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The Berry Fruit Açai (Euterpe oleracea Mart): Bringing Health Benefits and Exotism to the Modern Table

Farid Menaa

Department of Oncology and Food Nanotechnology, Fluorotronics Inc. & Co. San Diego, CA, USA

INTRODUCTION

The palm Amazonian fruit açai (Magnoliophyta: Arecaceae, Euterpe oleraceae Martius) has been applied in folk medicine. Nowadays, this exotic berry fruit is commonly used to make beverages (i.e. juices) and food preparations (e.g. ice creams).

Açai is widely distributed in northern South America where it is traditionally consumed. In the recent years, açai has gained popularity abroad as a food and functional ingredient. It has then considerable both nutritional and economic importance (e.g. exportation). This is mainly due to both its content in bioactive molecules beneficiating health.

Indeed, considerable research has been made on the fruit’s pulp of açai. Some highlights include: (i) the relatively high presence of certain polyphenols (e.g. flavonoids especially proanthocyanins and, in a lesser extent, anthocyanins such as cyandin 3-glucoside and cyanidin 3-rutinoside) as well as carotenoids, ascorbic acid (aka vitamin C); (ii) the subsequent relatively strong anti-oxidant activity (e.g. scavenging of free Radical Oxygen Species (ROS) such as superoxide (O$_2^-$) and peroxyl (ROO$^-$) radicals, which is discussed to contribute to the prevention of several inflammatory-state diseases (e.g. non-communicable pathologies such as diabetes, arthrits, cancers). In fact, it is commonly accepted that açai fruit represents an interesting functional food for disease prevention and therapy, and one of the berry fruits (along with blueberry and cranberry) that display the most anti-oxidant potency.

HEALTH BENEFITS OF THE AçAI FRUIT

Preventive and Therapeutic effects:

Açai’s health benefits are based on consistent experimental studies that range from cells (e.g. microglial, cancer cells) to animal models (e.g. flies, rodents, zebrafish). Nevertheless, there is still a paucity of reports using different parts of the açai fruit other than the pulp, and so, the assessment of their comparative effects in humans is not relevant yet.

Briefly, this exotic “super food” is recognized for its potential against:

(i) Inflammation (e.g. inhibition of NF-κB activation and MAPK pathway; inhibition of Cyclooxygenase (COX) 1/2 activities);

(ii) Aging (i.e. increased longevity in flies submitted to a high Saturated Fatty Acid (SFA) diet or deficient in enzymatic anti-oxidants such Superoxide Dismutase 1 (SOD1); dermatological care against disorders such as psoriasis, atopic dermatitis; cosmetic care);

(iii) Cancers (e.g. induced apoptosis of leukemia cells; prevention of chemically-induced esophageal, bladder, or colon cancer in rodents);

(iv) Cardiovascular disease (i.e. vasodilatation effect mediated by Nitric Oxide (NO)/cyclic
Conclusions: Challenges and Prospects

Açai is a valuable functional food for healthcare. Likewise for resveratrol, considered as the most potent antioxidant, the nanoe encapsulation of açai extracts, açai blends or pure açai-based alkaloids might improve the clinical outcome in patients with specific health conditions (e.g. skin disorders, inflammatory diseases). Nano-açai products may also be valuable for the development of innovative cosmetics. Eventually, human experiments from both açai extracts and derived-bioactive pure chemicals are requested in order to precisely evaluate their respective molecular effects in disease prevention, diagnosis and therapy as well in esthetics (e.g. açai-based cream formulations). If it is proven that açai extracts or derived pure molecules effects are valuable in humans, then it should be used in a routine clinical setting. Indeed, studies in rodent models are invaluable for understanding the potential cellular mechanisms for the pathogenesis of insulin resistance, and genomic responses in mouse models poorly mimic human inflammatory diseases. An explanation is that, in terms of evolution, large mammals display a lower mass-specific basal metabolic rate (m-BMR in g/ml of O₂ per h) when allometrically compared with small ones (e.g. human species showed a 93.6% decrease in mass-specific basal metabolic rate compared with the mouse species). Therefore, rather than over-relying on animal models to understand what happens in humans, isn’t time to embrace the human ‘model’ to move forward?

Acknowledgements

The author would like to thank Dr. Abder Menaa, MD, specialist in nutrition and anti-aging medicine, for having kindly accepted to review this article.

References


Prevalence of *Listeria* species in Fresh Salad Vegetables and Ready-to-Eat Foods Containing Fresh Produce Marketed in Canterbury, New Zealand

Qi Zhu and Malik Altaf Hussain

Centre of Food Research and Innovation, PO Box 6747, Lincoln University, Springs Road, Lincoln 7647, New Zealand

ABSTRACT

Fresh produce, the most common food, is source of high value nutrients in human diet. However, some pathogenic bacteria associated with fresh produce may cause illness. For example, *Listeria monocytogenes* is a microorganism that causes a disease of variable severity, such as mild gastroenteritis, severe infections of the blood stream and/or the central nervous system, and even leads to abortion in pregnant women. The aim of this study was to determine the prevalence of *Listeria* spp. in fresh produce. Four common fresh vegetables (lettuce, carrot, purple cabbage, green cabbage) were tested in the amount of *Listeria* spp. over a 5-week period, and eight individual samples of each fresh vegetable were collected from two fruit and vegetable markets each week. *Listeria* spp. were detected in all the purple cabbage samples collected from these two markets. *Listeria* spp. were also present in 60% and 100% of the green cabbage samples obtained from market 1 and market 2, respectively. *Listeria* spp. were found in the lettuce samples collected from both markets during week 1 to week 3. Carrot samples had the lowest percentage (20%) of *Listeria* spp. Generally *Listeria* spp. were present in all the four vegetables sampled. However, variations were recorded between vegetable types and sources. Selected ready-to-eat products containing fresh produce were also collected to test the presence of *Listeria* spp. and it turned out that two samples were *Listeria* spp. positive. Our data suggested that *Listeria* spp. were generally prevalent in fresh vegetables and Ready-to-Eat (RTE) food products containing fresh produce.

KEYWORDS: *Listeria monocytogenes*; fresh produce; enumeration.

INTRODUCTION

Fresh produce have high nutritional qualities and several health benefits attributed to them. From a nutritional point of view, fresh produce are low energy-dense foods relatively rich in vitamins, minerals and other bioactive compounds as well as being good sources of fiber. Consumption of fresh produce is encouraged by government health agencies, health groups and health professionals. Generally, fresh fruits and vegetables are believed to protect against a range of illnesses such as cancers and cardiovascular diseases. This campaign is helping to change dietary habits toward higher per capita consumption of fresh fruits and vegetables, particularly, in developed world. For instance the average intake of fruits and vegetables in New Zealand is nearly 400 gram per day. Without doubt, fresh fruits and vegetables are important components of a healthy and balanced diet, however, several new food safety issues are being associated with their consumption. One of the major issue is the emergence of pathogens that were not traditionally associated with raw produce. This situation has enhanced the potential for outbreaks associated with raw fruits and vegetables.
Bacterial pathogens such as *Salmonella*, *Bacillus cereus*, *Escherichia coli* O157:H7, *Clostridium botulinum*, *Shigella*, *Staphylococcus*, *Vibrio cholera*, *Aeromonas*, *Campylobacter jejuni* and *L. monocytogenes* were frequently isolated from fresh produce. Among all of these pathogens, *L. monocytogenes* is a kind of pathogenic bacterium that causes a serious disease called listeriosis. This infection has variable degree of health consequences, such as mild gastroenteritis, severe infections of the blood stream and/or the central nervous system, and even abortion for pregnant women. This illness is a relatively rare but has high fatality rates (30%). Among all of these pathogens, *L. monocytogenes* had previously been isolated from market produce i.e. cabbage, corn, lettuce, sprouts, potatoes, cucumbers, parsley, watercress and salad vegetables.

Several outbreaks of *L. monocytogenes* infection associated with fresh produce have been reported in various parts of the world. Recently *L. monocytogenes* was responsible for the deaths of 10 people in food poisoning outbreaks of chopped celery in 2010 and 30 people due to contaminated melon in 2011. The foodborne outbreaks caused by contaminated fresh produce are predicted to increase in coming years. Therefore, this study was planned to investigate the status of *Listeria spp.* contamination in salad vegetables and selected Ready-to-Eat (RTE) food products containing fresh produce. To our knowledge this is the first investigation on *Listeria spp.* contamination levels in fresh produce marketed in Canterbury region, New Zealand.

**MATERIAL AND METHODS**

**Sampling plan and samples collection**

Four most commonly consumed fresh vegetables, including green cabbage, purple cabbage, lettuce, and carrot, were selected in this study. These vegetables are consumed raw or in minimally processed form. Samples of these vegetables were collected from two different fruit and vegetable markets (reported as market 1 and market 2) located in Canterbury region of New Zealand over a 5-week period. All samples were bagged separately when purchased and brought to laboratory in a way normally consumers take home. Following the raw vegetables testing, samples of 12 different RTE products that contained fresh produce as ingredient were also tested to determine possible relationship between fresh produce and *Listeria* spp. detection. These RTE products were also collected from two different supermarkets (supermarket 1 and supermarket 2).

**Samples preparation**

Briefly, 100 g of each raw vegetable sample was weighed into a stomacher bag. An appropriate amount of 0.1% peptone water was added before stomaching for 5 min. Afterwards, 1 mL of the liquid portion of stomacher contents was used to prepare serial dilutions in 0.1% peptone water. One mL of selected dilutions were transferred onto each *Listeria* selective agar plate (in duplicate) and incubated at 35 °C for 24 h. Media and enumeration of *Listeria* spp.

*Listeria* selective agar (CM0856, OXOID) was used in this study. Media plates were prepared according to manufacturer’s instructions. Typical *Listeria* spp. colonies were brown color. Pure *Listeria monocytogenes* (strain V7) culture was used as a positive control (Figure 1) and 0.1% peptone water was the negative control. Confirmation of *L. monocytogenes* was not carried out in this study. The numbers of typical *Listeria* spp. colonies were counted after incubation and reported. Colony forming units (cfu/g) were calculated using the formula:

$$\text{cfu/g of sample} = \frac{A \times 10^n}{V_1} \times \frac{V_2}{m}$$

where A — number of colonies (average in two plates);

$10^n$ — level of dilution at which the counting was carried out;

V1 — volume of inoculum;

V2 — total volume of peptone water;

m — total sample weight

![Figure 1: Typical *L. monocytogenes* colonies on *Listeria* selective agar (CM0856, OXOID). (A) *L. monocytogenes* (strain V7) pure culture colonies (B) Colonies of *L. monocytogenes* from a test product sample](image)

**RESULTS**

**Listeria** spp. in selected salad vegetables

This study investigated the prevalence of *Listeria* spp. in four different vegetables, commonly used as salad, sampled from two fruit and vegetable markets in Canterbury region of New Zealand. All purple cabbage samples from both markets were consistently positive for *Listeria* spp. presence during the 5-weeks sampling period (Table 1). *Listeria* spp. were detected in 60% and 100% of green cabbage samples from market-1 and market-2, respectively. Lettuce samples collected from both markets during week 1 to week 3 were contaminated with *Listeria* spp., while carrot samples had the lowest percentage (20%) of positive samples. Generally *Listeria* spp. was present in all vegetable types tested in this study. However, purple and green cabbage samples mostly carried *Listeria* spp. and the samples collected in market-2 had more number of positive samples for *Listeria* spp. compared to market-1. Carrots had the lowest...
number of samples and frequency for *Listeria* spp. detection.

![Table 1: Comparison of *Listeria* spp. presence in vegetable samples collected from two different markets](image)

<table>
<thead>
<tr>
<th>Market</th>
<th>Sampling period</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Market-1</strong></td>
<td>Lettuce</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Green Cabbage</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Purple Cabbage</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Carrot</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Market-2</strong></td>
<td>Lettuce</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Green Cabbage</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Purple Cabbage</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Carrot</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

![Figure 2: Total number of *Listeria* spp. (cfu/g) detected in each of the vegetable sample from market-1 (A) and market-2 (B) over 5-week period. Error bars represent standard deviations of cfu detected each week.](image)

*Listeria* spp. in RTF containing fresh produce

Our curiosity to see whether *Listeria* spp. can be detected in RTE food products containing fresh produce led to collection of samples from two supermarkets (Table 2). Twelve samples were randomly collected once and two samples (Japanese style coleslaw and spinach and basil dip) were found in positive for *Listeria* spp. presence. This aspect of the study needs the detailed investigation. However, our results indicate there is potential risk associated with RTE products containing fresh produce as ingredient.

**DISCUSSION**

Many research reports indicated the existence of *Listeria* spp., *L. monocytogenes* in particular, on the surface of fresh vegetables. *L. monocytogenes* is an important pathogen and was detected in 28% of fresh vegetables positive for *Listeria* spp. presence.\(^{11}\) Althaus et al. (2012) found that the samples of RTE lettuce were infected by *L. monocytogenes*.\(^{12}\) In another report, Uzez and Adepoju (2013) showed that *L. monocytogenes* was present on salad vegetables (sliced cabbage, lettuce and carrot).\(^{13}\) The growth of *L. monocytogenes* was linked to the storage condition, especially temperature. Our results also strongly suggested that *L. monocytogenes* was prevalent in purple cabbage, green cabbage and lettuce. These observations are consistent with previous findings mentioned above.

According to guideline for the control of *L. monocytogenes* in RTE products, *Listeria* spp. level in fresh produce is expected to be as low as possible, and the maximal safety level limit should not exceed 100 cfu/g.\(^{14}\) Obviously, purple cabbage from market-1 sampled in the first week had the level of *Listeria* spp. (150 cfu/g). The lettuce sample collected in week-1 from market-2 also found to have numbers close to maximum limits. All the other vegetable samples had detected numbers within the range of safety limits. However, we need to keep in mind that the guidelines of the food safety agencies are for regularity purpose and ambiguity of *Listeria* makes it more dangerous, thus *Listeria* guidelines need frequent review. In our opinion, it is not the number of pathogenic cells rather...
Cordano and Jacquet (2009) tested monocytogenes. However, numbers were less than threshold L. basi dip collected from supermarket-1 were positive in its products (Table 2). Japanese style coleslaw and spinach-lowed by testing in RTE products containing L. monocytogenes. Microbiological analysis of raw vegetables was fol-
et al. 15 who reported 7.67±0.09 cfu/g of L. monocytogenes. Comparing the results of this study with Sant’Ana et al. 12.86%, 15.03% and 31.04% for farm, restaurant and home, respectively.16

Table 2: Listeria spp. detection in RTE food products containing fresh produce sampled from two different supermarkets.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Product name</th>
<th>Source</th>
<th>Listeria spp. detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cole Salad</td>
<td>Salad coleslaw</td>
<td>Supermarket 1</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Coleslaw salad</td>
<td>Supermarket 2</td>
<td>–</td>
</tr>
<tr>
<td>Slaw</td>
<td>Japanese style coleslaw</td>
<td>Supermarket 1</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Herbslaw</td>
<td>Supermarket 1</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Broccoslaw</td>
<td>Supermarket 2</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Crisp salad</td>
<td>Supermarket 2</td>
<td>–</td>
</tr>
<tr>
<td>Dips</td>
<td>Spinach and basil dip</td>
<td>Supermarket 1</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Spinach and feta dip</td>
<td>Supermarket 1</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Feta and spinach hummus</td>
<td>Supermarket 2</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Cucumber and mint yoghurt dip</td>
<td>Supermarket 2</td>
<td>–</td>
</tr>
<tr>
<td>Burger/ Sandwiches</td>
<td>Meat lover roll (burger)</td>
<td>Supermarket 2</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Ham triple sandwiches</td>
<td>Supermarket 2</td>
<td>–</td>
</tr>
</tbody>
</table>

Comparing the results of this study with Sant’Ana et al.15 who reported 7.67±0.09 cfu/g of L. monocytogenes in cabbage samples, we have detected much higher number. However, carrot samples had 6.15±0.02 cfu/g, much more than our result (i.e. 2 cfu/g). Another investigation on the risk assessment of lettuce samples from home, restaurant and farm in Korea found that average final contamination level before consumption after washing treatment in restaurant and farm were -1.50 log cfu/gor -0.146 log cfu/g, respectively. Using food safety limit for L. monocytogenes population on fresh produce (2 log cfu/g) as threshold value, contamination levels on lettuce greater than this threshold value were observed as 12.86%, 15.03% and 31.04% for farm, restaurant and home, respectively.16

Microbiological analysis of raw vegetables was followed by L. monocytogenes testing in RTE products containing fresh produce. L. monocytogenes was not present in majority of its products (Table 2). Japanese style coleslaw and spinach-basil dip collected from supermarket-1 were positive in L. monocytogenes. However, numbers were less than threshold value for RTE products. Cordano and Jacquet (2009) tested 154 samples of industrial minimally processed raw salads, and L. monocytogenes was not detected. However, Althaus et al. (2012) found 5 out of 223 samples of RTE lettuce contained L. monocytogenes. Number of Listeria spp. was less than 2 log cfu/g in each sample. Data from our study and several other reports strongly suggest that Listeria spp. could pose a serious threat to the safety of fresh produce and products that contain them as an ingredient. Therefore, it is important to use appropriate measures in order to reduce or minimize contamination well.

Prevalence is more serious problem that allows this pathogen to reach to immune-compromised groups of the population (elderly, infant, pregnant women, etc).

In conclusion, Listeria spp. have been frequently detected in market produce such as cabbage, corn, lettuce, sprouts, potatoes, cucumbers, parsley and watercress. In this study, all four fresh vegetables (lettuce, green cabbage, purple cabbage, carrot) were found in carrying the Listeria spp. more or less. These results indicate that food safety risks are associated with fresh produce consumption. However, RTE products (containing fresh produce) are relatively safe. Further, investigations are needed to establish level of risk associated with each product type and food safety threat to consumers.

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Red Natural Colors for High pH Applications

Deepti Dabas* and George Kean

Kalsec Inc, 3713 West Main Street, Kalamazoo, MI 49006, USA

ABSTRACT

It is said that we taste with our eyes. Colors have a tremendous influence on choice of foods. Natural colors are seeing a huge demand because of consumer trends shifting towards natural ingredients. Common natural colorants in the red category - anthocyanins and carmine - despite being stable and offering a host of advantages, have their limitations. Beet and lycopene colors were evaluated as colors of choice for coloring high pH food applications. Kalsec®Vegetone® Vivid Red beet color was successfully tried in hard candy and flour salt applications. The applications extend to confectionary and dry beverage mix applications respectively. Kalsec®Vegetone® Vivid Red beet color imparted a red color to candy whereas control beet product imparted a bluer appearance. Kalsec®Vegetone® Vivid Red beet color was darker in color than control when added to flour salt at the same concentration. Kalsec®Vegetone® Vivid Red beet color when added to a model beverage solution, showed more color retention compared to control beet when incubated under heat-also pointing towards a more stable product. Kalsec®Vegetone® Vivid Red beet color was also used in a cupcake indicating its potential as a stable colorant for baked applications. Kalsec®Vegetone® Rich Red lycopene color was tried in flour salt, beverages and retort applications demonstrating its versatility. Regulations of USA allow beet and lycopene to be used in food products however EU regulations have some limitations which should be followed before deciding on their use.

KEYWORDS: Beet; Lycopene; Natural colorants.

INTRODUCTION

Color has tremendous influence on our preference of food and beverages. How a product looks impacts the consumer’s purchase and even influences how food and beverages are perceived through taste. In recent years, food colorants have come under increasing global attention for their possible health effects from allergies, aluminium content and potential behavioral problems in children. In addition to synthetic colors, carmine despite being a natural colorant has also been under increased scrutiny. As a result, food manufacturers are reformulating recipes and incorporating ingredients which will enable products to display “cleaner labels”.

The growth of natural colors increased 29% between 2007 and 2011. In contrast the growth rate for synthetics was only 4%. Of the global colors share, natural colors increased from 34% in 2007 to 39% in 2011. The segment is now worth an estimated USD $570 million. Kalsec® has broadened its red color product portfolio for manufacturers who would like to switch to naturally-sourced colors. This study focuses on Kalsec®Vegetone® Vivid Red high stability colors formulated for a wide range of pH conditions. The primary natural sources of these new red colors are tomato and beet.

Limitations of Certain Red Colors

Research from Innova Market Insights indicates that red is the most challenging of colors to naturally produce. Even then, it comprises around 50% of natural colors market demand. Anthocyanins can provide a bright red to bluish-red hue and are a good replacement for synthetic dyes; however, anthocyanins are unstable at pH >4.5. At this relatively higher pH,
these pigments change from red to blue, are not stable and are rendered ineffective in low acid products. Carmine is another alternative but is derived from the cochinell insect and is not Kosher-approved. There are numerous consumer acceptability issues with carmine. Carmine is an aluminum salt of carminic acid, and the aluminum required to produce the carmine is a regulatory concern in some countries. Additionally, the extract can contain proteins which can cause severe allergic reactions in humans. Moreover, a FDA rule effective January 5, 2011 requires all foods containing cochineal to declare the ingredient on consumer food labels. Also, as of August 2014 the use of aluminum containing colors such as Carmine (E120) has been prohibited in certain foodstuffs within the EU. As a result, there remains a gap in naturally-sourced, consumer-friendly, stable red colorants that can be filled with Kalsec®Vegetone® Red colors. The prices for Red 40, a common synthetic colorant, have increased giving processors another reason to switch to their natural counterparts.

Red beet and lycopene fall under the exempt category in the US Code of Federal Regulations (CFR) and are approved for use in food as food additives. Kalsec®Vegetone® Red colors are stable at a wide pH range, when compared to anthocyanins, and provide more consumer-friendly attributes than carmine since they are derived from vegetable sources and are Kosher-approved.

Red Beet: Betalains

Red beet (Beta vulgaris L.) is a root crop that grows naturally in both tropical and temperate regions and is globally consumed in cooked or raw form. Red beet color is obtained by pressing, concentrating and subjecting the juice of beetroots to heat processing.

Structurally, betalains are indoxyl derivatives of betalamic acid and are categorized into either betacyanins or betaxanthins. Condensation products of betalamic acid and cyclo-Dopa [cyclo- 3-(3, 4-dihydroxyphenylalanine)] are commonly referred to as betacyanins due to their deep violet color. The maximum absorption of these compounds varies between 510-550 nm. Betanin is the major betacyanin present in red beet. Betaxanthins (yellow) are condensation products of betalamic acid and amino acids or amines, respectively. Depending on the particular structure of the amino compound, maximum absorption of betaxanthins varies between 460 and 480 nm.

Though relatively robust with respect to pH changes, betalains perform their best in the pH range 3-7. They are sensitive to intense heat processing including Ultra-high temperature (UHT). They perform better in lower water activity conditions and certain stabilizers, including chelating agents, improve their stability. They are prone to degradation under oxygen and are best preserved by excluding air. They also perform better at high pigment concentrations.

Lycopene

Lycopene is a member of the carotenoid family and is responsible for the characteristic red color of tomato, watermelon, pink grapefruit, orange and apricot. Lycopene has 11 conjugated double bonds and 2 unconjugated double bonds at each end. Kalsec®Vegetone® Vivid Red lycopene colors are extracted from tomatoes, a source allowed by FDA standards. Lycopene is a hydrophobic molecule and is not soluble in water. Lycopene’s solubility in vegetable oil is 0.2 g/L at room temperature which increases upon heating. Lycopene tends to aggregate into crystals in aqueous systems. This behavior, however, can be exploited and particle size can be reduced to form dispersion in aqueous foods. This enables the color expression to be red instead of the yellowish-orange which would occur if it were to be dissolved in a fat-based medium or on heating. Vegetone® Rich Red lycopene colors perform well for water-based applications. In the presence of higher amounts of oil and heat these pigments will dissolve and impart an orange color to a finished food.

Lycopene is heat stable and functions well in retorting and extrusion; with only limited pigment loss during processing. Like other carotenoids, lycopene is sensitive to light; therefore products should be protected with appropriate packaging.

EXPERIMENTAL

Kalsec®Vegetone® Vivid Red beet color - 57.01 was obtained in house. ‘Control’ was prepared from the raw material used to make 57.01. Only raw material and water were added and mixed to make the control. It was made similarly as 57.01 was made, except that no stabilizers were added. Betanin content for both Vegetone Vivid Red and control was 0.62%. Kalsec®Vegetone®Rich Red lycopene color - 59.01 with a 1.75% lycopene was obtained in house. A Kalsec® paprika oleoresin product and a Kalsec® paprika emulsion products were used. Both of these were formulated in house.

METHODS

Preparation of hard candy: Light corn syrup was obtained from Meijer Store (Grand Rapids, MI). Granulated sugar was obtained from Gordon Food Service (Wyoming, MI). 30% corn syrup, 14% water and 56% sugar were weighed and mixed in a beaker. The beaker was covered with a watch glass and heated until contents dissolved. After that watch glass was removed and temperature monitored. Heating was continued until temperature reached 150 °C. At this time, beaker was taken off heat and 57.01 were added at 0.2%. This mixture was then mixed thoroughly and poured onto a heat resistant mat and allowed to solidify. Candy was similarly prepared using control. Color at 0.2% was added as soon as beaker was taken off hot plate.

Plating on flour salt: Alberger Fine Prepared Flour salt was obtained from Zeeland (Cargill Foods). Retsch RM 200 mortar
Preparation of beverages: pH 5.0 buffer was used to create a model beverage. 0.025% citric acid and 0.075% sodium citrate dehydrate were added to water and pH maintained at 5.0 using 1% citric acid/0.5% KOH solution. To this 8% sucrose was added and mixed. Beverages with beet were dosed at 0.114% of Vegetone® Vivid Red beet or control. 10g of solutions were poured in scintillation vials. These vials were incubated at 35 °C. Absorbances at 530 nm were measured and % absorbance retention was calculated at different time points.

Another beverage system used for this study was at lower pH (3.1), 14.3 % High Fructose Corn Syrup (HFCS) 55 (Batory Foods, IL), 0.0165% sodium benzoate (Sigma, St. Louis, MO) and 0.063% citric acid (Sigma, St. Louis, MO) were mixed. This beverage was used for comparison of lycopene with paprika emulsion. Vegetone® Rich Red lycopene color was added at 0.035% or a paprika emulsion (ASTA value 453.75) was added at 0.072% - so as to match the color spectrophotometrically at respective absorbance maxima. The beverage was poured in 500ml HDPE bottles. Colorimetry was carried out using CM700 d (Konica Minolta) at regular intervals while incubation in light. A D65 source of illumination was used and a 10 degree observer was used. Instrument was used in SAV mode (aperture equals 3 -6 mm). This instrument has an integrating sphere of 40 mm. Specular Component Excluded (SCE) mode was used. Colorimetry was carried out non- invasively by holding colorimeter against a smooth surface of bottle while using a white background on one side of bottle. CIE 1976 L*, a* and b* values were obtained. SpectraMagic NX software was used to record the data.

RESULTS

Kalsec®Vegetone® Vivid Red beet stability data

Kalsec®Vegetone® Vivid Red beet color can provide increased heat processing and storage stability. Figure 1 shows the efficacy of stabilizers during the processing of hard candy. Product colored with Vegetone® Vivid Red beet color shows improved color expression (Figure 1A) whereas the control red beet colored product (no stabilizers) shows a bluer appearance (Figure 1B).
Vegetone® Vivid Red beet color provided darker color expression when plated on flour salt exhibiting a stronger red color (Figure 2A).

Vegetone® Vivid Red beet color and the control red beet color were added to pH 5.0 solutions and incubated at 35 °C. Vegetone® Vivid Red beet color performed better than the control red beet color during incubation as shown in Figure 3.

Vegetone® Vivid Red beet color was successfully used in a cupcake as shown in Figure 4.

Kalsec®Vegetone®Rich Red lycopene stability data

Beverages were made from Kalsec®Vegetone®Rich Red lycopene color and stability was compared to a standard paprika emulsion coloring. The beverages were incubated at 50 W/m² light intensity and colorimetric parameters were measured over time. The a* values were monitored and are plotted in Figure 5A. As can be seen below, Vegetone® Rich Red lycopene color was more stable through the duration of the study. The photo presented in Figure 5B demonstrates the stability of beverages with Vegetone® Rich Red lycopene color.

Vegetone® Rich Red lycopene colors and standard paprika were plated onto flour salt at the same concentrations (Figure 6) and their stabilities were studied under accelerated light conditions. The color was extracted at specific time points and % absorbance at respective absorbance maxima calculated in Figure 7. As can be observed in Figure 7(A) Vegetone® Rich Red lycopene color was found to be more stable than standard paprika under light. Stability under heat conditions (78 °C) for Vegetone® Rich Red lycopene color and standard paprika were also carried out and the results are presented in Figure 7(B). Vegetone® Rich Red Lycopene color outperformed paprika under these accelerated heating conditions.
Stability of Vegetone® Rich Red lycopene color was analyzed as a result of heat processing. Vegetone® Vivid Red lycopene color was added at two doses in a pH 5.0 beverage model systems. Figure 8 shows the product before and after retorting. Table 1 indicates that the loss of pigment is dependent on concentration added. The beverage maintained almost all of the color when added at 0.22%. The beverage although lost some color when lycopene was added at lower dose, however the hue was maintained, as can be seen in the Figure 8.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>% Retention of color after processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.11%</td>
<td>68.8±0.57</td>
</tr>
<tr>
<td>0.22%</td>
<td>99.8±0.65</td>
</tr>
</tbody>
</table>

Table 1: Effect of retorting on Vegetone® Rich Red lycopene in a pH 5.0 beverage model system. Vegetone® Rich Red lycopene color was added at two mentioned concentrations and color was measured at 478nm using THF.

DISCUSSION

Applications based on Kalsec®Vegetone® Vivid Red beet

As observed above, Kalsec®Vegetone® Vivid Red beet gave a distinct candy product when compared to control beet product. Also, in flour salt it gave a darker product. Thus same dose of this product when put in a dry beverage mix would give a darker color than the control product. In solution form, Kalsec®Vegetone® Vivid Red beet was found to be more stable than control product. Also it was used successfully in cupcake which is a common high pH application processed at high temperature. Use in this application demonstrates that beet can be a viable coloring option for different processed foods. Beet is generally recommended for frozen foods like ice-creams or foods for which minimal heat processing is required like chewing gum. This study demonstrates many more applications for beet. Kalsec®Vegetone® Vivid Red beet color can be used in a wide range of applications such as yogurt, ice-cream, sorbet, sausages, bakery products, frosting, hard candy and gummies. Also, it was shown to be more stable than control, which differentiates Kalsec®Vegetone® Vivid Red beet color from other products and further diversifies the domain of this product.

Kalsec®Vegetone® Vivid Red beet can be a suitable...
alternative to carmine for coloring sausages. Matinez et al. (2006) successfully used both red beet juice and commercial betanin 162 colors in sausages and demonstrated superior performance during processing and storage. They found red beet juice to be the best performer among the colorants.  

Kalsec®Vegetone® Rich Red lycopene

As was observed with the experiments with Kalsec®Vegetone® Rich Red lycopene, the product was used in a host of applications successfully - dry (flour salt) as well as beverage. The product was found to be more stable than another carotenoid- paprika- in both flour salt and beverage application. It also survived retorting which is an otherwise harsh process. Kalsec®Vegetone® Rich Red lycopene color is used in applications where an attractive red color is desired. Examples include dairy-based applications and soy milk systems where Vegetone® Rich Red lycopene color can provide an appealing red appearance. Currently, there are no alternatives for these applications and processors have to use synthetics.

Higher stability of Vegetone® Rich Red lycopene compared to paprika, a common carotenoid used in many applications, indicates that it can easily replace paprika and perhaps synthetics colors and can still provide a viable product. Also, lycopene provides a more attractive red as against the orange hue of paprika and thus may be more appealing.

Vegetone® Rich Red lycopene works well in the replacement of carmine in sausages. Examples include mortadella, bologna and salami. Studies have been carried out on application of lycopene in sausages. The fat content of food and processing conditions are also important factors in determining Vegetone® Rich Red lycopene addition.

CONCLUSION

The Kalsec® Red Color Collection can work at various pH ranges and can provide matching hues either individually or in customized blends. Vegetone® Vivid Red beet and Rich Red lycopene colors have more stability than standard beet colors and provide improved color expression in certain applications. Vegetone® Rich Red lycopene disperses well in applications to provide an attractive and stable red appearance. Using Kalsec’s® Durabrite® stabilization technology, continuous improvements in natural pigment stability provides the food industry with suitable alternatives to certified colors throughout the world. In addition, Kalsec® has over 50 years of experience in extraction and color formulation that can address specific concerns or challenging applications.

ACKNOWLEDGEMENT

I would like to acknowledge the help received from Tanushree Tokle and Tony Vanden Hombergh for experiments.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES


Comparison of Microbiological Food Safety Issues in New Zealand and Australia

Min Min and Malik Altaf Hussain

Centre for Food Research and Innovation, Lincoln University, Lincoln 7647, Canterbury, New Zealand

ABSTRACT

Microbiological foodborne outbreaks have become a major challenge in food safety, in general, and for safer food supply chains, in particular. In order to prevent foodborne diseases, many countries systematically monitor outbreaks. This paper investigated microbial food safety issues in Australia and New Zealand by collecting and analysing data published in annual or quarterly reports about foodborne outbreaks by ESR (New Zealand) and OzFoodNet (Australia), between 2007 and 2011. Foodborne pathogens, food vehicles or food preparation places associated with the high numbers of outbreaks, were compared. The most frequent foodborne outbreaks in Australia and New Zealand were caused by Salmonella typhimurium and norovirus, respectively. The highest numbers of outbreak cases in both countries occurred in restaurants, aged care facilities and private homes. The most frequently-implicated vehicles in the outbreaks were poultry products in New Zealand and eggs and egg-based dishes in Australia. Some similarities and differences existed in the microbiological issues faced by both nations. The implementation of food safety programmes in the food industry and improvements in hygiene education for people working with foods have effectively decreased or eliminated some foodborne diseases from both countries. Common concerns are the diversity of microorganisms and the ability of foodborne pathogens adapt to a new environment as these are likely to increase the degree of difficulty for surveillance and prevention of foodborne diseases.

INTRODUCTION

Food safety is a term that generally refers to ways and approaches to ensure that the production, preservation, distribution and consumption of food happen in a safe manner. Consumption of contaminated food could increase the risk of consumer acquiring an illness, caused a foodborne illness or disease. Every year, foodborne and waterborne diarrhoeal diseases kill about 2.2 million people, including 1.9 million children. The health care cost of foodborne diseases has become a heavy economic burden for countries. In Australia, 5.4 million cases of food poisoning per year were estimated to generate economic cost a AU$1.2 billion, while the estimated total costs of foodborne disease in New Zealand were NZ$ 1.6 billion in 2009. In order to decrease or eliminate foodborne diseases effectively, the WHO developed a global strategy named the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/food) to monitor and supervise foodborne diseases from different countries, including New Zealand and Australia.

Foodborne disease is normally associated with microbiological, chemical or physical hazards. Among these three major health hazards, foodborne diseases, caused by pathogenic microorganisms, have become a big health issue. One of the key reasons is that microbial contamination is hard to predict and track back to its origin. A better understanding of the...
characteristics and epidemiologist of foodborne pathogens may lead to a decrease in the occurrence of microbiological foodborne outbreaks. Preventive and management approaches such as Microbiological Risk Assessment (MRA) and Hazard Analysis and Critical Control Point (HACCP) have been successful in lowering the risks of foodborne diseases. The Australian New Zealand Food Standards (ANZFS) also direct food safety agencies to supervise and monitor foodborne pathogens in each country.

A foodborne outbreak refers to an incident where two or more people present a similar illness after consuming a common food or meal. In New Zealand, foodborne outbreak surveillance was commenced in 1997 by the Institute of Environmental Science and Research Ltd (ESR) on behalf of the Ministry of Health. In this surveillance programme the real-time data are collated, analysed and recorded on EpiSurv, a national notifiable disease surveillance system. The OzFoodNet, foodborne diseases surveillance systems was established by Australia governments in 2000 to collect and analyse foodborne data from the National Notifiable Disease Surveillance System (NNDSS) and enhanced surveillance data from OzFoodNet sites. Data on foodborne outbreaks in quarterly annual reports published by ESR and OzFoodNet are a sound and reliable source of information about microbiological food safety issues in Australia and New Zealand. Data analysis of foodborne outbreaks is recognised as one of the effective approaches in estimating how many human cases of specific enteric diseases are associated with a specific food item. However, some mild foodborne illnesses may be ignored by patients or medical institutions so that the numbers of national foodborne illness attributing to overall foodborne diseases would consequently be underestimated.

The purpose of this review was to gather information on foodborne outbreaks reported during 2007 to 2011 in Australia and New Zealand. A comprehensive comparison of the microbiological food safety issues and factors associated with foodborne outbreaks i.e. different foodborne pathogens, food types and preparation methods, was made. The outcome from our data analysis produced detailed information about the similarities and differences in the food safety challenges faced by each country. Finally, some recommendations and suggestions to decrease foodborne outbreaks were listed.

Information Collection and Data Processing

In order to compare microbial food safety issues in Australia and New Zealand between 2007 and 2011, information on foodborne outbreaks were collected and collated by searching online quarterly and annual reports published by OzFoodNet and ESR (New Zealand). Foodborne pathogens, food vehicles and food preparation methods were the key factors in gathering data. The useful data on foodborne outbreak were classified into four groups, which included the total numbers of outbreaks in both countries, foodborne pathogens involved in the outbreaks, food vehicles associated with outbreaks, and food preparation methods. The data were converted into tabulated sheets using Microsoft Excel and processed to generate into a presentable output. This allowed us to use recorded data to illustrate the microbial food safety issues in both countries through figures and graphics. However, it is important to notice the limitations of the data in the outbreak reports by ESR or OzFoodNet as, for example there may have been further changes and updates of information on foodborne outbreaks that occurred after outbreak reports were completed.

Following data collection and analysis, three electronic databases (Web of Knowledge, Google Scholar and Science Direct) were searched to explore information on microbiological foodborne pathogens, food vehicles and preparations methods associated with the highest numbers of outbreaks. The numbers of overall identified foodborne outbreaks from the national surveillance systems in each country assisted to recognize and rank the microorganisms responsible for the top five foodborne outbreaks as reported by ESR and OzFoodNet. In other words, the electronic databases were used to study the characteristics of these microorganisms as well as to understand possible the sources and pathways for them to infect human population through food vehicles and food preparation places. Finally, the impact of changes in food policies and standards as well as implementation of different food safety strategies in Australia and New Zealand to prevent outbreaks from occurring was discussed.

Comparison of Food Safety Issues Between Australia and New Zealand

A total of 13 published quarterly and/or annual reports on foodborne outbreaks by ESR and OzFoodNet from 2007 to 2011 were chosen for the study of microbiological food safety issues in Australia and New Zealand. Figure 1 shows number of total outbreaks recorded in Australia and New Zealand from 2007 to 2011. There were 122 foodborne outbreaks reported in New Zealand in 2011, a 39.3% increase from five years ago (2007). When looking at the situation in Australia during the same period (2007 to 2011) the numbers of reported outbreaks remained fairly consistent at around 150 in each year. Comparison of total numbers of foodborne outbreaks showed that overall outbreak numbers in both countries were similar in 2008, 2010 and 2011; however, Australia had slightly higher numbers of foodborne outbreaks than New Zealand. The Australian population was approximately five times more than New Zealand, therefore population adjusted outbreak numbers in Australia were more appropriate to be used for comparing with outbreak numbers in New Zealand in 2007 (Australia, 30 and New Zealand, 74), 2008 (Australia, 21 and New Zealand, 89), 2009 (Australia, 33 and New Zealand, 84), 2010 (Australia, 31 and New Zealand, 141), and 2011 (Australia, 30 and New Zealand, 122) (Figure 2). Therefore, these data indicated that the New Zealand population was at a higher risk of facing a foodborne outbreak than...
Association between Specific Food Service Sector and Outbreaks

Improvement in food safety practices could prevent foodborne disease and this is one of the most important elements of strategies to reduce outbreaks. The lack of food safety knowledge among food handlers has been widely recognised as problematic area in food safety. Figure 3 shows an association between food preparation places and foodborne outbreaks in both countries. In New Zealand, the most common food preparation places associated with foodborne outbreaks were private homes (26%), aged care facilities (26%), childcare centres (10%) and unknown preparation sites (11%). On the other hand, in Australia, the high risk food preparation places were restaurants (39%), aged care facilities (11.8%), private homes (10%), commercial caterers (8%) and takeaway businesses (7%).

It was noticed that the three main food preparation places linked to the highest number of outbreaks were the same in both countries i.e. restaurants, aged care facilities and private homes. Restaurants were responsible for the highest number (39%) of outbreaks in Australia, while in New Zealand, private homes (26%) and aged care facilities (26%) were leading places for the outbreaks. Restaurants associated with 11% outbreaks were the third major reason in New Zealand. The Top foodborne outbreak place (private homes) in New Zealand was the third major outbreak place in Australia. This clearly showed that both nations face different types of food safety challenges. The most important contributing factors in restaurant-associated foodborne disease outbreaks were food worker’s health and hygiene (responsible for the largest number of foodborne outbreaks occurring in the restaurants), food preparation practices within establishments and contamination outside the restaurant premises.

Aged care facilities were at a high risk of foodborne outbreaks due to the low-immunity status of the residents. Aged population are particularly vulnerable to food infections by foodborne pathogens, thus these places are required to take extra care to avoid incidents happening. In order to prevent foodborne outbreaks, Kirk et al. suggested the facilities needed to enhance handling processes of puree foods and not to provide raw or undercooked eggs to the elderly. In addition to the home being a risky place, poor food-handling and food safety knowledge still resulted in a high risk of foodborne outbreaks. A right attitude by the individuals towards food-handling practices and hygiene conditions is important to reduce outbreaks in private homes.

It was found that the pattern of the top five high-risk food preparation places in Australia was different from that in New Zealand except cooking at home. The high numbers of foodborne outbreaks occurred in Australian food service businesses probably suggested more tourists or visitors came to this country than to New Zealand. It also suggested that more numbers of Australian dined outside than New Zealanders did. One way to improve this situation in Australia is to implement food safety programmes in food service industry (restaurants, cafes and bars) and to monitor them more closely. Standard operating procedures (SOP) should also need to be developed for entire food service sector. In order to improve food safety at private homes, one of the highest risk food preparation places in New Zealand, the efforts are needed on educating the general population on safe food handling practices.
Overall, in order to protect consumers from food poisoning episodes and to decrease the potential risks of foodborne outbreaks in both countries, the government agencies have worked together to develop joint food standards for more than fifteen years. Now there are documented regulations (Australia New Zealand Food Standards Code) developed by Food Standards Australia and New Zealand (FSANZ). Furthermore, food service sector (including restaurants, takeaways and aged care facilities) in New Zealand need to comply with the new Food Act 2014 and the operator must have in place a system i.e., Food Hygiene Regulations 1974 or Food Safety Programmes (FSP) to ensure consumer’s safety. Although, these regulations are aimed to decrease risks of food poisoning incidents, the business operators in high-risk food preparation places play a crucial role to determine the food safety status of the final food products. Therefore, the external audit of these premises by the food regulatory authorities and food workers’ training and awareness of harmfulness microbiological hazards need to be improved through establishing an effective FSP in the food service sector.

Foodborne Outbreaks and Vehicle Foods

Food, as an ideal growth medium for microorganisms, is recognised as a transmission vehicle. Considering the vehicle or source implicated in foodborne outbreaks during the period from 2007 to 2011, there were 720 and 510 foodborne outbreaks associated with an identified vehicle or source in Australia and New Zealand, respectively (Figure 4). The top five foods or sources associated with foodborne outbreaks in New Zealand were shellfish, meat, poultry, fish and dairy, while consumption of contaminated eggs or egg-based dishes, fish/seafood, mixed dishes, poultry and meat or meat-based dishes were the top five food vehicles in Australia. Poultry and eggs, as major transmissions of foodborne pathogens Campylobacter and Salmonella were also noted in our analysis of the data. It has been suggested that the primary processing stage was the most cost-effective intervention site to decrease the risks of campylobacter contamination on poultry meat. Poultry, fish and meat were the common food vehicles identified in different outbreaks in both countries. Australia has specific problems with egg and egg-based foods whereas contamination of dairy foods was a particular issue in New Zealand. Most of the reported foodborne outbreaks in this article were noticed to be mainly associated with poor handling and contribute in lowering the potential risks of foodborne outbreaks.

Major Foodborne Pathogens Associated with Foodborne Outbreaks

Data analysis of foodborne outbreaks in Australia and New Zealand during a five-year period (2007-2011) revealed the responsible biological agents. (Figure 5) presents an overall picture of biological contamination, including the top five foodborne pathogens in both countries. Norovirus (20%), Campylobacter spp. (10%), Salmonella spp. (7%), Clostridium perfringens (5%) and Giardia spp. (3%) were top five foodborne pathogens in New Zealand; in Australia, the top five pathogens or agents were Salmonella typhimurium (31%), Norovirus (8%), Ciguatera fish poisoning and Salmonella spp. (5% for each), C. perfringens (4%). In majority of the cases casual agent was unidentifiable (43% and 34% of total number of foodborne outbreaks in New Zealand and Australia, respectively). This indicated that both nations have a common challenge to improve outbreaks monitoring and investigation system for identifying the casual agent.

Foodborne pathogen data also showed that both countries had different food safety risks to handle i.e. norovirus in New Zealand and S. typhimurium, in Australia, were recognised as the major foodborne pathogens during 2007 and 2011. Norovirus and Salmonella were also responsible for nearly 75% of foodborne outbreaks reported in the Unite State. The risk of Salmonella associated with eggs was investigated in South Australia and the authors found that was highly linked to egg shell instead egg contents. They accordingly suggested a good hygiene in food preparation and prevention of cross contamination especially after handling broken, cracked or dirty eggs and expected such practices could contribute to reduce risks caused by this pathogen. Looking at the high outbreak numbers associated with norovirus in New Zealand, consumption of raw shellfish specifically raw oysters (contaminated by polluted pipe water or sea water) was the major attributing factor. Nevertheless, serving raw or undercooked oysters is a well-accepted eating habit in New Zealand. Monitoring oyster growing water quality and educating people to eat thoroughly cooked oysters can be effective approaches for decreasing the norovirus outbreak numbers.

Norovirus: This is the most common contributor to acute non-bacterial gastroenteritis outbreaks in New Zealand. From 2002...
to 2009, more than 38% of shellfish-associated outbreaks were caused by norovirus; a total of 1206 norovirus outbreaks were identified by genotyping and 825 (68.4%) outbreaks was related to norovirus GI.4.31 A study showed food handlers would be potential transmission mode of norovirus GI.4 or GI.4 if a person had recovered from gastroenteritis prior to handling foods.32 Waste water was also found to be linked to two norovirus outbreaks occurred in commercially farmed oysters.35 Greening et al.31 suggested that tracking the variants of norovirus by country could give information about the various transmission modes and, thus would be useful in deceasing rate of international spread.

Salmonella: This microorganism causes salmonellosis, a major foodborne disease worldwide. The most common route of transmission of Salmonella was faecal-oral, which meant humans could be infected by consuming contaminated food or water by direct or indirect contact with faeces of an infected human or animal. In Australia and New Zealand, Salmonella typhimurium was the most common serovar followed by Salmonella enteritidis.34 S. typhimurium was the causative serotype in 78% of 172 outbreaks in New Zealand; S. infantis and S. typhimurium phage type 135 were most commonly identified.35 Non-typhoid salmonellosis was identified as a foodborne disease in New Zealand. However, it was not clear how this pathogen infected consumers.36 Moreover, changes in the Salmonella serovar spectrum and distribution were found from year-to-year, thus leading to the complexity of the global epidemiology of Salmonella.34 In Australia, Salmonella species were responsible for 36% of foodborne diseases from the consumption of unsafe eggs and egg-based dishes. However, Salmonella associated with eggs was not endemic in New Zealand.19

Campylobacter: New Zealand has one of the highest risks of campylobacteriosis in the world. High population of Campylobacter was commonly found in the environment and on some raw plant or animal origin foods, in particular, raw poultry meat.37 Identified routes of transmission were food (47%), direct animal contact (28%), overseas travel (7%), person-to-person transmission (4%) and water-related (3%).38 The reports also suggested that in summer there was a higher risk of campylobacteriosis than other seasons, however, the reasons for these observed seasonal trends were poorly understood.39 Additionally, the implicated food vehicles associated with campylobacteriosis may vary from country to country.

Clostridium perfringens: This microorganism is one of the most common causes of foodborne outbreaks in New Zealand and Australia. High occurrences of Clostridium perfringens were related to the gut of food animals and humans, and the production of an enterotoxin was encoded by the C. perfringens enterotoxin gene (cpe).40

Ciguatera fish poisoning: This is a global disease caused by the consumption of certain marine fish that have accumulated orally-effective levels of ciguatoxins from complex environmental origins, thus resulting in diverse and long-term human health effects.41 In Australia, the total number of foodborne outbreaks due to ciguatera fish poisoning were 43 and this placed in the third position in a total of 685 foodborne outbreaks from 2006 to 2010. There were only three outbreaks of ciguatera fish poisoning that occurred in New Zealand between 2008 and 2012 (data not included in this review).

Listeria: Listeriosis does not cause large numbers foodborne outbreaks, however, high mortality rates in immune-compromised individuals put it on the priority list of food safety authorities in New Zealand and Australia, so an enhanced listeriosis surveillance system was consequently established. In Australia, listeriosis cases were sporadic and limited to contaminated mussels, processed meats, sandwiches and fruit salads, in light of high protein, moderate water activity and low numbers of microflora in those foods.42

Changes of Food Policy and Standards

Foodborne pathogens are able to adapt and grow under adverse conditions, therefore, food policy and standards need to be updated in order to ensure their effectiveness in food safety plans. There have been significant changes in regulation and policies for Listeria control that have been changed from having no policy at all to zero tolerance for all foods in Australia, New Zealand, Italy and the Unite State.43 In New Zealand, the latest standard code states that L. monocytogenes control in food supply chain was unsuccessful of mainly due to the inconsistent requirements and legislation for the control of this pathogen. These authors also noticed that in the dairy and seafood industries L. monocytogenes could be controlled effectively by legislation and by the food safety programme of the business. Some specific control measures have been mandated through legislation in the food retail and service sectors.

Poultry meat has been scientifically established to be a significant exposure pathway in New Zealand.36,39 In order to prevent and decrease Campylobacter contamination, the policy changed from zero to low levels of contamination because researchers found that the current interventions (such as biosecurity measurements) were not able to eliminate Campylobacter in the poultry production chain completely.45 On the other hand, broiler chicken carcasses at the end of primary process was recognized as an important pathway for Campylobacter to infect human population in New Zealand; the Ministry for Primary Industries (MPI) consequently commenced and conducted “Campylobacter Risk Management Strategy” from 2006. This policy change
helped to drop the estimated foodborne cases of campylobacteriosis in New Zealand i.e., from 4500 cases per quarter in 2006 to 2000 cases per quarter in 2013.36

CONCLUSION

This paper reviewed foodborne outbreaks reported by ESR (New Zealand) and OzFoodNet, (Australia) between 2007 and 2011. The total numbers of these outbreaks during these five years were also summarised and compared, according to foodborne outbreaks associated with pathogens, transmission vehicles and preparation setting in each country. The most frequently implicated vehicles were seafood, meat and poultry, but the implicated foods relating to high risks of the outbreaks varied from each country. For example, a high risk of Salmonella infection associated with eggs and egg-based foods in Australia and dairy products in New Zealand. Private homes, restaurants and aged care facilities were associated with the highest number of outbreaks in both countries. Data analysis in this review suggested that the surveillance of foodborne outbreaks needed to be increased to develop food safety policies in the future.42 A research survey on trust in the Australian food supply indicated that participants had little knowledge, or interest, in understanding of food regulations even while they expressed high trust in the food system.37 Therefore, common control measures such as food safety education and good practices (cleaning and disinfection) would help to effectively reduce risks of microbiological foodborne illness in Australia and New Zealand.

REFERENCES


A Multicenter Investigation on Nutritional Risk and Nutrition Therapy in Chinese Teaching Hospitals with Nutritional Risk Screening 2002

Zhi-jun Zhou¹,²#, Bao-dong Tang³#, Hai-yan Mai⁴, Yu-Long He¹,², Shi Fang⁴* and Chang-Hua Zhang¹,²*

#Equally contributed

¹Department of Gastrointestinal Surgery, the First Affiliated Hospital of Sun Yat-sen University, 58 Zhongshan 2nd Road, Guangzhou, Guangdong 510080, China
²Gastric Cancer Center of Sun Yat-sen University, 58 Zhongshan 2nd Road, Guangzhou, Guangdong 510080, China
³Department of Gastroenterology, Eastern Hospital of the First Affiliated Hospital of Sun Yat-sen University, 183 Huangpu Road, Guangzhou, Guangdong 510080, China
⁴Department of Clinic Nutrition, the First Affiliated Hospital of Sun Yat-sen University, 58 Zhongshan 2nd Road, Guangzhou, Guangdong 510080, China

ABSTRACT

Objective: Nutritional Risk Screening 2002 (NRS-2002) is recommended by ESPEN for nutrition screening. We aimed to compare the prevalence of nutritional risk and the rate of nutrition therapy based on NRS-2002 between surgical and internal medicine wards.

Methods: Patients admitting to four Chinese teaching hospitals were enrolled from April to December 2008. Patients were divided into surgical wards (Departments of GI Surgery, hepatic surgery, Breast & thyroid surgery and Thoracic Surgery) and internal medicine wards (Departments of Gastroenterology, Pulmonology, Neurology and Nephrology). The Nutritional Risk Screening (NRS) 2002 tool was used for nutritional risk screening. Prevalence of malnutrition, nutritional risk and implementation rate of nutritional therapy were compared between patients in surgical wards and those in internal medicine wards.

Results: A total of 1278 patients were in the final analysis. Among them, 551 patients were in surgical wards (the surgical group) and 727 patients were in internal medicine wards (the internal group). Compared to the internal group, the prevalence of nutritional risk was significantly lower in the surgical group (27.22% vs 39.48%, p=0.0001). There were no significantly differences in prevalence of malnutrition between surgical and internal groups (15.06% vs 16.23%, p=0.5702). In the internal group, more patients without nutritional risk underwent nutrition therapy (22.27% vs 13.97%, p=0.0019) and fewer patients with malnutrition or nutritional risks underwent nutritional therapy (p<0.05).

Conclusions: Nutritional risk and inappropriate nutritional therapy are more common among patients in internal medicine wards than those in surgical wards. The NRS-2002 is a worthwhile nutritional risk screening tool for hospitalized patients.

TRIAL REGISTRATION: ClinicalTrials.gov Identifier: NCT00289380.

KEYWORDS: Nutritional risk; NRS-2002; Nutrition therapy; Hospitalized patient.
INTRODUCTION

Nutritional status of hospitalized patients has been recognized as an important factor on clinical outcomes. Patients with nutritional risk or malnutrition faced higher risk of worse outcomes including increased length of hospital stay, healthcare costs, morbidity and mortality, and readmission rate. Malnutrition was associated with a weakened immune system and higher incidence rate of infection and infectious disease. The prevalence of malnutrition in hospitalized patients was reported to be 8%-80% and varied with different diseases. Old patients and cancer patients had higher nutritional risks. However, there were no reports comparing nutritional risk and nutritional therapy between surgical and internal medicine wards till today.

The prevalence of malnutrition varied largely among different reports which should be partially related to different screening tools used. Currently, there were several optional screening tools such as Nutritional Risk Screening 2002 (NRS-2002), the Malnutrition Universal Screening Tool (MUST), the Subjective Global Assessment (SGA) and the Mini Nutritional Assessment (MNA). NRS-2002 established by Kondrup et al. has shown superior performance in regard to sensitivity and specificity for predicting complications compared with other screening tools and was recommended by the European Society of Parenteral and Enteral Nutrition (ESPEN) for nutritional assessment in hospitalized patients. A multicenter, prospective study involving 26 hospital departments from more than 10 countries showed that nutritional risk defined by NRS-2002 was an independent predictor of poor clinical outcome. NRS-2002 was first introduced in China in 2007 and we formerly reported the prevalence of nutritional risk defined by NRS-2002 in Guangzhou teaching hospitals was 41.5%.

Nutritional screening and assessment could quantify a patient’s degree of malnutrition, and monitor the adequacy of nutritional supplements as well as identifying patients at risk of malnutrition. Early detection of nutritional risk would allow for early intervention and better outcomes. One reason for not doing such screening might be lack of time since nutritional screening is one of several time-consuming procedures in a busy hospital and may be easy to neglect. Studies showed that nutritional therapy based on nutritional screening results significantly reduced length of hospital stay and morbidity rate. Inappropriate implementation of nutritional therapy was found in China and other countries, but the difference on nutritional therapy between surgical and internal medicine wards was not clear.

In this study, we aim to compare nutritional status, the prevalence of nutritional risk, and nutritional therapy between patients in surgical wards and those in internal medicine wards. We found that nutritional risk and inappropriate nutritional therapy were more common among patients in internal medicine wards than those in surgical wards. Patients with internal diseases should be intensively evaluated for nutritional risk and nutritional therapy. Our results showed that NRS-2002 was a worthwhile nutritional risk screening tool for hospitalized patients and implementation of nutrition therapy should be based on nutritional screening results.

METHODS

Study design

The present study is a prospective observational cohort study for patients admitted to four teaching hospitals including the First and the Sixth Affiliated Hospital of Sun Yat-sen University, the First Affiliated Hospital of Guangzhou Medical University, and Guangzhou Red Cross Hospital. Each hospital included surgical wards (Departments Gastrointestinal Surgery, hepatic surgery, Breast & thyroid surgery and Thoracic Surgery) and internal medicine wards (Departments Gastroenterology, Pulmonology, Neurology and Nephrology). All patients gave written informed consent. The trial was approved by the Ethics Committee of all four teaching hospitals (Register No S054, Clinical trial register No NCT00289380). The study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki.

Patient enrollment

 Patients were interviewed with a questionnaire composed of the items in NRS-2002 within 24 hours after admission. Inclusion criteria were as following: 1) age of 18-80 years old at entry; 2) length of hospital stay >1 day; 3) not given surgery within 24 hours after admission; 4) good orientation to time and place; 5) able to speak and understand Chinese. Exclusion criteria included coma patients, uncooperative patients and patients underwent surgery within 24 hours after admission.

Nutritional risk screening and data collection

General information on changes of body weight, feeding habits, and fluid retention (presence or absence of edema) were obtained after admission. The patients’ height and body weight at admission were measured using a calibrated scale to calculate individual body mass index (BMI) values (BMI = Weight[kg]/(Height[m])²). Weight was weighted with the subjects in light clothes and no shoes using a mechanical scale to the nearest 0.1 kg. Height was measured using a fixed tape to the nearest 0.1 cm. According to the Chinese criteria by Working Group on Obesity in China, patients were defined as malnutrition if a BMI<18.5 kg/m² combined with an impaired general condition, overweight if a BMI was between 24 kg/m² and 28 kg/m², and obesity if a BMI>28 kg/m². NRS score was calculated by adding the nutritional status score (0 to 3) to the disease severity score (0 to 3), plus a score of 1 for age of patient ≥70 years old. The nutritional status score was based on weight loss, reduced food intake, and BMI, as described previously. The severity of disease was categorized as absent, mild, moderate or severe.
severe (score 0-3) according to prototype provided previously and converted to a score of 0-3. Patients with an NRS score ≥3 were considered nutritionally “at risk”. Parenteral Nutrition (PN) was defined as nutrients administered intravenously that contain a combination of carbohydrate, amino acids or fatty acids. Enteral Nutrition (EN) was defined as oral nutrient supplements or tube feeding. Patients who received EN or PN for at least 3 days were included in the nutritional-therapy group. All primary data were confirmed within 24 hours after the patient was discharged and put into the Epidata database where the software was able to perform logical check and finally determine an object database.

**Statistical analysis**

Statistical analysis was performed with SPSS 16.0 (Chicago, IL, USA). Descriptive data were presented as mean ± SD or percentages. Student-t test or F test were used for the comparison of continuous variables among different groups. Chi-square analysis was used for the comparison of incidence rate among different groups. A p-value<0.05 was considered statistically significant.

**RESULTS**

**General data**

Between April to December 2008, a total of 2602 patients were investigated and 1278 patients were in the final analysis (Figure 1). Patient demographics and nutritional risk status at admission in each ward were shown in table 1. Among them, 551 patients were in surgical wards (the surgical group) and 727 patients were in internal medicine wards (the internal group). There was no difference in gender between the two groups (Table 2). Mean age in the internal group was about 8 years older than that in the surgical group (61.21±15.64 versus 53.10±16.30, p<0.0001).

[Figure 1: Participant flow]

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>GI surgery</th>
<th>Hepatic surgery</th>
<th>Breast &amp; thyroid surgery</th>
<th>Thoracic Surgery</th>
<th>Gastroenterology</th>
<th>Pulmonology</th>
<th>Neurology</th>
<th>Nephrology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>129</td>
<td>14</td>
<td>33</td>
<td>147</td>
<td>159</td>
<td>147</td>
<td>61</td>
<td>73</td>
</tr>
<tr>
<td>Female</td>
<td>86</td>
<td>12</td>
<td>76</td>
<td>54</td>
<td>103</td>
<td>53</td>
<td>72</td>
<td>59</td>
</tr>
<tr>
<td>Age (years),mean±SD</td>
<td>52.38±16.23</td>
<td>55.38±19.23</td>
<td>49.12±16.51</td>
<td>55.72±15.46</td>
<td>56.45±16.39</td>
<td>61.59±15.2</td>
<td>65.44±12.73</td>
<td>65.8±14.85</td>
</tr>
<tr>
<td>Age≥70 years,%(n)</td>
<td>18.60% (40)</td>
<td>30.77% (8)</td>
<td>15.60% (17)</td>
<td>18.91% (38)</td>
<td>22.90% (60)</td>
<td>39.50% (79)</td>
<td>48.12% (64)</td>
<td>50.76% (67)</td>
</tr>
<tr>
<td>BMI(kg/m2),mean±SD</td>
<td>21.3±3.22</td>
<td>21.53±3.01</td>
<td>22.46±2.81</td>
<td>21.85±3.5</td>
<td>21.79±3.21</td>
<td>20.53±4.09</td>
<td>22.36±2.7</td>
<td>22.79±4.11</td>
</tr>
<tr>
<td>Undernutrition,%(n)</td>
<td>16.74% (36)</td>
<td>11.54% (3)</td>
<td>5.50% (6)</td>
<td>18.91% (38)</td>
<td>11.07% (29)</td>
<td>31.50% (63)</td>
<td>5.26% (7)</td>
<td>14.39% (19)</td>
</tr>
<tr>
<td>Normal,%(n)</td>
<td>63.72% (137)</td>
<td>76.82% (20)</td>
<td>61.47% (67)</td>
<td>50.75% (102)</td>
<td>61.83% (162)</td>
<td>51.50% (103)</td>
<td>67.67% (90)</td>
<td>52.27% (69)</td>
</tr>
<tr>
<td>Overweight,%(n)</td>
<td>15.81% (34)</td>
<td>7.69% (2)</td>
<td>30.28% (33)</td>
<td>26.37% (53)</td>
<td>23.28% (61)</td>
<td>13.50% (27)</td>
<td>24.81% (33)</td>
<td>23.48% (31)</td>
</tr>
<tr>
<td>Obesity,%(n)</td>
<td>3.72% (8)</td>
<td>3.85% (1)</td>
<td>2.75% (3)</td>
<td>3.96% (8)</td>
<td>3.82% (10)</td>
<td>3.50% (7)</td>
<td>2.26% (3)</td>
<td>9.85% (13)</td>
</tr>
<tr>
<td>nrs≥3,%(n)</td>
<td>29.3% (63)</td>
<td>42.3% (11)</td>
<td>10.1% (11)</td>
<td>32.3% (65)</td>
<td>24.0% (63)</td>
<td>58.0% (116)</td>
<td>36.1% (48)</td>
<td>45.5% (60)</td>
</tr>
<tr>
<td>nrs&lt;3,%(n)</td>
<td>70.7% (152)</td>
<td>57.7% (15)</td>
<td>89.9% (98)</td>
<td>67.7% (136)</td>
<td>76.0% (199)</td>
<td>42.0% (84)</td>
<td>63.9% (85)</td>
<td>54.5% (72)</td>
</tr>
</tbody>
</table>

Table 1: Patient demographics and nutritional risk status at admission in each ward
The prevalence of malnutrition and nutritional risk

Prevalence of malnutrition was 16.23% in the internal group which was similar to that in the surgical group (16.23% versus 15.06%, p=0.5702). Incidence rate of nutritional risk was significantly higher in the internal group compared to the surgical group (39.48% versus 27.22%, p<0.0001). These results demonstrated that NRS-2002 could detect difference existed in the two groups (Table 2).

<table>
<thead>
<tr>
<th>Nutritional therapy</th>
<th>Internal medicine wards</th>
<th>Surgical wards</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrition therapy</td>
<td>196(26.96%)</td>
<td>141(25.59%)</td>
<td>0.5819</td>
</tr>
<tr>
<td>No</td>
<td>531(73.04%)</td>
<td>410(74.41%)</td>
<td></td>
</tr>
<tr>
<td>NRS≥3 and nutritional therapy</td>
<td>98(34.15%)</td>
<td>85(56.67%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>No</td>
<td>189(65.85%)</td>
<td>85(43.33%)</td>
<td></td>
</tr>
<tr>
<td>NRS&lt;3 and nutritional therapy</td>
<td>98(22.27%)</td>
<td>56(13.97%)</td>
<td>0.0019</td>
</tr>
<tr>
<td>No</td>
<td>342(77.73%)</td>
<td>345(86.03%)</td>
<td></td>
</tr>
<tr>
<td>BMI&lt;18.5 and nutritional therapy</td>
<td>47(39.83%)</td>
<td>45(54.22%)</td>
<td>0.0438</td>
</tr>
<tr>
<td>No</td>
<td>71(60.17%)</td>
<td>38(45.78%)</td>
<td></td>
</tr>
<tr>
<td>BMI≥18.5 and nutritional therapy</td>
<td>149(24.47%)</td>
<td>96(20.51%)</td>
<td>0.125</td>
</tr>
<tr>
<td>No</td>
<td>480(75.53%)</td>
<td>372(79.49%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Nutritional risk status of patients at admission in surgical and internal medicine wards

Nutritional therapy for patients in surgical and internal medicine wards

Among patients with malnutrition, 39.83% patients in the internal group and 54.22% in the surgical group underwent nutritional therapy (Table 3). More patients in the surgical group received nutritional therapy than in the internal group (p=0.0438). Similar results were observed to patients with nutritional risk (Table 3). More patients with nutritional risks were given nutritional therapy in the surgical group than that in the internal group (56.67% vs. 34.15%, p<0.0001). Fewer patients without nutritional risks underwent nutritional therapy in the surgical group than in internal group (13.97% vs. 22.27%, p=0.0019). Figure 2 showed clearly that more patients without nutritional risk (16.8% vs. 8.45%) and fewer patients with nutritional risk (13.48% vs. 15.42%) underwent nutritional therapy in the internal group compared to those in the surgical group.

Figure 2: Distribution of patients with or without nutritional risk underwent nutrition therapy or not. NR is abbreviation of nutritional risk and NT represents nutrition therapy.
CONCLUSION/DISCUSSION

The NRS-2002 was used as the screening method in this study. Differences on nutritional status between the surgical and internal groups were not detected by BMI but by NRS-2002. It demonstrated that NRS-2002 was more sensitive to detect difference among different groups of hospitalized patients. NRS-2002 was also found to be a sensitive tool for hospitalized patients by other studies. An NRS score showed both nutritional status and the severity of disease. Repeated point prevalence surveys could reflect changes in nutritional status and disease severity. In our study, some patients were excluded for BMI score missed. We could not measure weight and height for these patients because of incapable to or unwilling to cooperate. As van Bokhorst-de van der Schueren, et al. mentioned that not one single screening or assessment tool was capable of adequate nutrition screening as well as predicting poor nutrition related outcome. Kuppinger, et al. found that the NRS-2002 alone was insufficient to precisely predict complications and a modified NRS-2002 classification might be required to preoperatively identify patients at a high nutritional risk for surgical patients with non-abdominal diseases. However, Kuppinger et al. did not deny the value of NRS-2002. They modified the value of NRS-2002 to “>2 or <2”, rather than “>3 or <3” as it is currently classified. Most importantly, the theory proposed by Kuppinger et al. has not been widely recognized, while NRS-2002 is widely recognized and is recommended by European Society of Parenteral and Enteral Nutrition (ESPEN). In order to make our study more comparable with other studies, we decided to take NRS-2002 as the assessment tool.

By using the NRS-2002 screening tool, we found that prevalence of nutritional risk was significantly higher in the internal group compared to the surgical group. One reason for this difference should be partially related with the difference in mean age between the two groups. It is well known that older patients have higher risk of malnutrition. Mean age in internal wards was 8-year older than that in surgical wards. Another reason might be better patient performance in surgical wards compared to internal medicine wards. For those patients with worse performance might not be suitable for an elective surgery, they usually admit to the internal medicine ward for preoperative preparation. Malnutrition could cause impaired immunity and increased complications such as stress ulcer, infection, organ dysfunction, etc. Malnutrition and nutritional risk were recognized as major causes of morbidity and mortality for hospitalized patients. Thus, patients with malnutrition and nutritional risk should be given nutrition therapy, even underwent surgery after two weeks nutritional therapy.

Nutritional screening has been recommended as the first step to individualized nutritional treatment in some clinical guidelines. Researches demonstrated that implementation of nutritional therapy based on nutritional screening results significantly improved clinical outcomes. ESPEN guideline suggested that nutritional therapy should be started if undernutrition already existed or an inadequate food intake (<60% of estimated energy expenditure) was anticipated for more than 10 days. However, these positive results and guidelines have not gained wide recognition, since the most recent studies showed that adequate dietary intake and/or adequate artificial nutrition support was almost non-existent among undernourished patients. In most cases, this was not due to the disease process but rather due to lack of involvement of hospital and department managements, combined with a lack of knowledge of elementary theoretical and practical aspects of clinical nutrition among staff members. In current study, we found that more non-nutritional risk patients and fewer nutritional risk patients in internal medicine wards underwent nutritional therapy compared to those in surgical wards. Both surgical wards and internal medicine wards had less than 60% patients in nutritional risks underwent nutritional therapy. These denote a lack of appropriate implementation of nutrition therapy and adequate daily energy and protein intake for undernourished hospitalized patients. These results implied that implementation of nutrition therapy should be based on nutritional screening results as clinical guidelines suggested.

In summary, the NRS-2002 was an optional screening tool for hospitalized patients. Nutritional risk and inappropriate nutrition therapy were more common among patients in internal medicine wards than those in surgical wards. Different diseases may be the main reason for this difference besides patient’s age and performances. Nutritional risk and nutrition therapy should be intensively evaluated among patients with internal diseases. Implement a rational nutrition therapy should be based on nutritional screening results.

COMPETING INTERESTS

The study was funded by the National Science & Technology Pillar Program. None of the authors report competing interests.

AUTHORS' CONTRIBUTIONS

ZZJ and TBD are equal contributors to this work. ZCH, FS and HYL participated in setting up the research design and interpret resulted. ZCH, TBD and MHY carried out the studies, data collection and interpreted results. ZZJ, MHY and ZCH participated in statistical analysis and drafted the manuscript. All authors read and approved the final manuscript.

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Crosstalk between Autophagy and Obesity: Potential Use of Avian Model

Alissa Piekarski, Elizabeth Greene, Nicholas B. Anthony, Walter Bottje and Sami Dridi*

Center of Excellence for Poultry Science, University of Arkansas, Fayetteville AR 72701, USA

ABSTRACT

Autophagy, a self-eating mechanism for recycling cellular constituents, is essential for maintaining cellular homeostasis and its malfunction is associated with diverse diseases including neurodegeneration, cancer, immunity and metabolic syndrome. Human Obesity is a devastating multifactorial disease with continuous increasing prevalence and, thus, there is a need for more extensive research using several experimental models to understand its underlying molecular mechanisms. Emerging evidence indicates a key role of autophagy in the development of obesity and has been a focus of research interest in recent years. This review will briefly describe the autophagy processes and provide insight into metabolic characteristics of avian species that make birds a model of choice for investigation of autophagy particularly with respect to obesity.

KEYWORDS: Autophagy; Obesity; Avian species.


INTRODUCTION

Autophagy is a highly conserved cellular mechanism that is responsible for the degradation and recycling of damaged organelles. In recent years though, autophagy has appeared to play critical roles in several cellular functions and physiological processes. Although originally described in 1960 by Christian de Duve, the founding father of autophagy,1 first autophagy-related proteins were only recently identified (in 2008) which makes autophagy a relatively new and thriving field of study. Autophagy was used to distinguish the ‘eating’ (phagy) of part of the cell’s self (auto) from the breakdown of extracellular material (heterophagy).2 The name was coined from the observation of electron microscopy studies that showed novel single or double-membrane vesicles containing organelles in various stages of degradation and, therefore, distinguishes it from the ubiquitine (Ub)-proteosome pathway that is specific for the degradation of short-lived proteins.

There are three major types of autophagy; micro-, macro-autophagy, and chaperone-mediated autophagy.3,4 Micro- and macro-autophagy can selectively engulf large structures such as mitochondria and endoplasmic reticulum (referred to as mitophagy or reticulophagy, respectively4,6) or by non-selective mechanisms (e.g. bulk cytoplasm), whereas chaperone-mediated autophagy degrades only soluble proteins.4 Micro-autophagy refers to the sequestration of cytosolic components directly by lysosomes through invaginations in their limiting membrane. However, macro-autophagy that we will address in the present review refers to
the sequestration of material within an autophagosome, a unique double membrane cytosolic vesicle. Autophagosomes fuse with late endosomes and lysosomes, promoting the delivery of organelles, aggregated proteins and cytoplasm to the luminal acidic degradative milieu that enables their breakdown into constituent molecular building blocks that can be recycled by the cell.9

Although autophagy was first observed in mammalian cells, the molecular mechanisms were delineated in yeast. A number of protein complexes and signaling pathways that regulate autophagy have been identified in yeast and many of these have mammalian orthologs. A breakthrough for studying the molecular basis of this pathway was through identifying the Atg (Autophagy-related) genes.10 There are currently more than 30 Atg genes that have been identified in yeast as well as functionally characterized orthologs of the Atg gene products in higher eukaryotes including: mammals, insects, worms, and plants.11,12

Knowledge gained over the past decade about the mechanisms that underpin autophagy has provided a universal framework for studies of diverse (patho-)physiological processes. Of particular interest is the emerging role of autophagy in the regulation of energy homeostasis.13 Dysregulation of autophagy might contribute to the development of metabolic disorders such as obesity and insulin resistance. Using different experimental animal models may shed light on these underlying molecular mechanisms and may help to develop new therapeutic strategies.

In the following sections we will briefly describe the autophagosome formation from initiation to maturation, their interaction with nutrition in the development of metabolic syndrome and unique metabolic characteristics of avian species that argue for birds becoming a model of choice to study the molecular mechanisms involved in obesity.

**Autophagosome Process**

The autophagy process contains genes that function in key stages of the pathway: initiation (or induction), elongation, maturation, and fusion with the lysosomes are shown in figure 1. First, Atgs are concentrated on single lipid bilayer membranes called phagophores that bud from pre-existing mitochondria or ER and modulate membrane elongation to form cup-shaped structures that engulf cytoplasmic components to generate spherical autophagosomes.14

![Figure 1: Steps of autophagosome formation](image1)

**Figure 1:** Steps of autophagosome formation: Autophagosome formation can be initiated via mTOR inhibition or AMPK activation during starvation or nutrient limitation. This results in the activation of ULK1 which in turn phosphorylates Atg13, Atg101 and FIP200. When autophagy is activated, Beclin 1 is liberated from Bcl-2 and is associated with Vps34, Vps15 and Atg14. ULK1 phosphorylates also AMBRA, a component of the PI3K CIII complex enabling it to relocate from the cytoskeleton to the isolation membrane. The activation of Vps34 generates PI3P which catalyzes the first of two types of ubiquitination-like reactions that regulates membrane elongation. Firstly, Atg5 and Atg12 are conjugated to each other in the presence of Atg7 and Atg10. Attachment of the Atg5-Atg12-Atg16L1 complex on the isolation membrane induces the second complex to covalently conjugate PE to LC3 which facilitates in turn the closure of the isolation membrane. The complex Atg5Atg7Atg18 cycles between endosomes, the Golgi and the phagophore possibly carrying lipid components for membrane expansion. LC3-I is formed by LC3 phagophores to its lipid target PE and Atg4 removes LC3-I from the outer surface of newly formed autophagosome, and LC3 on the inner surface is degraded when the autophagosome fuses with lysosomes. AMBRA, autophagy/Beclin-1 regulator 1; Atg, autophagy-related genes; LC3, microtubule-associated protein light chain; PE, phosphatidylethanolamine; PI3K, phosphatidylinositol 3 kinase; PIP3, phosphatidylinositol 3-phosphate; ULK1, UNC51-like kinase 1.
The autophagosome-lysosome fusion releases the autophagosome content into the lysosome lumen for degradation. Under fed (normal nutrient-energy adequate) state, the nutrient sensor mechanistic target of rapamycin (mTOR) is activated and in turn phosphorylates ULK1 and thereby sequestering the ULK1-Atg13-FIP200 complex in an inactive state at the mTOR complex. In contrast when nutrients are limited (e.g. during stress or starvation), the energy sensor AMPK is activated. AMPK activation inhibits mTOR activity leading to a reduced ULK1 phosphorylation and consequently releases the ULK1-Atg13-FIP200 complex from mTOR to the site of autophagosome formation and induction of autophagy. In the second step of autophagy, Beclin1 forms a lipid kinase complex with vacuolar sorting proteins Vps15, Vps34 and Atg14 that phosphorylates phosphorylated phosphatidylinositol (PI) to form inositol-3-phosphate (PI3P) and is essential for induction of autophagy.14 Accumulation of PI3P in specific sub-domains of the ER increases membrane curvature at the site of autophagosome formation. The elongation step involves two ubiquitin-like reactions of the pre-autophagosomal structures. First, the ubiquitin-like protein Atg12 is conjugated to Atg5 by the action of Atg7 and Atg10 after which Atg16 multimerizes to form the Atg12-Atg5-Atg16 complex. Next, Atg4 cleaves soluble microtubule-associated protein light chain 3-1 (LC3-I) to form the membrane-bound LC3-II. Both steps complete the formation of the autolysosome and its lysis, that releases proteins and amino acids that can be used as an energy source during times of low energy availability or increased energy demand (stress) for the organism (Figure 1).

Autophagy and Development of Obesity

Obesity is a devastating multifactorial disease that continues to rise and has become an epidemic in the world with rates in the U.S. being among the highest.20 Fat accumulation and fat cell hypertrophy in human are strongly associated with autophagy, and autophagy elevation is particularly prominent in the omental fat of individuals who developed obesity-related insulin resistance.21 Using the chicken and egg analogy, it is not known which came first; obesity or adipose autophagy.

Current evidence implicates autophagy in the regulation of lipid stores within the two main organs involved in maintaining lipid homeostasis, namely the liver and adipose tissue.22 In the hepatocytes, cytoplasmic lipid droplets were found to be subject to breakdown by autophagy, a process referred to as lipophagy. Pharmacological or genetic inhibition of Atg5 increased triglyceride levels and decreased β-oxidation in a rat hepatocyte cell line.23 Moreover, hepatic lipid droplets were found to be co-localized with LC3 under basal and autophagy-induced conditions. Specific inhibition of hepatic Atg7 caused an accumulation of lipid within the mouse liver23 indicating that autophagy regulate hepatic lipid turnover.

In adipocytes, however, autophagy appears to have an opposite effect on lipid stores. Indeed, autophagy is required for adipocyte differentiation and lipid droplet accumulation.24,25 Atg7 adipocyte-specific knockout reduced white adipose tissue and increased brown adipose tissue along with increased rate of β-oxidation. Recent studies showed also that autophagy regulates lipid metabolism through lipoprotein assembly and apoB degradation.26-27 ApoB was found to be co-localized with autophagosomes in docosahexaenoic acid-treated McA cells, and knockout of Atg7 gene increased apoB recovery.28

It is well known that a high fat diet and increased obesity induces insulin resistance in liver, adipose tissue and muscle resulting in hyperglycemia.29 It has been shown that an excess in nutrient supply down-regulated autophagy via insulin signaling.30 Recent studies found that hepatic autophagy is defective and its up-regulation improves insulin sensitivity in common rodent obese models (ob/ob, db/db and HFD).31-33 Furthermore, insulin inhibited autophagy via mTOR activation and mTOR inhibition by rapamycin lead to a hyperinsulinemic and hyperglycaemic states in rat skeletal muscle.34,35 These results indicate that insulin and autophagy might participate in a feedback mechanism of reciprocal regulation. Thus, autophagy may regulate insulin sensitivity in a tissue specific manner. For instance, HFD decreased and increased LC3-II levels in mouse liver and adipose tissue, respectively.21

Autophagy and Avian Species: A Model of Choice

As the incidence of obesity or metabolic syndrome continues to rise, there is a clear demand to identify new and efficient therapeutic strategies. Therefore, insights into the molecular mechanisms of this devastating disease using different experimental models are of uppermost interest. Rodents are very useful models for the study of obesity, but it could be suggested that another equally good model for this study would be chickens (Gallus gallus).

Whereas lipogenesis occurs in both adipose tissue and liver in rodents,36,37,38 chickens are similar to humans in that lipogenesis occurs exclusively in the liver and is exported via the circulatory system to adipose tissue.39 In addition, chickens are characteristically hyperglycemic compared with mammals, with their blood glucose levels averaging three times that found in humans (300 vs. 100 mg/dl).40 Genetic selection for production ef-
ficiency (rapid growth rate and feed efficiency) necessitates feed restriction in commercial meat-type chicken (broiler) breeders that are hyperphagic, heavy, and prone to obesity. Broilers voraciously consume approximately 4.1 kg of feed to achieve a 40-fold increase in body weight that is concomitant with tremendous increase in muscle development as well as abdominal fat during a period of 42 days. Both meat and egg producing chickens (broilers and layers) are insulin resistant and lack the glucose transporter protein GLUT4. They require insulin doses greater than four times that required in mammals to achieve hypoglycaemia.

Obesity is often considered to be a result of either excessive food intake or insufficient energy expenditure. Brown Adipose Tissue (BAT), a specialized fat that dissipates energy to produce heat, plays an important role in the regulation of energy balance. Interestingly, chickens do not have functional BAT. Thus, these unique metabolic characteristics make chickens an interesting model for understanding the role of autophagy in obesity development.

Recently, we characterized several genes involved in autophagosome initiation, elongation and maturation in chickens (Gallus gallus) and Japanese quail (Coturnix coturnix japonica). These genes are ubiquitously expressed and showed high similarity with their mammalian orthologs indicating that autophagy is a conserved mechanism in different species. Interestingly, we found that the expression of autophagy-related genes is tissue- and gender-dependent. For instance, it was found that hypothalamic Beclin1, UVRAC, Atg9a, Atg13, Atg4b, Atg7 and Atg5 mRNA levels were significantly higher in female compared to male chickens suggesting that hypothalamic autophagy might be involved in the regulation of feed intake. Recently, Kaushik and colleagues showed that autophagy regulates lipid metabolism within hypothalamic neurons which in turn modulate neuropeptide levels that control feed intake and energy homeostasis. Furthermore, using two experimental male quail lines divergentely selected over 40 generations for low (resistant line) or high (sensitive line) stress response it was found that the expression of several autophagy-related genes are higher in several tissues including adipose tissue in the resistant compared to the sensitive line. Since autophagy has been shown to play a key role in stress response and fat metabolism and since the regulation of energy homeostasis and the stress response are coupled physiological processes, the differential expression of autophagy-related genes between the two quail lines indicated that these lines would be a very useful model to study the interaction between stress response, autophagy and fat metabolism.

In conclusion, studies using avian models may provide critical information on the role of autophagy in lipogenesis and lipid metabolism because the liver is the primary site of de novo fatty acid synthesis in chickens and may help for targeting new therapeutic strategies to treat obesity and its related diseases.

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