Peptan®
Collagen peptides for a healthy lifestyle

The building blocks for innovative products

With recognized health benefits supported by scientific studies and the widest range of origins and options, Peptan® is the premier bioactive protein. Odorless, flavorless and colorless, it is the ideal ingredient for functional foods, beverages and supplements. Peptan® is manufactured in France and Brazil by Rousselot, a world leader in collagen peptides.
AlgeaFood Phyto for shaping your waistline

In Norway, beyond the Arctic Circle, in one of the purest habitats in the world, we have been harvesting and processing seaweed with great passion for over 75 years. We harvest this Arctic alga following a strict process to preserve its quality, with tools and technologies advanced, that can ensure minimal noise and low environmental impact. From this expertise, AlgeaFood is born, the line of food ingredients which meets our body’s many needs.

AlgeaFood Phyto is one of these ingredients, a phytocomplex, from pure Ascophyllum nodosum with a natural content of marine active compounds. It is rich in anti-oxidants and algal carotenoids such as phlorotannins, high-quality polyphenols and fucoidans that can help to boost your product in reshaping the silhouette, reducing waist circumference and, moreover, help to maintain normal level of triglycerides.

We tested the efficacy of our ingredient with a clinical test (randomized double blind study active versus placebo following cross-over design), an efficacy trial and other tests, write us to have more information on tests. As well as efficacy test of our ingredients, we also ran several tests including functional foods and supplements, getting really positive results.
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Calorie reduction & well-being

ID-alG™ is a brown seaweed extract supported by clinical, in vivo and in vitro studies. ID-alG™ is a brown seaweed extract rich in long chain polyphenols (phlorotannins) that block the activity of the two main digestive enzymes: up to 54% inhibition of the lipase activity & up to 58% inhibition of the amylase activity.

Clinical and in vivo studies have showed the following results:
• 2.8kg average weight loss (moderate BMI of 25 to 30)
• Weight loss is directly correlated to fat mass reduction
• No side effect on transaminase

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Hitting the mark in the functional food market with collagen peptides

Today’s consumers are becoming increasingly proactive in looking after their general well-being and interested in the many benefits nutritionally enhanced foods and drinks can provide. To tap into these growing opportunities, manufacturers are looking for cost-effective and appealing ingredients which offer proven health benefits. Delivering a wide range of scientifically varied benefits, collagen peptides are an example of such a solution.

Collagen: the body’s building block

Representing almost 30 per cent of human protein content, collagen ensures the cohesion, elasticity and regeneration of skin, cartilage and bones.

Collagen peptides are the hydrolysed form of collagen. Manufactured using a gentle enzymatic process, they offer a unique combination of key amino acids which provide specific nutritional properties not found in any other protein source. Highly digestible and bioavailable, 90 per cent of hydrolysates are available within the connective tissues just a few hours following ingestion. This ensures the efficient delivery of essential peptides and amino acids to the body and stimulates the endogenous collagen synthesis.

Rousselot’s Peptan® is the most extensively researched range of collagen peptides available on the market. The proven efficacy of the range is supported by an impressive body of scientific evidence.

Beautiful, younger looking skin

Collagen is the main structural element of skin, accounting for between 70 and 80 per cent of its dry weight. It provides a support structure that ensures skin elasticity, suppleness and hydration. Collagen fibres, which are constructed within fibroblast cells, are responsible for the maintenance and resistance of skin tissues. As we age, collagen synthesis decreases and less new collagen fibres are produced. Collagen in the skin also becomes more cross-linked and fragmented. This results in tougher, drier skin and the formation of wrinkles.

Exogenous collagen peptides may act to stimulate fibroblast cell production and increase fibroblast density, promoting younger-looking and suppler skin. A daily dosage of Peptan collagen peptides can increase skin hydration by 28 per cent after eight weeks and suppleness by 19 per cent after 12 weeks. A recent clinical study performed by COSderma in France has also demonstrated the effectiveness of Peptan in restructuring and regeneration of skin collagen.

Joint function

Collagen fibres make up between 70 and 95 per cent of cartilage. Following ingestion, collagen peptides rapidly accumulate in cartilage and promote endogenous collagen synthesis. This process can act to reduce the cartilage degrading effects of osteoarthritis.

A 2013 clinical trial found that an 8g daily dose of Peptan can significantly lower joint pain and increase joint function and flexibility. The improvements were recorded after three months of intake and further enhanced after six months. Numerous additional clinical studies have shown similar effects and trials also demonstrated that subjects with severe joint deterioration benefit even further from the effects of collagen peptides.
Hitting the mark in the functional food market with collagen peptides

Dense and strong bones
Osteopenia, the pre-stage to Osteoporosis, is a condition in which bone mineral density is lower than normal levels. Early diagnosis and treatment has been demonstrated to improve quality of life.

Collagen is a key bone component, responsible for the structural framework upon which minerals are deposited. Collagen peptides have been shown to stimulate the production of collagen by bone cells, leading to higher levels of new bone tissue formation. Additionally, in vivo studies have demonstrated that Peptan dietary supplementation improves bone metabolism and promotes denser, stronger bones. It can also be combined with calcium and vitamin D to offer further benefits.

Combating muscle deterioration
The consumption of adequate dietary protein is crucial for counteracting the effects of sarcopenia – the loss of lean muscle mass and strength with age. An international expert panel has suggested elderly people can benefit from increased protein, recommending an intake of 1.2 g/kg per day – often higher than the typical diet of an older person.

Collagen peptides are an easily digestible protein source which maintains nitrogen balance well in the elderly. It may therefore preserve lean body mass better than equivalent doses of whey protein when a diet has relatively low protein content.

Optimal nutritional support
The most satiating macronutrient, proteins can play an important role in supporting weight management programmes. Collagen peptides in particular have been found to have a more effective satiating effect than other common protein sources, such as casein, soya or whey.

Multiple studies have also been published on the benefits of bioactive proteins – and collagen peptides in particular – for sports performance and recovery. An effective supplement for maintaining and restoring protein content in muscles following exercise, research has also identified that collagen peptides may support creatine synthesis in the body. This organic acid aids muscular contraction during periods of high intensity exercise.

Added appeal
Imparting no distortion in the taste or odour of finished products, Peptan can be easily and cost-effectively incorporated into a wide range of functional foods, beverages, and nutraceuticals. The 100 per cent natural range meets the highest international quality standards and is free from any preservatives or additives.

Conclusion
Demand for solutions which support healthier and more active lifestyles is continuing to grow. Manufacturers are therefore searching for ingredients which can help them deliver scientifically endorsed and appealing products. Collagen peptides are the perfect choice for creating innovative solutions which satisfy the requirements of health aware consumers.

*Study references available upon request

Rousselot® is a leading manufacturer of gelatine and collagen peptides to the food, pharmaceutical and technical industries. With a staff of 2,400 people, the company benefits from a global sales and production network of 13 plants and 10 sales offices located in Europe, North America, South America and Asia. Rousselot is a brand of Darling Ingredients Inc.

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Food Labelling: Underutilised opportunities to improve public health

In the last few years food labelling legislation has been revised drastically in the European Union (EU) and we are quickly approaching December 2014 when most articles of Regulation (EU) No 1169/2011 on the provision of food information to consumers (FIR) (1) will start to apply. At a time when the food industry is dealing with labelling challenges and chasing regulation deadlines, I welcome the opportunity to discuss the importance of the existing food labelling regulations from a public health perspective.

General food labelling

Key changes in the area of general food labelling include highlighting allergens on the list of ingredients of pre-packed foods, and extending the mandatory labelling of allergens to foods that are not pre-packed; ensuring better legibility; and the mandatory labelling of the origin of some unprocessed meats. The compulsory labelling of a nutrition declaration on all processed pre-packed foods will also be introduced in December 2016.

Nutrition labelling of pre-packed foods is a cost-effective population-level intervention with unparalleled reach (2), enabling consumers to be informed about the composition of foods and helping them make informed choices. An audit of over 37,000 food products in EU countries revealed that, on average, 85% of the products were labelled with nutrition labelling or related information, although significant differences were observed between the countries (3). For example, the penetration of nutrition information on food labels was 97% in Ireland and only 70% in Slovenia. From a public health perspective, the introduction of mandatory nutrition information will ensure that consumers in all EU countries will have equal opportunities for informed food choices. However, to utilise the potential governments will need to further explore the formats and different types of information content to ensure that nutrition information is accessible and understandable (2). There are different approaches to achieving this, including the introduction of a standardised graphic presentation of a nutrition declaration (as practised in the United States) and various front-of-pack labelling schemes, such as multiple traffic light label, GDA and health symbols (4,5). However, despite considerable research on nutrition labelling, it has proven difficult to find a front-of-pack labelling system which is informative with regard to product healthfulness across various situations.

Labelling with nutrition and health claims

Consumers are sensitive to health-related communications and the use of nutrition and health claims is a convenient tool for marketing ‘healthy foods’. The use of health claims was harmonised in the EU in 2006 following the acceptance of Regulation [EC] 1924/2006 (6). Being aware of the challenges of this area, the European Commission (EC) supported cooperation and research projects within the Seventh Framework Programme (FP7), such as CLYMBOL and REDICLAIM. To support informed choice, promote healthy eating, and strengthen the competitiveness of the food industry, consumers must understand health claims and symbols correctly. Further, claims and symbols will only affect healthy eating if they impact consumer purchasing decisions in a healthier direction. The objective of CLYMBOL is to determine how health-related symbols and claims, in their context, are understood by consumers and how they affect purchasing and consumption taking into account both individual differences in needs, wants, motivation and attitude, as well as country-specific differences (7). On the other hand, REDICLAIM seeks to understand the main issues concerning the substantiation and use of health claims, and the level of awareness about legal obligations related to health claims among the relevant stakeholders (8). The project focuses on the substantiation process, health research and innovation in the food chain, and nutrition economic models to determine possible health impacts.
To avoid the situation in which the use of claims on foods could mask their overall nutritional composition and confuse consumers when trying to make healthier food choices, the legislation provides the introduction of nutrient profiles. This part of the legislation has not yet been implemented (9). While it is clear that introducing nutrient profiles would have a big impact on the food market, the last available response from the EC is that this issue is still on the agenda (9). Further research on nutrient profile models is needed to provide a reliable and operative system for the classification of foods based on their nutrient composition, but the final decision here is likely to be a political one.

Botanicals

Another major problem with health claims concerns botanicals – plant and herbal substances with a long tradition of being used in both food and medicine (10). As a medicinal product, these can be registered using a simplified traditional use registration and sold as a Traditional Herbal Medicinal Product (THMP) without the requirement for clinical trials on the product’s effectiveness. On the contrary, when botanicals are put on the market as foods or food supplements, any health claims must be substantiated by scientific evidence of the highest possible standard. In 2010, the EC decided it was not possible to continue assessing health claims for botanicals and the European Food Safety Authority (EFSA) was asked to discontinue its assessment of claims for botanicals with the result that these, together with a number of already assessed botanicals, have been put on hold (10). This decision has enabled the further use of almost 2,000 unauthorised health claims for botanicals. While some of those claims might actually be scientifically substantiated, this is generally not the case. The sanctioning of the use of such unsubstantiated health claims poses a problem for the authorities in most member states, putting botanicals in a privileged position over other food ingredients without authorised claims. A prompt decision on this issue is needed to assure a high level of consumer protection and the effective functioning of the EU market. A recent opinion of the EFSA indicates what further evaluations might look like. In the opinion, a cause-and-effect relationship was confirmed between the consumption of hydroxyanthracene derivatives (from the root and rhizome of Rheum palmatum L. and from other plant sources) and an improvement in bowel function (11). What distinguishes this opinion is the use of EMA and WHO monographs as a main source to support the “well established effect” of hydroxyanthracene derivatives on bowel function and to propose conditions of use (12). However, this opinion raised a series of safety-related issues between member states and the question arises of whether the claim will in fact be authorised. There are currently big differences among the member states concerning the safety assessment and classification of products containing botanicals. Harmonising this area on the EU level would be very beneficial not simply from a public health perspective, but also to ensure a single market and the free movement of goods.

REFERENCES AND NOTES

[1] Regulation (EU) No 1169/2011 on the provision of food information to consumers (FIR)
[7] CLYMBOL: Role of health-related claims and symbols in consumer behaviour. URL: www.clymbol.eu
[8] REDICLAIM: Understanding the impact of legislation on “Reduction of Disease risk” CLAIMs on food and drinks. URL: www.rediclaim.eu
Which protein is the best fit for recovery drinks for recreational athletes?

Based on casein protein versus whey protein; both protein sources have a high quality both protein sources have a high quality. Whey protein has a slightly higher leucine content but casein has a slightly higher PDCAAS.

Evening work-out
Most recreational athletes have their work-out in the evening and have at least 12 hours to recover for their next training, with a night of sleep in between. So what protein source fits best? It is widely described that casein protein acts as a slow protein. The amino acids will be appearing in the blood more slowly, but the response lasts longer than with fast proteins like whey (1). When comparing a plant based protein such as soy to milk, Wilkinson (2) found that milk (80% casein) was better able to increase muscle protein synthesis. He suggests this could be because milk proteins provide a slower pattern of amino acid delivery which ultimately leads to differences in muscle protein synthesis.

This slow absorption of casein could be an advantage for longer recovery periods. Two studies show the muscle protein synthesis (MPS) during the night with elderly (3) and athletes (4). Both found an increase in MPS after the ingestion of casein. Multiple studies show muscle protein synthesis increases most after ingestion of whey protein (5). But most studies (including Tang, Figure 1, only measure the effect on MPS during the first 180 minutes after exercise.

A study which did research a longer period after exercise (6) did not find a difference between casein or whey protein 300 minutes after resistance exercise. A more recent study(7) Figure 2 did not find a significant difference in MPS, 6 hours after exercise and ingestion of 20 grams of whey protein or caseinate. So the longer the recovery period, the less differences are shown. When you add to this, the benefit that casein provides better nitrogen
retention and utilization (8), casein comes very close to whey protein for the recreationally active athlete. When building a tasteful high protein drink it is not necessary to compromise on nutritional quality. Studies show that the longer the recovery period, the less differences in muscle protein synthesis occur. To make the best high protein drink, both from functional and nutritional perspective, casein is definitely something to consider.

References


5) Tang JE, Moore DR, Kujbida GW, Tarnopolsky MA, Phillips SM. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest.


Observers could say one of the world’s largest dairy ingredient groups is “getting fit” – all to help global food and beverage processors create the next generation of performance and lifestyle nutrition foods. “Although people always have exercised, more and more—at all ages—are taking sport to a higher level and aspire to be top athletes,” says Ramon Mommersteeg, a FrieslandCampina DMV marketer. “Another group has become convinced that making smart decisions about weight management and lifestyle will make a real difference to their health and happiness.”

DMV’s Performance and Lifestyle Nutrition market team includes 12 professionals, including a mix of scientists and nutritionists and market trend researchers. They help customers integrate dairy ingredients with recipe development and market intelligence—all to create new foods or beverages targeting sports nutrition, weight management and/or healthy aging.

Royal FrieslandCampina

Every day Royal FrieslandCampina provides around 1 billion consumers all over the world with food that is rich in valuable nutrients. With annual revenue of 11.4 billion euro FrieslandCampina is one of the world’s five largest dairy companies.

FrieslandCampina supplies consumer products such as dairy-based beverages, infant nutrition, cheese and desserts in over 100 countries worldwide. FrieslandCampina also supplies ingredients and half-finished products to manufacturers of infant nutrition, the food and nutrition industry and the pharmaceutical sector around the world. FrieslandCampina has offices in 28 countries and employs a total of 21,186 people.
Effects of anthocyanins as nutraceuticals

KEYWORDS: anthocyanins, nutraceutical, neuro-protective, cardio-vascular protective, anti-inflammatory, hepatic health

Abstract

Anthocyanins are bioactive compounds with strong nutraceutical potential. They are a widely distributed class of flavonoids and can be defined as glycosides of anthocyanidins. Anthocyanins are usually present as pigmented compounds in fruits and vegetables including cherries, plums, strawberries, raspberries, blackberries, grapes, redcurrants, blackcurrants, vegetable roots, legumes and cereals. There are various reports about the biological properties and nutraceutical potential of anthocyanins. This review discusses some recent reports on the neuro-protective effects, cardio-vascular benefits, liver health improvement and anti-inflammatory effects associated with the consumption of anthocyanins compounds, either in pure or complex forms.

INTRODUCTION

It is a well established fact that diet has direct relationship with the health and most of the health promoting effects is associated with different kinds of nutrients and bioactive compounds present in fruits and vegetables. The magnitude of different types of chronic diseases that may be related to age including cardiovascular problems, neurological disorders, diabetes, and cancers is increasing. These facts lead the researchers and health practitioners to explore and recommend use of plant derived health promoting foods. The ability of plant derived products in reducing or preventing chronic health diseases is linked mostly to their non-nutrient secondary metabolites or phytochemicals which have been studied to exert wide range of actions in living organisms. The phytochemicals may not be as quick in action as the synthetic pharmaceuticals however a long term use can significantly improve the health of consumers. These include sulphur containing compounds, terpenoids (carotenoids, monoterpenes, and phytoesters), and different polyphenolic groups (anthocyanins, flavones, flavan-3-ols, isoflavones, stilbenoids, eicagic acid, etc.) (1). Consumers these days look for products in the market that contain bioactive compounds or extracts derived from plant or natural sources. Such products have close affiliation with the pharmaceuticals and hence cannot be classified simply as “food” instead a new term that carries impression of being nutrients as well as pharmaceuticals, ‘nutraceuticals’, has been used for the identification of this class of product. Nutraceuticals can be referred as supplements to normal diet and carry a bioactive agent derived or originated from a food, and usually contained in a non-food matrix. The purpose is to deliver certain kinds of bioactive compounds in quantities much higher than that obtained in regular food material. The objective of this review is to focus on up to date available information on anthocyanins from plant sources with relation to their biological effects and applications as nutraceuticals. Anthocyanins have been studied to possess different biological functions such as antioxidants, anti-inflammatory, antimicrobial and anti-carcinogenic activities. They have also been associated with vision enhancement, induction of apoptosis and neuro-protective effects (2). The objective of this review was to collect most resent information on structure, sources and health benefits imparted by anthocyanins.

STRUCTURE OF ANTHOCYANINS

Chemically anthocyanins are glycosides of anthocyanidins and usually present in pigmented compounds in fruits and vegetables. There are complex glycosylation patterns which result in many type of anthocyanins compounds however only some of the aglycones (anthocyanidins) types have been characterized. Anthocyanins are a widely distributed class of flavonoid compounds that are water-soluble and nontoxic pigments and being studied extensively because of their biological or antioxidant properties (3). The most common anthocyanins are based on six anthocyanidins that include cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin (4) and their structures are presented in Figure 1.
The position and amount of hydroxylation and methoxylation in the B ring can be regarded as crucial for biological properties of anthocyanins (3). Delphinidin that contained three hydroxylations in the B ring possessed a higher antioxidant activity whereas the pelargonin had the lowest antioxidant activity among six common anthocyanidins. The pattern of glycosylation in anthocyanins can also affect their biological properties (5).

**NUTRACEUTICAL EFFECTS OF ANTHOCYANINS**

Anthocyanins are regarded as the most important types of flavonoids in plant based foods due to their strong potential as antioxidant and other valuable physicochemical and biological properties. These are primarily responsible for colour in leaves, stems, flowers, roots and fruits of different plant sources (6).

**SOURCES OF ANTHOCYANINS**

There are different plant sources of anthocyanins and a summarized list of some of these sources and food products based on such sources is presented in Table 1 along with approximate proportion of anthocyanins in each case. Almost 600 anthocyanins compounds have been isolated and identified from different plant sources including fruits, vegetables, roots, legumes and cereals (4). There may also be many other sources of anthocyanins which have been either identified or need yet to be qualified. The consumption of natural sources rich in anthocyanins is important for its health benefits. Populations not aware about the nutraceutical potential of this particular phytochemical can also get its benefits due to presence of anthocyanins in routine human diet. The estimated consumption on daily basis is from 3 to 215 mg (6). The daily anthocyanins consumption was studied in Europe by Zamora-Ros et al. (7) and it was observed that total anthocyanins consumption by men was 19.3 to 64.88 mg/day, whereas it was 18.73 to 44.08 mg/day for women. The daily anthocyanins consumption in USA has been reported to be 180–215 mg/day. A recent study however showed an even lower consumption range from around 3 to 15 up to 150 mg/day in USA (4). It is recommendable therefore to increase the consumption of foods rich in anthocyanins in order to prevent certain chronic disorders which are being discussed in subsequent sections.

<table>
<thead>
<tr>
<th>Food material</th>
<th>Scientific names of plants</th>
<th>Anthocyanins contents (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apples red delicious with peel</td>
<td>Matus domestica</td>
<td>4.59</td>
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<tr>
<td>Apples red delicious without peel</td>
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<td>Avocados</td>
<td>Persea americana</td>
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<td>Bananas</td>
<td>Musa paradisiaca</td>
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<td>Purple accal berries</td>
<td>Euterpe oleracea</td>
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<td>Blackberries</td>
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<td>Blueberries</td>
<td>Vaccinium myrtillus</td>
<td>163.3</td>
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<td>Cranberries</td>
<td>Vaccinium oxycoccos</td>
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<tr>
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<td>Red wine vinegar</td>
<td>-</td>
<td>0.66</td>
</tr>
<tr>
<td>Red table wine</td>
<td>-</td>
<td>19.27</td>
</tr>
<tr>
<td>White wine</td>
<td>-</td>
<td>0.06</td>
</tr>
<tr>
<td>Red dessert wine</td>
<td>-</td>
<td>109.29</td>
</tr>
</tbody>
</table>

Table 1. Anthocyanins contents of some commonly consumed foods and beverages (6).
plants which may depend on pH structural characteristics of these compounds (3). Anthocyanins are involved in important roles in plant–animal interactions and can be regarded as antioxidants, phytoalexins or plant’s chemical defense mechanism against different infections (8). Increased consumption of foods rich in anthocyanins has been shown to have potential health beneficial effects against different diseases such as cancer, aging, neurological diseases, inflammation, diabetes as well as reported to act as anti-bacterial agents (3).

**Neuro-protective effects**
Polyphenolic compounds have been associated with their free radicals scavenging ability; however the fact that their peak concentrations in the brain are lower than endogenous glutathione levels has led to their possible ability to reduce neuro-degeneration through additional protective mechanisms (9). Based on this very assumption the polyphenols have been observed to exhibit different neuro-protective activities other than their abilities to scavenge free radicals (10). The neuro-protective effects include reduction of oxidative stress via effects on mitochondrial respiratory chain function and increase in inflammatory responses linked to glial activation (11). Recent epidemiological findings suggest that the consumption of berries (e.g. blueberries, strawberries) rich in anthocyanins may reduce the risk of Parkinson’s disease (12). Brief descriptions of some of the recent examples of neuro-protective effects of anthocyanins in relation to the possible mechanisms are given in Table 2. Previous in vivo studies have shown that extracts from berries rich in anthocyanins can impart protection to brain function as a result of reduction in oxidative ischemic damage and memory enhancement (13). There was decrease in blood sugar levels in animals fed with anthocyanins and reduction in their body weight which means its ability to control diabetes and obesity (14). The rats which were fed with high quantity of lipids showed weight which means its ability to control diabetes and obesity (15). Neuro-protective effects include reduction of oxidative stress via effects on mitochondrial respiratory chain function and increase in inflammatory responses linked to glial activation (11).

**Cardio-vascular effects**
The protective effects of anthocyanins against cardio-vascular disease have been revealed in various studies. These types of protective effects depend on the structural nature of anthocyanins and the degree of polymerization (19). These effects may include reduction of oxidative stress, improvement of endothelial dysfunction, mediation of vasodilatation, anti-inflammatory effects related to cardiac system, metabolic effects related to heart health and decrease in hypertension as shown in Table 3 (6). The commercially available chokeberry extract consumption along with statin for a period of six week in cardio-vascular patients led to a significant decrease in levels of serum iso-prostanes and oxidized LDL; increase in the level of adiponectin and reduction in blood pressure (20). Herrera-Arellano et al. (21) studied that Hibiscus sabdariffa-based nutraceutical product, enriched with anthocyanins was able to cause significant reductions in blood pressure and plasma angiotensin converting enzyme activity in hypertensive patients.

**Hepatic health benefits**
The liver plays vital role in different metabolic and detoxification activities against different materials entering human body. Different factors such as toxic chemicals, alcohol intake and viral diseases result in liver damage and liver malfunction. Such diseases are common now days and become one of the important health concerns (22). Hou et al. (23) observed that anthocyanins-rich black rice bran extract containing cyanidin-3-glucoside (Cy-3-G) and peonidin-3-glucoside, can impart significant benefits on liver health, and that Cy-3-G was predominant anthocyanins in black rice bran exerting this effect. The antioxidant potential of anthocyanins was the main reason of black rice bran extract to exert hepatic health benefits. Naturally occurring anthocyanins in pigmented extract obtained from purple sweet potato was tested for its effectiveness in improving the fasting blood glucose level, glucose and insulin tolerance through reduction of ROS and restoration of glutathione (GSH) contents (24). This extract was observed to prevent the endoplasmic reticulum stress in livers of high fat diet treated mice. Anthocyanins restored, to a notable extent, the impairment of the insulin receptor substrate-1/ phosphoinositide 3 kinase/protein kinase B (Akt) insulin signaling in the livers of mice. In was concluded that anthocyanins from purple sweet potato can impart protection against high fat diet induced hepatic insulin resistance and the main mechanisms were reported to be the decrease in ROS level and resistance to ROS-mediated endoplasmic reticulum stress.

**Anti-inflammatory effects**
Inflammation can be regarded as a part of a complex series of physiological reactions to a

<table>
<thead>
<tr>
<th>Source</th>
<th>Neuro-protective effects</th>
<th>Reported by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthocyanins from grape</td>
<td>Anthocyanins treatment with seven days prevented memory impairment in rats caused by caused by scopolamin administration.</td>
<td>Gutierrez et al. (16)</td>
</tr>
<tr>
<td>skin</td>
<td>Anthocyanins were able to regulate cholinergic neurotransmission and restore the NaN+, K+ ATPase and Ca2+ ATPase activities.</td>
<td>Tremblay et al. (17)</td>
</tr>
<tr>
<td>Anthocyanins from blue</td>
<td>The anthocyanins showed neuro-protective effects in PC-12 cells in vitro against oxidative stress in a dose-dependent manner.</td>
<td>Im et al. (18)</td>
</tr>
<tr>
<td>berries</td>
<td>The major anthocyanins for their neuro-protective effects were identified as cyanidin 3-O- sambubioside, cyanidin 3-O-glucoside, cyanidin 3-O- xylosylrutinoside, and cyanidin 3-O-rutinoside in increasing order of amounts.</td>
<td>Im et al. (18)</td>
</tr>
<tr>
<td>Anthocyanins from ripe</td>
<td>Fruit extracts rich in anthocyanins showed neuro-protective activity and a number of individual anthocyanins interfered with retinene neurotoxicity.</td>
<td>Strathearn et al. (11)</td>
</tr>
<tr>
<td>Miquel (Rubus coreanus)</td>
<td>It was suggested that anthocyanins rich botanical extracts may alleviate neuro-degeneration in Parkinson’s disease via enhancement of mitochondrial function.</td>
<td>Strathearn et al. (11)</td>
</tr>
<tr>
<td>fruit</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Some recent examples of neuro-protective effects by anthocyanins.
leucocytes (27). It was further observed that these extracts from grapes significantly reduced arthritis scores and cachexia in rats, and such decrease was more prominent in rats which received continuous low doses over a longer period in comparison to those treated with high doses five times only. The ability of anthocyanins to reduce inflammation can also be related to their ability for treatment of gastric ulcer. In a recent study carried out by Kim et al. (28) in vitro and in vivo experiments were conducted to demonstrate the palliative effects of anthocyanins on gastric ulcer in rats (ulcer was induced by naproxen dosage). It was observed that ROS produced by naproxen were effectively reduced by anthocyanins treatment which relieved the oxidative stress. The treatment using anthocyanins also showed significant reduction in lipid peroxidation products and increments in the quantities of antioxidant enzymes which included catalase, superoxide dismutase, and glutathione peroxidase. It was concluded that gastric ulcer therapy using anthocyanins is an effective approach in rats. Decendit et al. (26) observed that malvidin-3-O-b glucoside which a major anthocyanins compounds in grape has no toxicity on human peripheral blood mononuclear cells. It reduced the transcription of genes that are responsible for encoding the inflammatory mediators. This was demonstrated by the inhibition of TNFα, IL1, IL-6 and iNOS-derived nitric oxide (NO) secretions from activated macrophages. Anthocyanins also significantly reduced the inflammatory cachexia and arthritic paw scores in rats at both therapeutic and preventive levels. It was also reported that malvidin-3-O-b glucoside has a strong potential to be considered as an anti-inflammatory agent through in vitro and in vivo, trials.

DIRECTIONS FOR ACTION

In general the quality of a nutraceutical is assessed by using different in vitro assays. However these assays may not be enough to elaborate the health benefits of a nutraceutical unless qualified using well designed in vivo assays. There are also labels on nutraceutical products that show recommended doses however the scientific evidence regarding such recommendations is still lacking. The daily recommended dose of dietary anthocyanins may vary from well above 100 mg and go up to 1000 mg. It is also essential that consumers are well aware about the risks associated with over dosage of such compounds. It is worth

<table>
<thead>
<tr>
<th>Main mechanisms</th>
<th>Effects induced by anthocyanins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic effects</td>
<td>Anthocyanins decreases LDL-cholesterol, increase HDL-cholesterol, prevent LDL oxidation, improve fecal expression of acidic and neutral steroids, change and improve the sugar and lipid metabolism, enhance insulin resistance, reduce NFkB levels, suppress iNOS and COX2 expression</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Prevention of oxidative stress by anthocyanins is accomplished by superoxide radicals and hydroxyl radicals scavenging, improvement of antioxidant enzyme activity, ROS reduction and NO reduction</td>
</tr>
<tr>
<td>Cardiomyocytes and the endothelium</td>
<td>Anthocyanins improves endothelial dysfunction and vasodilation</td>
</tr>
<tr>
<td>Anti-inflammatory effects</td>
<td>There is decrease in P-selection, MCP-1, TNF-α and IL-10 expression, reduction in VEGF and ICAM-1 expression on endothelial cells, reduction of VLA-4, CD40 and CD36 expression on monocytes as a result of consumption of anthocyanins</td>
</tr>
<tr>
<td>Anti-ischemic and hypertension effects</td>
<td>Anthocyanins decrease the magnitude of ischemia–reperfusion injury and reduce hypertension</td>
</tr>
</tbody>
</table>

Table 3. Health benefits of anthocyanins in relation to cardio-vascular protective effects (6).
mentioning that higher doses of such compounds may not be beneficial for increasing the activity or effects. The optimal quantities of specific polyphenolic compound required to yield certain amounts of specific metabolites for desired health effects, still need to be explored (1). The present review focused on some of the major health benefits associated with anthocyanins; however they may also be useful in prevention and cure of other diseases and in the manufacture of functional foods. The potential of these compounds as natural colorants for different food products also needs to be elaborated.

In spite of the knowledge accumulated in the recent few years, more scientific work is required on the nature and detection of possible anthocyanins derivatives formed in vivo: metabolites and breakdown products originated under physiological conditions or from the colonic micro-flora activity, as well as their tissue distribution. There may be also be many more health beneficial properties of anthocyanins which still need to be explored along with the discovery of new sources and best techniques for the optimal recovery of high quality anthocyanins from plant source. More efforts are needed in future to conduct systematic studies for development of nutraceutical products based on anthocyanins with easy availability, acceptability and faster benefits for improving human health.

ACKNOWLEDGEMENTS

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SODB reduced obesity markers in hamsters
Beneficial effects in hepatic and adipose tissue by modulating oxidative status

KEYWORDS: adipose tissue, antioxidant defences, insulin sensitivity, liver, melon superoxide dismutase, metabolic syndrome

Abstract
Obesity-related metabolic syndrome is often associated with an increased oxidative stress and a decrease of insulin sensitivity, inducing several modifications, particularly in adipose tissue. However, dietary antioxidants could prevent oxidative stress and insulin-induced damage. In this context, we evaluated the effects of a 1-month curative supplementation with SODB, a melon SOD, on the liver and adipose tissue of obese hamsters. SODB reduced body weight and obesity markers. These beneficial effects could be due to the increased expression of tissular antioxidant defense proteins. These findings suggest that SODB could exert its antioxidant properties by inducing the endogenous antioxidant defense. The mechanisms underlying this induction need to be further investigated.

INTRODUCTION

The metabolic syndrome is a constellation of dysfunctions including glucose intolerance, central obesity, dyslipidemia, and leptin/adiponectin imbalance (1). Dyslipidemia is characterized by low levels of plasma HDL cholesterol, high levels of LDL cholesterol and hypertriglyceridemia (2). Moreover, dysregulated production of adipocytokines (leptin and adiponectin) by adipocytes participates in the pathogenesis of obesity-associated metabolic syndrome. Indeed, a decrease in plasma adiponectin is causative for insulin resistance in obesity. Insulin resistance corresponds to a reduction of the gluco-regulating activity of the insulin (3). Insulin resistance could be caused by alterations in insulin receptors and glucose transports (4). Thus, glucose assimilation is decreased and it accumulates in blood circulation, causing hyperglycemia.

The metabolic syndrome can occur in several forms, depending on the combination of these various components, and it is now well established that it increases the risk to develop cardiovascular disease, type 2 diabetes, and cancer (5).

The current lifestyle, called modern Western lifestyle (6), including stress, positive energy balance, low-quality food, and the disruption of chronobiological function/rhythms, contributes to the increase of metabolic syndrome incidence. In fact, human obesity arises from a complex interaction of multiple nutritional and lifestyle-related factors directly linked to the excessive consumption of industrial era foods (7). Therefore, modeling the metabolic disorders of human obesity in animals is better with diets consisting of palatable industrially processed foods (named cafeteria diets), compared to traditional high-fat diets. Indeed, these cafeteria models lead to a phenotype of exaggerated obesity and related disorders (8), particularly in white adipose tissue, which is the main organ involved in obesity.

The pathogenesis of obesity-related metabolic syndrome is associated with an increased risk to develop insulin resistance (4), which causes several physiological modifications in adipose tissue, such as the disruption of lipogenesis (9) and lipolysis (10).

Some studies have shown a negative correlation between obesity and tissue or plasma antioxidant capacity (11, 12). Recent studies have suggested the potential therapeutic role of dietary antioxidant supplementation in the reduction of body weight and its beneficial effects in several obesity-related disorders (13, 14).

An original way to increase antioxidant capacity could be by supplying antioxidant enzymes, which have longer lasting effects because of their lower rate of exhaustion than mere metabolites.
In this context, we investigated the influence of SODB, a gastroresistant encapsulated melon concentrate particularly rich in superoxide dismutase (SOD), in a Golden Syrian hamster model of obesity (15, 16). In short, we induced obesity in these hamsters using a diet consisting of high-fat, high-sugar, and high-salt supermarket products and measured markers of obesity as well as oxidative status in the liver and adipose tissue.

MATERIALS AND METHODS

SOD by Bionov (SODB; Avignon, France) is a non-GMO melon concentrate particularly rich in SOD, resulting from a patented extraction process. For nutraceutical applications, SODB is coated with palm oil in order to protect SOD activity from digestive enzymes. In this study, it contains 14 U SOD/mg powder measured according to the method of Zhou and Prognon (17). Detailed information about the antioxidant content of SODB has been published in a previous study (18).

Seventeen 3-week-old male Golden Syrian hamsters (Janvier, Le Genest-St-Isle, France) were used. They were housed at 23°C, subjected to a 12-h light/dark cycle with free access to food and water. After an 18-day adaptation period, the hamsters were randomly divided into three groups. Two groups of hamsters (n = 5 each) were assigned for 19 weeks to a cafeteria diet consisting of nine types of palatable industrially processed foods designed for human consumption and selected for their high energy, fat, sugar, and/or salt content (cake, potato crisps, sweets, cheese, cola, etc.). After 15 weeks, one of the two groups of obese animals was given SODB (10 U SOD/day) orally for the next 4 weeks (SODB group), while the other group was maintained on the cafeteria diet alone (CAF group). Another group of hamsters (n = 7) was fed a standard pellet diet and served as controls (STD group).

Food intake and body weight were recorded daily. At the end of the experimental period, the hamsters were deprived of food overnight. Fasting blood samples were collected and plasma and erythrocytes were separated.

The left tibia was measured in order to standardize the liver and adipose tissue weight, for comparison. Plasma glucose, cholesterol, HDL-cholesterol, and triglyceride levels were analyzed by enzymatic methods. Insulin, adiponectin, and leptin levels were assessed using immunoassay kits. The homeostatic model assessment for insulin resistance (HOMA-IR) was determined according to Matthews et al. (19): HOMA-IR = (fasting glucose (mmol/L) x fasting insulin (μU/L))/22.5

RESULTS

Obesity and metabolic syndrome plasma markers

The cafeteria diet induced a significant threefold increase in body weight (Table 1). SODB supplementation decreased body weight (5 percent lower than in the untreated CAF group), although food intake was not affected.

The cafeteria diet led to increased plasma triglycerides, LDL-cholesterol, glucose and leptin levels, and decreased adiponectinemia (Table 1). SODB treatment corrected the increases in LDL-cholesterol and glucose, by 55 and 35 percent, respectively (Table 1). Insulinemia and HOMA-IR were increased fivefold by the cafeteria diet, but were significantly attenuated by SODB treatment (25 percent and 50 percent respectively; Table 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight gain at 15 weeks (g)</th>
<th>Final body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STD</td>
<td>34 ± 2a</td>
<td>109 ± 3b</td>
</tr>
<tr>
<td>CAF</td>
<td>107 ± 2a</td>
<td>190 ± 2b</td>
</tr>
<tr>
<td>SODB</td>
<td>183 ± 2c</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1. Obesity and metabolic syndrome markers. Values are means ± SEM. Means in a row with superscripts without a common letter differ significantly, p < 0.05.
SODB supplementation reduced adipose tissue weight by decreasing adipocyte size and improved adipocytes lipolysis

In CAF group, the adipose tissue weight was fourfold higher than in STD group (Table 1), and decreased significantly after SODB treatment (by 22 percent compared to CAF group). Cafeteria diet induced hyperplasia and hypertrophy of abdominal adipocytes (Figure 1a, Table 1). Adipocytes size was significantly reduced by 54 percent after SODB supplementation (Figure 1a). Cafeteria diet seemed to alter lipolytic activity of adipose tissue by reducing the expression of HSL (by 43 percent), but SODB supplementation restored HSL expression (Figure 1b).

Liver and adipose tissue expression of antioxidant enzymes was modified by the cafeteria diet (Table 2). However, SODB treatment raised the expression of SODs, GPx and CAT, compared to the untreated CAF animals, in both liver and adipose tissue (Table 2).

**DISCUSSION**

The cafeteria diet induced obesity and related disorders in hamsters, including dyslipidemia, insulin resistance, and oxidative stress in the liver and adipose tissue. Here, we show that curative supplementation with SODB decreases adipose tissue weight probably by activating adipocytes lipolysis and thus reducing their size. Finally, we demonstrate that SODB administration increases the expression of endogenous antioxidant enzymes and thus reduces oxidative stress and insulin resistance.

As expected, the cafeteria diet induced several markers of metabolic syndrome in our hamsters (CAF group), such as increased plasma glucose, cholesterol and triglycerides, an altered leptin–adiponectin balance and insulin resistance. Several models of obesity display a decrease in insulin sensitivity. Indeed, visceral fat accumulation is a major contributor to the development of insulin resistance (20). SODB treatment induced a decrease in major of these alterations, including an improvement of insulin sensitivity.

The body weight gain induced by the high-fat, high-sugar, and high-salt diet (CAF hamsters) was higher than by the standard diet. Cafeteria diet induced a significant increase in adipose tissue weight by both hyperplasia and hypertrophy, as shown in numerous animal models of...
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through the nuclear-factor-E2-related factor (Nrf2)/antioxidant response element (ARE) pathway [28, 29]. Finally, the induction of several antioxidant enzymes avoids imbalance, which could be involved in some diseases, such as Down syndrome (30). Indeed, CAT and GPx remove the H$_2$O$_2$ produced after the dismutation of O$_2$ $^.$ by SOD. This global induction of endogenous defense, possibly by the activation of Nrf2/ARE pathway, suggests that SODB could have potential applications in several situations in which oxidative stress is enhanced.

**REFERENCES AND NOTES**


obesity (21). SODB oral administration led to adipose tissue weight reduction, which is mainly due to a decrease of the adipocyte size, while adipocyte number remained at the elevated level achieved during the period of weight gain. Indeed, in case of weight loss, adipocytes can shrink but often remain constant in number (22). The decrease of adipocyte size and thus the loss in abdominal fat could be explained by an increased lipolysis, as shown by others authors (14, 23). Lipolysis modulation seems to be closely linked to insulin signaling and the interplay between adipose tissue state and insulin sensitivity has been pointed out by numerous studies (24, 25), showing that insulin inhibits adipocyte lipolysis and enhances adipocyte differentiation. Hence, lipolysis (HSL expression) is decreased in the obese insulin resistant animals. Therefore, the decrease of adipocyte lipolysis in CAF hamsters could be explained by hyperinsulinemia and insulin resistance induced by cafeteria diet. Moreover, the increase of lipolysis (HSL expression) after SODB supplementation is probably linked to the correction of insulin signaling in SODB animals.

The cafeteria diet also induced liver and adipose tissue oxidative stress, clearly evidenced in CAF group by the overproduction of O$_2$ $^.$ and the increase in F(8)-IsoP levels, an in vivo reference marker resulting from arachidonic acid peroxidation [26]. These oxidative stress parameters were significantly decreased after SODB supplementation. The main result of this study is the induction of endogenous antioxidant enzymes expression after SODB supplementation. Indeed, the expression of SOD, GPx, and CAT was enhanced in SODB group. Such an induction could explain the decrease in ROS production and F(8)-IsoP levels, and then the improvement of insulin sensitivity. Indeed, overproduction of ROS has been implicated as an important contributor to the pathogenesis of obesity-associated insulin resistance [13]. These findings suggest that the decrease of O$_2$ $^.$ production after SODB supplementation, could restore insulin sensitivity, and then improve adipocyte lipolysis.

Although SOD cannot be absorbed, our results suggest that SODB could act by triggering a cascade of events from the intestine till the induction of antioxidant enzymes in other tissues. Other exogenous SODs seem to act by inducing endogenous antioxidant defense in the tissues, as reviewed by Carillon et al. [27], and some hypothesis have been proposed even if the precise mechanism is still unknown. Indeed, it could be hypothesized that the induction of antioxidant enzymes is regulated at the transcriptional level

### Table 2. Oxidative status in adipose tissue and liver. Values are means ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>STD</th>
<th>CAF</th>
<th>SODB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adipose tissue</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superoxide anion production (RLU x 10$^3$/mg prot)</td>
<td>299 ± 39a</td>
<td>781 ± 202b</td>
<td>259 ± 100a</td>
</tr>
<tr>
<td>SOD (%) of STD group</td>
<td>100 ± 0a</td>
<td>72 ± 4b</td>
<td>98 ± 10a</td>
</tr>
<tr>
<td>GPx (%) of STD group</td>
<td>100 ± 0a</td>
<td>46 ± 8b</td>
<td>84 ± 22a</td>
</tr>
<tr>
<td>CAT (%) of STD group</td>
<td>100 ± 0a</td>
<td>58 ± 9b</td>
<td>74 ± 16ab</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superoxide anion production (RLU x 10$^3$/mg prot)</td>
<td>29 ± 4a</td>
<td>61 ± 4b</td>
<td>52 ± 5c</td>
</tr>
<tr>
<td>SOD (%) of STD group</td>
<td>100 ± 0a</td>
<td>106 ± 13a</td>
<td>157 ± 18b</td>
</tr>
<tr>
<td>GPx (%) of STD group</td>
<td>100 ± 0a</td>
<td>93 ± 16a</td>
<td>146 ± 15b</td>
</tr>
<tr>
<td>CAT (%) of STD group</td>
<td>100 ± 0a</td>
<td>95 ± 11a</td>
<td>146 ± 19b</td>
</tr>
</tbody>
</table>

Means in a row with superscripts without a common letter differ significantly. $p < 0.05$.
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Creatine timing on muscle mass and strength: Appetizer or Dessert?

**KEYWORDS:** supplements, creatine, strength, muscle mass, timing

**Abstract**

Resistance training is a potent stimulus to enhance skeletal muscle hypertrophy and strength. Combining creatine supplementation with resistance training may be an effective strategy to enhance the physiological adaptations from resistance training alone. Emerging evidence suggests that the timing of creatine supplementation may be an important regulator of muscle hypertrophy and strength. Creatine ingested before and after resistance training sessions appear to be an effective strategy to increase muscle mass and strength, with slightly greater benefits if creatine is consumed after exercise compared to before. This brief review will evaluate the literature pertaining to the strategic ingestion of creatine and resistance training resulting in practical creatine supplementation strategies.

**INTRODUCTION**

It is well established that the mechanical stimuli from resistance training increases muscle protein synthesis (1). Although the machinery for stimulating muscle protein synthesis is increased after resistance training (2), the anabolic response may be delayed post-exercise (3). The combination of creatine supplementation and resistance training may lead to greater muscle benefits than resistance training alone in young and older adults (4, 5). Furthermore, the timing of creatine ingestion may be an important factor for creating an anabolic environment for muscle growth (5). Emerging evidence suggests that creatine supplementation, in close proximity to resistance training sessions, may provide superior benefits compared to creatine intake at other times of the day (6, 7). While the mechanistic actions explaining the greater benefits from timed creatine ingestion are unknown, it is possible that blood flow kinetics and creatine transport are involved (8, 9). Therefore, the purpose of this review is to 1) briefly outline the potential beneficial effects of creatine supplementation, 2) review the emerging evidence involving the timing of creatine supplementation combined with resistance training, and 3) outline creatine supplementation strategies.

**CREATINE SUPPLEMENTATION**

Creatine, methyl-guanidino acetic acid, is a naturally occurring nitrogen-containing compound (5, 10, 11). Creatine excretion occurs at a rate of ~2 g·d⁻¹ (12). Creatine can be replaced via endogenous synthesis (1-2 g·d⁻¹) in the kidneys, liver, and pancreas or through dietary intake, typically ~1-3 g·d⁻¹ (11, 12). Creatine is found in high concentrations in red meat and seafood (12). Ninety-five percent of creatine is stored in skeletal muscle, of which 60-70 percent is phosphorylated (i.e. phosphocreatine) (13). Phosphocreatine rapidly resynthesizes adenosine diphosphate to help maintain adenosine triphosphate (ATP) during high intensity exercise such as resistance training (13). Theoretically, elevated phosphocreatine stores (via creatine supplementation) may increase exercise training intensity and the volume of work performed leading to greater muscle accretion and strength (reviewed in Branch (14); Rawson & Volek (15)). Several purported mechanisms exist which may help explain the typical increase in muscle mass and strength from creatine (4, 5, 10). Creatine supplementation elevates skeletal phosphocreatine and total creatine stores (16) which increases phosphocreatine resynthesis (17) and exercise fatigue resistance (18). Creatine may also influence myocellular water retention due to increased intracellular osmolarity and increase muscle glycogen storage (19). Subsequent muscle cell swelling may stimulate genes regulating various anabolic pathways (20). Furthermore, creatine has been shown to increase satellite cell differentiation (21), activity (22), and content (23); transcription factor activity (24), hormonal secretion (e.g. IGF-1; (25)), muscle protein kinetics (26), and decrease inflammation (27).
CREATINE TIMING

The timing of creatine supplementation is proving to be an important regulator of muscle growth (Table 1). The strategic ingesting of creatine immediately before and after resistance training sessions appears more important than ingesting creatine at other times of the day. For example, in the most recent study, we showed that creatine (0.1 g·kg⁻¹) immediately before and immediately after resistance training sessions for 8 months produced similar gains in muscle mass and strength. However, compared to placebo, only post-exercise creatine resulted in greater improvements in whole body lean tissue mass (8.2 percent vs. placebo = 1.4 percent) and leg press strength (creative after = 28.3 percent vs. 3.4 percent; unpublished findings). The slightly greater benefit from post-exercise creatine supplementation indirectly supports the findings of Antonio and Ciccone (28) who found a greater muscle benefit from post-exercise creatine supplementation (5 g) in young adults compared to pre-exercise creatine supplementation. We previously found no differences between creatine supplementation (0.1 g·kg⁻¹) immediately before vs. after resistance training sessions for 12 weeks in older adults (29). However, a major limitation of the studies by Antonio and Ciccone (28) and Candow et al. (29) was that a placebo (control) was not used for comparison to creatine. Consuming creatine immediately before (0.05 g·kg⁻¹) and immediately after (0.05 g·kg⁻¹) resistance training sessions (3 days/week, 10 weeks) resulted in greater muscle accretion (2.0 ± 0.3 cm) compared to placebo (0.8 ± 0.3 cm) and resistance training in healthy older males (59-77 years) [30]. These results support previous findings of a significant increase in lean tissue mass (6 percent), type II muscle fibre area (29 percent), and insulin growth factor-1 (78 percent) in adults (19-55 years) who ingested creatine before (0.03 g·kg⁻¹) and after (0.03 g·kg⁻¹) resistance training [6 days/week, 8 weeks] [25, 31]. Interestingly, in comparing the effects of creatine ingestion before (0.5 g·kg⁻¹) and after (0.5 g·kg⁻¹) resistance training (10 weeks) to creatine ingestion in the morning and evening on training days, Cribb et al. [6] showed that creatine ingestion before and after exercise resulted in significantly greater intramuscular creatine content, lean tissue mass, and muscle cross-sectional area of type II fibres. Although it is difficult to compare results across studies, it has been theorized that these positive results from creatine ingestion before and after exercise may be due to an increase in blood flow and delivery of creatine to exercising muscles (8), an upregulation of the kinetics involved in creatine transport (9), and by an increase in Na⁺/K⁺ pump function during exercise [9].

Based on the limited studies performed thus far, it appears that creatine supplementation before and after resistance training sessions is important for muscle and strength. Post-exercise creatine ingestion may provide slightly greater benefits than pre-exercise creatine supplementation.

REFERENCES AND NOTES


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Probiotics to reduce antibiotic side effects

KEYWORDS: probiotics, lactobacillus, bifidobacterium, antibiotic side effects, antibiotic associated diarrhoea

Abstract
One of the oldest health benefits of probiotics is reduction of diarrhoea. Indeed, probiotics appear to be particularly effective in reducing the risk for side effects of antibiotic therapy. Here we summarise the effect a combination of four probiotic strains has on maintaining intestinal microbiota composition and reducing risk for antibiotic associated diarrhoea. Despite these successes, it is difficult to make reference to these benefits in many jurisdictions. It is therefore important that reference to alternative endpoints is made such as maintenance or restoration of microbiota composition or reduction of potential pathogens. This is important, not only for improving quality of life of patients on antibiotic use but also from a health economic perspective; the cost of probiotics is relatively small compared to the cost of antibiotic associated diarrhoea.

INTRODUCTION
Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host [1]. It may therefore not seem like a good idea to combine probiotics with antibiotics. However, one of the most convincing health benefits of probiotics has been the reduction of antibiotic associated diarrhoea (AAD) [2]. Diarrhoea is one of the oldest health targets for probiotics. There may be various reasons why probiotics are effective in AAD despite the expected incompatibility of probiotics with antibiotics.

1. Probiotics may be resistant to the antibiotic in question. Bacteria commonly show resistance to some antibiotics. When most members of a particular species exhibit resistance to a certain antibiotic it may be due to intrinsic resistance; the target for the antibiotic maybe missing or may be different and therefore the bacteria are not affected by the antibiotic (Figure 1). This type of resistance cannot be transferred and is therefore not a safety concern.

2. Alternatively, probiotics may have acquired antibiotic resistance. This will usually be found only among a limited number of strains within a species. This resistance may have emerged through a mutation causing a change in the target of the antibiotic which renders the organism insensitive to the antibiotic (Figure 1). This type of antibiotic resistance is usually not transferable and is therefore not a safety concern.

3. Acquired antibiotic resistance may also involve specific mechanisms to ‘neutralise’ the antibiotic; e.g. by enzymatically inactivating the antibiotic (beta-lactamase is the best known example of this) or pumping the antibiotic out of the bacterial cell (Figure 1). This type of antibiotic resistance is encoded for by specific resistance genes which may be transferred and is thus a potential safety concern. Even when probiotics are sensitive to antibiotics, they may survive the contact with the consumed antibiotics, as antibiotics are usually only effective against growing bacteria. When the probiotic becomes active further down the intestinal tract, it may be that the antibiotic is already absorbed and thus has limited influence on the probiotic.

PROBIOTICS AND MICROBIOTA MAINTENANCE
Antibiotics do not only result in reduction of certain pathogens but can diminish the microbial diversity in the gut. The disturbance of the intestinal microbiota by antibiotics can cause diarrhoea by different mechanisms. Firstly, the ability of the gut to resist the colonisation of opportunistic pathogens is reduced when the microbial diversity is decreased in the gut. This can lead to an overgrowth of certain pathogens, e.g. Clostridium difficile, C. perfringens or Salmonella, resulting in diarrhoea. Secondly, antibiotics have been shown to reduce colonic bacterial carbohydrate metabolism and reduce the levels of short chain fatty acids (SCFAs) [3]. The accumulation of non-absorbable carbohydrates and reduced levels of SCFAs increase the water retention into the lumen and may contribute to osmotic...
diarrhoea. In addition, antibiotics may reduce the number of dehydroxylating bacteria in the gut resulting in increased levels of primary dehydroxy bile acid (3). Dehydroxy bile acids are potent colonic secretory agents and can therefore cause diarrhoea. The exact mechanism of probiotic action is yet unknown, but the preventative effects on diarrhoea are most likely related to maintaining the stability of the gut microbiota (4). Restoring the baseline microbiota prevents changes in the carbohydrate and bile acid metabolism and therefore leads to a reduction of osmotic diarrhoea. Probiotics may also competitively inhibit the growth of pathogens. The related protective effects of probiotics include the production of bacteriocins or defensins, inhibition of adherence or translocation of pathogens and reduction of luminal pH (5).

In clinical settings, probiotics have been shown to stabilize the intestinal microbiota during antibiotic supplementation (6). In a randomised controlled trial 40 healthy subjects were administered a probiotic combination or placebo during and after a 7-day treatment with amoxicillin and clavulanate. The similarity of the faecal microbiota of the subjects was determined by terminal restriction fragment length polymorphism and culturing, and measured by comparing data from baseline to post-treatment. The similarity of the bacterial community to baseline was decreased significantly during antibiotic treatment. However, the intestinal microbiota of the probiotic group was less disturbed and showed a more rapid return to the baseline microbiota composition compared to the placebo group. The levels of Bifidobacterium, Bacteroides and Enterobacteriaceae were also increased in the probiotic group. Accordingly, other studies have shown that probiotics minimise the decrease in the diversity of microbiota (7) and increase the levels of antibiotic-reduced Bifidobacterium (7), and Bacteroides (8). Both Bifidobacterium and Bacteroides are important saccharolytic groups in the colon.

There is evidence from studies using both culturing and molecular approaches that antibiotic treatment changes our intestinal microbial community and function (9, 10). Also the effects of probiotics on microbiota during and after antibiotic treatment have been studied using more traditional methods (8). However, the knowledge on how probiotics affect the antibiotic-related changes in the gut is still limited. Clinical studies using new molecular based methods for investigating the faecal and mucosal microbiota could provide more insight into the impact of probiotics during antibiotic therapy.

**PROBIOTICS AND REDUCTION OF ANTIBIOTIC SIDE EFFECTS**

The incidence of AAD, as reported in the literature, ranges between 5 percent and 39 percent and varies according to individual susceptibility, the patient environment, and the class of antibiotics administered (2). Risk factors include age (<6 years and >65 years), comorbidities, immunological status of the patient; type, dose and duration of antibiotic administered, and duration of hospitalization (2). However, data on the incidence of AAD in age groups other than children and the elderly are very limited (3). Diarrhoea is defined as three or more loose stools in 24 h, not all patients using antibiotics will develop diarrhoea, but may nevertheless have gastrointestinal complaints. The number of patients experiencing side effects is thus far greater than the reported 5-39 percent of subjects with AAD. In a recent study (11), we observed a substantial fraction of subjects with loose stools (but not diarrhoea) and subjects with abdominal pain (may or may not suffer from diarrhoea). Figure 2. A recent meta-analysis indicated that probiotics given together with an antibiotic give a relative risk for AAD of 0.58 (95 percent confidence interval 0.50-0.68) probiotics in general are thus highly effective in reducing the risk for AAD (2). But, there is a lack of sufficiently large studies and dose response studies. Two dose response studies have so far been published on probiotics and AAD. A study with a probiotic combination (L. acidophilus CL1285® and Lactobacillus casei LBC80R®) demonstrated dose-response effects on incidence of AAD and various symptoms of AAD (20). The lower dose in this study (5×1010 CFU) was able to significantly reduce both AAD and CDAD and the higher dose (1011 CFU) reduced these even more. This study was done in middle-aged/older subjects (50-70 years of age) who are more sensitive to developing AAD. The second study investigated the effect of lower doses (4.17 × 109 and 1.7 × 1010 CFU of a four strain probiotic combination HOWARU Restore) in younger adults (20-70 years of age) (11). The younger mean age resulted in less AAD than in the former study. Although the lower dose of HOWARU Restore reduced the incidence of AAD, this was not significant. The high dose, however, did significantly reduce AAD incidence. Although the lower dose reduced fever and bloating, this did not reach significance. The high dose, however, did reduce bloating, fever, and abdominal pain while both high and low dose reduced duration and number of liquid stools, compared to the placebo group. Although different probiotic strains were used in both studies, and slightly different population, it is tempting to speculate that the lowest dose tested in these studies was below what is required to reduce AAD or CDAD incidence; but enough to still reduce some of the associated symptoms. The doses above 1010 CFU/day were sufficient. This, however, does not mean that higher probiotic cell counts are always better, nor does it answer the question whether eventually a plateau is reached, above which no further benefits are to be expected.

Besides AAD, the use of antibiotics has been suggested to be associated with an increased risk for the development of irritable bowel syndrome (IBS) (12). This is interesting since at the same time, Rifaximin has been suggested as a treatment for IBS (13). This, in any case, suggests an involvement of the intestinal microbiota in IBS. This may suggest an additional benefit for the use of probiotics during antibiotic therapy; reduced risk for post-antibiotic IBS; a target that would deserve further attention. Even more recent, microbiota changes induced by low dose antibiotics have been suggested to be involved in obesity; in a mouse model (14).

**FUTURE OF PROBIOTICS IN AAD**

Despite the convincing benefits probiotics can provide to antibiotic users and the expected cost savings that can be made by society, there is no general recommendation to use probiotics as complement to antibiotic therapy; which is unfortunate. The number needed to treat has been reported to be around 8 (2, 11); i.e. to prevent one case of AAD, 8 subjects have to consume probiotics, which is quite low. Although the risk factor for AAD in some jurisdictions could possibly be used to support a health claim; e.g. reduction of intestinal pathogens, only a limited number of known pathogens causes AAD. An alternative approach would be to use a more holistic approach and assess maintenance or recovery of the faecal microbiota as a basis for a health claim; using molecular techniques that assess the whole microbiota; not just a limited number of "good" and/or "bad" genera or species. While everyone’s microbiota is unique, it is likely that each individual’s microbiota is appropriate for him or her and maintenance or recovery of this microbiota can be considered a health benefit. It is, however, not known how legislators would interpret such data. Furthermore, because subjects consuming
dose response studies that are sufficiently powered further support the use of probiotics as a complementary treatment with antibiotic use (11). Besides an improved quality of life for the patient, substantial savings can be expected for society (15). It is therefore unfortunate that to date no claims are allowed on this topic or general recommendations made. Legislators and professional organisations do not like to give recommendations on broad product groups. It is therefore important to establish which probiotic products are efficacious in the management of AAD (and other health benefits for that matter).

REFERENCES AND NOTES

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Functional properties of collagen hydrolysates from the jellyfish (Chrysaora sp.)

KEYWORDS: collagen, collagen hydrolysate, functional properties, ribbon jellyfish, Chrysaora sp.

Abstract

Collagen from the jellyfish Chrysaora sp. was isolated by limited pepsin digestion. After extraction, collagen was further hydrolysed using three different enzymes: trypsin, alcalase, and protamex. Properties of the collagen hydrolysates, including degree of hydrolysis, molecular weight distribution, and functional properties, were then measured. Alcalase digestion resulted in the highest degree of hydrolysis (45 percent), followed by protamex (39 percent) and trypsin (35 percent). The molecular mass of alcalase and protamex hydrolysates was ~8–10 kDa, and the value for the trypsin hydrolysate was ~10–20 kDa. All hydrolysates exhibited high water absorption, water binding, water holding capacity, and oil absorption capacity, and they also had good emulsifying and moderate foaming properties.

INTRODUCTION

Jellyfish have been used as food in East Asian countries for more than a thousand years (1, 2). Edible jellyfish are limited to 11 species in 5 families in the order Rhizostomeae and class Scyphomedusae (3). Jellyfish have a unique texture, high nutritional value, and pharmacological properties. In Chinese traditional medicine, jellyfish have been used to treat diseases such as high blood pressure, arthritis, back pain, gastric ulcers, asthma, bronchitis, and burns (1, 4-7), and the effectiveness of the treatment for some of these conditions has been confirmed by recent studies (1, 8-10).

Collagen is the main component of jellyfish (11), thus it is assumed to be the component responsible for the aforementioned benefits. If a considerable amount of collagen can be obtained from this marine resource, it may prove to be an alternative source of collagen and collagen derivatives. In fact, jellyfish can be extremely abundant, particularly when they occur in blooms. When abundant, they can clog the cooling equipment of power plants, contaminate fish catches (12), overload and burst fishing nets, sting humans, and cause environmental pollution when they accumulate as waste in coastal areas (13). Thus, finding a use for this abundant marine resource may mitigate these problems while also providing a source of collagen.

Collagen is the most abundant protein in the animal body, as it accounts for about 30 percent of total body proteins; it is found in skin, bone, tendon, cartilage, and many other connective tissues (14, 15). This biological material has wide range of applications in the food, cosmetic, pharmaceutical, nutraceutical, and biomedical industries (4, 11, 16, 17). Collagen also can be enzymatically hydrolysed to biologically active peptides, which may have antioxidant activity, angiotensin-I converting enzyme (ACE) inhibitory activity (10, 18), and an anti-fatigue effect (1). It has also been reported to be effective in treating diseases such as osteoarthritis, osteoporosis (9), and rheumatoid arthritis, and it is commonly used as a nutritional supplement to relieve symptoms of these conditions (8). Compared with artificial antioxidants and chemical medications, bioactive peptides have no health risks, are safe for human consumption, and can be used in the food industry (e.g., as food preservatives, functional food components, and nutritional supplements) as well as in the nutraceutical, pharmaceutical, and biomedical industries (10).

Depending on the source of protein and degree of hydrolysis, protein hydrolysates exhibit some important functional properties (e.g., water and oil absorption capacity, water holding capacity, foaming and emulsification capacity) that differ from those of their parent protein (10, 19). For example, compared to collagen, collagen hydrolysates are more water soluble due to their lower molecular weight, thus they can be good foaming or emulsifying agents (10). Collagen hydrolysates also do not form a gelatin gel after cooling, which consists completely out of denatured collagen (9). The subject of this study was the ribbon jellyfish, Chrysaora sp.1, which is a newly identified species of jellyfish found in the coastal area of Penang Island, Malaysia (20). The umbrella diameter of this species is about 8–12 cm, and the oral arms under the bell are flat, ribbon-like, and 20–50 cm long. Chrysaora sp. may be totally white (morphotype 1) or have a brown stripe around the umbrella (morphotype 2) (20). The objective of this study was to extract collagen hydrolysate from ribbon jellyfish (Chrysaora sp., morphotype 1) and to measure its functional properties in order to explore its potential applications in the food and nutraceutical industries.
MATERIALS AND METHODS

Materials and chemicals
Specimens of Chrysaora sp. were collected from the offshore areas of Penang Island, Malaysia. The umbrella was dissected, washed with distilled water, cooled on ice, transported to laboratory, and stored at −80°C until use. Pepsin (EC number: 3.4.23.1, 400 U/mg pro), trypsin (EC number: 3.4.21.4), and standard collagen type II from chicken sternum were purchased from Sigma-Aldrich Inc. (St Louis, MO, USA). Alcalase (EC number: 3.4.21.62) and proteamex™ (a Bacillus protease complex, EC numbers: 3.4.21.62 and 3.4.24.28) were supplied by Novozymes A/S (Bagsvaerd, Denmark). SDS-PAGE chemicals and molecular weight markers were purchased from Bio-Rad Laboratories (Hercules, CA, USA). All chemicals and reagents were of analytical grade.

Isolation of jellyfish pepsin solubilized collagen (JPSC)
Collagen was extracted according to the method of Nagai et al. (21) with some modifications as described in our previous report (22). All procedures were performed at 4°C. Jellyfish umbrellas were thawed at 4°C for 4–5 h, cut into small pieces (0.5 cm × 0.5 cm), and washed with distilled water. To remove non-collagenous substances, each sample was treated with 0.1 M NaOH at a sample/solution ratio of 1:10 (w/v) with gentle stirring for 2 d (the solution was changed once a day). After being centrifuged at 10,000 g for 30 min, the remaining insoluble matter was washed with distilled water until neutral pH and digested using 10 percent (w/w) pepsin in 0.5 M acetic acid (10 volumes v/w) with gentle stirring for 3 d. The final viscous liquid was centrifuged at 20,000 g at 4°C for 1 h. Digestion step was repeated on the remaining precipitate for another 3 days. The supernatants were combined and dialyzed against 10 volumes of 0.02 M Na2HPO4 (pH: 8.8) for 3 d to inactivate the enzyme. The dialyzed sample was centrifuged at 20,000 g at 4°C for 1 h. The resulting precipitate was dissolved in 0.5 M acetic acid and salted out by adding NaCl to the final concentration of 1 M, followed by centrifugation at 20,000 g at 4°C for 1 h. The resulting precipitate was dissolved in 0.5 M acetic acid and dialyzed against distilled water for 2 d. The sample was lyophilized and stored at −80°C until further analysis.

Preparation of the collagen hydrolysate
The isolated collagen (100 mg) was suspended in 100 ml distilled water and digested using one of three different enzymes (trypsin, alcalase, and proteamex) at an enzyme-to-substrate ratio of 2 percent (w/w). Digestion was carried out at 50°C for 5 hours at optimum pH for each enzyme as suggested by the manufacturer and previous research as follows: alcalase and trypsin at pH 8 (23) and proteamex at pH 7 (1). The hydrolytic reaction was stopped by heating the samples at 95°C for 10 min. The samples were cooled to room temperature and centrifuged at 3000 rpm for 30 minutes. The resultant supernatants were lyophilized as jellyfish collagen hydrolysate (JCH) and stored at −80°C for further analysis.

Degree of hydrolysis
The Hoyle et al. (24) method was used to determine the degree of hydrolysis (DH) of the isolated collagen. Trichloroacetic acid (TCA, 20 percent) was mixed with an equal volume of the hydrolysate solution. TCA-soluble materials (10 percent) were collected after centrifugation at 7000 × g (20 minutes, 10°C). The following formula was used to compute the DH:

\[
\text{percent DH} = \left(\frac{10 \text{ percent TCA} - \text{soluble nitrogen}}{\text{total nitrogen in the sample}}\right) \times 100
\]

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)
SDS-PAGE for both JPSC and JCH was performed following the method of Laemmli (25). Samples (freeze-dried form) were dissolved in Laemmli sample buffer (Bio-Rad Laboratories) and heated for 5 minutes at 95°C with and without b-mercaptoethanol (5 percent v/v). SDS-PAGE analysis was conducted on a polyacrylamide gel consisting of 7.5 percent resolving gel and 4 percent stacking gel for JPSC and a gel consisting of 15 percent resolving gel and 4 percent stacking gel for JCH. A sample containing 20 µg of JPSC or 3 mg of JCH was loaded into each well, and electrophoresis was run at 80 V for 10 minutes followed by 120 V for 1 hour. After electrophoresis, protein bands were stained with Coomassie brilliant blue R-250. A high molecular weight pre-stained marker (Bio-Rad Laboratories) was used to estimate the approximate molecular weight of proteins. Type II collagen from chicken sternum (Sigma-Aldrich Inc.) was used as the reference protein.

Functional properties of jellyfish collagen hydrolysates
Water absorption capacity
Water absorption capacity (WAC) of JCH was determined according to the method of Tao (1997) with modifications. First, each sample (0.5 g) was spread on a culture dish (diameter = 5 cm) and incubated at 30°C at 85 percent relative humidity. The dish was weighed every 6 hours until an almost constant weight was reached. WAC was calculated using the following equation:

\[
\text{WAC (g/g)} = \frac{\Delta W}{W}
\]

where ΔW is the weight of absorbed water and W is the initial weight of the sample.

Water binding capacity
Water binding capacity (WBC) was determined according to the method of Petravi et al. (26) with modification. First, 5 mg of each sample were transferred into a graduated tube. Next, 40 µl of distilled water were added to the samples, which were then stirred for 30 seconds. The resulting solutions were allowed to rest for 10 minutes. Mixing and resting were repeated 10 times. The solution was then centrifuged at 3000 rpm at room temperature for 25 minutes. The supernatants were removed and the tubes were placed downwards at an angle of 20° at 50°C for 25 minutes to drain. After cooling to room temperature, the tubes were weighed. WBC was expressed as the amount of water absorbed by 1 g of sample as follows:

\[
\text{WBC} = \frac{\Delta W}{W_0}
\]

where ΔW is the amount of absorbed water and W₀ is the weight of the dry sample.

Water holding capacity
Water holding capacity (WHC) was determined according to the method of Tao (27) with slight modification. First, 5 ml of each sample was dried at 100°C for 24 hours and then reweighed. The water holding capacity (WHC) was calculated using the following equation:

\[
\text{WHC} = \left(\frac{W_{s1} - W_{s2}}{W_0}\right) \times 100
\]

where W₀ is the weight of the dry sample, Ws1 is the weight of the sample after drying at 100°C for 24 hours, and Ws2 is the weight of the sample after drying at 100°C for 24 hours and then reweighing.

Materials and methods
The following formula was used to compute the DH:

\[
\text{percent DH} = \left(\frac{10 \text{ percent TCA} - \text{soluble nitrogen}}{\text{total nitrogen in the sample}}\right) \times 100
\]
sample (1 percent w/v in distilled water) were added to a culture dish and then incubated at 37°C at 75 percent relative humidity. Samples were weighed every 10 minutes for 70 minutes in order to measure the remaining water in the samples. Glycerol and distilled water were used as controls. WHC was calculated as follows:

\[ \text{WHC} (%) = \frac{\Delta W}{W_0} \times 100 \]

where \( \Delta W \) is the amount of water each time and \( W_0 \) is the initial amount of water.

**Oil absorption capacity**

Oil absorption capacity (OAC) was determined according to the method of Dachuan et al. [28] with modification. First, 50 mg of each sample were mixed with 400 \( \mu \)l of soybean oil in a centrifuge tube. Samples then were allowed to stand at room temperature (25 ± 1°C) for 30 minutes, followed by centrifugation at 3000 g for 30 min at room temperature. The volume of the supernatant (free oil) was measured, and OAC was calculated as follows:

\[ \text{OAC (ml/g)} = \frac{V_f}{W} \]

where \( V_f \) is the volume of free oil, \( V_0 \) is the initial volume of oil, and \( W \) is the weight of the dry sample.

**Foaming properties**

Foaming ability (FA) and foam stability (FS) of collagen hydrolysates were measured according to the method of Shahidi et al. [29] with modification. First, 25 ml of each sample (0.1 percent, 0.5 percent, and 1 percent w/v solution, pH: 7) were homogenized at 28,000 rpm with an Omni TH Homogenizer (Omni International, NW, Kennesaw, GA, USA) for 2 minutes. The total volume was measured at 0, 1, 5, 10, 20, and 30 minutes after whipping. FA and FS were calculated according the following equations:

\[ \text{FA} (%) = \frac{V_f - V_0}{V_0} \times 100 \]

where \( V_f \) is the volume after whipping (ml) and \( V_0 \) is the volume before whipping (ml).

\[ \text{FS} (%) = \frac{V_2}{V_0} \times 100 \]

where \( V_2 \) is the volume at 1, 5, 10, 20, and 30 min after whipping (ml) and \( V_0 \) is the volume before whipping (ml).

**Emulsifying properties**

Emulsifying activity (EA) and emulsion stability (ES) were determined according to the method of Dachuan et al. [28] applied by Li et al. [30] with modification. First, 50 ml of each sample (7 percent solution in distilled water) (pH 7.0) were homogenized at 18,000 rpm for 30 seconds using the Omni TH Homogenizer. The sample was then mixed with 50 ml of soybean oil and homogenized at 18,000 rpm for 5 minutes. The emulsion was divided into two tubes. One of the tubes was centrifuged at 1500 g for 5 minutes, and the volume of the emulsified mixture was measured. The second tube was placed at 25°C for 5 hours and the volume of the emulsified mixture was recorded every hour. EA and ES were calculated using the following equations:

\[ \text{EA} (%) = \frac{V_e}{V_0} \times 100 \]

where \( V_e \) is the volume of the emulsified mixture and \( V_0 \) is the total volume of the mixture.

\[ \text{ES} (%) = \frac{V_2}{V_1} \times 100 \]

where \( V_2 \) is the volume of the residual emulsion after each hour of resting and \( V_1 \) is the volume of the initial emulsion.

**RESULTS AND DISCUSSION**

**Degree of hydrolysis**

Degrees of hydrolysis for the alcalase, protamex, and trypsin hydrolysates were 45 percent, 39 percent, and 35 percent, respectively. Thus, alcalase was more effective at hydrolyzing collagen than trypsin and protamex, which is in agreement with reports for other proteins [18, 31]. According to SDS-PAGE pattern and FTIR spectra of JPSC, the collagen structure was almost intact [22]. Thus, it is assumed that the hydrolysis process has been occurred only through application of trypsin, alcalase and protamex.

**SDS-PAGE profile**

Figure 1a shows the electrophoretic pattern of JPSC (under reducing and non-reducing conditions). There was no difference between the reducing and non-reducing patterns, suggesting that this type of collagen does not contain disulfide bonds. This observation was confirmed by the results of the elemental analysis, which showed only a trace amount of sulphur in the sample (data not shown). The a-chain band was the major component of the JPSC electrophoretic pattern (~136 kDa). High molecular weight b- and g-components with molecular mass of around 227 and 296 kDa, respectively; were also observed. The only a band found in JPSC was α1, which is characteristic of type II collagen. In addition, the JPSC pattern was similar to that of standard collagen type II extracted from chicken sternum. Thus, the data suggest that the major collagen in the Chrysaora sp.1 umbrella was type II collagen composed of a homotrimer of three α1 chains.

Type II collagen from marine resources is rare, with only a few reports of isolation of type II collagen (e.g., from cannonball jellyfish [32] and Cyanea nozaki kishinouye [33]). However, Nagai et al. [2000] reported that collagen from the rhizostomous jellyfish Rhopilema asamushi consisted of α1 and α2 chains, and collagen from Stomolophus nomurai [34] and Stomolophus melagris...
hydrodynamic water, bound water, and physically entrapped water (35). WBC is one of the most important functional properties of proteins, as it can determine their industrial applications, especially in food. WBC was highest for the alcalase-digested JCH (8.94) followed by the protamex-digested JCH (8.64) and the trypsin-digested JCH (8.16) (Table 1). WBC can be affected by the presence of polar and non-polar groups, which can bind to water (35). Differences in WBC among the three sample types might be due to differences in the availability of the aforementioned groups, which depends on the type enzyme used and the amino acid sequence of the released peptides (particularly at the N- and C-terminals). However, the WBC did not differ much among the sample types, as they were derived from the same source and the amino acid composition was similar.

Data show the mean value ± standard deviation from three separate samples. Different superscripts in the same column indicate significant differences (p < 0.05).

Water holding capacity
WHC is a key property in food products such as meat and bakery products. All hydrolysates exhibited a high WHC (Figure 2). Among the samples, the alcalase-digested JCH had the highest value, followed by the protamex-digested JCH and trypsin-digested JCH. A higher WHC appears to be due to production of lower molecular weight peptides, which have greater hydrophilic properties than larger sized peptides (36). It is also possible that digestion by alcalase and protamex resulted in retention of more hydrophilic amino acids compared to digestion by trypsin (36). WHC of all samples was higher than that of distilled water and lower than that of glycerol.

Functional properties of jellyfish collagen hydrolysates

Water absorption capacity
WAC in this study was defined as the amount of moisture absorbed by dry samples at a certain humidity. Table 1 lists the WAC of the collagen hydrolysates in the form of grams of absorbed water per gram of sample. WAC was significantly higher for trypsin/protamex-digested JCH than alcalase-digested JCH. All three samples, however, exhibited higher water absorption capacity compared to pig skin and yak bone collagen, possibly due to a higher content of hydrophilic groups in the JCHs (30). This result suggests that JCH may be a useful alternative source of collagen hydrolysate for applications in which water absorption is required.

Water binding capacity
WBC, which is described as water absorption capacity in some references, is the ability of a sample to hold water in the form of
Emulsifying properties

Emulsifying properties of proteins are important in the food industry (35). Figure shows the EA and ES of the samples. Trypsin-digested JCH had the highest (52.1 percent) followed by protamex-digested JCH (49.3 percent) and alcalase-digested JCH (42 percent). ES was monitored for 5 hours. The ES value decreased by the third hour and remained constant up to the fifth hour. Trypsin-digested JCH had the highest ES followed by alcalase-digested JCH and protamex-digested JCH, which had almost the same values.

Molecular size, peptide composition, and the lipophilic-hydrophilic arrangement affect emulsion properties (30), and there is a direct relationship between length of peptide and emulsifying properties (38). Therefore, the higher EA and ES of trypsin-digested JCH might be due to its higher molecular mass (Figure 1b). This result is in agreement with that reported for gelatin hydrolysate from the skin of sole and squid (10), but the JCH had a higher EA. The EA of samples at concentrations of 0.1 percent and 1 percent was slightly higher for trypsin-digested JCH than for alcalase-digested JCH and protamex-digested JCH. No significant difference was observed between EA of samples at the concentration of 0.5 percent.

Figure 3a shows the stability of the generated foams from different hydrolysates. After 30 minutes, no significant difference was observed between different hydrolysates at the same concentration. The foam of the 1 percent concentration samples lost approximately 25 percent of its initial volume, whereas this value was around 75 percent for the 0.5 percent and 0.1 percent concentrations. Thus, the higher concentrations of hydrolysate exhibited higher FS, possibly due to the formation of a stiffer network around air particles (30). However, no significant difference was observed between concentrations of 0.1 percent and 0.5 percent.

Foaming properties are influenced by molecular size, protein structure, surface charge, flexibility and hydrophobicity of the hydrolysate, extent of protein-protein interaction, and Proline and Hydroxyproline contents, which are related to the parent protein and the hydrolysis conditions (10, 30). In this study, all hydrolysates were derived from the same parent protein. Therefore, similarities in foaming properties are reasonable, and the slight differences observed likely were due to different molecular sizes of polypeptides and possible differences in the amino acid sequence of peptides obtained by digestion with the various enzymes.
agreement with the OAC results, which is known to be in direct relation with the emulsion properties.

CONCLUSION

JPSJC was successfully isolated from the umbrella of Chrysaora sp.1 and identified to be type II collagen. The type of enzyme used to hydrolyse collagen was shown to affect the properties of the resulting hydrolysate. All three types of JCH exhibited good functional properties and likely will prove to be useful in enhancing physicochemical properties of the products when used as bioactive reagents in functional foods, as pharmaceutical and biomaterial component, and in nutraceutical industry.

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VITAMIN D IN HEALTH AND DISEASE

KEYWORDS: vitamin D, calcium, bone health, immunomodulation, insulin

Abstract

Vitamin D is the precursor of the steroid hormones 1,25-dihydroxy-vitamin D (1,25(OH)2D). Most of the biological effects of 1,25(OH)2D derive from its interaction with vitamin D receptor (VDR), an intracellular transcription receptor. VDR regulates genes that influence bone mineral homeostasis, immunomodulation, insulin secretion, cell cycle and the detoxification of exogenous and endogenous compounds. The most investigated function of 1,25(OH)2D is the regulation of calcium and phosphate homeostasis and, in turn, the bone turnover rate in skeletal tissue. Recently, also the extra-osseous activity of 1,25(OH)2D has received increasing attention. Nevertheless, the lack of definition of optimal status and dosage of vitamin D limits the knowledge derived from available studies and highlights the need for further studies on these topics.

INTRODUCTION

Vitamin D is a group of fat-soluble seco-sterols of which the most common in foods are cholecalciferol or vitamin D3 and ergocalciferol or vitamin D2. Nutritionally, the two forms are similarly metabolized in humans and for a long time were considered equivalent, however, recent studies have revealed a different half-life of resulting metabolites (see below). Cholecalciferol is produced in the skin of animals, including humans, when light energy is absorbed by a precursor molecule, the 7-dehydrocholesterol, however, because any excess previtamin D3 or vitamin D3 is destroyed by sunlight, excessive exposure to sunlight does not cause vitamin D3 intoxication (1). Ergocalciferol is the vegetable form of the vitamin, is obtained also from the UV irradiation of the yeast sterol ergosterol and is found naturally in sun-exposed mushrooms (2). Both vitamin D2 and vitamin D3 are used for food fortification and in vitamin D supplements (3). Vitamin D is thus not a true vitamin, because individuals with adequate exposure to sunlight do not require dietary supplementation (4), however, the use of sunscreen with a sun protection factor of 30 reduces vitamin D synthesis in the skin by more than 95 percent (3). Dietary sources of vitamin D include some fatty fish, egg yolks, milk and a number of plants and fungi, however, natural diets typically do not contain adequate quantities of the vitamin, thus exposure to sunlight or consumption of foodstuffs purposely supplemented (especially, milk and cereals) and/or dietary supplements are necessary to prevent deficiencies. The bioavailability of vitamin D depends on intestinal absorption capacity, liver health of the individuals, and their fat storage because adipose tissue easily absorbs it. Although an adequate exposure to sunlight is the main source of vitamin D for humans, the large number of factors affecting its synthesis and/or bioavailability and the carcinogenic potential of ultraviolet radiation increase the importance of diet in maintaining adequate levels of this vitamin. Hypovitaminosis D is a worldwide problem with health consequences including musculoskeletal and non-musculoskeletal disorders (5). Risk factors for hypovitaminosis D in developing countries are similar to those of Western countries and include extremes of age, female sex, winter season, dark skin pigmentation, malnutrition, lack of sun exposure, a covered clothing style and obesity (6).

BIOCHEMISTRY

Vitamin D is a pro-hormone that is metabolized within the body to the hormonally-active forms 1,25 dihydroxyergocalciferol and 1,25-dihydroxycholecalciferol (also known as calcitriol). When vitamin D3 enters the circulation after UV exposure is primarily associated with vitamin D binding protein (DBP), whereas after intestinal absorption, vitamin D is coupled with both DBP and lipoproteins. Vitamin D from either route is delivered initially primarily to the liver, where is hydroxylated to 25-hydroxychole(ergo)calciferol (25 OH-D) and in this form returns into the circulation associated with DBP and then is released to the kidney where the dihydroxylated forms (1,25(OH)2D) are obtained after a second hydroxylation reaction. The renal synthesis of 1,25(OH)2D is tightly regulated by calcium levels and two hormones, parathyroid hormone (PTH), with up-regulatory action, and fibroblast-like growth factor-23, with down-regulatory effect. Moreover, it has been recently shown that many other tissues (breast, colon, skin, brain, ovary, prostate, etc.) contain the two hydroxylases necessary for vitamin D activation thus achieving an autocrine production of 1,25(OH)2D (7-10). Most of the biological effects of 1,25(OH)2D derive from its interaction with an intracellular transcription receptor called vitamin D receptor (VDR), a member of the steroid nuclear receptors.
superfamily. VDR have an ample distribution throughout the body and regulates several hundred genes, however its biological activity in most tissues remains somewhat controversial. VDR has been found in the endocrine glands (pituitary, pancreas, parathyroid, gonads, and placenta), cardiovascular tissues (endothelial cells, vascular smooth muscle cells, and cardiomyocytes) and in hematolymphopoietic cells, where 1,25(OH)\textsubscript{2}D\textsubscript{3} has been shown to regulate cell differentiation, the production of interleukins and cytokines and, consequently, immune system response and balance (4). VDR modulates gene expression, via heterodimerization with the retinoid X receptor. The complex formed by 1,25(OH)\textsubscript{2}D, RXR, and VDR can recognize the vitamin D responsive elements (VDREs) of genes regulated by 1,25(OH)\textsubscript{2}D producing effects in bone mineral homeostasis, immunomodulation, insulin secretion, detoxification of exogenous and endogenous compounds, and cell cycle (11).

VITAMIN D STATUS

Vitamin D is bound to DBP much less strongly than 25 OH-D, thus vitamin D is more accessible than 25 OH-D for internalization into several cell types with the exception of kidney and parathyroid gland that provide the vitamin D endocrine system and use the megalin-cubilin endocytic system for 25 OH-D uptake (7). Stable isotope 25 OH-D measurements have revealed that 25 OH-D\textsubscript{2} half-life is shorter than 25 OH-D\textsubscript{3} half-life, and these half-lives are affected by DBP concentration and genotype (12). The blood concentration of 1,25(OH)\textsubscript{2}D\textsubscript{3} is 1000 times lower than that of 25 OH-D and the half life of the farmer is by far lower than that of latter (4-6 hours vs. 15-20 days); for these reasons 25 OH-D is used as indicator of nutritional and functional status of vitamin D. The optimal serum levels of 25 OH-D are controversial, however the most accepted classification of vitamin D status is the following: deficient below 20 ng/mL, insufficient between 21 to 29 ng/mL, and sufficient between 30 to 44 ng/mL (3, 4). The most common disorders related to low vitamin D serum levels are secondary hyperparathyroidism and, as a consequence, childhood rickets, osteomalacia, increased risk of minimal trauma fractures, nonspecific muscle pain, poor muscle strength and low bone mineral density (BMD). Suboptimal vitamin D status has also been associated with an increased risk of several non-musculoskeletal disorders, such as infectious diseases, in particular respiratory infections, and an increased risk of common cancers, autoimmune diseases, hypertension, diabetes mellitus and possibly cardiovascular diseases (6), although causal links between vitamin D insufficiency and the above diseases still need to be established. Melanin is extremely efficient in absorbing UVB radiation, and, thus, increased skin pigmentation markedly reduces vitamin D\textsubscript{3} synthesis and increases hypovitaminosis risk for people with dark skin living in a temperate climate. The risk of vitamin D Insufficiency or deficiency was high across all age groups, but children and the elderly are those most exposed to these risks. A major risk factor for infants is maternal vitamin D deficiency, while for the elderly the age-dependent decrease in concentrations of 7-dehydrocholesterol, the precursor of vitamin D3 in the skin (1). Obesity also increases the risk of lower 25 OH-D levels probably because of the sequestration of vitamin D by the large body fat pool (4). Antiseizure medications, glucocorticoids and fat malabsorption are other common causes of deficiency (5). As for the other liposoluble vitamins, an excess of vitamin D causes hypervitaminosis (4). Vitamin D deficiency is treated by supplementation with vitamin D\textsubscript{2} or vitamin D\textsubscript{3} (3).

DINATORY SOURCES AND RECOMMENDED INTAKES

Foods, in particular vegetables, contain very low amounts of vitamin D. Table 1 report the vitamin D the content of selected foods. Foods from animal origin contain above all, vitamin D\textsubscript{3} together with its metabolite 25 OH-D\textsubscript{3}, while vegetable vitamin D\textsubscript{3} together with its metabolite 25 OH-D\textsubscript{3} (13). Dairy contains also varying amounts of vitamin D\textsubscript{3} and its metabolite 25 OH-D\textsubscript{3} (13). Fish, in particular oily fish, and fish products are regarded as the major dietary source of vitamin D (0 to 30 µg vitamin D/100 g (13). When exposed to UV radiation, also mushrooms become an abundant source of vitamin D\textsubscript{3} (2, 14).

Recently, the availability of fortified foods with vitamin D has increased worldwide. In the United States and Canada, milk, orange juices, cereals, yogurts, cheeses and some bread products are fortified with vitamin D (3, 15). Italy, most countries do not fortify milk with vitamin D, because in the 1950s there were many cases of vitamin D toxicity in young children, and, as a result, the fortification of foods with vitamin D was forbidden by law (3). However, Sweden and Finland now fortify milk, and many European countries add vitamin D to cereals, breads, and margarine (3). Fortification of foods with vitamin D\textsubscript{3} or vitamin D\textsubscript{3} has been shown to be a safe and effective way to increase 25 OH-D levels in children and adults (16-18). Dietary recommendations to prevent vitamin D deficiency vary between countries. The recommended dietary intakes for the Italian population are the following: 10 µg/day for infants; 15 µg/day for individuals aged 1-74 years and 20 µg/day for individuals aged more than 74 years (19). Since, as above mentioned, an excess of vitamin D causes hypervitaminosis, the European Food Safety Authority set the tolerable upper intake level for vitamin D for adults at 50 µg Vitamin D/day (20).

VITAMIN D IN CALCIUM, PHOSPHATE AND BONE MINERAL HOMEOSTASIS

The regulation of serum calcium and phosphate homeostasis and, in turn, the bone turnover rate in skeletal tissue is the most investigated and known function of 1,25(OH)\textsubscript{2}D\textsubscript{3}. Plasma calcium normal levels are also necessary for the proper functioning of vasodilatation, neuromuscular junctions, nerve transmission, and hormonal secretion, in particular of parathyroid hormone (PTH). 1,25(OH)\textsubscript{2}D\textsubscript{3} exerts these actions primarily through three different mechanisms: the stimulation of calcium and phosphate...
absorption in the intestine and, in association with PTH, the renal re-absorption of calcium and the mobilization of calcium from bone. Without vitamin D, only 10 to 15 percent of dietary calcium and about 60 percent of phosphorus are absorbed. Vitamin D sufficiency enhances calcium and phosphorus absorption by 30–40 percent and 80 percent, respectively. Moreover, 1,25(OH)\(_2\)D regulates the transcription of genes encoding for calcium-transporting proteins and bone matrix proteins, such as osteocalcin and osteopontin, and promotes osteoclastogenesis and bone re-absorption. The elevation of serum calcium concentration decreases PTH secretion through the parathyroid calcium receptor and calcitriol that suppress PTH synthesis in the parathyroid gland through the activation of VDR and the subsequent suppression of parathyroid gene expression and cell proliferation. 1,25(OH)\(_2\)D, despite the promotion of bone re-absorption, over the long term through the control of PTH secretion supports bone formation.

In large scale epidemiological studies, serum 25 OH-D levels were associated with BMD with varying limits of 25 OH-D (from 20 ng to 36 ng), from which the BMD reaches a plateau, depending on the target population and the geographical region (4). It is important to note that these 25 OH-D concentrations were also those that determine the lower PTH levels. The specific thresholds of circulating 25 OH-D for optimal bone health has not yet been defined because of the imprecision of different 25 OH-D assays (21) and the polymorphism of genes involved in cholesterol synthesis, hydroxylation, and vitamin D transport (22). Nevertheless, the results of numerous studies indicate that serum 25 OH-D levels of at least 20 ng (50 nmol L\(^{-1}\)) are required for normalization of PTH levels, to minimize the risk of osteomalacia and for optimal bone cell function (23). However, Priemel et al. (24) by analyzing 25 OH-D serum levels and transflect crest bone biopsies from 675 patients without secondary bone disorders of northern Germany, showed that manifest mineralization defects of bone were absent in all individuals with 25(OH)D serum levels above 30 ng/mL (75 nmol L\(^{-1}\)). In the last decades numerous clinical studies that address the effects of vitamin D deficiency or supplementation on bone health have been performed. The last critical review of these studies published in 2007 showed that the trials on vitamin D alone were scarce and in most studies the effects of vitamin D and calcium supplementation could not be separated (21).

Furthermore, the authors found that the supplementation with vitamin D alone did not produce significant effects, while led to some positive result in combination with calcium. In particular, this meta-analysis showed that vitamin D\(_3\) at a dose of at least 700 IU/day with calcium supplementation had a small beneficial effect on bone mineral density compared to placebo and reduced the risk of fractures only in specific population subgroups, such as older individuals in institutional settings and postmenopausal women (21). More recently, Gorter et al. performed a meta-analysis of the studies on the effects of vitamin D deficiency or supplementation on a particular bone health-related factor, i.e. fracture healing. Even in this case, the available data were too scarce and inconclusive to elucidate a possible role of vitamin D on fracture healing (25). These results highlight the need for additional high quality studies on these topics.

**EXTRA-OSSEOUS EFFECTS OF VITAMIN D**

Many of the extra-osseous effects of vitamin D appear to be triggered by locally produced 1,25(OH)\(_2\)D in a number of cell types expressing VDR, such as skin, adipocytes, cells of the immune system, colon, pancreas, and the vasculature (11). Extra-osseous activity of vitamin D is not defined completely, but it appears that 1,25(OH)\(_2\)D, likely cooperating with other regulators, improves barrier function, exerts immunoregulation, antimicrobial defense and xenobiotic detoxification, and, through the controls cell cycle and insulin secretion, could be involved in cancer and type II diabetes prevention (11).

Vitamin D regulates the body’s defence systems at various levels. 1,25(OH)\(_2\)D upregulates genes encoding proteins required for tight junctions, gap junctions and adherents junctions, thus improving the physical barrier effect of epithelial cells in the skin, gut, respiratory and urinary tract and protecting from injury or invasion by pathogens (26). The role of the vitamin in immunomodulation has long been known, but it is only in the last years that its potential role in normal human immune function has been recognized, through a series of genome-wide analyses that revealed the induction of vitamin D system by pathogens, and the resulting intracellular machinery able to promote both innate and adaptive immune responses (27). 1,25(OH)\(_2\)D have an opposite effect on adaptive and innate immune response: generally it inhibits T helper cell proliferation and B cell immunoglobulin production, and, in contrast, it promotes the proliferation of immunosuppressive regulatory T cells and their accumulation at sites of inflammation (28, 29).

VDR, together with other ligand-activated nuclear receptor transcription factors (pregnane X receptor, constitutive androstane receptor and farnesoid X receptor) regulates the inducible expression of genes that encode crucial drug- and bile-acid–transporter proteins and several key enzymes of drug and xenobiotic metabolism in enterohepatic tissues (30). The expression of these genes influences patient drug response and eventual clinical outcome and can be conditioned by the seasonal changes in vitamin D status (31). 1,25(OH)\(_2\)D also modulates the transcription of cell cycle proteins, which decrease cell proliferation and increase cell differentiation of a number of specialised cells of the body, such as osteoclastic precursors, enterocytes, keratinocytes, etc. (32-34). Furthermore, numerous epidemiological and experimental studies have showed that 1,25(OH)\(_2\)D elicits growth inhibitory and pro-differentiating effects in several malignant cell types and retards tumour growth in animal models (32-34). Moreover, numerous clinical trials have demonstrated that sufficient dosing and exposure to 1,25(OH)\(_2\)D is critical for achieving antitumor effects during intermittent regimens (32).

Vitamin D seems to be also involved in the regulation of fat depot in adipocytes and insulin signalling, thus participating in obesity and type 2 diabetes mellitus prevention. Observational studies have showed a negative association between indicators of obesity and/or type 2 diabetes mellitus and serum levels of vitamin D (4). However, it is important to remember that, as above mentioned, vitamin D can be easily absorbed into adipose tissue, and this contributes to the reduction of 25 OH-D serum levels. Cell and animal studies have demonstrated that in adipocytes (including human adipocytes) vitamin D inhibits the active form of adipogenic transcription factors and lipid accumulation during cell differentiation (35, 36). Furthermore, 1,25(OH)\(_2\)D activates the transcription of the human insulin receptor gene and peroxisome proliferator activator receptor, stimulates the expression of insulin receptor, and enhances insulin-mediated glucose transport in vitro (37). Nevertheless, there is a general lack of consistency in studies that have evaluated the effects of vitamin D on insulin secretion and sensitivity, because the effects disappeared after adjustment for adiposity (4).
CONCLUSIONS

Vitamin D, as steroid hormone precursor, has a range of physiological functions and its deficiency contributes to the pathogenesis of several major diseases. The ambiguity of most favourable therapeutic concentrations of serum 25 OH-D and the lack of optimal vitamin D dosing regimens limit the knowledge derived from available intervention studies and highlight the need of future well-designed studies on these topics. Furthermore, taking in account that the estimation of the risk of deficiency is very high in both industrialized and developing countries, the seasonal and geographical dependence of endogenous synthesis of vitamin D, and the insufficient vitamin D content of diet it becomes evident the need to increase the availability of fortified foods [16-18].

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KEYWORDS: Lupinus spp., nutraceutics, bioactive ingredients, Horizon 2020, Lupin Conference, EXPO-2015

Abstract
Lupin seeds are becoming a real food alternative both as nutritious and healthy whole food and source of nutriti- and techno-functional ingredients, especially the protein fraction which is the seed main component. Still, issues related to develop standardized, high-quality and health-promoting lupin ingredients and design new lupin-based food products have to be faced. Moreover, lupin component full potential to the prevention and treatment of many civilization diseases has not thoroughly been investigated yet. Two recent opportunities may contribute making significant steps toward the solution of these problems. One is the proposal of a lupin protein-devoted Horizon-2020 EU project in the frame of ‘Proteins of the future’ call. The other one is the body of activities associated to the 14th edition of the International Lupin Conference to be held in Milan, Italy under the auspices of EXPO-2015. Both initiatives are discussed in their relationships with the most advanced and recent scientific findings in the area.

INTRODUCTION
The growing world population, the crucial need of sustainable crop production strategies, the food security and safety issues, the key role of disease prevention, rather than therapy, all these aspects require dramatic and urgent answers by the institutional bodies and the scientific community. In this respect, one of the emerging problems to be faced concerns the demand for nutrient-rich food sources. Among the nutrients, proteins play a major role for the supply of amino acids, nitrogen and carbon skeletons to the body and for their emerging, and still under investigation, role of metabolism modulators. If many different food and protein sources, including insects, algae, unconventional marine sources, bacteria and unicellular eukaryotes, minor or exotic crops, can in principle be considered, nevertheless consumer acceptance of new and/or improved foods, as well as other factors related to market uptake, require proper consideration, if global food security together with environmental and socio-economic sustainability is to be ensured.

For these and other reasons, lupin seeds represent a real food alternative, both as nutritious and healthy whole food and as a source of nutri- and techno-functional ingredients, especially the protein fraction which is the lupin seed main component. Still, issues related to develop standardized, high-quality and safe lupin ingredients and design new lupin-based food products, for a sustainable and competitive lupin seed exploitation, have to be faced. Moreover, lupin ingredient full potential to the prevention and treatment of many civilization diseases has not thoroughly been investigated yet and the need of clinical intervention studies has become crucial.

RECENT ADVANCES IN THE INVESTIGATIONS ON LUPIN (PROTEIN) BENEFICIAL HEALTH EFFECTS
Various reviews have previously dealt with lupin beneficial health effects (1-4). However, this area is rapidly expanding and new knowledge is accumulating from day to day. One reason is the burst of the consumers’ interests toward nutritional/dietary issues. Another reason is the growing availability of analytical in vivo/in vitro methodologies, which allow more accurate analyses even on minor food components or when they are available in small amounts. Moreover, the search of a direct cause/effect relationship between food components and bioactivities, which is the underlying principle of “molecular nutraceutics” (1), is providing a deeper understanding of the effects themselves and the related mechanisms of action.

Lupin proteins, beyond their role as amino acid suppliers, positively impact on key physiological parameters, including serum glucose and lipid concentrations and blood pressure levels (3). As far as fibre and fibre/protein associations are concerned, their effect on the satiety control, as well as in the facilitation of food intestinal transit, seems extremely relevant too.

Table 1 reports a synopsis of the scientific literature concerning lupin biological activities in the last decade. As it can be seen, most of the works concern human and animal studies carried out to elucidate mainly the lupin serum glucose- and lipid-lowering activities, the remaining being cellular and molecular studies mostly aimed at assessing the mechanism involved in the described positive health effects. Interestingly, the published results arise from several independent
laboratories, which, though with different methodologies and approaches, all agree on the beneficial effects of lupins in the different reported areas. A difficulty in the comparison of results obtained by the different laboratories emerges from Table 2, where the type and formulation of lupin samples is shown to span from the whole flour to specific components or fractions. Anyway, proteins seem to play pivotal roles, especially in the control of plasma glucose and lipid concentrations. The lupin active protein responsible of plasma cholesterol and triglyceride decrease has not been ascertained as yet, while the glucose-control active lupin protein has unequivocally been identified in the protein termed γ-conglutin (5, 6). In addition to the synopses of Tables 1 and 2, it seemed worth to report here the most recent advances in the field with greater details and the corresponding references. To do this, we divided the scientific contributions of the last four years basing on three main areas of interest, that are: concentration of plasma lipids and hypertension, which are relevant to cardiovascular diseases, and glycaemia control, which is relevant to all glucose impairment syndromes, including diabetes and metabolic syndrome.

**Effects on plasma lipid concentration**

The reduction of total cholesterol levels in moderately hypercholesterolemic individuals by using lupin protein/fibre combinations has been described by Sirfoni et al. (7). As a matter of facts, these subjects seem to be the best targets for this approach, which candidates lupin proteins and fibre as preventive as well as therapeutic effective agents. More recently, Baehr et al. (8) have shown that the administration in 72 hypercholesterolemic subjects of 25 g lupin protein per day, included in complex food products, significantly lower the levels of total and LDL cholesterol, triacylglycerols, homocysteine and uric acid. The observed effect is not solely to be attributed to arginine, abundant in lupin proteins, which Jobgen et al. (9) suggested to be a lipoprotein metabolism modulator as nitric oxide donor. A study by Parolini et al. (10) demonstrated a marked cholesterol-lowering activity of proteins from *L. angustifolius* in rats. Lupin protein-fed rats displayed higher hepatic mRNA levels of SREBP-2, a major transcriptional regulator of intracellular cholesterol levels, and CYP7A1, the rate-limiting enzyme in bile acid biosynthesis. Radtke et al. (11) investigated whether lupin protein isolates may modulate sterol excretion and mRNA expression of intestinal sterol transporters by the use of pigs. Data revealed that the relative mRNA concentrations of intestinal sterol transporters involved in cholesterol absorption were lower and that the cholesterol-lowering effect could then be attributed to an increased faecal excretion of cholesterol and a reduced intestinal uptake of cholesterol.

An example of the use of hydrolyzed lupin proteins, in combination or not with lupin insoluble fibre, is the study by Kapravelou et al. (12) in a diet-induced animal experimental model of hypercholesterolemia. Lupin hydrolyzate resulted effective to reduce plasma and hepatic triacylglycerols and showed promising effects on glucose metabolism, as well as protection against dietary-induced renal alterations. Such health benefits can be complemented by lupin protein hydrolyzate and insoluble fibre effects on large intestine physiological status, due to its fermentative and water holding capacity. The dietary inclusion of both functional ingredients, along with adequate physical activity and, when needed, pharmacological treatments, has great potential in the prevention and treatment of lipid and glucose metabolism disorders.

**Effects on hypertension**

A lupin hydrolyzate obtained by pepsin digestion showed angiotensin-converting enzyme (ACE)-inhibitory activity, which is related to blood pressure control, as described by Boschin et al. (13). The findings of this work demonstrated that in particular two major storage proteins, namely α- and β-conglutins, are the main sources of ACE-inhibitory peptides.

**Effects on plasma glucose concentration**

After the identification of lupin γ-conglutin as the molecule responsible for glucose-lowering properties in lupin seeds (14), recently also lupin based foods were tested in glucose
controlling activity trials. This is the case of the inclusion of selected lupin proteins in pasta matrices subsequently used to fed rats made hyperglycaemic (15). Pasta supplemented with isolated lupin protein fractions reduced body weight gain and food intake of rats and the g-conglutin enriched-pasta in particular showed a significant decrease of plasma glucose concentration upon glucose overload trial. The effect observed with g-conglutin is therefore unrelated to the already known low glycemic index of lupin seeds, which do not contain starch. These results evidence the potential of supplementing traditional foods with exogenous nutraceutical seed proteins. Dove et al. (16) assessed the acute effects of lupin-based beverages on glucose and insulin responses in type 2 diabetic individuals. Compared to the control beverage, the 4 h post-beverage glucose response was lower and the insulin and C-peptide responses were higher for lupin treated subjects. Whether this effect was attributable to a specific action of g-conglutin has not been ascertained in this work. Keogh et al. (17) monitored a lower post-prandial glucose and insulin response after a lupin bread breakfast compared with a white bread breakfast in human subjects. Higher fullness (satiety) responses were also recorded.

THE OPPORTUNITIES FOR FURTHER INVESTIGATIONS

Future lupin (protein) developments strictly depend on a number of factors, among which availability and stability of the seed production, set up and use of sustainable industrial processing strategies, development of tradition-inspired new food items, clinical evaluation of the beneficial effects, market evaluation and penetration are of utmost importance. Clearly, all these and other multifaceted aspects cannot properly be addressed by a unique public or private institution. On the other hand, gathering different teams to implement these activities also raises some problems, including the intellectual property ones or the adoption of new industrial strategies, which are not easy to deal with. A great opportunity, in this respect, has recently been given by the opening of the R&D project call entitled: “Proteins for the future” in the framework of EU Horizon 2020 programme. Indeed, this specific call aims at increasing quality and sustainability of food protein EU production, strengthening industrial cooperation among research institution and SMEs, opening new market opportunities in the area, as well as unveiling the positive impacts of novel food proteins on human health, environment and biodiversity.

Based on these main goals, a group of lupin-experienced research teams from the academy and private companies has decided to set up a lupin protein project with the underlying principle to implement the complete valorisation of this autochthonous food protein source. Indeed, lupin seed represents a real alternative to other existing food protein sources or exotic and non-native potential alternatives, which are far from the traditional dietary habits of the European consumers. The project is entitled “LUPIN Sustainable and Healthy Protein” (LUSH Protein); it is coordinated by a Spanish team and it gathers 23 teams from 10 EU and non-EU countries. The work-plan is strongly multidiscipline-oriented and consists of 7 work packages, which are reported and inter-connected in the diagram of Figure 1. As pointed out in the project foreword, while the world population continues to grow, the global demand of meat, dairy and fish products for human consumption is rising, thus becoming unsustainable. Therefore, a trend towards diets containing more plant protein seems not just strongly recommended, but inescapable. The LUSH Proteins consortium has been brought together to implement lupin-based protein products as a sustainable alternative protein source in the diet of European populations and clearly state real benefits to human well-being and health. By gathering the most experienced teams in the field, with a strong industrial involvement and international cooperation with third countries, the project has the ambition to give a boost to lupin protein, as an excellent candidate for the sustainable supply of food protein in Europe.

The Project has already undergone the first step of evaluation and it has been invited to the next and last evaluation stage.

THE OPPORTUNITIES TO DISSEMINATE AND MAKE USE OF THE NEW FINDINGS

In this scenario, the need of both a scientifically-correct and public-oriented dissemination plan of the recent findings and achievements is acute.

Thirty-five years ago, the interest in lupin as a useful crop and its seed components has led some forerunner scientists to establish the International Lupin Association (ILA). ILA is a multi-national organism, presently based in New Zealand, which represents a variety of scientific interests related to the lupin biology, growing and utilization. The main institutional function of ILA is the constant public-oriented dissemination plan of the recent findings and achievements is acute. The next ILC edition will be the 14th one and it will be organized in Milan on the 21st-26th of June, 2015 by our team. This is the first Italian edition in 35 years. There are two main reasons that led to choose this location. The first is that in Italy a growing industrial commitment to the exploitation of plant bioactive compounds and the formulation of novel foods, linked to the Mediterranean tradition, is taking place in the recent years. In this respect, a
great expectation for the outcomes of the Conference is being recorded. The second reason is that the Conference will take place during the course of the Universal Exposition EXPO-2015, entitled “Feeding the planet—Energy for life”. Indeed, the ILC 14th edition does match the mission and the expectations of this global event.

The title of the 14th ILC is “Developing lupin crop into a modern and sustainable food and feed source”. An official web site of the Conference has been set up (18). The scientific sessions of the 14th ILC will cover all aspects of lupin growth and utilization with the unique multidisciplinary approach typical of the former Conference editions. About 60 lectures by authoritative speakers in any field and from many countries are planned for this Conference edition. The Conference Organizing and Scientific Committees will guarantee a top-quality congress program, including key and regular lectures and poster exhibitions. Post-congress thematic satellite meetings may also be organized, according to the delegate expressions of interest. These will give the participants a tailored offer of updated and requested topics.

In detail, the topics that will be dealt within the Conference sessions are: i) Genetics, Genomics and Molecular Breeding; ii) Agronomy, Farming; iii) Taxonomy, Biodiversity and Agro-ecology; iv) Biochemistry, Biotechnology, Proteomics and Metabolomics; v) Physiology, Plant development and Symbiosis; vi) Pathology and Protection; vii) Food, Feed and Non-food uses; viii) Health Benefits. Given these facts, main intents of this ILC edition, in the unique traditional multidisciplinary format, will be the following ones: i) To improve our science-based understanding of lupin world; ii) To disseminate innovation in lupin growth, protection and utilization by asserting the sustainability of lupin crop; iii) To update our knowledge on design, formulation, preparation and value of lupin-based food, drink and feed; iv) To review and explore the opportunities of lupin seeds and derivatives in human nutrition and well-being; v) To help creating links and synergies among lupin stakeholders.

A visit to EXPO-2015 installations will complete the technical/scientific activities of the Conference. Figure 2 reports the drawing designed by our team and implemented by the Artist Chiara Cataldi to condense next Lupin Conference messages in a witty and impactive cartoon.

CONCLUSIONS

In the case of lupin seed, its proteins represent an innovative, though not new, nutrient source providing cost-effective and resource-efficient alternatives to animal or other established sources, with more positive impacts on human health and the environment. The integrated R&D approaches, possibly granted by the Horizon 2020 programme, can provide support to EU policies on agriculture, nutrition, health, environment, sustainable food development and security by increased market uptake of an existing wealthy and healthy source. While focusing our present and future initiatives on lupin seed as an actual and feasible alternative, we are also aware that all issues mentioned in this manuscript fully match EXPO-2015 mission by: i) providing deeper knowledge on the use and beneficial properties of a legume seed which, together with all other legume grains, does belong to the components of the so-called Mediterranean diet; ii) envisaging an international dissemination scheme represented by the ILC and related initiatives; iii) being strictly related to the EU call “Proteins for the future” which de facto is a pillar of the EXPO-2015 mission. As a matter of facts, the large scale and integral utilization of lupin as a food, feed and non-food source is one of the feasible answers to EXPO challenges.

If the main barrier to the wider diffusion and utilization of lupin is the still scarce demand from users and final consumers, which in turn prevents industrial/commercial investment plans, these international dissemination events can effectively contribute breaking the vicious circle and overcoming the bottleneck. These perspectives and related solutions will benefit of EXPO large platform and, in turn, provide to the Universal Exposition innovative and sustainable strategies and approaches to the “Feeding the planet” mission.

REFERENCES AND NOTES

KEYWORDS: yield, cost, income, production, genetically modified crops, pesticide, carbon sequestration, no tillage, environmental impact quotient

Abstract
This paper summarises the economic and key environmental impacts that crop biotechnology has had on global agriculture. The analysis shows that there have been very significant net economic benefits at the farm level amounting to $18.8 billion in 2012 and $116.6 billion for the seventeen year period 1996-2012 (in nominal terms). These economic gains have been divided roughly 50 percent each to farmers in developed and developing countries. GM technology have also made important contributions to increasing global production levels of the four main crops, having added 122 million tonnes and 230 million tonnes respectively, to the global production of soybeans and maize since the introduction of the technology in the mid-1990s. In terms of key environmental impacts, the adoption of the technology has reduced pesticide spraying by 503 million kg (-8.8 percent) and, as a result, decreased the environmental impact associated with herbicide and insecticide use on these crops (as measured by the indicator the Environmental Impact Quotient (EIQ)) by 18.7 percent. The technology has also facilitated a significant reduction in the release of greenhouse gas emissions from this cropping area, which, in 2012, was equivalent to removing 11.88 million cars from the roads.

INTRODUCTION
This paper provides insights into the reasons why so many farmers around the world have adopted crop biotechnology and continue to use it in their production systems since the technology first became available on a widespread commercial basis in the mid-1990s. The paper draws, and is largely based on, the considerable body of peer reviewed literature available that has examined these issues. It specifically focuses on the farm level economic effects, the production effects, the environmental impact resulting from changes in the use of insecticides and herbicides, and the contribution towards reducing greenhouse gas (GHG) emissions.

The report is based on extensive analysis of existing farm level impact data for biotech crops. Whilst primary data for impacts of commercial cultivation were not available for every crop, in every year and for each country, a substantial body of representative research and analysis is available and this has been used as the basis for the analysis presented. This has been supplemented by the authors' own data collection and analysis. The analysis of pesticide usage also takes into consideration changes in the pattern of herbicide use in recent years that reflect measures taken by some farmers to address issues of weed resistance to the main herbicide (glyphosate) used with herbicide tolerant biotech crops. For additional information on the methodology, data sources and references (1), readers should consult a detailed examination of these issues in Brookes G and Barfoot P (2014) GM crops: global socio-economic and environmental impacts 1996-2012, available at www.pgeconomics.co.uk.

ECONOMIC IMPACTS
GM technology has had a significant positive impact on farm income derived from a combination of enhanced productivity and efficiency gains (Table 1). In 2012, the direct global farm income benefit from GM crops was $18.8 billion. This is equivalent to having added 5.6 percent to the value of global production of the four main crops of soybeans, maize, canola and cotton. Since 1996, farm incomes have increased by $116.6 billion.

The largest gains in farm income in 2012 have arisen in the maize sector, largely from yield gains. The $6.7 billion additional income generated by GM insect resistant (GM IR) maize in 2012 has been equivalent to adding 6.6 percent to the value of the crop in the GM crop growing countries, or adding the equivalent of 3 percent to the $226 billion value of the global maize crop in 2012. Cumulatively since 1996, GM IR technology has added $32.3 billion to the income of global maize farmers.
Substantial gains have also arisen in the cotton sector through a combination of higher yields and lower costs. In 2012, cotton farm income levels in the GM adopting countries increased by $5.5 billion and since 1996, the sector has benefited from an additional $37.7 billion. The 2012 income gains are equivalent to adding 13.5 percent to the value of the cotton crop in these countries, or 11.5 percent to the $47 billion value of total global cotton production. This is a substantial increase in value added terms for two new cotton seed technologies.

Significant increases to farm incomes have also resulted in the soybean and canola sectors. The GM HT technology in soybeans has boosted farm incomes by $4.8 billion in 2012, and since 1996 has delivered over $37 billion of extra farm income. In the canola sector (largely North American) an additional $3.66 billion has been generated (1996-2012).

Table 2 summarises farm income impacts in key GM crop adopting countries. This highlights the important farm income benefit arising from GM HT soybeans in South America (Argentina, Bolivia, Brazil, Paraguay and Uruguay), GM IR cotton in China and India and a range of GM cultivars in the US. It also illustrates the growing level of farm income benefits being obtained in South Africa, the Philippines, Mexico and Colombia.

In terms of the division of the economic benefits obtained by farmers in developing countries relative to farmers in developed countries, Table 3 shows that in 2012, 46.2 percent of the farm income benefits have been earned by developing country farmers. The vast majority of these income gains for developing country farmers have been from GM IR cotton and GM HT soybeans. Over the seventeen years, 1996-2012, the cumulative farm income gain derived by developing country farmers was 49.9 percent ($58.15 billion).

Examining the cost farmers pay for accessing GM technology, Table 4 shows that across the four main GM crops, the total cost in 2012 was equal to 23 percent of the total technology gains (inclusive of farm income gains plus cost of the technology payable to the seed supply chain (2)).

For farmers in developing countries the total cost was equal to 21 percent of total technology gains, whilst for farmers in developed countries the cost was 25 percent of the total technology gains. Whilst circumstances vary between countries, the higher share of total technology gains accounted for by farm income gains in developing countries, relative to the farm income share in developed countries, reflects factors such as weaker provision and enforcement of intellectual property rights in developing countries and the higher average level of farm income gain on a per hectare basis derived by developing country farmers relative to developed country farmers.

Notes: All values are nominal. Others = Virus resistant papaya and squash and herbicide tolerant sugar beet. Totals for the value shares exclude ‘other crops’ (ie, relate to the 4 main crops of soybeans, maize, canola and cotton). Farm income calculations are net farm income changes after inclusion of impacts on yield, crop quality and key variable costs of production (eg, payment of seed premia, impact on crop protection expenditure)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Increase in farm income 2012</th>
<th>Increase in farm income 1996-2012</th>
<th>Farm income benefit in 2012 as a percentage of total value of production of those crops in GM adopting countries</th>
<th>Farm income benefit in 2012 as a percentage of total value of global production of crop</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM herbicide tolerant soybeans</td>
<td>4,797.9</td>
<td>37,998.6</td>
<td>4.6</td>
<td>4.6</td>
</tr>
<tr>
<td>GM herbicide tolerant cotton</td>
<td>1,187.9</td>
<td>5,414.7</td>
<td>1.2</td>
<td>0.9</td>
</tr>
<tr>
<td>GM herbicide tolerant canola</td>
<td>417.0</td>
<td>1,956.4</td>
<td>4.3</td>
<td>1.3</td>
</tr>
<tr>
<td>GM insect-resistant cotton</td>
<td>6,727.8</td>
<td>32,377.2</td>
<td>6.6</td>
<td>3.5</td>
</tr>
<tr>
<td>GM insect-resistant canola</td>
<td>5,323.5</td>
<td>36,377.2</td>
<td>15.1</td>
<td>11.2</td>
</tr>
<tr>
<td>Others</td>
<td>46.5</td>
<td>450.7</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Total</td>
<td>15,764.4</td>
<td>115,896.6</td>
<td>13.6</td>
<td>10.6</td>
</tr>
</tbody>
</table>

Notes: All values are nominal. Others = Virus resistant papaya and squash and herbicide tolerant sugar beet. Totals for the value shares exclude ‘other crops’ (ie, relate to the 4 main crops of soybeans, maize, canola and cotton). Farm income calculations are net farm income changes after inclusion of impacts on yield, crop quality and key variable costs of production (eg, payment of seed premia, impact on crop protection expenditure).

Table 1. Global farm income benefits from growing GM crops 1996-2012: million US $
PRODUCTION EFFECTS OF THE TECHNOLOGY

Based on the yield impacts used in the direct farm income benefit calculations above and taking account of the second soybean crop facilitation in South America, GM crops have added important volumes to global production of maize, cotton, canola and soybeans since 1996 (Table 5).

<table>
<thead>
<tr>
<th>1996-2012 additional production (million tonnes)</th>
<th>2012 additional production (million tonnes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybeans</td>
<td>16.7</td>
</tr>
<tr>
<td>Maize</td>
<td>1.6</td>
</tr>
<tr>
<td>Cotton</td>
<td>16.2</td>
</tr>
<tr>
<td>Canola</td>
<td>2.0</td>
</tr>
<tr>
<td>Sugar beet</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Note: GM HT sugar beet only in the US and Canada since 2008

Table 5. Additional crop production arising from positive yield effects of GM crops

The GM IR traits, used in maize and cotton, have accounted for 97.1 percent of the additional maize production and 99.3 percent of the additional cotton production. Positive yield impacts from the use of this technology have occurred in all user countries (except for GM IR cotton in Australia [3]) when compared to average yields derived from crops using conventional technology (such as application of insecticides and seed treatments). The average yield impact across the total area planted to these traits over the 17 years since 1996 has been +10.4 percent for maize and +16.1 percent for cotton.

The primary impact of GM HT technology has been to provide more cost effective (less expensive) and easier weed control, as opposed to improving yields. The improved weed control has, nevertheless, delivered higher yields in some countries. The main source of additional production from this technology has been via the facilitation of no tillage production system, shortening the production cycle and how it has enabled many farmers in South America to plant a crop of soybeans immediately after a wheat crop in the same growing season. This second crop, additional to traditional soybean production, has added 114.3 million tonnes to soybean production in Argentina and Paraguay between 1996 and 2012 (accounting for 93.5 percent of the total GM-related additional soybean production).

ENVIRONMENTAL IMPACT FROM CHANGES IN INSECTICIDE AND HERBICIDE USE

To examine this impact, the study has analysed both active ingredient use and utilised the indicator known as the Environmental Impact Quotient (EIQ) to assess the broader impact on the environment (plus impact on animal and human health). The EIQ distils the various environmental and health impacts of individual pesticides in different GM and conventional production systems into a single ‘field value per hectare’ and draws on key toxicity and environmental exposure data related to individual products. It therefore provides a better measure to contrast and compare the impact of various pesticides on the environment and human health than weight of active ingredient alone.

Readers should, however, note that the EIQ is an indicator only (primarily of toxicity) and does not take into account all environmental issues and impacts. In the analysis of GM HT technology we have assumed that the conventional alternative delivers the same level of weed control as occurs in the GM HT production system.

GM traits have contributed to a significant reduction in the environmental impact associated with insecticide and herbicide use on the areas devoted to GM crops (Table 6). Since 1996, the use of pesticides on the GM crop area was reduced by 503 million kg of active ingredient (8.8 percent reduction), and the environmental impact associated with herbicide and insecticide use on these crops, as measured by the EIQ indicator, fell by 18.7 percent.

In absolute terms, the largest environmental gain has been associated with the adoption of GM Insect Resistant (IR) technology. GM IR cotton has contributed a 25.6 percent reduction in the volume of active ingredient used and a 28.2 percent reduction in the EIQ indicator (1996-2012) due to the significant reduction in insecticide use that the technology has facilitated, in what has traditionally been an intensive user of insecticides. Similarly, the use of GM IR technology in maize has led to important reductions in insecticide use, with associated environmental benefits.

The volume of herbicides used in GM maize crops also decreased by 203 million kg (1996-2012), a 9.8 percent reduction, whilst the overall environmental impact associated with herbicide use on these crops decreased by a significantly larger 13.3 percent. This highlights the switch in herbicides used with most GM herbicide tolerant (HT) crops to active ingredients with a more environmentally benign profile than the ones generally used on conventional crops.

Important environmental gains have also arisen in the soybean and canola sectors. In the soybean sector, herbicide use decreased by 4.7 million kg (1996-2012) and the associated environmental impact of herbicide use on this crop area decreased, due to a switch to more environmentally benign herbicides (-15 percent). In the canola sector, farmers reduced herbicide use by 15 million kg [-16.7 percent reduction] and the associated environmental impact of herbicide use on this crop area fell by 26.6 percent (due to a switch to more environmentally benign herbicides).

In terms of the division of the environmental benefits associated with less insecticide and herbicide use for farmers in developed countries relative to farmers in developing
countries. Table 7 shows a 54 percent:46 percent split of the environmental benefits (1996-2012) respectively in developed (54 percent) and developing countries (46 percent). About three-quarters (73 percent) of the environmental gains in developing countries have been from the use of GM IR cotton.

It should, however, be noted that in some regions where GM HT crops have been widely grown, some farmers have relied too much on the use of single herbicides like glyphosate to manage weeds in GM HT crops and this has contributed to the development of weed resistance. There are currently 28 weeds recognised as exhibiting resistance to glyphosate worldwide, of which several are not associated with glyphosate tolerant crops (www.weedscience.org).

For example, there are currently 14 weeds recognised in the US as exhibiting resistance to glyphosate, of which two are not associated with glyphosate tolerant crops. In the US, the affected area is currently within a range of 20 percent-40 percent of the total area annually devoted to maize, cotton, canola, soybeans and sugar beet (the crops in which GM HT technology is used).

In recent years, there has also been a growing consensus among weed scientists of a need for changes in the weed management programmes in GM HT crops, because of the evolution of these weeds towards populations that are resistant to glyphosate. Growers of GM HT crops are increasingly being advised to be more proactive and include other herbicides (with different and complementary modes of action) in combination with glyphosate in their integrated weed management systems, even where instances of weed resistance to glyphosate have not been found.

This proactive, diversified approach to weed management is the principal strategy for avoiding the emergence of herbicide resistant weeds in GM HT crops. It is also the main way of tackling weed resistance in conventional crops. A proactive weed management programme also generally requires less herbicide, has a better environmental profile and is more economical than a reactive weed management programme.

At the macro level, the adoption of both reactive and proactive weed management programmes in GM HT crops has already begun to influence the mix, total amount and overall environmental profile of herbicides applied to GM HT soybeans, cotton, maize and canola and this is reflected in the data presented in this paper.

### IMPACT ON GREENHOUSE GAS (GHG) EMISSIONS

The scope for GM crops contributing to lower levels of GHG emissions comes from two principal sources:

- Reduced fuel use from less frequent herbicide or pesticide applications and a reduction in the energy use in soil cultivation. The fuel savings associated with making fewer spray runs (relative to conventional crops) and the switch to conservation, reduced and no-till farming systems, have resulted in permanent savings in carbon dioxide emissions. In 2012 this amounted to about 2,111 million kg (arising from reduced fuel use of 791 million litres). Over the period 1996 to 2012 the cumulative permanent reduction in fuel use is estimated at 16,736 million kg of carbon dioxide (arising from reduced fuel use of 6,268 million litres):
  - The use of ‘no-till’ and ‘reduced-till’ (4) farming systems. These production systems have increased significantly with the adoption of GM HT crops because the GM HT technology has improved growers ability to control competing weeds, reducing the need to rely on soil cultivation and seed-bed preparation as means to getting good levels of weed control. As a result, tractor fuel use for tillage is reduced, soil quality is enhanced and levels of soil erosion cut. In turn more carbon remains in the soil and this leads to lower GHG emissions. Based on savings arising from the rapid adoption of no till/reduced tillage farming systems in North and South America, an extra 6,706 million kg of soil carbon is estimated to have been sequestered in 2012 (equivalent to 24,613 million tonnes of carbon dioxide that has not been released into the global atmosphere). Cumulatively, the amount of carbon sequestered may be higher than these estimates due to year-on-year benefits to soil quality; however it is equally likely that the total cumulative soil sequestration gains have been lower because only a proportion of the crop area will have remained in no-till and reduced tillage. It is, nevertheless, not possible to confidently estimate cumulative soil sequestration gains that take into account reversions to conventional tillage because of a lack of data. Consequently, our estimate of 203.560 million tonnes of carbon dioxide not released into the atmosphere should be treated with caution.

Placing these carbon sequestration benefits within the context of the carbon emissions from cars, Table 8 shows that:

- In 2012, the permanent carbon dioxide savings from reduced fuel use were the equivalent of removing 0.94 million cars from the road;
- The additional probable soil carbon sequestration gains in 2012 were equivalent to removing 10.94 million cars from the roads;
- In total, in 2012, the combined GM crop-related carbon dioxide emission savings from reduced fuel use and additional soil carbon sequestration were equal to the removal from the roads of 11.88 million cars, equivalent to 41.38 percent of all registered cars in the United Kingdom;
- It is not possible to confidently estimate the probable soil

<table>
<thead>
<tr>
<th>Change in field EQ impact (in terms of million field EQIha units): developed countries</th>
<th>Change in field EQ impact (in terms of million field EQIha units): developing countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM HT soybeans</td>
<td>-4,170.9</td>
</tr>
<tr>
<td>GM HT maize</td>
<td>-5,585.5</td>
</tr>
<tr>
<td>GM HT cotton</td>
<td>-351.0</td>
</tr>
<tr>
<td>GM HT canola</td>
<td>-508.1</td>
</tr>
<tr>
<td>GM IR maize</td>
<td>-1,574.4</td>
</tr>
<tr>
<td>GM IR cotton</td>
<td>-805.5</td>
</tr>
<tr>
<td>GM HT sugar beet</td>
<td>-2</td>
</tr>
<tr>
<td>Total</td>
<td>-13,601.8</td>
</tr>
</tbody>
</table>

Table 7. GM crop environmental benefits from lower insecticide and herbicide use 1996-2012: developing versus developed countries
CONCLUDING COMMENTS

Crop biotechnology has, to date, delivered several specific agronomic traits that have overcome a number of production constraints for many farmers. This has resulted in improved productivity and profitability for the 17.3 million adopting farmers who have applied the technology to 160 million hectares in 2012.

Overall, there is a considerable body of evidence, in peer reviewed literature, and summarised in this paper, that quantifies the positive economic and environmental impacts of crop biotechnology. Readers are encouraged to read the peer reviewed papers cited in the references section of the main report this summary is taken from, and to draw their own conclusions.

ACKNOWLEDGEMENT

The authors acknowledge that funding towards the researching of this paper was provided by Monsanto. The material presented in this paper is, however, the independent views of the authors – it is a standard condition for all work undertaken by PG Economics that all reports are independently and objectively compiled without influence from funding sponsors.

REFERENCES AND NOTES

1. The total number of reference sources used totals about 150, most of which are from peer reviewed journals
2. The cost of the technology accrues to the seed supply chain including sellers of seed to farmers, seed multipliers, plant breeders, distributors and the GM technology providers
3. This reflects the very good levels of Heliothis/Helicoverpa (boll and bud worm pests) pest control previously obtained with intensive insecticide use. The main benefit and reason for adoption of this technology in Australia has arisen from significant cost savings (on insecticides) and the associated environmental gains from reduced insecticide use
4. No-till farming means that the ground is not ploughed at all, while reduced tillage means that the ground is disturbed less than it would be with traditional tillage systems. For example, under a no-till farming system, soybean seeds are planted through the organic material that is left over from a previous crop such as corn, cotton or wheat
The role of sensory perception and sensory evaluation in the development of reduced sodium foods

KEYWORDS: sensory evaluation, consumer sensory testing, sodium reduction, salt, food and drinks

Abstract

Food and drink manufacturers are under increasing pressure to reduce the sodium (generally referred to as salt) content of their products. One of the factors making this difficult is the negative effect on the sensory profile of many products when salt (NaCl) content is reduced. Sensory evaluation is a scientific discipline that aims to research and understand the sensory properties of products and the hedonic responses to them. This article is a selective introduction and review into how and why knowledge about sensory perception and the use of sensory evaluation tools are important for food and drink salt reduction efforts. The main objective is to introduce the reader to this area, rather than critique the strengths and weaknesses of particular techniques.

INTRODUCTION

Many disease risk factors are associated with ‘unhealthy’ food and drink consumption, including excessive consumption of sodium or salt. According to the Centres for Disease Control and Prevention, too much sodium can increase blood pressure and the risk for a heart attack and stroke (1). In January 2013, the World Health Organisation (WHO) published guidelines saying that adults should consume no more than 2g of sodium, or 5g of salt per day. According to the WHO, public health measures to reduce sodium can include negotiating with food manufacturers to reduce the amount of salt in processed foods (2). Government enforced limits on salt use have become a reality with the March 2013 signing of legislation requiring mandatory sodium reductions in several foodstuffs in South Africa by June 2016 (3). The possibility of similar legislation being implemented in other countries or regions is a topic of some discussion within the media, and political and commercial circles.

Along with a range of other challenges (such as safety, cost, process, shelf-life, etc.), the sensory properties of reduced salt products are often key to their success. In the modern world, many consumers will expect to be able to purchase foods that are healthy and that also taste good. According to Dr. Leon Bruner, Chief Science and Regulatory Affairs Officer for the Grocery Manufacturers Association; “Reducing sodium in products without negatively affecting consumer acceptance must be taken into consideration, because a ‘healthy food’ will not promote health if it is not purchased or eaten” (4). Understanding the sensory profile of food and drinks and how this relates to liking, acceptance and choice is therefore a key tool in the challenge to develop reduced salt foods.

The effect of salt reduction on the sensory profile of food and drinks

The most obvious effect of reducing salt in foods is to reduce saltiness, but other effects are also likely. For example reducing salt may increase bitterness, decrease sweetness, and decrease positive flavours associated with saltiness and sweetness (5). The problem is compounded by the fact that some salt substitutes or replacements can taste bitter, particularly potassium chloride (KCl) (6). Reduced salt foods will often need to be reformulated to ‘add back’ missing flavours, or with new ingredients that will mask bitterness.

In addition to taste and flavour, reducing salt can also affect appearance and/or texture of some food products. For example, Pietrasik and Gaudette (7) found that salt reduction adversely affected some textural characteristics of restructured hams, and that hams containing reduced amounts of salt had different colour attributes compared to that of the control salt level ham.

FACTORS OF RELEVANCE TO SALTY TASTE PERCEPTION AND LIKING

There are a range of factors which can inform both the design of reduced salt food and drinks, and research
to develop these. These factors include thresholds and sensitivities, inherent vs. learnt preferences, adaptation and habituation, personal habits and motivations, and cultural norms and practices.

The detection threshold, recognition threshold, and supra-threshold perceived taste intensity for a given concentration of a basic tastant (including salt), will vary from person to person. Thresholds and perceived intensities will also vary depending on the food and drink context.

In general, liking for most context-appropriate taste stimuli, will show something like an inverted U-shaped trend, increasing with concentration up to a certain point, flattening out, and then decreasing. Therefore, finding the optimum level and its concentration range for a particular food or drink formulation and/or target consumer group can be very important for successfully designing reduced salt foods.

It is believed that environmental factors, including exposure to salty foods may have a large influence on salt taste preference [8, 9]. This is different for example, from sweetness for which there is a clear preference from birth [10]. A potential implication of any learnt liking for saltiness is the importance of children’s diets in establishing their preferences for salty taste: But there is also the possibility that changes (reductions) in salt level in the diet later in life could also affect adult preferences for salty taste [11].

It has been reported that a preference for salt in children that is greater than that in adults emerges between three and eleven years of age, and possibly peaks in the teens [12]. On the other end of the life span, studies of changes in taste perception of basic tastes with ageing are considered by some researchers as not conclusive. The confounding variables include the medium in which tastants are evaluated, and the diversity of older adults [13]. On the other hand, in a systematic review, Methven et al. [14] found that salt detection thresholds increased with age, although the average threshold for older people was found to be below the levels of salt found in most foods.

Although variation in sensations from salt have been found to be associated with differences in hedonic responses to high sodium foods and thus sodium intake [15]; it should be noted that given the complexities of individual tastes and perceptions, it is not certain that a reduction in saltiness perception will necessarily always affect pleasure or acceptance of a food or drink.

The difference threshold is the amount of salt that needs to be added or taken away for a difference in saltiness to be detected. The difference threshold will vary depending on the individual, the food and drink context, and the original salt concentration. The situation is quite complex in foods because there are mixture effects; reducing saltiness may cause other tastes and flavours to become more prominent and potentially mask the salt that is present. If more is known about salt difference thresholds within various foods, it can be easier to design reformulations.

Long term habituation is also important factor to consider. Psychological and physiological adaptation to saltiness levels in specific foods and drinks over longer periods of time may allow consumers to get used to less salt. This is likely to be more successful where changes are made gradually over time, and is commonly referred to as the ‘stealth approach’ [16].

Short term physiological adaptation can reduce saltiness perception [17]. Structure of foods may also result in some salt not being readily accessible to the taste receptors. The result is that much of the salt that is consumed within a food or drink product may not be efficiently perceived. Research into food and salt structures, and their effects on sensory perception, may help to reduce adaptation and/or create structures that are effective for salty taste delivery.

Habits and cultural norms and practices are a related issue. Individuals need to be motivated and informed to reduce sodium in their diet to look for, buy and consume reduced sodium foods. Some individuals may add salt to foods because it is what they have always done, or that is how the society in which they live prepares or serves specific foods and drinks. Individuals may not be consuming salt at the optimum level for their own tastes, and may often not know how much salt or sodium they are ingesting. For example, a recent eight country study [18] revealed that participants largely underestimated their individual salt intake and they also showed difficulties in identifying the main dietary sources of salt.

OVERVIEW OF SENSORY EVALUATION

Sensory evaluation is a scientific discipline that aims to research and understand the sensory properties of products and the hedonic responses to them. In general, sensory evaluation helps to characterise how the attributes and sensory profile of a product are linked to perception, consumer liking, and ultimately consumer choice.

Within the context of salt reduction, the type of questions that sensory evaluation may help to answer, include:

- How is salty taste perceived and how can salty taste be enhanced?
- How much can salt be reduced without consumers noticing or rejecting product?
- What can be used as a substitute for salt? How much of the substitute is needed?
- Which structures/formulations promote inherent saltiness?
- What do consumers like? What do they expect?

Within the discipline of sensory evaluation there are two main approaches that can be used in the context of sodium reduction: These are objective methods and consumer sensory methods.

Objective sensory research methods

Methodologies and techniques that use screened and/or trained assessors to objectively describe products and their attributes include two key areas:

- Discrimination testing – tests which are designed to determine if there is a significant difference between the sensory attributes or profile of two products.
- Common discrimination tests include triangle, duo-trio and paired comparison.
- Sensory profiling - methods that are designed to describe and quantify the attributes of food products and the differences between these products.

Reducing salt is likely to affect the perception of a product within the timescale of consumption, and
therefore time-based methods can also be important. The major time-based methods include measurement and evaluation of a single sensory attribute within a bite or sip or during consumption of a portion (time-intensity), measurement of the most dominant sensations at discrete time points over the course of a bite or sip or during consumption of a portion (temporal dominance of sensations), and measurement of the entire profile of a product at various points during consumption of a portion or a meal, etc. (progressive profiling). Panels of assessors are often used in objective sensory evaluation, and key outputs are choice frequencies or average scores. Numbers of assessors are chosen to establish a desired level of statistical confidence, and can vary from as low as eight in some sensory profiling applications, to approximately 60 for some discrimination testing situations, such as paired comparison testing (19).

**Consumer sensory research methods**

Methodologies and techniques that use consumers reporting on how much they like products and specific product attributes, or which products they prefer, are generally referred to as consumer sensory testing. Three of the most common types of consumer sensory research methods which can be used in the area of ‘reduced’ product development include:

- **Paired preference** – a simple test in which consumers are asked to choose their most preferred product/samples of two options.
- **Overall liking scales** – quantitative scales that give a statistical indication of overall liking or acceptance and whether there are significant differences between samples.
- **Diagnostic measures** – methods such as specific attribute liking scales, Just-About-Right scales, check all that apply techniques, and ideal profiling. These methods use consumers to determine if product attributes are at their optimum level and give an indication of any changes in formulation that may be necessary.

In general, consumer sensory testing is carried out with demographically appropriate consumers. Enough individuals are recruited to establish a desired level of statistical confidence in the results (this often means at least 100).

**Combining objective techniques and consumer response**

Objective sensory evaluation is useful for understanding sensory profiles and attribute intensities, whereas consumer sensory testing gives information about liking and acceptance. The output of objective and consumer sensory research methods can be combined to understand key drivers of liking for individuals and consumer segments, and to optimise sensory profiles and formulations or processes (see Figure 1). These combined methods are often referred to as preference mapping or modelling.

**Sensory evaluation in recent research**

Below are a few illustrative examples of the use of objective sensory and consumer sensory methods within the development of reduced salt products:

- Bubowski and Vickers (20) determined a set of sequential difference thresholds for sodium chloride reduction in plain water, and in water with added tastes to simulate a more complex-flavoured broth, using paired comparison tests and assessors from a trained descriptive panel. From these thresholds, two series of concentrations were established: a 26-step reduction for salt in water, and a 12-step reduction for salt in water with added stimuli. According to the authors, the difference in the number of steps illustrates the importance of product complexity in determining sensitivity to sodium reduction and provides basic information for manufacturers interested in gradually decreasing salt content of foods without being noticed by consumers.

- Canto et al. (21) investigated the sensory attributes and consumer acceptance of low-sodium restructured caiman steaks containing microbial transglutaminase (MTG) and salt replacers (KCl and MgCl$_2$). Sensory profiles were determined by eight experienced and trained assessors using eleven clearly defined attributes. Consumer panellists evaluated cooked steaks using liking and Just-About-Right scales. According to the authors, their findings suggest that the combination of MTG, KCl, and MgCl$_2$ can be employed as a suitable salt reduction strategy in restructured caiman steaks without compromising sensory attributes and consumer acceptance.

- Vella et al. (22) used a sensory time-intensity technique to evaluate the temporal profile of salty taste of seven varieties of sea salt and a Kosher, table salt, control. There were few differences in the maximum salt taste intensity, but some differences in the salts’ time-intensity profiles. The authors concluded that, based on the fact that the salts did not show large differences in taste intensity, and many of the salts did not contain less sodium than the Kosher control, using the studied sea salts as a sodium reduction strategy was not viable.

**SENSORY INTERACTIONS AND IN VIVO TECHNIQUES**

An interesting area of research is sensory interactions and cross-modal effects. The key question of relevance is: How do perceptions in one sensory modality affect another, and can these interactions be used to help reduce sodium? For example; how might odour, colour or texture increase salty taste or reduce bitter taste? Sensory and consumer research methodologies are being
developed to answer these type of questions. Using odour to enhance salty taste looks promising. For example, Nasri et al. (23) showed that a salt-associated food odour (sardine) can increase the salty taste in a reduced salt solution by 25 percent. The data was collected from sixty-four panellists who scored the odour intensity and taste intensity (sourness, bitterness, saltiness, and sweetness) of samples on linear scales from 0 to 10. The assessors were asked to evaluate a range of sample characteristics to avoid ‘dumping’ all sensations into the saltiness intensity.

In-vivo methodologies measure taste, flavour, or texture components in real time, in human subjects; using analytical, empirical, or sensory measurement tools. These techniques can be used to study what is actually happening to food and drinks and their components during consumption. When sensory evaluations are paired with analytical or empirical quantifications, the combination can give invaluable insights into sensory interactions, structure and contrast effects, etc.

Some interesting work can be done by looking at saliva. Tian and Fisk (24) conducted an evaluation of the delivery of salt from salted crisps to the saliva through measurement of perceived saltiness, saliva conductivity and sodium content by tongue swabbing over a 60 s period after a crisp was chewed but not swallowed. A measured peak in sodium delivery after 20 to 30 s correlated with a perceived saltiness peak. The results highlighted that during normal eating patterns, a proportion of sodium is likely to be consumed without being perceived.

Pflaum et al. (25) found (using in-mouth measurements and a mastication simulator) a coarse-pored bread gave significantly faster sodium release than a fine-pored bread of the same sample weight. Corresponding experiments with constant sample volumes revealed a significantly enhanced saltiness in the coarse-pored bread despite similar amounts of extracted sodium during the first seconds of chewing. According to the authors, therefore, saltiness was influenced both by the velocity of sodium release and by crumb texture.

THE IMPORTANCE OF CONTEXT

In traditional sensory evaluation, context issues were largely designed out of studies, but now are generally acknowledged as potentially very important. Context effects could be related to the product itself (amount, texture, formulation), how it is eaten (container, cutlery, etc.), what it is eaten with (other foods and drinks), the environment (temperature, lighting, sound, etc.), the volume consumed and frequency of consumption, and type and level of social interaction. Different contextual elements associated with eating are likely to affect perception of saltiness and/or acceptance of reduced sodium food and drinks.

Although traditional sensory methods are often based on sampling small amounts of product, in one consumption event; some newer approaches look at preferences and sensory profiles of products over longer time periods and in terms of consumption of realistic quantities. For example, Methven et al. (26) investigated the effect of repeated exposure on liking of no added salt soup. 37 participants, previously assessed for their preferred salt level in soup, were allocated to either an exposure group that received 20 ml soup samples with no added salt, to a group that received a 280 ml bowl of this soup, or to a control group that received 20 ml soup samples containing salt at a normal commercial range. Soups were presented on eight occasions, at approximately daily intervals. Increases in liking of the no-added salt soup were evident starting from the third daily exposure.

In addition to repeated exposure and amount of food, eating context has been shown to potentially have more of an effect on liking of foods than the salt content itself. For example Lucas, et al. (27) found that liking of hash browns was influenced by whether testing was in a laboratory or dining room environment. In a dining room environment, large decreases of sodium content of food were achievable with only minor decrease in liking and no effect on consumption of the food. The ability to perform well-designed and controlled studies, and observe eating and choice behaviour in realistic environments is important. Some research organisations (such as the Institut Paul Bocuse in France, and Wageningen University in the Netherlands) have designed facilities that play a dual role; serving both as a restaurant, and as a sensory/consumer research laboratory.

As well as the environment of a meal, the cutlery or crockery used to consume food could have an effect itself. Colour, shape and form, is an area where interactions and context may help to design reduced sodium foods or enhance consumer’s acceptance of existing products. Harrar and Spence (28) investigated the shape of the cutlery on taste perception of cheese. Participants identified cheese sampled from a knife as saltier than that eaten from a spoon, toothpick, or fork. The authors hypothesised that in cheese shops, samples are often given directly from the knife, and cheese shops often sell more aged, therefore saltier cheeses; so therefore eating cheese from the knife may have enhanced perceived saltiness.

Some recent research has used behavioural economics tools, combined with more traditional sensory evaluation methodologies, to gain a better understanding of how much individuals value ‘reduced’ or healthier foods and why. For example, Aimil and Hersleth (29) investigated salt replacement and brine injection in smoked salmon from objective descriptive, and sensory hedonic perspectives. In addition to liking, consumer participants also evaluated their willingness to pay within an experimental auction type procedure. Descriptive results showed brine injection samples differed in appearance, taste and texture from dry-salting samples, while NaCl + KCl samples obtained the same sensory profile as NaCl samples. Consumers preferred dry-salting samples, but did not discriminate between salt types, neither in liking nor in willingness to pay. According to the authors, the results indicate a market potential for partially salt-replaced smoked salmon.

CONCLUSIONS

Sensory evaluation, which measures perception and liking of the sensory properties of food and drink, is now seen as an important function in many organisations within the
food chain. This article has attempted to introduce the role of sensory perception in this area, and to explain how sensory evaluation is a vital addition to the tools available for the development of successful reduced salt food and drinks. The wide range of sensory evaluation techniques means that there is the opportunity to examine the effect of salt reduction on perception in both fundamental and applied ways.

REFERENCES AND NOTES

Aspartase activity of *Acinetobacter caFFacoaceticus* isolate from semi-arid alkaline soils of Comarca - L, North-East México

**KEYWORDS:** aspartase, *Acinetobacter calcoaceticus*, semi-arid soils

**Abstract**

Eight bacterial strains showing aspartase activity were isolated from the semi-arid soil samples of the Laguna region of North-East Mexico, using a selective culture medium with ammonium fumarate as an inducer for the production of aspartase. The enzyme activity of the strains was determined by the consumption of ammonium fumarate 0.5 M, pH 8.0 and 37°C. From the eight strains, ASP1 and ASP3 strains showed the highest specific enzyme activity (U/mg of protein), and were used for further studies and their molecular characterization. The production of aspartic acid by these strains was confirmed by paper chromatographic analysis. In order to identify the bacterial strains, primers specific to aspartase were used to amplify a conserved region of 430 bp. The amplicons were sequenced by the Sanger method and ASP3 and ASP1 strains were identified as *Acinetobacter calcoaceticus* PHEA-2 by BLAST search.

**INTRODUCTION**

There is a great demand for amino acids by various industries and nearly half of the global production of amino acids is used in the food industry, where L-glutamic acid, L-aspartic acid and L-phenylalanine are considered of great importance (1, 2). Various methods such as extraction, chemical synthesis, fermentation or enzymatic synthesis are used for the production of amino acids. The use of microorganisms in a fermentation process for producing amino acids using economical sources of carbon is becoming a profitable process (3), apart from various other advantages such as simplification of the process, decrease the cost of raw materials, energy, easy separation of the product, waste reduction and recycle of enzyme for various production cycles (4). In actual, amino acid L-aspartic acid, is produced on a large scale by the use of the enzyme aspartase and is widely used for the elaboration of aspartame (5). Aspartase or aspartate ammonia lyase (EC 4.3.1.1.) is associated with cell protein complex (6) and also can catalyze the reversible deamination of amino acid L-aspartic to fumarate (7), and hence both the L-aspartic acid and fumarate can function as substrates. The increase in the use of microorganisms and enzymes for bioprocesses has resulted in search for microorganisms from novel and unique environments. Therefore, the present work is focused on bioprospection and identification of bacteria with aspartase activity from the semi-arid soils of Laguna region, North-East Mexico and optimization of its production.

**EXPERIMENTAL**

**Purification and maintenance of bacterial strains with aspartase activity**

Soil samples were collected from the Laguna region and a selective culture medium with the following composition (g/L): 1.0 NaCl, 1.0 KCl, 2.5 yeast extract, 5.0 casein peptone, 1.0 ammonium fumarate and 2.0 bacteriological agar; pH 8.0 was employed. Spread plate technique was used for the isolation of bacterial strains and the plates were incubated at 37 °C for 24 h. The isolates were purified and their purity was confirmed by cell morphology and Gram staining. The strains were stored at 4°C in slants using the same agar described above.

**Growth of bacterial strains**

The bacterial strains were grown in 125 ml Erlenmeyer flasks containing 30 ml of broth containing nutrients at the composition mentioned previously. Three hundred µl of bacterial cell suspension (1.2 X10⁶ cells/ml) was inoculated and the flasks were incubated at 37°C with an agitation of 200 rpm for 8 hours. After the incubation, the cells were separated by centrifugation at 10,000 rpm, 20°C for 20 min and the cell pellet was washed twice with phosphate buffer at pH 7.0 and used for subsequent analyzes.

**Aspartase activity of the bacterial isolates**

The obtained cell pellet (2.6 X10⁹ cells/ml) was resuspended in phosphate buffer, pH 7.0 and used for the enzyme assay.
The reaction mixture contained 3 ml of 0.5 M ammonium fumarate, magnesium chloride 0.005 g and 0.024 ml toluene at pH 8.0. The reaction was initiated by adding 0.5 ml of the resuspended cell pellet. The reaction mixture was placed in a water bath at 37°C with constant stirring at 200 rpm. Aliquots of 6 µl were taken during each hour, diluted to 15 ml and the reaction was stopped by heating the samples in a water bath at a temperature of 80-100°C for three minutes. The consumption of substrate was determined by measuring the absorbance of the samples at 240 nm (8).

One unit of the Enzyme activity (U) is defined as the amount of enzyme which consumes 1 µmol of fumarate per hour at 37°C, pH 8.0 and the specific enzymatic activity (U/mg) is expressed as the units of enzyme activity per milligram of cell protein.

In the case of cell protein, 0.5 ml of cell suspension was hydrolyzed with 0.5 ml of 1N NaOH in a hot water bath at 100°C, centrifuged and the supernatant was analyzed by using dye binding assay (9). The concentration of samples were determined against a calibration curve prepared using egg albumin as standard (10). Ten replications were maintained for each strain and the results obtained were statistically analyzed with the Centurion XVI Statgraphics version 1.16.18.

Analysis of aspartic acid formation using paper chromatography

Whatman No.1 chromatography paper was used with a mixture of n-butanol, acetic acid and water (4:1:2) as mobile phase (11). Five µl of sample after the enzymatic reaction was applied on the chromatography paper along with L-aspartic acid (Sigma) at 500 ppm as standard. At the end of the run, the paper was sprayed with 0.2 percent ninhydrin in 75 percent ethanol and dried at 50°C for 30 minutes. The Rf value of samples were compared with the standard L-aspartic acid to confirm the aspartic acid production by the bacterial strains ASP3 and ASP1.

Molecular characterization of the bacterial strains.

DNA of the bacterial strains was extracted with QIAamp Mini Kit from Qiagen and were amplified using degenerate oligonucleotides designed on based upon the highly conserved region of aspartase reported previously in Bacillus sp. YM55-1 (12), E. coli (13) and Pseudomonas fluorescens (8). Forward (5’-AARATGGGIMGHACICAIYTDCARGAYGC-3’) and reverse (5’-GGRTHACYTTIWHCCGCAT-3’) primers were diluted to a concentration of 25 pmol/µl and used. The PCR mix contained 3 µl of DNA template, 8 µl of TDmH buffer containing dNTP’s at 25 pmol/µl and 1.5 mM MgCl2, 1 µl of Taq Polymerase, 1 µl each of forward and reverse primers and the total volume was adjusted to 25 µl. The DNA samples were amplified for 30 cycles under the following conditions: 1 min 94°C, 2 min 57°C, 1 min 72°C (14) using Health Care Esco Swift thermocycler Max Pro.

The PCR products of the strains ASP1 and ASP3 were purified and sequenced at the Instituto Potosino de Investigación Científica y Tecnológica (IPICYT) by the Sanger method using the 3130 Genetic Analyzer sequencer (Applied Biosystems). The obtained sequences were analyzed and compared with NCBI database using the BLAST (Basic Local Alignment Search Tool) for identification of the strains. Bio Edit Sequence Alignment Editor version 7.1.3.0 was used for comparing the obtained amplified sequence with aspartase related genes reported earlier.

Components of culture medium, biochemical reagents and enzymes used in this study are of analytical grade and obtained from Sigma-Aldrich Co., Mexico.

RESULTS AND DISCUSSION

Isolation and selection of the bacterial strains with aspartase activity.

From the eight different bacterial strains isolated, three strains ASP1, ASP3 and ASP8 were selected based on the activity in broth with ammonium fumarate as inducer. Of these strains, ASP1 and ASP3 showed higher activity than ASP8 (Table 1) and were chosen for further studies in this work.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Aspartase activity (U)</th>
<th>Specific Aspartase activity (U/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASP1</td>
<td>69,000</td>
<td>68,793</td>
</tr>
<tr>
<td>ASP3</td>
<td>34,998</td>
<td>33,174</td>
</tr>
<tr>
<td>ASP8</td>
<td>3,000</td>
<td>1,662</td>
</tr>
</tbody>
</table>

Table 1. Aspartase activity of ASP1, ASP2 and ASP3 bacterial isolates based on the consumption of ammonium fumarate 0.5 M; pH 8.0, agitation 200 rpm and at 37°C.

Aspartase activity of the isolated strains

Results on the kinetics of substrate consumption showed that ASP1 recorded higher consumption than ASP3 (Figure 1).

Based on the substrate consumption, aspartase activity and specific aspartase activity was determined as mentioned previously in the experimental section. ASP1 and ASP3 bacterial isolates recorded aspartase activity of 69,000 U and 34,998 U respectively. A similar trend was observed with respect to specific aspartase too. ASP1 showed specific enzyme activity of 68,793 U/ mg of cell protein, whereas ASP3 showed only 33,174 U/ mg of cell protein. Results clearly demonstrated that there is significant difference (P <0.05) between the strains with respect to enzyme activity. Previously whole cells of Escherichia coli ATCC 11303, has been reported with an enzyme activity of 11,290 U at pH 8.5 (15). However,
the same strain under immobilized conditions was reported with an activity 68,000 U [16]. It can be observed that free living cells of ASP1 of this present study showed almost similar levels of aspartase activity [69,000 U]. It has been observed in previous studies that aspartase loses its activity in acid pH and as well as pH greater than 8.5 [17] and the presence of magnesium ions at an alkaline pH of 8.0 provided stability to the enzyme [18].

Identification of L-aspartic acid in extracts of the medium
The products of the enzymatic reaction with whole cells of ASP1 and ASP3 were analyzed by paper chromatography and the formation of L-aspartic acid was confirmed by paper chromatography analysis. There is significant difference between the enzyme activity of ASP3 and ASP1 and the enzyme activity of ASP3 was nearly two times superior to ASP1. Both ASP1 and ASP3 strains were identified as Acinetobacter calcoaceticus PHEA-2.

PCR amplification of DNA
PCR product obtained was analyzed by gel electrophoresis and the corresponding amplicon (430 bp) was observed for ASP1 and ASP3.

Sequence analysis of the amplified fragments ASP1 and ASP3 strains.
The PCR products of ASP1 and ASP3 were sequenced at the Instituto Potosino de Investigación Científica y Tecnológica (IPICYT). The sequences obtained were analyzed and compared to the database at National Center for Biotechnology Information (NCBI) by means of BLAST and conservation of bacterial strains and during kinetic studies of this work.

REFERENCES AND NOTES

CONCLUSIONS
Whole cells of ASP1 and ASP3 bacterial strains isolated from soil of Laguna region showed aspartase activity with 0.5M ammonium fumarate, pH 8.0. Formation of aspartic acid as a result of the enzymatic reaction by these two strains was confirmed by paper chromatography analysis. There is an interesting metabolic diversity (20).
GMOs in Italy
The Regional Administrative Court of Lazio clarifies the concept of “emergency situation” that allows Member States to ban GMOs already authorized at EU level

On 23 April 2014 the Regional Administrative Court of Lazio (hereafter TAR Lazio) ruled that a Member State may prohibit the cultivation of genetically modified organisms (GMOs), although they have been authorized at European level, when the European Food Safety Authority (EFSA) has changed its original positive opinion and - meantime - the European Commission has not amended or suspended its authorization. Specifically, the TAR Lazio highlighted that the “emergency situation” and the precautionary principle, which allow Member States to take distances from the EU authorization system, shall be respected even when such National derogation has been adopted to resolve a procedural stalemate at European level, which prevents the adoption of a European legislation based on the most recent scientific findings of EFSA.

This judgment has been released in a particular moment for the GMOs European regulatory framework, increasing Member States’ choice to restrict or prohibit the cultivation of GMO’s on their territory.

In its judgment of the 23 April 2014 the TAR Lazio clarified the compulsory conditions that Member States must fulfil in order to introduce temporary bans on genetically modified organisms, already authorized at European level. The dispute stems from a decree, adopted on 12 July 2013 by the Minister of Health, together with the Minister of Agriculture, Food and Forestry and the Minister of Environment, which - on the basis of an “emergency situation” - prohibited the cultivation and sale of the biotech corn MON 810. The owner of a farm cultivating this kind of corn challenged the decree.

The TAR Lazio held that, on the basis of the principles already established by the Court of Justice of the European Union in case C-36/11, Pioneer Hi Bred Italy (paragraphs 69, 70 and 71), a Member State cannot autonomously ban the cultivation of GMOs, for health or environmental reasons, if they are already authorized in the Regulation (EC) No. 1829/2003, and reported in the list of the Council Directive 2002/56/EC.

However this legal framework provides some exceptions, as the one in Article 34 of Regulation (EC) No. 1829/2003, which states that in the context of emergency measures, an authorization can be modified or suspended, in case of serious risks for human and animal health, and for the environment, as long as Member States comply with the disclosure requirements of Article 53 and 54 of the Regulation. Also, it is required that the adopted measures shall be provisional. This exception was the legal basis for the adoption of the contested decree.

The TAR Lazio correctly noted that the emergency measures, together with the safeguard clauses of Article 23 of the Council Directive 2002/56/EC, are the only two cases that can justify an exception in that matter. In particular, according to TAR Lazio, the condition of “serious risk” (as analysed in case C-236/01, Monsanto Agriculture Italy SpA, paragraphs 106 and 107) shall be interpreted as a risk that lays down new elements based on consistent scientific data. Consequently, the judge excludes that these extraordinary measures can be adopted on the basis of hypothetical risks, based on mere suppositions that are not yet scientifically verified. In this regards, it is noted that Member States can take the interim safeguard and preventive measures whether the risk is assessed and completely demonstrated.

Hence, on the basis of the criteria offered by the Court of the EU, the TAR Lazio pointed out that a procedural stalemate at European level may legitimize Member States in adopting specific waivers, providing that there are real risks for human and animal health, and for the environment.

The TAR Lazio considered that the contested decree of the Italian government was in line with the precautionary principle, which legitimizes the protective measures, suspending also authorizations, when there is uncertainty on risks for human health. Such protective measures are allowed until the risks are fully demonstrated. In this sense, the judge concluded noting that a Member State may suspend or modify an authorization of cultivation and sale, even when the EFSA, changing the first assessment, reveals, on the basis of new scientific data, alarming aspects for
human and animal health, and for the environment. This is rightly allowed although the Commission has not yet taken specific action in light of the raised concerns.

Indeed, this judgment has been released in a particular moment for the GMOs regulatory framework, increasing Member States’ choice to restrict or prohibit the cultivation of GMOs on their territory. In fact, during the Council of the European Union of 12 June 2014, the Ministers of Environment have reached a political agreement on amendments to the Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms.

As a consequence Member States will better take into account their National context when deciding on GMOs cultivation. Those amendments also secure a Member State’s legitimate right to adjust its decision to restrict or ban cultivation during the 10 years GM authorisation period, if new objective circumstances arise.

Lawyer- Michela Velardo – Bruxelles
IMMUNE SYSTEMS OF HEALTHY CHILDREN
RESULTS OF STUDY IN 310 CHILDREN PUBLISHED IN PEDIATRICS

A study demonstrating that a follow-up formula containing a combination of DHA, a prebiotic blend of PDX and GOS, and yeast β-glucan supported the immune systems of healthy three- and four-year-olds was published in the June issue of Pediatrics, the official journal of the American Academy of Pediatrics. Children who consumed the follow-up formula (FUF) designed for this study by Mead Johnson Nutrition had fewer episodes and shorter duration of acute respiratory infections (P = .007) than children consuming an unfortified cow’s milk-based beverage. They also required fewer antibiotic treatments (P = .01) and missed fewer days of day care due to illness, the study found. In addition, the FUF group had both higher interleukin-10 and white blood cell (WBC) counts at the end of the study, suggesting an anti-inflammatory mechanism and/or an increase of effector immune cells (WBC counts remained in the normal range). In a double-blind, randomized, controlled, prospective trial, 3-4 year old children were fed 3 servings per day of either a FUF containing 25 mg DHA, 1.2 g PDX/GOS, and 8.7 mg yeast β-glucan (Wellmune WGP®) per serving or an unfortified, cow’s milk-based beverage (control) for 28 weeks. Fecal and blood samples were collected to assess immune markers and iron/zinc status. Incidence of acute respiratory infections, diarrheal disease, and antibiotic treatment were obtained from medical records.

www.wellmune.com

POLARIS INGREDIENTS RECEIVE APPROVAL FOR SALE DELIVERED BY BRAZILIAN AUTHORITIES

POLARIS is expanding its international export business by obtaining the authorisation to sell its products onto Brazil’s nutraceutical market. With this authorisation, the company is consolidating its international growth strategy with the opening of new markets, such as Brazil, with its marine oils rich in Omega 3. The Brazilian ministry of agriculture “MAPA” (Ministério da Agricultura, Pecuária e Abastecimento) has given its positive opinion and therefore authorises the sale of natural and concentrated marine oils from Polaris’ Omegavie® range in Brazil. Already present on the French market with customers in the nutraceutical, food and animal health sectors, Polaris now wants to explore other markets. Polaris is reinforcing its presence in South America by appointing a distributor in Brazil. “BRIC countries, and especially Brazil, are on our radar as they are showing growth. Consumer habits in Brazil are similar to the ones in France: they are very targeted at beauty and health. Our ingredients have got their place on this market”, says Stéphane Lozachmeur, Polaris CEO. Thanks to its high-tech unit for the production, concentration and purification of marine oils rich in Omega 3, POLARIS will start selling in Brazil in the coming months.

www.polaris.fr

FLEETWOODGOLDCOWYARD

FleetwoodGoldcoWyard has been presented with the 2013 KUKA System Partner of the Year Award at the Annual System’s Partner Summit in May. The teamwork of FleetwoodGoldcoWyard’s sales organization, engineering and electrical groups with the support of the KUKA organization assisted in achieving this award four of the last five years. Gary Mulski, Robotics Product Champion for FleetwoodGoldcoWyard commented: “The integration of robotics has really expanded our product line offerings to our customers and opened many new opportunities for us. We now offer a more flexible and innovative solution to our customers with robotic technology. We could not have done this without the tremendous sales and customer support we receive from KUKA Robotics. Thank you to everyone on the team that makes it all happen!” Jim Krapes, Case Palletizing and Robotics Leader for FleetwoodGoldcoWyard added: “It is an honour to receive this award from KUKA for the third straight year. It is a testament to the dedication and commitment from the FleetwoodGoldcoWyard and KUKA teams to provide our customers with the finest robotic technical solutions for their packaging requirements”. FleetwoodGoldcoWyard has supplied approximately 200 robotic systems worldwide which are either currently in operation or being manufactured. Installations include can end palletizers and accumulators, case and bag palletizers, depalletizers to unwrappers/debaggers.

www.fgwo.com

THE CHOC-A-LIKE™ PRODUCT PORTFOLIO

Barry Callebaut presents Choc-a-like™, its extended range of compounds for professional bakers, ice cream manufacturers and chocolatiers. The Choc-a-like™ product portfolio offers different flavours of smart compound solutions that combine the sensation of delicious chocolate taste, easy workability and perfect food appeal. The extended technical flexibility of Choc-a-like™ products allows chocolate professionals to put their creative ideas into practice while increasing workability and minimizing production costs. Barry Callebaut’s Choc-a-like™ product range allows food processing professionals to go beyond the technical limits of regular chocolate while retaining an authentic chocolate taste, look and feel. In some specific cases, for example the creation of pralines, the making of pastry products or adding coatings to ice cream, working with chocolate can be technically very challenging. Possible issues include fat blooming or very complex processing. The new Choc-a-like™ compounds offer the crack and shine of regular chocolate in a wide variety of textures for different mouth feel and taste experiences. Rapid cooling is possible as Choc-a-like™ compounds only require basic cooling methods: in most cases simply shock cooling is required. Sofie De Lathouwer, Marketing Director Western Europe for Barry Callebaut states: “Choc-a-like™ compound products give our customers a significant competitive edge thanks to a broad array of technical possibilities and flexibility. This way, Barry Callebaut continues to be a partner in chocolate solutions for food professionals”.

www.barry-callebaut.com
**ALLMA ARRIVES TO SHAKE UP THE MICROALGAE MARKET WITH PREMIUM SUN-GROWN CHLORELLA**

A major new global player with a premium-quality proposition has entered the thriving market for microalgae ingredients suitable for use in food, beverage and dietary supplement products. Allma, based in Portugal, supplies natural, sun-grown Chlorella powder that is free from contaminants and rich in phytouniters, proteins, vitamins and minerals – creating a wealth of opportunities for new product development. Allma’s Chlorella is grown at the state-of-the-art Algafarm production unit in Leiria, 100km north of Lisbon, in closed food-grade production systems, called photo-bioreactors. The photo-bioreactors are exposed to sunlight to encourage photosynthesis, mimicking the growth of microalgae as it occurs in nature. Sun-grown Chlorella is highly valued because it is exceptionally high in nutrients that develop naturally as a result of exposure to the sun. João Navalho, Director of Allma, said: “The market for microalgae ingredients is very buoyant but most of what is available today is sold as a commodity with very little focus on quality. Our Chlorella breaks the mould in the sense that while it is produced in the high volumes the industry requires, it is grown to be far superior in terms of nutrient content and purity. Our entry into this market is a significant development because, for the first time, companies will be able to access the very best microalgae ingredients in the quantities they require, at competitive prices and produced in accordance with demanding European food safety laws”. He continued: “Our mission is to take Chlorella to the next level in terms of its value as a food ingredient. We aim to be more than a company that sells Chlorella, but also a good partner that works closely with customers to find the perfect Chlorella solution for their products”.

www.allma.com

**NEW INGREDIENT COST-EFFECTIVELY LOWERS GLYCEMIC INDEX OF CARBOHYDRATE-RICH FORMULATIONS IN SUPPORT OF BLOOD SUGAR MANAGEMENT**

PLT Health Solutions, Inc. and Horizon Science have announced the launch of Benecarb® Glycemic Balance Complex in North America. The ingredient, based on molasses phytouniters, can reduce the glycemic impact of a broad range of everyday foods and beverages – with particular advantages in carbohydrate-rich formulations. High in natural antioxidants, minerals and polyphenols, Benecarb has been clinically shown to reduce the glycemic of index (GI) of foods and beverages by up to 20 percent at addition levels of 4-6 percent of total carbohydrate content. These low use levels correspond to a low impact on the organoleptic properties of food and beverage formulations and contribute to cost-effectiveness in use. Benecarb is a 100percent natural, clean-label liquid ingredient that can be added to existing formulations using existing processing equipment, with very little impact on food and beverage labelling. Use of Benecarb can support product claims of lower glycemic index, and consumer claims centered on support for blood sugar management and health.

www.PLTHealth.com/products/benecarb

**RANGE OF CARAMEL ALTERNATIVES WITH NATURAL BROWN SHADES BY SENSIENT**

Sensient Food Colors Europe has launched a comprehensive line of colours that provide a natural alternative to traditional caramel colours. Sensient’s dynamic range of natural brown shades for food and beverage applications includes plant-based products that are available as colouring foodstuffs complying with the new EU guidance notes on colouring food. One of the biggest challenges has been to produce rich natural brown shades for use in confectionery to meet the demanding product and processing conditions, such as in pan coatings and hard-boiled candies, explains Dr. Andreas Klingenberg, Technical Director, Sensient Colors Europe GmbH: “Sensient has developed the technologies and know-how to meet these processing and stability challenges, using natural components as building blocks to create high performance solutions”. Sensient’s new product range incorporates a variety of natural raw materials formulated for specific product applications. Sensient’s product line includes apple-based products offering several attractive brown shades particularly suitable for the beverage and confectionery industries. For dairy products, alternatives based on malt and burnt sugar are now available, and a range of oil soluble products are included for use in savoury and snack applications.

www.sensient-fce.com

**ADVANTAME APPROVED IN EUROPE**

In a move that will make it easier for food and beverage companies to meet commitments to reduce calories and sugar, the European Commission has approved Advantame, the new, low calorie sweetener from Ajinomoto. Advantame is a new ingredient with a clean, sugar-like taste and can be used to reduce calories, while maintaining the excellent sweet taste that consumers expect. In the words of the Commission Regulation, Advantame “will provide manufacturers with greater flexibility in formulating energyreduced foods with a similar taste profile as the full-caloric equivalent”. A spokesperson for Ajinomoto Co., Inc. said: “We welcome the approval of Advantame by the European Commission. Advantame blends well with sugar and other caloric sweeteners, providing food and beverage companies with an opportunity both to reduce calories and to manage sweetening costs”. Advantame was approved for general use as a sweetener in foods and beverages by the US Food & Drug Administration on May 19. Advantame is also FEMA GRAS approved in the United States for use in frozen dairy applications, milk products, beverages and chewing gums. The ingredient has found success in North America and Asia in applications where it is used to enhance various flavours. Advantame also extends the duration of the sweet taste in confectionery. Ajinomoto continues: “The need to address the over-consumption of calories is self-evident, not just in Europe, but throughout the developed world. With the approval of Advantame, we can provide a unique, value-added ingredient to food and beverage companies”.

www.aminosweet.eu
INDENA: ENOVITA® GETS GRAS ASSESSMENT
Indena has announced that its grape seed extract Enovita® obtained self- affirmed GRAS (Generally Recognized as Safe) status for use as food ingredient in a number of food categories including beverages and beverage bases, breakfast cereals, fats and oils, frozen dairy desserts and mixes, grain products and pastas, milk – whole and skim – milk products, and processed fruits and fruit juices, at levels up to 291 mg per person per day. The safety assessment focused on the addition of the extract to a variety of conventional foods to function as an antioxidant and/or emulsifier. The safety profile of Enovita® is supported by broad-based documentation, including animal studies, metabolism studies, human experience, in vitro studies and refined chemical characterization. Enovita®’s production facility has been inspected by the Centre for Food Safety and Applied Nutrition of the FDA for compliance to the Food Safety Modernization Act (FSMA) and ensures full traceability from grape harvest to the finished product.

www.indena.com

DSM NUTRITIONAL PRODUCTS & ISOBIONICS
DSM Nutritional Products Ltd. - a division of Royal DSM N.V., the global life sciences and material sciences company - and the natural ingredients company Isobionics B.V. have announced an exclusive partnership to distribute Valencene and Nookkatone grades to the flavour and fragrances market. “The current supply chain of many natural compounds is unstable and characterized by high volatility regarding availability, quality and pricing. With our proprietary fermentation technology, which is similar to brewing beer, we create the stability and reliability of supply that the market expects. I am pleased with the worldwide distribution capabilities and know-how of DSM’s Aroma Ingredients Business and confident about the success of our cooperation”, said Toine Janssen, CEO and founder of Isobionics. Arnold Gloor, Senior Director Aroma Ingredients of DSM Nutritional Products, commented: “We are pleased to extend our portfolio with exciting new aroma ingredients such as Valencene and Nookkatone, produced through an innovative fermentation technology by Isobionics to bring stability into the market place”.

www.dsm.com

COSUCRA: INVESTMENTS IN ENVIRONMENT
Cosucra’s mission is to make a contribution to safe and healthy food using the traditional wealth of locally grown vegetable crops. As a responsible company, they also strive to act as environmental-friendly as possible. Apart from investments in solar panels and a new anaerobic water purification system recently, Cosucra also set up an environmental management system at the chicory sites Warcoing Industrie and Socode in 2012. After an audit in March 2014, they now have obtained an ISO 14001 certification for both sites.

www.cosucra.com

NEW ANTI-FOG CONCENTRATE FOR POLYPROPYLENE (PP) BY CRODA
Atmer 7373 by Croda is a unique anti-fog concentrate designed especially to deliver outstanding anti-fog performance in both hot and cold food packaging. Scientifically formulated for clear polypropylene films and containers, Atmer 7373 prevents droplet formation on plastic surfaces, keeping food looking fresher for longer. Polypropylene is extensively used in food packaging due to its excellent clarity and low temperature processing. In a crowded and ever competitive retail environment, food must look appealing to stand out from the competition. The unique formulation of Atmer 7373 effectively disperses water droplets on plastic surfaces in both hot and cold fog applications, making food packaging clearer and giving food a fresh appeal. Key benefits: - Excellent hot and cold anti-fog performance; - Food contact approved; - No observable effect on haze; - Supplied as concentrate for easy dosing. Applications: - Salad and fruit display packaging; - Ready meal packaging; - Sandwich display packages; - Hot deli food packaging.

www.croda.com

DUPONT: NEW TECHNICAL SERVICE CENTER
DuPont Nutrition & Health has opened a new technical service centre to support dairy manufacturers in the growing Eastern European market. Expanding the company’s global service network, the centre specializes in optimizing starter culture performance in fresh fermented dairy production. Yogurt and other fresh fermented dairy products are important drivers of the dairy market in Eastern Europe, where Euromonitor International forecasts value sales will grow 6 percent a year up to 2018. Located in Kiev, Ukraine, the technical service centre will focus primarily on issues related to bacteriophages. These viruses impact the acidification performance of yogurt starter cultures and are a common cause of production failures or product quality issues. The centre will also work with basic microbial analyses. “Efficient strategies against bacteriophage development are essential to ensure stable batch-to-batch production and premium quality products”, says Dmitrij Shulmeister, Sales Director SAFI South-Eastern Europe & Balkans, DuPont Nutrition & Health and continued: “In addition to routine analyses of customers’ product samples, our application specialists can identify the optimum starter cultures for use in rotary combinations and provide prompt assistance when bacteriophage issues arise”. DuPont has similar technical service centres in place in France, China and the USA. The new centre in Kiev is another step in completing the global network of regional outposts.

www.danisco.com
The industry’s gathering point where science & strategy intersect

For more than 15 years, SupplySide West has been the world’s largest expo gathering ingredient suppliers and finished product manufacturers. Today the show offers more than ever before. You’ll find cutting-edge scientific innovations and strategy-centered educational opportunities everywhere you turn. Register for this one-of-a-kind opportunity to find the solution for your business that will drive revenue, market share, and consumer loyalty.

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When you’re searching for the best natural sources, you’d better be prepared to get your hands (and feet) dirty. If that means climbing up an impossibly steep hillside in West Africa to find green coffee beans exceptionally rich in chlorogenic acid—well, it’s nothing we haven’t done before. It’s that kind of obsession with the highest standards of quality that has made Nexira a global leader in such a wide range of naturally sourced ingredients.

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Green Coffee Bean (Coffea robusta)