain group, West Mountain group and Non-GI group. The BLC from East Mountain are closer to West Mountain samples compared to Non-GI group. The distribution of East Mountain samples is a little overlapped with West Mountain samples due to their close geographical location. However, the DTBLC samples can be separated from Non-GI [illegible] with a solid line circle). The clustering results based on PCA gave a satisfactory result to distinguish DTBLC

To better visualize the relative distribution of the BLC according to their geographical origin, CA was performed using 28 elements with significant differences. The samples were grouped into clusters in terms of their nearness or similarity. The measurement of the similarity was based on the squared Eucludean distance. The smallest distance indicates the highest degree of relationship, therefore, those objects are considered to belong to same group. All samples from different regions were separated into four clusters based on the dendrogram cut at a distance of 7.5. The first cluster was composed of West Mountain samples (n = 4). The second cluster was composed of samples from Dongting Mountain (including East Mountain (n = 19) and West Mountain (n = 8)). The third cluster was composed of only two East Mountain samples. The Non-GI samples entirely dominated the fourth clusters (n = 23). To observe the relationships of BLC from the three regions, the greater closeness of a cluster reflects a higher degree of sample association. The adjacent location of cluster I and cluster II implies that the quality of DTBLC from West Mountain and East Mountain are similar. In the same way, there is two DTBLC samples from East Mountain are similar to Non-GI BLC samples. The CA shows that the Non-GI BLC samples formed an independent group from DTBLC samples (including East and West Mountain). Some samples from East Mountain were clustered together with samples from West Mountain due to their close geographical location. The cluster analysis results implied that multi-element information could be suitably utilized to classify DTBLC from Non-GI BLC regions which is consistent with the results from PCA. Determination the geographical origin of DTBLC from East Mountain and West Mountain are not achieved based on this method.

For achieving better classification and identification of the BLC samples from different regions, LDA was performed on the basis of the content of 28 elements with significant differences ($p < 0.05$). The stepwise discriminant procedure was carried out to extract best discriminant variable separating BLC teas from different origins, which enters or removes variables by analyzing their effects on the discrimination of the groups based on the Wilks' lambda criterion. As a result, four elements (Fe, Mn, Sc and As) were selected and two discriminant functions were constructed on the basis of Wilks' lambda values. The two functions explained the 100% of the variance (Function 1 explained 85.0% of the total variance, and function 2 explained 15.0%). Discriminant functions were shown as follows,

Function 1 = 0.0967Sc – 10.729As + 0.004Mn + 0.013Fe – 8.189

Function 2 = 0.0257Sc + 4.982As - 0.004Mn + 0.007Fe + 0.297

The statistical significance of each discriminant function was evaluated on the basis of the Wilks' Lambda factor. This parameter ranges from 1.0 (no discriminatory power) to 0.0 (perfect discriminatory power). Wilk's Lambda value were 0.022 and 0.306 for function 1 and 2, respectively ($p = 0.000$) which implied that there is a significant difference among different regions. The separation of BLC from East Mountain, West Mountain and Non-GI region was checked by plotting the two functions scores (Fig. 4). It is clearly shown that BLC from different regions was well distinguished from each other. The DTBLC is successfully classified from Non-GI BLC. Moreover, the DTBLC from different protection areas was also well distinguished in which only one sample from East Mountain was incorrectly assigned as West Mountain. The results confirmed that metal element contents provided the useful information for BLC classification. In order to investigate the contribution of rare earth elements to the recognition ability of BLC samples from those three groups. The LDA classification results based on trace metal elements and trace metal plus rare earth elements are summarized in Table 2. The discriminant functions achieved recognition ability (% of the objects belonging to the training set correctly classified) of 96.4% based on the trace metals, in which 2 samples from East Mountain was incorrectly assigned as West Mountain. After rare earth elements added into the data set, the recognition ability was increased to 98.2% of the original data set. Furthermore, the DTBLC and Non- GI BLC were 100% correctly classified. To evaluate the predictive capacity, the generated model was then validated by the leave-one out cross-validation method. The predictive ability of this model (% of the objects belonging to the testing set correctly classified using the developed model) was 96.4%, indicating a satisfactory performance of this model for the classification of BLC samples from different origins. Fisher's linear discrimination functions for East Mountain, West Mountain and Non-GI groups were as follows:

East Mountain group = 1.491Sc + 0.174Fe + 0.057Mn - 57.28As - 91.104

West Mountain group = 1.043Sc + 0.099Fe + 0.057Mn - 35.265As - 57.594

NonGI group = 0.735Sc + 0.068Fe + 0.029Mn + 26.32As - 35.807

A high percentage of correct classification represents the stability and the strong relationship between the extracted profiles and the origins, while a high predictive percentage of the validated set indicates the high ability to classify the origin of an unknown sample based on the relative profiles of five elements. It is demonstrated that BLC from different regions can be separated based on LDA analysis. Fig. 5 shows the medians and ranges of four selected elements (Fe, Sc, Mn and As) pointed out by LDA. As we can see from Fig. 5 (aed), The DTBLC from East Mountain with the highest concentration of Fe is significantly distinguished from the other two regions. Sc allows differentiating DTBLC from Non-GI regions. In addition, Mn concentration of West Mountain group is the highest in the three regions. Moreover, the highest concentration of As allow discriminating BLC from the rest Non-GI region samples.

Stepwise discriminant analysis was employed to classify the other green teas from Zhejiang province, China based on 37 metal elements analysis. It is clearly shown that the green tea samples can be separated into three groups according to their geographic origin. High accuracy rates were achieved for the training set (100%) and cross-validation set (96.4%). These results indicated that the green teas from different origins can be classified based on the combination of metal elements analysis and statistical analysis. The method of determining the geographic origin of DTBLC was also suitable for other green teas from close regions.

SERIE E – TRACCIA 3

1. Introduction

Nutraceuticals and functional foods have gained consumer demand because of the increased awareness of food and health relationship. The therapeutic potential of foods is because of the presence of some components (polyphenols, dietary fibers, bioactive peptides, etc.) that are either present as such or are released during storage and processing. These special components may also provide calories apart from offering health-promoting effects. Dietary proteins may be considered one among them, as they are able to release bioactive peptides along with the calories and amino acids. Bioactive peptides (BPs) are protein fractions that impart beneficial effect on human health and conditions. Due to high target specificity, wide spectrum of action, low toxicity, small size, and high structural diversity of food-derived peptides, these are thus considered as suitable therapeutic agents. BPs have demonstrated various health benefitting properties including antihypertensive, anti-proliferative, anti-microbial, antioxidant, immuno-modulating, mineral binding, and others. Wide ranges of food materials have been found as a source of BPs including milk, egg, cheese, corn, soy, etc4. BPs are generated through microbial fermentation, gastric digestion, in-vitro protein hydrolyzation using different proteases (pepsin, chymotrypsin, bromelain, papain, etc.). Among these processes, microbial fermentation is the most suitable method for the production of bioactive peptides in dairy foods. Because milk proteins produce potent BPs by the action of proteases released by the cultures used for the production of fermented milk products. Probiotics that mainly belongs to Lactic acid bacteria (LAB) are mostly used for production of fermented milk products. And various LABs have been reported to release bioactive peptides with profound physiological effects during milk fermentation. Fermented milk products including cheese are considered as the most popular foods for probiotic consumption. Kalari cheese is one of the most famous traditional Himalayan cheese. It is a fresh cheese made from buffalo buttermilk and fermentation is carried out with natural flora present in the milk. The cheese has great importance in the region because of its socio-economic and nutritional value. Each artisanal cheese has its unique characteristics due to the technology employed, the source of milk used, and microflora that release specific volatiles and peptides by enzymatic hydrolysis. Inverse relationship between the development of chronic diseases and dairy foods consumption is reported by various epidemiological studies. And valuating the nutraceutical properties of unexplored dairy products may pave the ways for managing the lifestyle diseases like cancers, diabetes, and immune disorders. To the best of our knowledge no studies are carried out to investigate in-vitro the health promoting potential of kalari cheese (immunomodulatory, anticancer, antidiabetic, and antimicrobial activities) prepared by addition of probiotic LAB strains. The present study highlighted the impact of added probiotics on health benefitting properties of under-explored kalari cheese. Thus the objective of the study was aimed to assess the anticancer, immuno-modulatory and anti-diabetic potential of water-soluble extracts of kalari cheese added with probiotics [Lactobacillus plantarum (NCDC 012), Lactobacillus casei (NCDC 297), and Lactobacillus brevis (NCDC 021)] and influence of added probiotic strains on these activities. In our previous study, the viability of these probiotic strains during storage was evaluated and the results demonstrated that kalari cheese is effective in maintaining the desired viability for conferring health benefits during refrigerated storage. Further, adding probiotics did not influence the physicochemical and sensory attributes of the cheese.

2. Materials and methods (omissis)

3. Results

Microstructure. Effect of different probiotic strains on microstructure of kalari cheese was determined by using scanning electron microscopy. Micrographs showed no significant difference between matrices of CLC, CLP, CLB, & CTR. The SEM micrographs of all the samples depicted uniformly distributed, compact and interconnected protein matrices. Peculiar microstructure of kalari cheese i.e., interwoven protein fibers with hollow channels possessing other soluble fractions including microorganisms was observed to remain unaffected with addition of different probiotic strains. The non-prominent fat globules in all the samples could be attributed to the composition of the cheese (low fat content) and are similar to those of low fat cheese where cheese matrix is more compact with proteins and low number of fat globules. **Attenuated total reflectance-fourier transform infrared (ATR-FTIR) spectroscopy.** ATR-FTIR was used to determine the effect of probiotic strains on molecular structure of crude peptides extracted from kalari cheese inoculated with different probiotic strains. The repeat units of polypeptide and protein chains give nine characteristic IR absorption bands, namely, amide A, B, I, II, III, IV, V, VI and VII. Absorption region between 3700 and 2800 cm− 1 corresponds to amide A and B group with bands at near 3300 cm− 1 are assigned to N-H stretching of amide A group. A distinctive peak of this region at 3264 cm− 1 for CTR was affected by probiotic strain addition as the peak was shifted to around 3270 cm− 1. The shifting of bands indicated the effect of microbial proteases on peptide chains. The region between 3000 and 2800 cm− 1 was observed in samples extracted from cheese with probiotics. Emergence of this region with a peak at around 2980 cm− 1 are attributed to the alkyl group exposure of peptide chains due to protein hydrolysis. FTIR spectrum of CTR extract shows a sharp amide II and I region between 1700 and 1500 cm− 1. The peaks at 1636\ cm− 1 seen in the spectra could have resulted from stretch vibrations of C=O group of peptide linkages. However, the spectra of extracted peptides from samples with added probiotics had shown new band regions around 1400-1200 cm−1. These regions correspond to the amide III group vibrations of side chain amino acids due to the protein hydrolysis. The changes in the absorption bands, especially the mergence of amide B and III of the samples with added probiotics established a link between the probiotics and protein structural conformations. And structural conformation of peptides, determine their bioactivity that were further validated through different assays like anti-microbial, anti-diabetic, immunomodulatory, and anti-proliferative. **Differential scanning calorimetry.** The thermal properties are presented for WSE of CLC, CLP, CLB, &CTR. The DSC curves show distinct variation in onset temperature (Tonset), peak temperature (Tpeak) and endset temperature (TEndset) between CTR WSE and WSE of CLC, CLP, & CLB. For all the samples obtained from added probiotic strains showed lower Tonset, Tpeak and TEndset compared to WSE of sample obtained from cheese without added probiotics. The lowering of onset temperature (Tonset), peak temperature (Tpeak) and endset temperature (TEndset) indicates degradation of protein into low molecular weight peptides. Protein degradation is an endothermic process and less the energy needed to denature a protein smaller is the molecule. Hence, the decrease in the Tonset, Tpeak and TEndset, indicates the degradation of proteins due to addition of probiotics having proteolytic activity. The indication of protein hydrolysis

to the production of bioactive peptides by proteclytic enzymes of added probiotics. **Antimicrobial activity.** The antimicrobial activity of the WSE has been assessed by, determining their potential to, inhibit the growth of selective pathogenic microorganisms (Escherchia coli, Pseudomonas perfringens, Micrococcus luteus, Enterococci faecalis, Staphylococcus aureus, Proteus vulgaris and Salmonella typhii) and the results recorded are presented. The results show that the WSE of cheese samples added with different probiotic strains were able to inhibit the growth of all tested pathogens (both gram positive and gram negative) and the inhibition increased in dose dependent pattern (data not shown). This could be evident from the inhibition zone diameters (IZD) given. IZD of CTR was significantly lower than CLC, CLP, & CLB but dose-dependent increase in IZD was shown by all the samples including CTR. Non-significant difference in IZD of CLC, CLP or CLB was observed at each concentration. This indicates that the addition of probiotic strains improved the bioactivity of the kalari cheese. The antimicrobial activity of the WSE could be attributed to the generation of peptides by proteolytic enzymes secreted by probiotics during fermentation. Our results are in accordance with previous data where have reported that addition of probiotic cultures to cheddar cheese enhance the bioactivity of its WSE. Also milk-derived peptides have been reported to exhibit antimicrobial activity against both gram positive and gram-negative pathogenic bacteria (Escherichia coli, Aeromonas hydrophila, Salmonella typhi, Bacillus cereus, Salmonella typhimurium, S. enteritidis, Staphylococcus aureus). Similarly many Italian cheese watersoluble extracts have shown antimicrobial activity against various pathogenic bacteria including E. coli, S. aureus Listeria innocua, and Bacillus megaterium. **Anti-proliferative activity**. The WSE of the cheese samples (CTR, CLC, CLP, & CLB) were evaluated for their growth inhibition effects on different cancer cell lines MCF-7, HCT-116, IMR-32 & HEK-T at concentration of 0, 10, 20, 30 and 50 mg/mL. The results indicated that the WSE of CLC, CLP, & CLB had profound % inhibition activity on MCF-7, HCT- 116, IMR-32 & HEK-T. Further, dose-dependent increase in the inhibition activity was shown by all the samples including CTR. However, the inhibition activity of CTR was significantly lower than CLC, CLP, & CLB on the entire tested cancer cell lines at all concentrations. However non-significant difference in proliferation inhibition of WSE added with probiotics was observed. Similar results were where non-significant difference in proliferation inhibition of camel milk fermented with different Lactobacillus spp. strains. The results validate the anti-proliferative activity of kalari cheese with added probiotics against different cancer cell lines. The significant increase in the anti-proliferation of WSE of CLC, CLP and CLB could be owed to the generation of the bioactive peptides during fermentation by added probiotics. The apoptotic mechanism where cascade of caspases cleavage the motifs containing aspartic acid may be involved which has lead to the death of the cancer cells used in the anti proliferative activity assay. Another possible mechanism could be the competition between the peptides and growth factors for cancer on receptors of cancer cell membrane. The peptides generated might have induced apoptosis because of specific cell toxicity against cancer cell lines. This hypothesis may also be considered for the results obtained for anti-proliferation against different cancer cell lines used. The bioactivity of the probiotic cheeses was also reported where the Lactobacillus casei containing prato cheese exhibited antioxidant and anti hypertensive activity and was attributed to generation of ACE-inhibitory and antioxidant peptides by proteolytic enzymes of added probiotic strain. 4.6. Immuno-modulatory activity (NO determination) shows the NO production in medium containing monocytes and WSE of kalari cheese. The results show that the absorbance (directly proportional to NO production) of WSE of CTR was low at all the concentrations (10, 20, 30 & 50 mg/ml) whereas NO level in the supernatant of cells incubated with WSE of CLC, CLP and CLB was significantly higher than CTR at all the concentrations. There was dose dependent increase observed in the NO production in all the samples including CTR. The results suggest that fermentation using different probiotics affect the immunomodulatory activity of kalari cheese. The increase in immuno-modulatory activity as evident from NO production could be related to the production of peptides produced during protein hydrolysis by proteolytic enzymes of added probiotics. Several cytochemical studies have shown that casein and whey proteins are able to generate immunomodulatory peptides using one of the methods viz. in-vivo digestion, fermentation and in vitro hydrolysis. These immuno-modulatory activities are associated with stimulation and growth of lymphocytes, macrophages, cytokine regulation, and antibody production. **α-glucosidase and α-amylase inhibition activity.** The ability of WSE of kalari cheese added with different probiotics to inhibit α –glucosidase and α-amylase in-vitro was evaluated. The WSE of probiotic added cheese was found to have higher α-glucosidase and α-amylase inhibition activity when compared to the cheese without added probiotics at all concentrations. However each sample showed dose dependent increase in % inhibition of α–glucosidase and α-amylase. The inhibitory activity of WSE of cheese with added probiotics against α–glucosidase and α-amylase could be because of the presence of higher amount of bioactive peptides generated during fermentation. The inhibition of α-glucosidase and α-amylase enzyme activity could be attributed to the bioactive peptides generated by the proteolytic enzymes of probiotics used. Different studies have demonstrated profound inhibitory potential of foodderived bioactive peptides against α–glucosidase and α-amylase because of their specificity of action and affinity towards molecular target. The possible mechanism of action by which peptides inhibit the enzymes is that they interact via hydrophobic bonds at active site of the enzyme, however the exact mechanism involved for their inhibitory activity is still unknown. The α-glucosidase and α-amylase inhibitory potential of peptides was owed to the hydrophobic amino acids as confirmed by Ren et al. **ABTS radical scavenging activity**. The radical scavenging activity of the WSE of kalari cheese without added probiotics was significantly ($p < 0.05$) lower than WSE of kalari cheese added with probiotics. Dose dependent increase in scavenging activity was observed in all the tested samples. Scavenging ability of CTR ranged between 11 and 30% at 10–50 mg/mL of WSE. Foods particularly fermented dairy products contain bioactive components that play a vital role in alleviating the effect of reactive oxygen species produced by oxidative stress cells. The bioactive constituents particularly peptides derived by protein hydrolysis have the potential to neutralize free radicals by donating electrons to free radicals. Antioxidant activity of peptides depends on the presence and position of hydrophobic amino acid residues in the peptide chain. Anti-oxidative peptides prevent essential fatty acids from peroxidation.

Consiglio Nazionale delle Ricerche

BANDO N. 368.32 RIC - Area strategica Produzioni alimentari e Alimentazione
Concorso pubblico per titoli ed esami per l'assunzione con contratto di lavoro a tempo pieno e indeterminato di n.5 unità di personale con profilo di Ricercatore – III livello professionale - presso strutture del Consiglio Nazionale delle Ricerche

PROVA SCRITTA A CARATTERE GENERALE DEL 27 MAGGIO 2019 – ORE 14.00

Il candidato, dopo aver scelto un elaborato tra i tre allegati, ciascuno estratto da un articolo pubblicato su rivista, dovrà definire un adeguato titolo e il riassunto in inglese (minimo 200, massimo 300 parole) attendendosi strettamente al contenuto dell'estratto.

SERIE D – TRACCIA 1

1. Introduction

Major problem concerning the food industry is microbial spoilage of food and severe economic losses are incurred as result of microbial spoilage and/or contamination of food items with pathogens. Quorum sensing (QS), a bacterial cell communication system is often associated with the bacterial spoilage of food products. Considering the importance of QS in food microbial ecology, development of novel food preservatives, packing materials that can specifically block QS and prevent losses due to spoilage of food is the need of the hour. Quorum sensing or cell-to-cell communication between bacteria commonly associated with contamination of food takes place by the production of signaling molecules called autoinducers and this bacterial cross talk can be intra as well inter species specific. Quorum sensing is known to regulate several proteolytic, lipolytic, chitinolytic, and pectinolytic activities associated with the deterioration of foods. Moreover, several types of signaling molecules have been detected in different spoiled food products. There is an urgent need to understand the role of quorum-sensing signaling molecules involved in food spoilage and develop novel, safe QS inhibitors that can interfere with bacterial signaling system and prevent food spoilage and biofilm formation by food-related bacteria. Cell to cell communication (QS) occurs across both Gram-positive as well as Gram-negative bacteria. Three major types of autoinducers have been recognized: acyl-homoserine lactones (AHLs), autoinducing peptides (AIPs) and autoinducer-2 (AI-2s) molecules5. These signal molecules regulate the production of various functions like pectinase, protease, siderophore-mediated iron chelation, characteristics associated with food spoilage. AHL based QS regulates the production of violacein pigment in Chromobacterium violaceum, virulence in Pseudomonas aeruginosa, flagellar motility in Listeria monocytogenes, bioluminescence in Vibrio harveyi and V. fischeri, sporulation, cell differentiation and community organization which lead to the development of the mature biofilms. Numerous studies have linked QS to biofilm formation in food-related bacteria. QS plays a key role in all the stages of biofilm formation including the initial attachment of the bacteria to the maturation of the biofilm. Biofilms formed on food surfaces are a major problem area as they act as carriers of bacterial contamination leading to spoilage of food and health hazards. Eradication of biofilms from food contact surfaces and other equipments is difficult and has attracted the attention of the scientific community worldwide. Compounds inhibiting or interfering with bacterial QS and biofilm are gaining importance as a novel class of next-generation food preservatives as well as antimicrobial agents. QS inhibitors target the virulence mechanism of the bacteria without inhibiting its growth. Hence, reducing the chances of development of resistance as no selective pressure is exerted on the pathogen. Potential of nanotechnology has revolutionized the food packaging industry and safe packaging material with improved properties have developed. Further, integration of antimicrobial agents, antioxidants, and nanosensors for monitoring the quality of food are expected to provide advanced packaging solutions. Nanoparticles (NPs) of metals and their oxides are being exploited by the industrial sector and several medical; pharmaceutical applications are well known. Synthesis of NPs has been reported using various chemical and physical methods but recently biological synthesis has gained importance because of the rapidity, safety, stability, and economical attributes associated with the method. This biogenic green synthesis approach involves the biomolecules such as proteins, amino acids, enzymes, vitamins, alkaloids, phenolics, saponins, tannins, and terpinoids, present in plant extracts, for reduction and stabilization of metal ions. This has prompted us to use Nigella sativa (NS) plant extract for synthesis of zinc oxide nanoparticles (ZnONPs). Zinc oxide has been chosen because of its wide spectrum applications in cosmetics, paints, plastic and rubber manufacturing, pharmaceutical products, diagnostics and microelectronics. The ZnONPs have also been used in heavy metal removal from water, and in dental applications. They have also been shown to exhibit strong protein adsorption properties, which can be used to modulate cytotoxicity, metabolism and other cellular responses. Also, due to its low toxicity, ZnO has been listed as "Generally Recognized as Safe" (GRAS) by the US Food and Drug Administration. Therefore, N. sativaseed extract has been used to synthesize NS-ZnNPs, by reduction of ZnNO3 without involving any supplementary chemicals. These seeds are used as spice, food additive and as a preservative. It exhibits wide pharmacological properties such as diuretic, antihypertensive, antidiabetic, anticancer and anti-inflammatory properties. Previously, extract of this plant has been used for synthesis of silver NPs and gold NPs.

2. Materials and methods (omissis)

3. Results and discussion

Nigella sativa seed extract was used for generating green zinc nanoparticles. X-ray diffraction (XRD) measurements were performed to determine the crystalline phase of the samples. The XRD patterns of ZnO sample was indexed using POWDER-X software as the ZnO wurtzite structure and well matched with the standard data. It can be clearly seen that it showed a single phase nature with hexagonal wurtzite structure. All diffraction peaks of the product show stronger peak intensities, indicating that the obtained ZnO nanoparticles have high crystallinity. The TEM image of ZnO nanoparticles are homogeneous and agglomerated with a particle size ~24 nm. The High-resolution transmission electron microscopy (HRTEM) image shows clear lattice fringes indicating the highly crystalline ZnO wurtzite structure

absorption spectrum of ZnO nanoparticles show well-defined exciton band at ~402 nm (calculated band gap of ~3.08 eV) which is red shifted by ~29 nm relative to the bulk exciton absorption (373 nm). The reason of the shifting of absorption band could be due to the oriented attachment of the nanoparticles by microwave irradiation, may lead to defect formation in the nanoparticles. Minimum inhibitory concentrations (MICs) of NS-ZnNPs was assessed against all pathogens (CV12472, CVO26, PAO1, L. monocytogenes, E. coli). The MICs of ZNP1 were found to be 512, 512, 128, 512, 256 µ g/ml against CV12472, CVO26, PAO1, L. monocytogenes, E. coli, respectively. Concentrations below the MIC level were considered as Sub-MICs and were used throughout the study to assess the anti-QS and biofilm inhibitory properties of the NS-ZnNPs. Anti-quorum sensing potential of NS-ZnNPs was screened using disc diffusion assay with bio-indicator strain Chromobacterium violaceum 12472, which produces the AHL-regulated violet-colored 'violacein' pigment. Concentration dependent violacein inhibition effect of NS-ZnNPs was recorded. Highest inhibition was recorded at 400 µ g/ml followed by 200, 100 and 50 µ g/ml while no pigment inhibition was observed at lower concentrations. Findings of the assay with C. violaceum 12472 were further confirmed by colorimetric determination of violacein production in C. violaceum CVO26. NS-ZnNPs at all tested concentration exhibited a statistically significant decrease in violacein content without inhibiting bacterial growth. At the concentration of 50 µ g/ml NS-ZnNPs reduced violacein production up to 41% in comparison to untreated control. Dose dependent increase in the inhibitory activity was observed with increasing concentration of NS-ZnNPs to a maximum of 91% at 400 µ g/ml concentration. Inhibition of PAO1 virulence by sub-MICs of NS-ZnNPs was evaluated. Effect of sub-MICs of synthesized NS-ZnNPs on the QS regulated virulence factors (elastase, total protease, pyocyanin production and alginate production) in PAO1 was assessed. Dose dependent decrease in the production of elastase, total protease and pyocyanin production was recorded. Alginate extracted from untreated and treated cultures of PAO1 was quantified. Gradual drop in alginate production was observed with increasing concentration of nanoparticle concentration. NS-ZnNPs demonstrated 20–73% reduction in alginate production of PAO1 at sub-MICs ranging from 10–80 µ g/ml. Effect of 10–40 µ g/ml concentrations of NS-ZnNPs on lasB and pqsA transcriptional activity was examined using β -galactosidase assay. NS-ZnNPs at 10, 20, 40 and 80 µ g/ml concentrations significantly reduced the lasB transcriptional activity by 35, 55, 78 and 85%, respectively. Moreover, 41–84% down regulation in pqsA was also recorded at concentration ranging from 10–80 µ g/ml concentration of NS-ZnNPs. The reduction in transcriptional pqsA activity was significant at all tested concentrations. The addition of sub-MICs of NS-ZnNPs showed a dose dependent decrease in the swarming migration of all the tested pathogens. The maximum inhibition in swarming migration was recorded at 1/2 x MIC against all the bacterial pathogens. The synthesized nanoparticle demonstrated reduction in motility behavior of C. violaceum, P. aeruginosa PAO1, E. coli and L. monocytogenes, at concentrations ranging from 1/16 x MIC− 1/2 x MIC. Spectrometric analysis of the extracted exopolysaccharide (EPS) revealed that the concentration of EPS decreased with increasing concentration of NS-ZnNPs. Statistically significant reduction in the EPS production was recorded at all sub-MICs tested. The biogenic nanoparticle (NS-ZnNPs) at 1/2 x MIC exhibited 95%, 91%, and 86% ($p \leq 0.001$) decrease in EPS production of L. monocytogenes, P. aeruginosa PAO1, and E. coli, respectively. Biofilm inhibitory properties of NS-ZnNPs were examined against foodborne pathogens L. monocytogenes, E. coli, C. violaceum, and P. aeruginosa PAO1. NS-ZnNPs reduced the biofilm biomass significantly in a dose-dependent manner without affecting the bacterial growth against all pathogens tested. The results of biofilm inhibition correlated positively with swarming and EPS reduction as these play an important role in adhesion and maturation of biofilms. In-situ visualization of biofilm inhibition was performed. Untreated biofilms grown on glass coverslips showed a thick layer of biofilm, stained easily with CV, and visualized under the light microscope. However, NS-ZnNPs treated coverslips exhibited dose dependent impairment of biofilm formation of L. monocytogenes, P. aeruginosa PAO1, C. violaceum and E. coli. Results of microscopic analysis revealed the maximum level of reduction in number ofmicrocolonies at the 1/2 x MIC against all tested food pathogens. Further, CLSM images also showedloose biofilm architecture and reduced biofilms of all foodborne pathogens after treatment with respectivesub-inhibitory concentration in comparison to untreated control. Effect of NS-ZnNPson preformed biofilms of L. monocytogenes, P. aeruginosa PAO1, C. violaceumand E. coli was assessed by allowing biofilm formation for 16 h and inducing disruption for 8 h by addition of respective 1/2 Å~ MIC of NS-ZnNPs. Crystal violet staining of disrupted biofilm revealed statistically significant reduction of 72%, 66%, 68% and 78% in the preformed biofilms of L. monocytogenes, E. coli, C. violaceum, and P. aeruginosa PAO1, respectively. The NS-ZnNPs were evaluated for their antimicrobial activity against test pathogens in two food media models. The results demonstrate significant growth inhibition of L. monocytogenes, P. aeruginosa PAO1, C. violaceum and E. coli at 1000, 256, 1000 and 512 µ g/ml concentration of NS-ZnNPs, respectively. Results show the reduction in growth rate for all the microbes tested after incubation with respective concentrations of NS-ZnONPs in beef extract media. Similarly, in the lettuce leaf model media significantly impaired growth of the test pathogens was recorded after treatment with NS-ZnNPs as compared to the untreated control. Antibacterial activity of the synthesized NS-ZnNPs was similar in both the model food mediums and it is envisaged that these zinc nanostructures have broad-spectrum antibacterial property.

1. Introduction

Food fraud is a form of criminal behaviour, no matter the definition of crime. The consequences of food fraud are devastating. Food companies and their reputation are damaged, stories go viral, whole supply chains are painted with the same brush, consumer confidence erodes, markets collapse, and management and/or employees are fired, prosecuted, and locked up. The general effects show similarities with other corporate frauds. Losses for individual businesses may include social losses & punishments, third party losses (e.g. extra testing), confidence losses, sales losses & over payment, as well as recall losses. If we take the horsemeat affair as an example, it certainly had a huge economic impact: widespread product recalls and serious effects on all ground beef sales across Europe. There is some popular belief that food fraud is mostly an external threat caused by organized crime groups seeking to permeate the food supply chain. Although politically convenient, in reality it is more often a problem within the food system itself and committed by legitimate food supply chain actors who make the most of criminal opportunities that arise. Fraud is the result of the interaction between motivated offenders, and the opportunities presented by victims and by those entrusted with controlling risks. Fraud vulnerability results from openings for undesirable events resulting from weaknesses or flaws related to the system. Further, criminogenic incentives can differ for the various tiers in production and distribution chains. An assessment of the factors affecting this vulnerability is, therefore, the first step towards fraud prevention and mitigation. Food fraud vulnerability is defined by three key elements: opportunities, motivations, and control measures. These elements can be subdivided into technical opportunities, opportunities in time and place, economic drivers, culture and behaviour, technical control measures, and managerial control measures. A food fraud vulnerability assessment (FFVA) can identify areas in the food chain where vulnerabilities might exist. It can also determine the key drivers and enablers of the vulnerability in supply chains. From the food fraud vulnerability concept of the three key elements described above, an FFVA tool was developed, and was made available as a free downloadable app. Some commodities seem to be more associated with food fraud than others. An inventory of reports in the three global food fraud databases over the period 2008–2013 revealed that the six most frequently reported commodity groups were spices and herbs, olive oil, seafood, dairy products, meat, and other oils and fats. The present study deals with fraud vulnerability across supply chains that have been reported often in the food fraud inventories since it appears that fraud is occurring in those chains. Fraud vulnerabilities in the fish, meat, milk, olive oil, organic banana, and spice supply chains were examined in order to understand the contributions of various fraud factors to the overall fraud vulnerability. Furthermore, we evaluated the differences and similarities between commodity supply chains, as well as between groups of actors (tiers) across chains (e.g. processors, retailers).

2. Materials and methods (omissis)

3. Results and discussion

Fraud vulnerabilities were assessed in the fish, meat, milk, olive oil, organic banana, and spice supply chains consulting the actor groups wholesalers, processors, and retailers, which resulted in scores for fifty questions for each business interviewed. The most frequently selected vulnerability levels per question (the mode) allows a first view of the differences and similarities across the commodity supply chains. A green box refers to a low vulnerability mode, orange to a medium vulnerability mode, and red to high vulnerability mode. It appears that generally scores for opportunities are higher than scores for motivations, whereas we notice that control measures vary across commodity supply chains. Since the motivations questions touch on sensitivities with regard to a potential offender, they may be answered slightly more reservedly than the opportunities related questions. Furthermore, a number of the motivations questions concern the own company, which may be an even more sensitive topic. This would be in agreement with the 'alien conspiracy theory' which describes that crime is often perceived as not being part of the own direct environment and shaped by the environmental circle itself, but rather a problem of threatening external parties. To circumvent this aspect the statistics applied involve relative comparisons. Some general prime fraud drivers and enablers can be distinguished by selection of those fraud factors that were assigned scores above average of the respective opportunities or motivations group. These 13 factors are: (Q2/4) availability technology and knowledge to adulterate raw materials/final products; (Q3/5) fraud detectability in raw materials/final products; (Q10/11/29) historical evidence of fraud in raw materials/final products/branch of industry; (Q12) supply and pricing of raw materials; (Q13) valuable components or attributes of raw materials; (Q25) corruption level country supplier; (Q26) economic conditions branch of industry; (Q30) level of competition branch of industry; and (Q31) price asymmetries. In this group of prime drivers and enablers both opportunities and motivations related fraud factors are included, which underlines that both opportunities and motivations need to be considered. Regarding mitigation of fraud by individual businesses, two options exist. One can avoid vulnerability by reducing opportunities and motivations, i.e. ceasing or not commencing activity to remove the source. Alternatively, the vulnerability can be lowered by implementation of control measures. The control measures are

When looking more in detail into the control measures which counteract the opportunities and motivations, it appeared that ~70% of all the businesses interviewed had any form of a fraud monitoring system for raw materials in place, but only ~45% had a similar system for final products. Furthermore, businesses answered that ~75% of their suppliers had such a monitoring system in place. From the businesses ~30% and from their suppliers only ~10% had an elaborate fraud monitoring system in place, i.e. a systematic, evidence-based sampling plan for fraud-related analyses, specific fraud screening methods and systematic use of fit-for-purpose confirmatory techniques (in house or in collaboration with accredited laboratories), customized procedures for fraud monitoring and handling of non-conformities, and systematic record keeping and detailed documentation of fraud monitoring procedures & systems. Approximately 80% of the businesses had an information (mass balance) system available, and ~75% of their suppliers. Similarly 85% had a tracking and tracing system present and 90% of their suppliers. On the contrary the managerial (soft) controls were not as widely available as the technical controls. Although ~80% had a code of conduct, only ~50% had a form of employee integrity screening, and ~65% had whistle blowing facilities. Ninety percent of the interviewed businesses indicated that there was some form of social fraud control in the supply chain, but only ~15% reported extensive social control. Fraud industry guidelines were lacking according to ~50% of the businesses. Furthermore, 45% and 65% businesses respectively, felt that the national food policy and enforcement were insufficiently covering fraud. To explore the data of the individual businesses, all FFVA data were subjected to multiple correspondence analysis (MCA). Results indicate a clear grouping of actors in the same commodity supply chain. The milk and bananas (right hand side) chains are separated from the fish and olive oil chains (left hand side) in the first dimension. The spices and meat chains separate in the second dimension. The plots show that the general FFVA patterns are very much determined by the type of commodity chain. The (dis)similarity between the chain actors interviewed was further examined by agglomerative hierarchical clustering (AHC). We can distinguish three main clusters. One group (blue) shows generally relatively low scores for opportunities and motivations, and high scores for control measures. This low vulnerability, blue group mainly consists of fish chain and organic banana chain actors. The remainder of the businesses are clustered in two groups. Both show relatively high scores for opportunities and motivations related fraud factors. However, the red group (predominantly meat) shows high scores for control measures and thus has counteract measures in place, whereas for the green coloured group (predominantly fish, olive oil, spice) generally lower scores for control measures are observed. The AHC results are in line with the MCA results, which also showed the commodity chain groupings and associated fraud factors. The assignment to the clusters is not fully according to commodity chain though; some businesses end up in other groups than their chain counterparts. Obviously, assessment patterns are not only determined by the commodity supply chain. Therefore, we will examine the impact of the supply chain and the actor group on individual fraud factors in greater detail in the following sections. The impact of the commodity chain as well as actor groups of the present study was examined simultaneously using a multi-factor ANOVA. The results show the 23 fraud factors presenting significant differences in scores across the commodity chains. Four opportunities related fraud factors (Q8/9/10/11), nine motivations related fraud factors (Q13/18/19/22/23/24/25/27/28), and ten control measures related fraud factors (Q34/35/36/39/41/42/43/47/48/49) revealed significant differences across the commodity supply chains. Thus, the scores of about half (46%) of the fraud factors contributing to fraud vulnerability appear to be significantly influenced by the commodity chain. Meat shows highest scores for opportunities related fraud factors, such as access to production lines and historical evidence (Q8/10/11). Meat and olive oil show highest scores for motivations related questions, such as valuable components and criminal offences of customers (Q13/27). On the other hand, the fish and spice chains show greatest lack of (adequate) control measures, e.g. for the availability of a fraud monitoring system for the final products and fraud preventing contractual requirements of suppliers (Q34/41). Taking all of the above into account, the order in fraud vulnerability from low to high in the current study is organic banana, milk, fish, meat, olive oil, with spice ranking highest. Although the chains were selected based on their fraud reports, it appears that we can adequately characterize and distinguish them by the fraud factors contributing to their overall fraud vulnerability. Using multi-factor ANOVA, the significant differences across actor groups were examined simultaneously with the impact of the commodity chains. Seven fraud factors showed significantly different scores between the actor groups: one opportunities related fraud factor (chain transparency: Q9), two motivations related fraud factor (financial strains supplier: Q19; criminal offences customer: Q27), and four control measures related fraud factors (companies' information system: Q36; and ethical code of conduct: Q39; contractual requirement suppliers: Q41; enforcement local chain: Q48). The latter are managerial control measures only. All these fraud factors are also affected by the commodity chain, so there is an effect on these scores by both the actor group and the commodity chain.

1. Introduction

Recently, increasing interest has been developed to isolate and investigate novel bioactive components from natural resources with health beneficial effects. Saffron is an antioxidant herbal product containing significant ingredients, such as crocin and safranal, with potential anti-atherosclerotic properties. Crocus sativus L. (Iridaceae), known as saffron and Krokos in Greece, tends to become extremely popular in modern therapeutics and preventive medicine. Until nowadays, it is commonly used in food supplements and as herbal tea. However, a wide-spectrum of potential pharmaceutical applications has been reported for saffron with the most remarkable effects being attributed to its main natural carotenoids, crocin and crocetin. In parallel, an increasing number of research studies on the possible clinical uses of saffron have been conducted, mainly against malignancies and Alzheimer's disease, as well as regarding its anti-atherosclerotic and cardioprotective effect. Experiments conducted in rodents, showed that orally administered crocins are hydrolyzed to crocetin before being incorporated into blood circulation and that crocetin is likely to be metabolized to glucuronide conjugates both in the intestinal mucosa and in the liver of mice. To explain the chemical and pharmacological properties of saffron, the chemical profile of the extract deriving from the stigmatic lobes of the C. sativus L. flower should be evaluated. According to literature data, the plant contains three main categories of components: (1) picrocrocin, which is the main substance responsible for saffron's bitter taste; (2) safranal, which is the volatile component responsible for the characteristic saffron aroma; (3) crocins (cis and trans forms of the molecule), which are saffron coloured compounds (unusual water-soluble carotenoids due to high glycosyl contents). All possible pharmacological properties are attributed to the above components. Antioxidant properties are mainly due to all-trans crocin and more specifically due to its in vivo hydrolysis product crocetin, whereas safranal is reported to be effective against respiratory diseases and picrocrocin seems to possess sedative effects on spasms and lumbar pains. Consequently, the evaluation of the precise composition of the extract is of great pharmacological importance. Despite the existing literature on saffron's extracts possible pharmacological effects, its serum pharmacokinetics (PK) and tissue distribution have not yet been investigated and therefore the selection of the administered doses is in most cases empirical. Most of the relevant PK studies are conducted after administration of the main active constituents namely crocin or its main metabolite crocetin, while there is only one study where serum crocetin levels are measured with a developed HPLC method in the plasma of four healthy volunteers after consumption of one cup of saffron tea. We recently performed preliminary pharmacokinetic experiments for the selection of the proper dose range to study the anti-atherosclerotic and cardioprotective effects of saffron. However, a systematic study and evaluation of basic serum and tissue pharmacokinetic parameters after administration of saffron extract are missing and could help to explore its possible pharmaceutical applications. Accordingly, the aim of this study was (1) the preparation, chemical profile characterization and storage stability study of an aqueous saffron extract (SFE), (2) the pharmacokinetic evaluation of the produced SFE by measuring serum and tissue levels of crocetin (unconjugated, conjugated and total) after intravenous and oral administration to C56/Bl6J mice.

2. Materials and methods (omissis)

3. Results and discussion

In vitro studies.SFE chemical profile characterization. According to the chromatograms obtained from the HPLC-PDA analysis, several fractions were collected at time points shown (totally 14 fractions). The identification and matching of each peak were based on the retention time of each fraction in comparison to bibliography, while NMR spectra were also obtained using >CD3OD as a solvent for the aqueous extract. Based on both chromatographic and 1HNMR results, SFE was found to consist mainly of the following secondary metabolites: picrocrocin mixture with a- and b-glucose (Rt = 3.2 min), all-trans-crocin (Rt = 3.4 min) and picrocrocin aglycon (Rt = 6.5 min), while the rest of the peaks did not reveal any substance due to low amounts. Possible safranal content was also determined and was found equal to 0.34 (0.02)% w/w. The measured very low safranal content supports the NMR analysis results, where the typical signals for safranal in the 1H NMR spectrum at d H 5.90–5.80 are missing, probably due to the aqueous nature of the SFE resulting in poor content with respect to less polar constituents such as safranal or being overlapped by the signals of the polar glycosidic constituents, such as crocins and picrocrocin. **SFE stability studies.** The content of all-trans crocin (%w/w) in the SFE was found to remain constant (91.0 ± 0.3% of the initial value) for at least 1 month, while it gradually decreased to 70.4 ± 0.2% of the initial crocin content after 6 months at room temperature and remained unchanged at this value up to the end of the study, 15 months from its preparation. After reconstitution in WFI, SFE's crocin content remained constant (95.6 ± 1.5% of the initial value) for 24 h and decreased to 90.2 ± 1.3% of the initial content after 48 h, when kept at 4°C. Accordingly, the reconstituted in WFI SFE can be used within 24 h after reconstitution kept in the refrigerator. **In vivo studies, Crocetin HPLC-PDA bioassay.** Crocetin serum and tissue bioassay were based on a previous method. The analytes were well-separated, with retention times 3.5 and 9.5 min for crocetin and IS, respectively, for serum and tissue samples and no peak distortions were visible. Calibration curves for crocetin (concentration range of 0.05–5 μg/ml) were linear (r2≥0.999), and the limit of detection and quantitation was 0.02 and 0.04 μg/ml, respectively. No differences were found between calibration curves constructed in serum and tissues homogenates. **Pharmacokinetic analysis. Compartmental PK modelling.** Pharmacokinetics analysis revealed a one-compartment model to describe serum pharmacokinetics of crocetin (unconjugated, parent) after iv administration of SFE, while for the crocetin metabolite (conjugated), a first-order input kinetic parameter (ka) was incorporated to describe the rate of crocetin biotransformation to its conjugated form. For the oral administration, the one-compartment model with first-order absorption was found to adequately describe experimental data for both crocetin and crocetin metabolite. A multiplicative residual error model was used in all cases to describe residual unexplained variability. The peros model parameters

Since crocetin is not directly administered but it is formed in vivo after SFE administration, its absolute bioavailability cannot be estimated. Therefore, the calculated F value is the relative bioavailability of crocetin after oral and i.v. administration of the same dose of SFE and is equal to 1.28. The higher crocetin levels measured after oral administration compared to the intravenous administration of the same dose of SFE could be probably attributed to the extended hydrolysis of the main active SFE constituent, crocin, in the gastrointestinal lumen and the intestinal mucosa, to form crocetin which is rapidly absorbed in the blood circulation through the portal vein. This suggestion is also supported by previous results where it was found in vitro, that crocin is subjected to extensive intestinal hydrolysis to form the deglycosylase trans-crocetin which is subsequently absorbed by passive diffusion from the intestinal epithelium to a high extent and rapidly enters the blood circulation. They also concluded that crocin deglycosylation to crocetin is mainly attributed to enzymatic processes in the epithelial cells rather than deglycosylation by the faecal microbiome which was found to contribute only to a very minor extent. On the other hand, crocin transformation to crocetin after i.v. administration of SFE seems to be very rapid, as was shown by the good fitting of the one-compartment i.v. bolus model to the experimental data without the need of an apparent formation constant to describe the kinetics of the transformation process, as in the case of crocetin metabolite. This, in conjunction to the rapid glucuronidation of crocetin and formation of the conjugated form (metabolite), as reflected on the respective high Cl value, may contribute to the lower plasma crocetin concentrations measured after i.v. administration of SFE, compared to those after peros administration of the same dose of SFE. The extent of crocetin and its metabolite tissue distribution is reflected on the estimated values for the volume of distribution, Vd. These values when compared to the total blood volume of the experimental animal model used in this study (58 ml/kg or approximately 1.5 ml for mean body weight of 25 g), reveal high tissue distribution of both crocetin and its metabolite after either i.v. or peros administration of the same dose of SFE. The ratio Vd_crocetin/Vd_metabolite is 6.56 after i.v. and 0.30 after peros administration of the same dose of SFE, probably reflecting extensive glucuronidation due to liver first-pass effect. Non-compartmental PK analysis. Serum and tissue crocetin pharmacokinetics calculated using NCA are presented in. The calculated serum AUC values for crocetin metabolite and total crocetin were 1.5–2.5 times higher compared to crocetin, reflecting the biotransformation of crocetin to its conjugated derivatives both after i.v. and oral SFE administration. The calculated serum Cl values of crocetin and crocetin metabolite were 55 and 30.6 ml/h, and 40.8 and 28.2 ml/h after i.v. and oral administration of the same dose of SFE, respectively. These values are in agreement with those estimated by the native pooled PK analysis in Phoenix taking into account the different methodologies applied. Relative oral bioavailability (F) of crocetin after administration of SFE was calculated. This value agrees with the value predicted with the 1-compartment PK model that was found to describe crocetin serum pharmacokinetics after Phoenix_ PK analysis. An equation was also applied to calculate F for total crocetin as more indicative for the fraction of dose absorbed, and was found equal to 1.17, very close to unity, reflecting rapid and complete intestinal absorption of crocetin after SFE administration. Non-compartmental PK analysis was also applied to calculate basic tissue PK parameters of crocetin, total crocetin and crocetin metabolite. A rough estimation of the tissue/serum distribution can be obtained calculating the ratio of the estimated AUC tissue to the estimated AUC serum (defined as Kpapp) after either iv or SFE administration (60 mg/kg). For crocetin, the calculated Kpapp values are higher than unity for the liver and kidneys, the main organs of crocetin biotransformation and elimination. No measurable levels of crocetin could be detected in lungs and heart, probably due to the rapid glucuronidation to its conjugated form. Regarding crocetin metabolite (conjugated), the calculated Kpapp values ranged from approximately 0.3 to 1.0 showing low distribution and higher Cl values compared to crocetin to the studied tissues. It should be mentioned, however, that steady state measurements would give more inside the tissue distribution kinetics and possible accumulation of crocetin after SFE i.v. or oral administration. To the best of our knowledge, this is the first study of crocetin (parent, total and metabolite) serum and tissue pharmacokinetics after i.v. and oral administration of SFE and the calculation of crocetin's oral bioavailability. Literature search revealed pharmacokinetic studies after administration of pure crocin or crocetin to animals and humans, while in one study saffron extract was administered as tea consumption and only crocetin levels in brain were measured. Furthermore, the intestinal permeation of saffron's crocin and crocetin as well as the crocin's intestinal conversion to crocetin was investigated. It should be mentioned, however, that the results of the present PK study are in agreement with the previously reported and show that oral administration of SFE (60 mg/kg, or 16.7 mg/kg of crocin) led to serum crocetin levels (Cmax = 2.77 lg/ml, %CV 2.17) comparable to those measured in healthy volunteers following oral administration of pure crocetin at doses of 7.5–22.5 mg or 0.11–0.32 mg/kg (Cmax = 100–280 ng/ml, %CV 24–50), as well as to those measured after oral administration of crocetin 40 nmol or 0.37 mg/kg (Cmax = 300 nM or 0.098 lg/ml) and crocin 40 nmol or 1.12 mg/kg (Cmax = 100 nM or 0.033 lg/ml) to mice, taking into account the different doses administered and the observed variability. The results of the present work, in conjunction to the previously published showing the cardioprotective and anti-atherosclerotic effect of SFE, great evidence of the pharmacological properties of SFE and can be used to support the study and exploration of its potential therapeutic applications.

Consiglio Nazionale delle Ricerche

BANDO N. 368.32 RIC - Area strategica Produzioni alimentari e Alimentazione
Concorso pubblico per titoli ed esami per l'assunzione con contratto di lavoro a tempo pieno e indeterminato di n.5 unità di personale con profilo di Ricercatore – III livello professionale - presso strutture del Consiglio Nazionale delle Ricerche

PROVA SCRITTA A CARATTERE GENERALE DEL 27 MAGGIO 2019 – ORE 14.00

Il candidato, dopo aver scelto un elaborato tra i tre allegati, ciascuno estratto da un articolo pubblicato su rivista, dovrà definire un adeguato titolo e il riassunto in inglese (minimo 200, massimo 300 parole) attendendosi strettamente al contenuto dell'estratto.

SERIE F – TRACCIA 1

1 INTRODUCTION

Trimethylamine-*N*-oxide (TMAO) is a naturally occurring small organic dietary compound that is abundant in fish or can be generated from other nutrients including choline (abundant in eggs) and carnitine (abundant in beef). Upon consumption of foods containing TMAO or its dietary precursors (choline and carnitine), it is proposed that gut bacteria generate trimethylamine (TMA; volatile, fish odor compound) and to a lesser extent, dimethylamine (DMA) with subsequent absorption via enterohepatic circulation. The majority of TMA is enzymatically converted to the odorless TMAO metabolite in a reversible reaction catalyzed by vitamin B2 dependent flavin-containing monooxygenase 3 (FMO3) in the liver. Loss-of-function mutations in this enzyme give rise to the rare genetic disorder known as trimethylaminuria or "the fish odor syndrome". Alternatively, TMA can be demethylated to DMA and methylamine (MA). The significance of TMAO in physiologic processes received early attention due to its function as an osmolyte. In addition, farmers recognized the benefits of adding TMAO to animal feed for the purposes of improving growth, carcass quality, and nutrient digestibility. More recently, TMAO has emerged as a predictive risk factor for heart disease in cardiac patients and colorectal cancer among postmenopausal women. However, very little is known about the effects of animal source foods on TMAO generation, absorption, and elimination in healthy adults. Furthermore, although there is an apparent role of the gut microbes in TMAO production, the gut microbiota composition in relation to TMAO production in humans has not been determined. Therefore, we aimed to test the hypotheses that (i) TMAO response to animal source foods would vary among healthy men and (ii) this response would be modified by their gut microbiota composition. To achieve these aims, we conducted a crossover feeding trial whereby healthy young men consumed study meals of fish, eggs, meat, and a fruit control in random order with 1-week washout periods. TMAO biomarker response to the study meals was quantified in plasma and urine, while gut microbiota composition was assessed in feces. Because the FMO3 G472A genetic variant may adversely influence TMA conversion to TMAO, men were genotyped for this polymorphism, which was considered as a covariate in the statistical models.

2 MATERIALS AND METHODS (omissis)

3 RESULTS

3.1 Participant characteristics and baseline metabolite concentrations

Forty healthy men had a mean age of 27.8 ± 1.0 years, BMI of 24.2 ± 0.4 kg/m2, and serum blood chemistry and blood cell counts within the normal range. Thirty-five percent of the participants were homozygous wild-type GG genotype for *FMO3* G472A, 55% were heterozygous GA, and 10% were homozygous variant AA, which is consistent with the distribution observed in the general population. TMAO and its derivatives did not differ across study meals.

3.2 Study meal TMAO content

The fish meal contained 650 times more TMAO, 200 times more TMA, and 1600 times more DMA compared to the egg and beef meals ($p < 0.0001$), whereas the fruit did not show any detectable levels of these metabolites. Food MA did not differ among the study meals. Total choline concentration was 125 times higher in eggs and 38 times higher in beef and fish ($p < 0.0001$) compared to the fruit control. Food betaine content was 27 times higher in beef and fish compared to egg and fruit meals ($p < 0.0001$). Carnitine was highest ($p < 0.0001$) in beef followed by fish and then eggs and not detected in the fruit control.

3.3 TMAO biomarker response to the study meals

Study meal and time interacted ($p < 0.0001$) to influence plasma concentrations of TMAO and its derivatives. As compared to egg, beef, and fruit control, fish consumption yielded plasma concentrations that were 48–62 times higher for TMAO ($p < 0.0001$), 8–14 times higher for TMA ($p < 0.0001$), and 4–5 times higher for DMA ($p<0.0001$), all of which peaked at 2 h and remained elevated until the end of the 6-h study period. MA concentrations were not detectable in plasma. Similar to plasma levels, study meal interacted with time ($p < 0.005$) to influence urinary TMAO and its derivative concentrations. Fish consumption resulted in urinary excretions that were 46–51 times higher for TMAO ($p < 0.0001$), 9–12 times higher for TMA ($p < 0.0001$), and six times higher for DMA ($p < 0.0001$) as compared to egg, beef, and fruit control during the 6-h study period ($p < 0.0001$). Because of the substantial TMAO response following consumption of the fish study meal, we were unable to detect differences in TMAO response among the egg, beef, and fruit study meals. Thus, we assessed TMAO response for eggs, beef, and fruit separately from fish, and compared the response to study baseline. A greater increase in plasma TMAO at each time point was detected for eggs and beef compared to the fruit control ($p < 0.05$). Likewise, the change in urinary TMAO excretion across the 6-h study period was greater following the consumption of eggs and beef compared to the fruit control ($p = 0.03$). Notably, the individual variations in urinary TMAO response after the consumption of eggs and beef ranged from –30% to 270%. We therefore stratified our participants into high-TMAO producers (n = 11; those with ≥20% increase in urinary TMAO in response to eggs and beef) versus low-TMAO producers (n = 15; those with <20% increase in urinary TMAO in response to eggs and beef) for the microbiome analyses (see Section 3.4). We also assessed for differences in the baseline characteristics of the TMAO response groups but none were detected.

3.4 Gut microbiota composition

To address whether microbiota composition was a determinant of TMAO response, we obtained a one-time baseline stool sample from each participant. Of the 40 stool samples, 26 samples were used in the analyses after amplification and quality control yielding 6,770,441 high-quality gene sequences with average sequences lengths (mean ± standard deviation) of 254

abundance of the bacteria showed distinct bacterial profiles between low-TMAO producers and high-TMAO producers ($p = 0.04$, $R2 = 0.11$ with 999 permutations by the anosim method). We then visualized gut microbiota differences between high-TMAO producers and low-TMAO producers using a heatmap. High-TMAO producers had 58.1% Firmicutes to 32.6% Bacteroidetes (≈2:1 Firmicutes: Bacteroidetes), whereas low-TMAO producers had 47.7% Firmicutes to 47.2% Bacteroidetes (1:1 Firmicutes: Bacteroidetes). High-TMAO producers were represented by Clostridiales within the Firmicutes phylum of which were Clostridiaceae, Lachnospiraceae, and Veillonellaceae. Low-TMAO producers were represented by Bacteroidales within the Bacteroidetes phylum of which were Bacteroidaceae and Prevotellaceae. The Archaea phylum was represented in the low-TMAO producers but absent in the high-TMAO producers.

4 DISCUSSION

This study sought to advance understanding of the effects of animal source foods on TMAO production, and to deter mine whether this response was influenced by an individual's gut microbiome. Three main findings emerged: (i) fish consumption yielded several-fold higher quantities of TMAO metabolites than either eggs or beef, (ii) dietary TMAO can be absorbed intact without processing by gut microbes, and (iii) high-TMAO producers (≥20% increase in urinary TMAO in response to eggs and beef) had more Firmicutes than Bacteroidetes and a less diverse gut microbiome.

4.1 Fish consumption yields the highest concentrations of plasma and urinary TMAO concentrations compared to all other study meals

Fish consumption yielded ≈50 times higher circulating concentrations of TMAO than either eggs or beef, which is consistent with previous reports of substantially higher urinary TMAO concentrations following the consumption of fish compared to meat, dairy, fruits, vegetables, or grain. Notably, plasma TMAO was elevated within 15 min of fish consumption indicating that the absorption of dietary TMAO may occur without processing to TMA by the gut microbes as previously proposed. The sustained elevation in circulating TMAO in response to fish consumption, despite substantial urinary excretion, suggests that a portion of the TMAO is retained by the body possibly due to its functions as an osmolyte and protein stabilizer. Other derivatives influenced by diet, albeit to a lesser extent than TMAO, included TMA and DMA, both of which showed higher levels in response to fish versus eggs and beef. In light of the recent proposed role of TMAO as a causative agent for cardiovascular disease, and a recent report of higher serum TMAO concentrations and accelerated aortic plaque formation in apoE null mice with increased fish intake, some researchers have advocated for the restriction of animal source foods that raise circulating TMAO concentrations. However, these animal source foods are enriched in nutrients that are required for optimal health, and fish consumption is well known for its cardioprotective attributes in humans. Moreover, in more recent studies, circulating TMAO and carnitine concentrations were inversely associated or showed no association with the development of chronic diseases. Thus, caution is warranted when proposing dietary recommendations that restrict the intakes of animal source foods because of their TMAO-raising characteristics.

4.2 TMAO response to dietary precursors may be a biomarker of the gut microbiota composition

Gut microbes are known to participate in modulating TMAO response to its dietary precursors and potentially chronic disease susceptibility. However, TMAO response is highly variable and it is unclear whether this variation arises from individual differences in gut microbiota composition. To address this question, we used urinary TMAO excretion to stratify our participants into "high" (≥20% increase) or "low" (<20% increase) TMAO producers, and examined differences in the gut microbiota composition between the two groups. Although differences in the gut microbiota were subtle at the individual OTU level, high-TMAO producers were characterized by enriched ratios of Firmicutes to Bacteroidetes compared to low-TMAO producers, which is consistent with previous reports that TMAO production potential is detected in Firmicutes but absent in Bacteroidetes. Furthermore, low-TMAO producers had more archaea Methanobrevibacter, which has been proposed to deplete host TMA levels. Lower α-diversity (within-individual difference) was also observed among high-TMAO producers indicating that TMAO production may be driven by a select set of bacteria (e.g., Firmicutes). Distinct gut microbiota profiles between high-TMAO compared to low-TMAO producers in response to its dietary precursors indicate that circulating TMAO may be a biomarker of the gut microbiota. As such, higher circulating concentrations of TMAO in a disease versus nondisease state may reflect differences in gut microbe composition, rather than indicating a causative role of TMAO in the disease process. A greater ratio of Firmicutes to Bacteroidetes has previously been associated with increased risk of obesity and metabolic syndrome, and this gut microbiota characterization may also be linked to atherosclerosis-associated dysbiosis. The significance of these findings is that circulating TMAO may be used to identify individuals with microbiota profiles that increase disease susceptibility, and thereby inform the development of dietary and pharmaceutical strategies aimed at increasing gut microbiota diversity and restoring the symbiotic relationship between the gut microbes and their host.

4.3 Study limitations and future directions

This was a short-term feeding study that focused on advancing current understanding of TMAO metabolism in healthy male adults. As such, the findings may not reflect long-term effects of the diet and may not be generalizable to other segments of the population including women and those with health conditions. In addition, predisease clinical endpoints, such as markers of inflammation, were not evaluated. More studies are needed to address these important issues and evaluate the clinical utility of lowering circulating concentrations of TMAO as a means to improve human health.

SERIE F – TRACCIA 2

1.Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large class of organic compounds that are produced through the incomplete combustion or pyrolysis of organic matter and are persistent, bio-accumulative, carcinogenic and mutagenic lipophilic organic contaminants. Individuals are mainly exposed to PAHs through ingestion, inhalation and skin contact. Among these, human intake of PAHs from food is higher than the other means of intake in daily life. Researchers have found that dietary intake contributes to more than 90% of all PAH exposure for the general populations in various countries, especially among non-smokers and non-occupationally exposed adults. PAHs manifest as contaminants of different food categories, (e.g., oils and fats, vegetables, fruits, seafood, tea, coffee, sugar, toasted bread, infant foods and smoked food products). PAH ingestion from edible oils accounts for one third of all ingestion from food constituting one of the main contributing sources. Thus, it is necessary to study and control PAH levels in edible oils. In 2005, the European Union (Commission Regulation No. 208/ 2005) set a maximum level of 2 mg/kg for benzo[a]pyrene in oils and fats for direct consumption or for use as an ingredient in foods and solicited further analyses of benzo[a]pyrene and other genotoxic compounds of 15 PAHs. However, ac- cording to comments made by the European Food Safety Authority (EFSA) in 2008 and according to Commission Regulation (EU) No. 835/2011 of August 19, 2011, benzo[a]pyrene is not a suitable marker of the presence of other PAHs in food; instead, four specific PAHs (benzo[a]pyrene, benzo[a]anthracence, benzo[b]floranthene and chrysene) are the most suitable indicators of PAHs in food. The maximum tolerable limit for these compounds has been set to 10 mg/kg. Some countries have established specific limits on concentrations of the following toxic and carcinogenic PAHs: benzo[a] anthracene, benzo[e]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene. The maximum limit for each individual PAH is 2 ppb, and a level of 5 ppb for the sum of eight heavy PAHs has been established. According to Chinese regulation GB2716-2005, there are no maximum limits on PAHs in edible vegetable oils except for those imposed on B[a]P (limit of 10 mg/kg). Due to the proven carcinogenic activities of PAHs, numerous studies have been conducted on PAHs found in edible oils. Most studies have focused on fresh crude edible oils and on fresh refined oils. However, few studies have analyzed the contents of PAHs found in cooked oils (e.g., deep-fried oils) despite the repeated use of edible oils, especially in small restaurants and at roadside stalls. Therefore, it is important to determine the levels and compositions of PAHs found in commonly used edible oils.

The objective of this study is to analyze features of the 16 PAHs found in fresh and cooked edible oils over varying deep-frying times. To achieve this objective, we examine four edible oils commonly used (rapeseed oil, soybean oil, peanut oil and olive oil) while deep-frying chicken nuggets and potatoes and obtain edible oils that have been deep-fried for 15, 30, and 45 min.

2. Material and Methods OMISSIS

Abbreviations

[(naphthalene (Nap, two-ring), acenaphthene (Act, three-ring), acenaphthylene (Acl, three-ring), fluorene (Flr, three-ring), phenanthrene (Phe, three-ring), anthracene (Ant, three-ring), fluoranthene (Flu, four-ring), pyrene (Pyr, four-ring), benzo[a]anthracene (B[a]A, four-ring), chrysene (Chr, four-ring), benzo[b]fluoranthene (B[b]F, five-ring), benzo[k]fluoranthene (B [k]F, five-ring), benzo[a]pyrene (B[a]P, five-ring), dibenzo[a,h]pyrene (DB[a,h]A, five-ring), indeno[1,2,3-cd]pyrene (Ind[1,2,3-cd]P, six-ring), and benzo[ghi]perylene (B[ghi]P, six-ring)

3. Results and discussion

- Variations in total PAH levels with cooking time for different edible oils. Variable levels of total PAHs were found among the different fresh edible oils and over different deep-frying periods. The concentrations of total PAHs are the sum values of the 16 PAH concentrations. For the fresh oils, the soybean oil and peanut oil samples generated the lowest (189.9 mg/ kg) and highest (2754.8 mg/kg) PAH concentrations, respectively. Vegetable oils are mainly free from PAHs, although they are contaminated by environmental pollutants, such as vegetable raw materials, and are also contaminated a result of seed drying. They are also contaminated through solvent extraction, soil burning, pack- aging material contact, mineral oil residue exposure and through migration from contaminated water or soils. In the present study, olive and peanut oils were found to contain higher concentrations of PAHs than the other oils. Total PAH levels in fresh oils detected in this study are similar to those found in other Chinese studies. However, the levels found in this study are higher than those reported by some other authors. Royo and Camargo (2012) determined levels of 13 PAHs found in crude soybean and deodorized oil samples, with concentrations ranging from 10 to 316 mg/kg and from 3 to 69 mg/kg, respectively. Regardless of food type, total PAH levels in the edible oils increased with increasing deep-frying time. This variable trend is related to the accumulation of PAHs in edible oils during the deep-frying process. However, total PAH concentrations increased slowly during deep-frying, especially during the deep-frying of chicken nuggets. Although the crude rapeseed oil sample presented the lowest PAH levels, these levels increased rapidly with increasing deep-frying time. In particular, only slight differences in total PAH levels were found among the four edible oils. As the peanut oil sample was deep-fried, total PAH concentrations increased considerably, reaching the highest levels found in this study. Total PAH levels in the edible oils differed slightly when different foods were deep-fried. When deep-frying potatoes, the concentration order of total PAHs in the edible oil samples was consistent with that in the crude oil samples. However, while deep-frying chicken nuggets, the peanut oil sample generated the highest concentration of total PAHs, followed by the rapeseed, soybean and olive oil samples. Thus, deep-frying periods and types of oils and ingredients used affect total PAH levels in edible oils. Four specific PAHs (B[a]P, B [a]A, B[b]F, and Chr) found in edible oils are considered to be the most suitable indices of edible oils according to the European Union. These four PAHs were not detected in the fresh oils. During the deep-frying process (with the exception of rapeseed oil), the other three oils were characterized by these four PAHs. Considering the values of these four PAHs detected in the deep-fried peanut and olive oil samples and the limits set by European regulations, the overall 4PAH levels found in these samples exceed established acceptable concentrations and increase rapidly with increasing deep-frying time. For peanut oil, the concentrations of overall 4PAH in the samples deep-fried for 45 min were 8.0- and 1.5- fold higher than those that were deep-fried for 15 min while deep- frying potatoes and chicken nuggets, respectively, reaching values of up to 11.1- and 7.4-fold higher than permitted limits, respectively. For the olive oil samples, mean levels of total 4PAH for those

were found in the sample that had be used to deep-fry potatoes for 45 min. Specific reasons for this result require further analysis. When considering food types, the results show that variation rates of total 4PAH levels in the deep-fried potato and chicken nugget samples differed with increasing cooking time, especially for peanut oil, suggesting that types of foods also affect the formation of the four PAHs.

- Effects of deep-frying periods on individual PAH concentrations. Four PAHs were detected in the fresh rapeseed oil sample. When potatoes were deep-fried, the 11 main substances rapidly became more plentiful, resulting in an increase in total PAH concentrations. Nap, Acl, Flu and Pyr levels in the potatoes deep-fried in oil for 45 min were 1.5-, 24.0-, 9.0- and 12.0-fold those of the fresh oil samples, respectively. The main PAH concentrations recovered from the oils used to deep-fry potatoes corresponded to the following contributors: Nap (42.4%), Act (13.9%), Ant (10.4%), and Flu (9.5%). For the rapeseed oil used to deep-fry chicken nuggets, the major PAHs found included the following: Nap (48.0%), Acl (15.8%), Ant (13.4%), Phe (9.9%), and Flr (9.0%). In addition, Acl, Phe, and Ant rapidly grew more plentiful during the deep-frying process. The PAH levels found in the fresh and deep-fried soybean oils. Nap, Phe and Ant were found in the fresh soybean oil sample at concentrations of 6.6, 37.0 and 146.3 mg/kg, respectively. Nine PAHs were found in the soybean oil sample after deep-frying. The B[a]P carcinogenic compound was found in the soybean oil sample from potatoes deep-fried for 45 min but was not detected in the other soybean oil samples. The major PAHs found in the soybean oil sample after deep-frying potatoes and chicken nuggets differed slightly. The fresh peanut oil sample contained 5 of the target PAHs. During the deep-frying process, 8 PAHs were found in the deep-fried peanut oils. However, the types of PAHs found after deep-frying the potatoes differed from those found in the chicken nugget samples. Moreover, in the fresh olive oil sample, 2 PAHs (Nap and Act) were detected at levels of 2230.7 and 123.0 mg/kg, respectively. During the deep-frying process, 8 and 7 PAHs were found in the olive oil samples used to deep- fry potatoes and chicken nuggets, respectively. Pyr and Ind[1,2,3- cd]P, which were not found in oils used to deep-fry chicken nuggets for 15 and 30 min, were detected in the olive oil samples used for 45 min of deep-frying at concentrations of 604.0 and 107.0 mg/kg, respectively. These data show that the species and concentrations of PAHs found in fresh peanut oil were the highest among the edible oils examined. The PAHs observed in the fresh oil samples were mainly low-ring PAHs (2- to 4-ring), with the exception of DB[a,h]A (5- ring), which was found in the fresh peanut oil sample. B[a]P (identified as group 1 by the International Agency for Research on Cancer) was not found in the four fresh oil samples. During the deep- frying process, the types and concentrations of PAHs increased relative to those of the fresh oils (especially for the high-ring PAHs (5-ring and above)), regardless of the oil type. Edible oils are contaminated by PAHs after cooking. Nap was especially prominent in all of the samples at concentrations of 6.6e2549.9 mg/kg, echoing previous results, who found Nap in all of the food samples that they analyzed. For the deep-fried rapeseed and soybean oils, no B[a]P was detected, although it was found in soybean oil used to deep-fry potatoes for 45 min. However, when deep-frying with peanut and olive oil, B[a]P was found at levels that exceeded acceptable limits at 2 and 10 mg/kg, respectively. In the peanut oil used to deep-fry potatoes and chicken nuggets, the average levels of B[a]P were 4.3- and 5.9-fold higher than the limit of 10 mg/kg and 25.5- and 38.5-fold higher the maximum permitted quantity of 2 mg/kg, respectively. For the olive oil samples used to deep-fry potatoes and chicken nuggets, B[a]P levels were 4- and 4.4-fold higher than Chinese standard levels and 24.0- and 26.2-fold higher than EU standard levels, respectively. The main PAHs collected from the oil samples examined were Nap, Act, Acl, Phe, Ant, and Pyr. By contrast, Chr, B[a]A, B[b]F, and B[K]F were found in only a few samples.

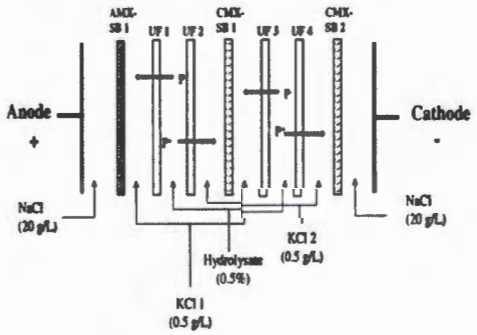
-Distributions of PAHs with different ring numbers in edible oils after different deep-frying periods. PAHs differ in toxicity levels depending on ring quantities; PAHs with more rings are more toxic. We thus categorized the 16 PAHs examined by aromatic ring quantities (2-, 3-, 4-, 5-, and 6-ring). The proportion of PAHs decreased with increasing ring quantities. Regardless of the edible oil, food product, and deep-frying period applied, the main PAHs found in the samples had 2e3 rings (Nap, Act, Acl, Flr, Phe, and Ant), accounting for 86.5% (ranging from 83.2% to 88.6%) of all PAHs found on average, echoing previous results. To further understand ring number distributions of PAHs in relation to previous research, 16 PAHs were classified into light-molecular-weight PAHs (LM-PAHs) and heavy-molecular-weigh AHs (HM-PAHs) based on quality levels. LM-PAHs and HM-PAHs consist of 2- to 4-ring and 5- to 6-ring PAHs, respectively. HM- PAHs are more toxic than LM-PAHs. The four fresh edible oils largely included no HM-PAHs. The exception was the uncooked peanut oil sample, which included a small number of HM-PAHs. LM-PAHs were predominant (>90% of the total PAH concentration) in all of the oil samples, in accordance with previously published results. Contributions from HM-PAHs, which include most carcinogenic PAHs, were less significant (mean of 3.4%). For example, while deep-frying potatoes in rapeseed oil, LM-PAHs and HM-PAHs accounted for 94.2% and 5.8% of all PAHs found, respectively. HM-PAH proportions found among all of the PAHs increased with increasing deep-frying time, although not for the rapeseed oil used to deep-fry chicken nuggets, for which HM-PAHs were not detected. Longer deep-frying periods resulted in increased PAH concentrations, possibly due to the repeated use of oil for deep- frying. This higher HM-PAH content level suggests that oil that has been deep-fried for a long time is more hazardous to human health than fresh oil. Regardless of the type of oil used, the percentages of HM-PAHs in total PAH from deep-fried potatoes were higher than those found for the deep-fried chicken nuggets. Therefore, food types also influence HM-PAH production.

SERIE F – TRACCIA 3

1. Introduction

It has been widely recognized that food protein-derived peptides are attracting increased interest due to their safety and multi-functional properties such as antioxidant, antibacterial, anti-tumor, antihypertensive, etc. These bioactive peptides are obtained from plant proteins like soy, peanut, corn and rice, as well as animal protein sources such as fish, milk, egg and chicken skin. Antihypertensive peptides have been shown to work through various mechanisms; however, modulation of the renin-angiotensin system (RAS) remains a key route. The physiological operation of RAS is maintained by two main enzymes, renin and angiotensin converting enzyme (ACE). The rate determining step involves renin-dependent proteolysis of angiotensinogen to produce an inactive angiotensin I (Ang I); ACE then catalyzes conversion of Ang I to form angiotensin II (Ang II), a potent vasoconstrictor. Under homeostatic conditions, Ang I and Ang II levels are maintained at optimal levels, which ensure balanced contractions and relaxations of blood vessels to maintain normal blood pressure. However, during metabolic disorders or under disease conditions, there is an excessive activity of renin and/or ACE, which leads to high blood levels of Ang II and a tendency towards increased higher rate of blood vessel contraction with less relaxation. This imbalance in blood vessel contraction and relaxation is one of the agents responsible for high blood pressure and the pathological condition of hypertension. Therapeutic management of hypertension has been traditionally carried out through the use of ACE or renin-inhibitory drugs but the occurrence of negative side effects has shifted attention to other potentially safer agents such as food protein-derived peptides There is already a copious amount of information on the production of antihypertensive food protein-derived peptides, but there is need for information on effective fractionation and peptide enrichment protocols. Following food protein hydrolysis, peptides with different charges and molecular weights are released to form the protein hydrolysate. The current approach towards obtaining high purity active peptide fractions is to combine several modern column chromatography separations, which can be time-consuming. Moreover, peptide column yields can be low and hence costly to operate. On the other hand, pressure-driven membrane separation technologies can be used to achieve large-scale bioactive peptides separation on the basis of differences in molecular weights. However, membrane separation is relatively poor for effective enrichment of target peptides with similar molecular weight due to its lower selectivity. In addition, high-throughput membrane separation can become regularly blocked, which causes reduced fractionation efficiency as a result of membrane fouling after repeated use. Electrodialysis with ultrafiltration (EDUF) membranes separation technology driven by electric field force is a useful invention for the large-scale separation of bioactive compounds. EDUF can be used to selectively separate peptides by setting the appropriate electric field, pH and ultrafiltration membranes according to the isoelectric point and molecular weight of the tar- get peptides, and has been tested for antibacterial, antioxidant, and antihypertensive peptide separations. Previous works have shown the blood pressure-reducing properties of rapeseed protein hydrolysates and peptides separated by laborious column chromatography techniques. However, information on potential enrichment of the rapeseed antihypertensive peptides by EDUF is not available.

2. Materials and methods OMISSIS



3. Results and discussion

- Membrane fouling evaluation. Changes in the ultrafiltration membrane conductivity and thickness are effective indicators to evaluate membrane fouling during the ultrafiltration process. Membrane conductivity values before and after 3 EDUF runs were similar with no significant difference ($p > 0.05$). Membrane thicknesses were also measured but there was no significant difference. Consequently, according to the evolution of conductivity and thickness values, no fouling was observed, indicating the integrity and efficiency of the membranes were not decreased after a total 18 h (3 consecutive runs of 6 h each) of rapeseed peptide separation by the EDUF technology.

- Peptide migration. Peptide migration is an important parameter to evaluate the efficiency of the EDUF selectivity in separating the target rapeseed peptides; this was directly monitored by determining peptide concentration in each compartment during the EDUF. At pH 7.0, the peptide concentration increased linearly with time for the recovery compartment KCl 1 and KCl 2, while that of FRPH (final or residual protein hydrolysate) compartment decreased gradually. This observation confirmed that rapeseed peptides indeed migrated to the electrodes driven by the electric field force, and was consistent with previous reports on peptide separation by EDUF. The results indicate that the original rapeseed hydrolysate contained a greater concentration of anionic peptides than cationic peptides at approximately pH 7.0. The higher migration tendency of anionic peptides is in accordance with results obtained by during separation of snow crab by-products hydrolysate by EDUF at pH 9.0. However, reported a higher cationic flaxseed peptides migration with a final concentration of 73.2 ± 4.5 lg/ml, at pH 7.0 when com- pared to the 55.2 ± 6.7

hydrolysis, which generated higher levels of cationic peptides that contain lysine and arginine. One of the advantages of EDUF is the ability to influence the type and quantity of peptides during migration by simple pH adjustment. For example, the migrations of snow crab peptides to the anode were found to be 52.9 lg/ mL, 152.8 lg/mL and 285.1 lg/mL at pH 3.0, 6.0 and 9.0, respectively. Therefore, further optimization of the EDUF process could enhance rapeseed peptides migration through pH adjustments. Based on the peptide concentrations, the entire EDUF process (n = 3) yielded 762 mg (2.54% of the initial hydrolysate feed) and 573 mg (1.91% of the initial hydrolysate feed) of the anionic (KCl 1) and cationic (KCl 2) peptides, respectively.

- **Amino acid profiles.** At the pH 7.0 separation condition, the total amount of positively charged amino acids (arginine, lysine and histidine) tended to be more in the cationic KCl 2 fraction (29.03%) than in the anionic KCl 1 fraction (5.80%). In contrast, negatively charged amino acids (Asx and Glx) tended to be more concentrated in the KCl 1 (43.57%) when compared to the KCl 2 fraction (17.95%). The results are consistent with expected peptide migrations to oppositely charged electrodes and similar to previous reports. In addition to arginine, the KCl 2 fraction also had higher levels of proline and hydrophobic amino acids when compared to KCl 1, which may contribute to in vitro ACE and renin-inhibitory properties. Overall, the essential amino acid content of KCl 2 tended to be higher than that of KCl 1, which indicates potential use of the EDUF technology to also improve nutritional profile of protein hydrolysates.

- **Peptide profiles.** Rapeseed peptides in the recovery compartments were identified according to the observed total ion chromatogram and MS/ MS spectroscopy data. The deduced peptide sequences were matched to the primary structure of rapeseed proteins, Cruciferin (P33525) and Napin (Q42469), which are available from the Uni- Prot protein database. There were 11 and 7 low molecular weight (Mr < 1000) peptide sequences identified as present in the respective anionic (KCl 1) and cationic (KCl 2) fractions. The results confirm the presence of high levels of low molecular weight peptides in the ORPH (original rapeseed protein hydrolysates). In a similar work, Doyen et al. (2012) reported that peptides in Mr. 300–600 range were the most abundant in snow crab hydrolysate fractions obtained after EDUF. Likewise, low molecular weight anticancer snow crab protein-derived peptides (Mr < 600) were recovered in the KCl 1 and KCl 2 fractions. In addition, the peptide net charges at pH 7.0 corresponded to their electrode migration, with the exception of zero net charge peptides such as NS, TF and LY. The pI values of NS, TF and LY are close to pH 7.0; therefore, minor pH fluctuations during EDUF may have conferred temporary negative charges and led to the observed anode migration. LY, TF, and RALP were the main antihypertensive peptides in rapeseed protein hydrolysate. The recovery of these three peptides in the KCl 1 and KCl 2 compartments confirms that EDUF as a useful technology to selectively separate and concentrate target peptides based on their molecular weight and charge properties.

- **ACE and renin inhibition of rapeseed protein hydrolysates after EDUF separation.** ACE-inhibitory activities of ORPH, FRPH, anionic peptides and cationic peptides are all concentration- dependent within the 0–0.5 mg/mL range. ACE-inhibitory activities of fraction KCl 1 and KCl 2 were significantly ($p < 0.05$) weaker (higher IC_{50} values) than that of ORPH and FRPH. The results suggest that the synergistic effects of peptides in ORPH and FRPH were probably higher than in the KCl 1 and KCl 2 fractions. ORPH and FRPH had similar amino acid composition, which may have contributed to their similar ACE-inhibitory activities. ACE-inhibitory activity of the cationic peptides was significantly ($p < 0.05$) stronger (lower IC_{50} value) than that of anionic peptides, suggesting that the higher abundance of proline in combination with higher levels of histidine, lysine and arginine in fraction KCl 2 may have been contributing factors. The protein hydrolysate samples also possessed concentration-dependent renin-inhibitory activities at 0–2.0 mg/mL. The results are higher than the 44.5% renin-inhibitory activity (at 7.4 mg/mL) that was reported for EDUF-separated flaxseed protein hydrolysate. The IC_{50} values of FRPH and cationic peptides were significantly ($p < 0.05$) lower than that of the original hydrolysate, indicating that the selective EDUF separation indeed improved in vitro renin inhibition by the rapeseed protein hydrolysates. Moreover, the renin-inhibitory activity of cationic peptides was significantly ($p < 0.05$) higher than that of anionic peptides, which indicates that hydrophobic amino acids and positively-charged amino acids are important contributing factors. The stronger renin-inhibitory activity of KCl 2 fraction may also be due to the presence of RALP, which we have previously shown to be the most potent with an IC_{50} value of 0.97 mol/L when compared to the 1.87 mol/L and 3.1 mol/L for LY and TF, respectively.

-Antihypertensive activity of rapeseed protein hydrolysates. All the samples displayed excellent BP- reducing effects after 6 h of oral administration when compared to the PBS. ORPH and FRPH (100 mg/kg bw) produced significantly ($p < 0.05$) better decreases in SBP (systolic blood pressure) by about 47 and 51 mmHg, respectively 6h after of oral administration. Interestingly, the FRPH was the most effective SBP-reducing agent, which may be due to the dual strong inhibitory effects against ACE and renin activities as shown by the IC_{50} values. The ORPH also had strong dual inhibitory effects against ACE and renin, which may have contributed to better SBP-reducing ability when compared to KCl 1 and KCl 2 fractions. There seems to be a direct correlation between observed ACE and renin inhibitory properties and SBP-lowering ability of the KCl 1 and KCl 2 peptide fractions. This is evident in the significantly ($p < 0.05$) higher SBP-lowering effect of KCl 2 (38 and 40 mmHg after 4 and 6 h, respectively), which also had stronger (lower IC_{50} values) ACE and renin inhibitions than KCl 1. The 100 mg/kg bw dose used for the SHR study will be equivalent to $1.15 g daily dose for a 70 kg human being in order to maintain effective blood pressure reduction. The SBP-reducing effects of peptides used in this work are better than those reported for flax-seed protein hydrolysates (200 mg/kg bw) separated by EDUF which reduced SBP of SHRs by about 18 and 12 mmHg, respectively at 2 and 4 h and 28 mmHg at 4–6 h.

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Concorso pubblico per titoli ed esami per l'assunzione con contratto di lavoro a tempo pieno e indeterminato di n.5 unità di personale con profilo di Ricercatore – III livello professionale - presso strutture del Consiglio Nazionale delle Ricerche

PROVA SCRITTA A CONTENUTO TEORICO-PRATICO DEL 27 MAGGIO 2019 – ORE 09.30

SERIE A

Il candidato sviluppi la proposta sintetica di un progetto, con un finanziamento di 1 milione di euro e per una durata di 2 anni, a scelta tra una delle tre tematiche qui di seguito tracciate:

A1) Microbiome applications for healthy nutrition and sustainable food systems
A2) Natural ingredients as alternatives to synthetic food preservatives
A3) Traceability and authenticity control for Italian food products

La proposta, scritta in lingua italiana o inglese, con un limite massimo di 4 facciate di foglio protocollo, ha anche l'obiettivo di saggiare la capacità di sintesi del candidato sui 5 aspetti del progetto che dovranno essere considerati, cioè:

1) Objectives
   Describe the overall and specific objectives for the project, which should be clear, measurable, realistic and achievable within the duration of the project. Objectives should be consistent with the expected impact of the project.
2) Relation to challenge and scope
   Explain the specific challenge and scope your proposal addresses.
3) Concept and methodology
   (a) Concept: describe and explain the overall concept underpinning the project. Describe the main ideas, models or assumptions involved. Describe the positioning of the project e.g. where it is situated in the spectrum from 'idea to application', or from 'lab to market'. Refer to Technology Readiness Levels where relevant.
   (b) Methodology: describe and explain the overall methodology, distinguishing, as appropriate, activities for research, demonstration, piloting, first market replication, etc.
4) Ambition
   Describe the advance your proposal would provide beyond the state-of-the-art, and the extent the proposed work is ambitious.
   Describe the innovation potential (e.g. ground-breaking objectives, novel concepts and approaches, new products, services or business and organisational models) which the proposal represents. Where relevant, refer to products and services already available on the market. Please refer to the results of any patent search carried out.
5) Expected impacts
   Please be specific and provide only information that applies to the objectives. Wherever possible, use quantified indicators and targets.
   Describe how your project will contribute to expected impacts that would enhance innovation capacity, create new market opportunities, strengthen competitiveness and growth of companies, address issues related to climate change or the environment, or bring other important benefits for society.
   Describe any barriers/obstacles, and any framework conditions (such as regulation, standards, public acceptance, workforce considerations, financing of follow-up steps, cooperation of other links in the value chain), that may determine whether and to what extent the expected impacts will be achieved.

BANDO N. 368.32 RIC - Area strategica Produzioni alimentari e Alimentazione

Concorso pubblico per titoli ed esami per l'assunzione con contratto di lavoro a tempo pieno e indeterminato di n.5 unità di personale con profilo di Ricercatore – III livello professionale - presso strutture del Consiglio Nazionale delle Ricerche

PROVA SCRITTA A CONTENUTO TEORICO-PRATICO DEL 27 MAGGIO 2019 – ORE 09.30

SERIE B

Il candidato sviluppi la proposta sintetica di un progetto, con un finanziamento di 1 milione di euro e per una durata di 2 anni, a scelta tra una delle tre tematiche qui di seguito tracciate:

B1) Innovative solutions for personalised nutrition
B2) Strategies for valorisation of fruit and vegetable by-products as sources of high value ingredients for food
B3) Emerging technologies for food-quality control

La proposta, scritta in lingua italiana o inglese, con un limite massimo di 4 facciate di foglio protocollo, ha anche l'obiettivo di saggiare la capacità di sintesi del candidato sui 5 aspetti del progetto che dovranno essere considerati, cioè:

1) Objectives
   Describe the overall and specific objectives for the project, which should be clear, measurable, realistic and achievable within the duration of the project. Objectives should be consistent with the expected impact of the project.
2) Relation to challenge and scope
   Explain the specific challenge and scope your proposal addresses.
3) Concept and methodology
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   (b) Methodology: describe and explain the overall methodology, distinguishing, as appropriate, activities for research, demonstration, piloting, first market replication, etc.
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BANDO N. 368.32 RIC - Area strategica Produzioni alimentari e Alimentazione

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PROVA SCRITTA A CONTENUTO TEORICO-PRATICO DEL 27 MAGGIO 2019 – ORE 09.30

SERIE C

Il candidato sviluppi la proposta sintetica di un progetto, con un finanziamento di 1 milione di euro e per una durata di 2 anni, a scelta tra una delle tre tematiche qui di seguito tracciate:

C1) Alternative sources of macronutrients for food and/or feeds
C2) Innovative solutions for sustainable food systems
C3) Emerging technologies for food processing and/or packaging

La proposta, scritta in lingua italiana o inglese, con un limite massimo di 4 facciate di foglio protocollo, ha anche l'obiettivo di saggiare la capacità di sintesi del candidato sui 5 aspetti del progetto che dovranno essere considerati, cioè:

1) Objectives
   Describe the overall and specific objectives for the project, which should be clear, measurable, realistic and achievable within the duration of the project. Objectives should be consistent with the expected impact of the project.
2) Relation to challenge and scope
   Explain the specific challenge and scope your proposal addresses.
3) Concept and methodology
   (a) Concept: describe and explain the overall concept underpinning the project. Describe the main ideas, models or assumptions involved. Describe the positioning of the project e.g. where it is situated in the spectrum from 'idea to application', or from 'lab to market'. Refer to Technology Readiness Levels where relevant.
   (b) Methodology: describe and explain the overall methodology, distinguishing, as appropriate, activities for research, demonstration, piloting, first market replication, etc.
4) Ambition
   Describe the advance your proposal would provide beyond the state-of-the-art, and the extent the proposed work is ambitious.
   Describe the innovation potential (e.g. ground-breaking objectives, novel concepts and approaches, new products, services or business and organisational models) which the proposal represents. Where relevant, refer to products and services already available on the market. Please refer to the results of any patent search carried out.
5) Expected impacts
   Please be specific and provide only information that applies to the objectives. Wherever possible, use quantified indicators and targets.
   Describe how your project will contribute to expected impacts that would enhance innovation capacity, create new market opportunities, strengthen competitiveness and growth of companies, address issues related to climate change or the environment, or bring other important benefits for society.
   Describe any barriers/obstacles, and any framework conditions (such as regulation, standards, public acceptance, workforce considerations, financing of follow-up steps, cooperation of other links in the value chain), that may determine whether and to what extent the expected impacts will be achieved.