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# Research, Development and Capacity Building for Food and Nutrition Security in Sub-Saharan Africa

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**Abstract**

This paper focuses on research, development and capacity building in relation to food and nutrition security (FNS) in sub-Saharan Africa (SSA). It looks at human capacity, education, teaching and learning, women empowerment, research, innovation and technology, research, indigenous knowledge (IK), institutional aspects, infrastructure, information and communication technologies (ICT), policies and finance. Professional bodies exist in many countries and the extent to which they engage in FNS awareness creation differs. Food and nutrition insecurity continues to affect people in Africa's 54 nations where the population is expected to double by 2050 with the expected doubling of food production to keep pace with population growth. Within the continent there is a substantial number of human capacity professionals who are global leaders in food, nutrition and related professions. Some research organisations in the continent directly or indirectly benefit from grants administered by developed economies but a challenge exists with brain drain and ageing of qualified and experienced experts. Increasing educational need, coupled with the growing population necessitates attention to ensuring a sustained supply of highly trained, adequately equipped and qualified professionals in the relevant fields of food and nutrition sciences. Higher educational institutions exist in especially those that fall within the 500 in world universities ranking. Research activities take place in the continent along with the translation of research outputs into commercialisable products. Research towards transforming agriculture for improved livelihoods is taking place in different parts of the continent. Education, governance, gender and rural development are the key challenges. Income growth and the impacts of climate change on food production have contributed to food insecurity. ICTs can play an important role for FNS. Strengthening research, development, capacity building and industry cooperation are critical for FNS in Africa.

***Keywords:*** Research; Human Capacity; Infrastructure; Policy; Food Security; Africa

## 1 Introduction

Food belongs at the heart of the culture of Africa, bringing families and friends together. At the same time, food and its nutrients are essential for our survival, and food-health issues are now regarded as being as, or even more, important than global warming.

Food security of households and individuals in the developing world is negatively impacted by a

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range of issues including chronic poverty, rapid population growth, declining per capita food output/ low food production, poor infrastructure, ecological constraints, limited arable land, inappropriate policies, parasites and diseases, poor water and sanitation, inadequate nutritional knowledge, civil war, and ethnic conflicts (Riely, Mock, Cogill, Bailey & Kenefick, 1999). Other factors militating against food security include: seasonal food shortages; high food prices; high unemployment; low level of nutrition education; and cultural factors and taboos that reduce access to food (Aworh & Egounlety, 2011). Of recent, income growth and the impacts of climate change on food production have contributed to food insecurity. This paper focuses on research, development and capacity issues in relation to food and nutrition security in Africa.

Education, gender and rural development remain a key challenge to the development of Africa. Food security is defined as 'when all people at all times have access to sufficient, safe and nutritious food to maintain a healthy and active life' (Clay, 2003). With the epidemic of obesity in the developed world (Baum, 2014), nutrition refers to the process involved from the choice and consumption of food up to its effects on health and well-being of individuals (Kropff, Van Arendonk & Löffler, 2013). From these definitions, food and nutrition security in low- and middle-income countries still needs significant inputs in terms of higher education, research and community engagement activities to have the desired impact (NORHED, 2013).

Food and nutrition insecurity continue to affect large numbers of people in Africa's 54 nations. FAO estimates indicate that 925 million people are undernourished, with 239 million from sub Saharan Africa (SSA) alone (FAO and WEP, 2010). Of the nine countries in the world having high population growth rates, five are from SSA (Table 1). Moreover, thirty-three countries in SSA (Figure 1) are among the forty-eight countries globally classified as Least Developed Countries (LDCs) by the United Nations Economic and Social Council (ECOSOC), with characteristics of low income, weak human resources and high economic vulnerability (UN, 2012). Research, development and capacity building are key requisites needed by Africa to be able to tackle the important challenges facing the continent (Table 4).

Recent research has revealed that the population in Africa is expected to double by 2050, and African nations will have to double their food production just to keep pace with population growth (Lartey, 2013). In the past, food production in Africa has lagged behind population growth, and the source of the problem has been low productivity on Africa's farms. Improving farm productivity requires, inter alia, a complex of policy and technological options, such as advocating for the adoption of good mixes of technologies in Africa; focusing on country-specific priorities, existing policies, availability of infrastructure for research and development; the role of diverse technologies that exist in Africa in ensuring adequate food security in the continent; nature and scope of indigenous technology options in Africa; examining the current food security situation in Africa; and the opportunities offered by different technologies in addressing the food security situation (Ozor & Urama, 2013).

Diversifying into biotechnology practices in food production will help the continent's growing population in food and nutrition security. Possibilities exist for genetic modification involving animals, plants, and microorganisms, e.g. genetically modified (GM) foods, GM food ingredients such as flavours and gums, and GM enzymes such as chymosin which contain DNA of bacteria & yeasts.

Some benefits of such Genetically Modified Organisms (GMOs) include: improved food quality including better processing qualities/functionality; dramatic increases in agricultural productivity, world food supply (e.g. maize, which is a major staple in many countries in Africa; protection of food (e.g. decreased crop losses due to insect herbivory); lowered production costs; grocery bill reduction to the consumer; and safer farm environment (through fewer/lower insecticide and herbicide use), and reduced exposure to pesticides (Jideani et al., 2013; Keats & Wiggins, 2014). Development and adaption of these technologies by Africa farmers requires considerable investment in research, education and capacity building. However, some countries are against the use of genetically modified crops.

As stated by Akinyele (2009) three strategic pillars of intervention include

1. support for national and regional centres of excellence;
2. support for infrastructure for higher education, science and technology; and
3. linking higher education, science and technology and the productive sector.

Such an approach will contribute significantly to the strengthening of African institutions of higher education (ADBG, 2008).

## 2 Human Capacity Building

Capacity is defined as 'the ability of people, organizations and society as a whole to manage their affairs successfully' (Bester, 2015). Capacity development is the process of unleashing, strengthening and maintaining of such capacity. Leadership matters in capacity development issues; as strong capacities with poor leadership can cause an organization/country to stumble. On the other hand, strong and positive leadership can bring about progress, even with low capacities (Pepping, 2010). Capacity building for food and nutrition security in Africa would require:

1. capacity development and infrastructure building,
2. government commitment,
3. private sector involvement – including industry, and
4. appropriate policies for reform.

It would also imply the promotion of national and regional innovation systems.

In human capacity, there are substantial number of professionals in food, nutrition and related professions in the continent. However, the growth in industries and demand from related international organisations like WHO, FAO, UNICEF, etc creates an apparent scarcity of professional experts in SSA countries. This calls for public-private partnerships (PPP) in SSA with inherent benefits like the supply of needed funds, exposure of local researchers to more skills, and the creation of an enabling environment for promoting research findings (SPORE, 2013). As stated by Hailu (2013) for the agricultural sector, and applicable to food and nutrition, forging innovative partnerships at different levels and building competencies of women and young people could bring about transformative changes in SSA.

Research in Food and Nutrition in SSA is facing the challenge of brain drain and ageing of highly qualified and experienced experts who will retire before the end of next decade. As reported in SPORE (2013), the goal set by the New Partnership for Africa's Development (NEPAD) of investing certain amount of agricultural GDP of a country in research and entrusted to the Forum for Agricultural Research in Africa (FARA), could help research among national, sub-regional and international organisations.

### 2.1 Education, Teaching and Learning

The increasing educational need, coupled with the growing population in SSA, particularly the youth, necessitates attention to ensuring a sustained supply of highly trained, adequately equipped and qualified professionals in the relevant fields of food and nutrition sciences. A good number of higher educational institutions (HEI) – universities, colleges and training centres - exist in most counties of SSA, the key ones are listed in Table 5. These HEIs offer training in Food Science/Technology and in Nutrition at Bachelors, Masters, and Doctoral levels.

The study of food and nutrition is a fast-developing discipline especially with the globalisation of the food industry; and is full of practical, technical and intellectual challenges. It draws knowledge from a range of disciplines including chemistry, biology, physics, psychology, geography, business and even art. A graduate of Food Science/Technology and Nutrition has the knowledge and skills to tackle real issues and problems on food faced by society and industry (Leeds, 2014).


Figure 1: The 33 Least Developed Countries in sub-Saharan Africa: Angola, Benin, Burkina Faso, Burundi, Central African Republic, Chad, Comoros, Democratic Republic of the Congo, Djibouti, Mali, Mauritania, Mozambique, Niger, Rwanda, São Tomé and Principe, Senegal, Sierra Leone, Somalia, Sudan, Equatorial Guinea, Eritrea, Ethiopia, Gambia, Guinea, Guinea-Bissau, Lesotho, Liberia, Madagascar, Malawi, Togo, Uganda, United Republic of Tanzania, and Zambia (UN, 2012)

Table 1: Nine countries with high population growth rates

| Country | Population in 2011 (in thousands) | Population in 2050 (projected) (in thousands) | Percent change |
|---|---|---|---|
| Afghanistan | 32,000 | 77,000 | 136 |
| Democratic Republic of the Congo | 68,000 | 149,000 | 120 |
| Ethiopia | 85,000 | 147,000 | 73 |
| India | 1,250,000 | 1,736,000 | 39 |
| Niger | 16,000 | 56,000 | 246 |
| Nigeria | 163,000 | 392,000 | 140 |
| Pakistan | 178,000 | 279,000 | 57 |
| Uganda | 35,000 | 95,000 | 173 |
| Yemen | 25,000 | 62,000 | 149 |

Source: UN, Annual letter from Bill Gates. Department of Economics and Social Affairs. Population Division, UN (2011) The Least Developed Countries: Things to know, Things to do. Office of the High representative for the Least Developed Countries, Landlocked Developing Countries and Small Island Developing States UN (2012). www.un.org/ohrlls. Visited 07-09-2013

The aim and objectives of most Food Science, Nutrition and related programmes in the education system are similar, in that they strive to:

1. strengthen the Biomedical-Social-Environmental Science Partnership;
2. facilitate the transfer of food and nutrition sciences and their partnerships to relevant technologies for human development and environmental sustainability;
3. build the capacity of individuals, institutions and the private sector to optimize food supply so that hunger is overcome, nutritionally-related diseases are prevented, and overall good health is promoted;
4. work with communities to deal with food and nutritionally-related diseases in ways that are culturally-sensitive, sustainable and effective; and
5. support sustainable food and nutrition policies based on sound science (Wahlqvist, 2006).

To achieve these objectives to their fullest extent, and to ensure that the study of food and nutrition sciences and cognate technologies have their impact on the human condition and on national economies, it is imperative that there is a greater flow of resources into the teaching and development of these sciences/technologies. Science/technology partnerships and mounting major internationally significant projects can help to provide part of the solution to these major challenges facing all African Universities, and training and research institutions.

A snapshot survey of food science and related curricula in 17 universities across 11 African countries by Minnaar, Taylor, Haggblade, Kabasa and Ojijo (2017) revealed the following fundamental problems:

1. given the tight links between the growing prevalence of processed foods and declining health status, solutions to Africa's emerging public health problems will require cross-disciplinary work linking food technology, human nutrition and public health;
2. yet collaborative degree programmes are almost completely lacking at national, regional and international levels for most countries in SSA;
3. too little extension work and training (non-degree education); and (iv) very few universities present BSc Nutrition/ BSc Food Science/Technology and Nutrition programmes.

Minnaar et al. (2017) also highlighted the following challenges faced by Food Science and Technology educators: lack of modern food processing equipment; lack of "state-of-the-art" analytical equipment; lack of technical support within institutions; and insufficient numbers of academics to run programmes. The low student numbers and poor quality of students (many potential students perceive Food Science and Technology to be the less attractive compared to other programmes). In addition to the above problems, a good proportion of students are not aware of the differences between Food Science, Nutrition and allied professions (Jideani et al., 2013). Other compounding problems include the facts that:

1. there is little nutrition education at school level (FAO, 2011a), and

2. most Food Security interventions rarely emphasise the issue of nutrition, and prevention of malnutrition.

Professional training in Food and Nutrition education and communication is needed not only by the new entrants into professions related to food security, but also by those already in practice, such as Nutrition Educators; Agricultural Economists; Agribusiness Executives and Managers; Rural Development Specialists, Parliamentary Portfolio Committee members on Agriculture and Fisheries, Food Biotechnologists, Agriculture Extension Officers, women farmers, officials from local and regional agricultural research organizations, Parliamentary Portfolio Committee members on Water and Environmental Affairs, agricultural scientists, officials of state and national departments of agriculture and fisheries officials, Cooperatives Development Centers, agricultural ICT experts, farmers and community organizations, Food Security experts / Information Specialists, academics and researchers from college and universities, officials from FAO, SADC, IFPRI, USAID, IFAD, NEPAD, CGIAR, scientists in agricultural research institutes, women working in agricultural research and development, staff of Food and Nutrition Associations, etc.

## 2.2 Women Empowerment for Food & Nutrition Security

Smallholder agriculture must have a central investment focus to support broad-based poverty reduction and food and nutrition security. Furthermore, gender issues must not be neglected, as African agriculture and food security are very much female-dominated in terms of the actual farm work (although not in terms of land ownership and financial benefits). Women make up more than 50% of the agricultural labour force in the LDCs (UN, 2012). Women therefore, can play an important part in helping to improve productivity, profitability and sustainability of smallholder farming. Experts agree that women are a critical part of expanding agricultural output, particularly in sub-Saharan Africa. There is therefore a need to

1. develop training and skills programmes in Food Science and Nutrition and agriculture for African women;

2. make funding available for African women to engage in Food Science and Nutrition research and development,

3. establish appropriate mechanisms at all levels to promote the advancement of African women economically, culturally and socially, through provision of equal access of women and girls to education, basic services, health care, economic opportunities, and decision-making at all levels (UN, 2012).

The Global Food Security Index (GFSI) showed a 0.93 correlation with the Economist Intelligence Unit's Women's Economic Opportunity Index, a measure of the global environment for female economic participation (Unit, 2012). Similarly, an FAO (2011b) report states that as women make up 43% of the world's farmers, women empowerment would increase total agricultural output in developing countries by 2.5% to 4% and reduce hunger globally by 12% to 17%.

Table 2: Number of researchers in research & development (R&D) per million population

| Countries | Number of Researchers |
|---|---|
| Brazil | 1176 |
| Russian Federation | 3101 |
| India | 215 |
| China | 1176 |
| South Africa | 1113 |
| United States | 4231 |
| Britain | 4299 |
| South Korea | 6899 |

Source: Qhobela (2018)

## 3 Research, Innovation and Technology Development

The number of researchers in research & development per million population is globally comparable (Table 2). In the continent there exist scientists "who are unequivocally recognised by their peers as leading international scholars in their field for the high quality and impact of their recent research outputs" (NRF/RISA, 2019). Hence, the funds made available to universities and research establishments for research and development have positive impact both in terms of excellent scientists, infrastructure and tangible products that are in the market. Numerous research activities are taking place in the continent. This brings to fore the need to translate research outputs into commercializable products and intellectual property. Hence, traditional ways of doing research in laboratories and experimental stations and publishing the papers in academic journals will not be enough (Hailu, 2013). As stated by Lartey (2013), priority research with the potential to address undernutrition in Africa must focus on how effective research interventions can be incorporated into country programmes and scaled up for maximum effect. Research and innovation are informed by development needs to make a good model. Most countries in Africa need national, regional and international partners, as well as the private sector to tackle food and nutrition needs and lead to development outcomes that can impact positively on the population. Increased research in nutrigenomics, i.e. the application of high-throughput genomic tools in nutrition studies and research, is needed in the continent, e.g. to help tackle a non-communicable disease, such as obesity. Such research will provide methods and tools for disease prevention and health promoting foods that match lifestyles, cultures and genetics of people living in SSA. Genes and their variants have been evidenced to play a role in obesity-associated metabolic complications through genetic association studies, including candidate gene and genome-wide association approaches in adults and children (Aguilera, Olza & Gil, 2013). A recent report by Baum (2014) states that the overweight population in developing countries has surpassed developed countries, with a higher number of overweight or obese adults in developing countries than in rich countries in 2008 (Stevens et al., 2012).

As it is for agricultural technologies, a significant reduction of food prices and food insecurity in developing countries (IFRI, 2014) will be achieved by the application of different, effective processing innovations and preservation technologies (fermentation, canning, drying, etc) to plant and animal foods. This can most significantly reduce food prices and food insecurity in SSA. As "no single silver bullet exists" in food and nutrition technologies that would provide enough food for the world in 2050, adapting a range of these technologies could yield maximum benefit for the continent and improve food security. However, a positive outcome depends on

SMEs gaining access to these technologies and learning how to use them in food processing operations. This underscores the need for improved food and nutrition education to ensure that processors can use the best available technologies for their region, location and resources.

### 3.1 Research at sub-regional and regional levels

Many regional and sub-regional research towards transforming agriculture for improved livelihoods is taking place in different parts of the continent. The collaborative project between the Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA) and the International Food Policy Research Institute (IFPRI) is a good example which aims at Strategic Priorities for Agricultural Development and Agricultural Research-for-Development in Eastern and Central Africa. The sources of funding for such projects are usually from development partners including the United States Agency for International Development's Regional Economic Development Support Organisation, the European Union, Swedish International Development Agency and the International Maize and Wheat Improvement Center, as well as other funding bodies like the FAO, United Nations Development Programme (UNDP), and New Partnership for African Development (NEPAD).
New research areas in Eastern and Central Africa include:

1. sustainable agricultural water productivity enhancement for improved food security and nutrition;
2. facilitating implementation of policies to enhance equitable access to input and output markets;
3. developing and upscaling technologies and innovations for the management of maize lethal necrosis disease;
4. development of smallholder wheat production systems and value chains;
5. crop-livestock-fish integration to enhance food security, nutrition and resilience of smallholder farms;
6. capacity development for sustainable plant genetic resources (PGRS) utilization and conservation;
7. strengthening the value chains of fruits and vegetables for improved production, processing, marketing and nutrition security (ASARECA, 2014).

Cassava which is mostly grown by poor farmers is vital for both food security and for income generation. Through a joint CIAT/ International Institute for Tropical Agriculture (IITA) project, efforts on Cassava Genetic Resources & Breeding, diversity for cassava germplasm have been centralized at regional research centers like the IITA in Nigeria. In 1996, IFAD and FAO initiated work to develop a global strategy for development of cassava considering its diverse utilisation (Table 3).
The Regional Universities Forum for Capacity Building in Agriculture (RUFORUM), which is located at Makerere University, is a consortium of 42 universities in an increasing number of countries. It tackles the oversee of graduate training and networks of specialization in the countries and universities by fostering innovative and responsive research; high performing and proactive graduates; a dynamic platform for university networking; advocacy for agricultural higher education; and university transformation for relevance. It aims to becoming an essential tool for anyone interested in agricultural research and training in Africa.

## 4 Indigenous and traditional knowledge

Most countries in Africa have a rich heritage of indigenous knowledge that is being exploited in food and nutrition interventions. One of such countries among others, is Kenya where the Indigenous Food Plants Programme, using locally available edible species to enhance community health, provides income and conserve biodiversity thereby ensuring for and nutrition security (Cousins & Witkowski, 2015). One of the aims is to compile a database of the indigenous food plants of Kenya through research in the

Table 3: Utilisation of cassava

| Major uses | Products |
|---|---|
| Human consumption | Raw cassava |
| | Boiled cassava |
| | Cooked cassava slices |
| | Fried cassava slices |
| | Fermented cassava |
| | Cassava flour |
| | Macaroni |
| | Fufu - porridges or pastes (West Africa) |
| | Gaplek |
| | Composite flour, bread |
| | Tapioca |
| | Gari (West Africa) |
| | Cassaripo or tucupa |
| | Cassava rice |
| | Beer |
| Livestock feed | Cassava pellets |
| | Cassava meal |
| | Cassava chips or slices |
| | Cassava leaf meal |
| | Broken roots |
| | Cassava silage |
| Industrial products | Starch, glues, binders |
| | Filler, stabilizer, food dusting agent |
| | Glucose, alcohol, acetone, dextrins |

Source: CIAT (2004)

field and at the East African Herbarium as well as the promotion of cultivation, consumption and marketing of these foods through field demonstrations, educational materials and the media. The programme has three components: research, extension and education. It was necessary as

1. people (e.g. younger generation) who took pride in their modern food consumption pattern) were despising their traditional foods in favour of exotic foods,

2. poverty, famine, and malnutrition were common in rural areas even though local foods were readily available, and

3. much local knowledge regarding the nutritional value and cultivation of local edible plants was being lost.

## 5 Institutional Aspects

Most countries have national professional bodies, and the extent to which they engage in food and nutrition security awareness creation differs (FAO, 2011b). Some of these organisations are listed in Table 5. They, along with others such as The African Union of Nutritional Sciences, help to create awareness of food and nutrition security through seminars, conferences and workshops. They also have linkages with similar organisations at the global level, namely the International Union of Nutritional Sciences (IUNS), and the International Union of Food Science and Technology (IUFoST). Regional nutrition leadership and institutional capacity building activities is increasingly gathering momentum, for instance the partnership between IUNS and the

Table 4: Challenges militating research, development and capacity towards food and nutrition security in Africa

| Infrastructure and Technology Issues | Production Issues | Finance and Policy Issues |
|---|---|---|
| Rudimentary technologies | Inconsistent quality & quantity | Poor market analysis |
| Inadequate supply of appropriate packaging | Safety & quality constraints | Policy failures - affecting domestic resource mobilization |
| Poor processing methods | Poor linkages between producers & processors | Inadequate fiscal reforms |
| Poor health facilities | Climatic conditions - global warming | Governance issues - |
| Lack of innovativeness and product diversification | Declining per capita food output | |
| Lack of technical expertise | Climatic conditions - global warming | |

United Nations University. Furthermore, inter-scientific union activities have strengthened, e.g. IUNS and IUFoST are collaborating in an online Food Science and Technology training initiative in Africa. The IUNS President chairs the International Science Council initiative on the Sciences for Health and Well-Being. This engages all major science unions, so allowing new science platforms and models to develop regarding contemporary and future needs (IUNS, 2013; Wahlqvist, 2006).

## 6 Infrastructure, Information and Communication Technologies (ICT)

Infrastructure, tools and equipment remains an area of challenge in most institutions of higher learning in SSA. However, some institutions exist that are as well equipped for food and nutrition research as in developed countries, especially those that fall within the top 500 in the World Universities Ranking (WUR, 2014); while others are a little less well equipped, such as some of those that fall within the top 50 in the Ranking Web (RW, 2014) of universities in Africa.

The development of renewable energy supply systems has considerable potential in many Africa countries. However, this requires major financial investment and technical know-how. This is another area where capacity building at all levels is critical (UN, 2012).

Information and Communication Technologies (ICTs) can play an important role for Food and Nutrition Security (FNS) in Africa. While the potential benefits of ICT in FNS are immense, ICT has not penetrated sufficiently in the various aspects of FNS. So far, ICT has remained at the level of mobile phones in practically every African village, however the other applications for FNS have remained largely untapped. Possibilities include improved and timely accessing and dissemination of information for supplementing the food value chain better and integrated production planning, monitoring and follow-up, access to the latest results of research, information on the latest agricultural production technology, markets, pest and disease control improvement of standards of food professionals, etc, all of which can contribute to enhancing productivity and reducing food insecurity in Africa and to meeting the challenges of modernized agro-processing technologies (FAO, 2011a). A major hindrance to the development of ICT is the inadequacy of a regular energy supply. In fact, 92% of rural households in African LDCs have no electricity.

## 7 Policies and Programmes

There have been several initiatives by SSA countries aimed at providing the necessary policy environment for addressing malnutrition in line with the MDG. The various policies articulate the fact that food and nutrition are an integral part of the overall national objective of improving nutritional status and socioeconomic well-

Table 5: Some institutions and societies relating to Food and Nutrition Sciences in sub-Saharan Africa

| Country | Institutions/ Societies |
|---|---|
| Benin Republic | Higher Educational Institutions: Universite d'Abomey-Calavi;l'Université Nationale du Cotonou; Agronomiques del'Université de Bénin,lomé |
| Botswana | Botswana Dietetic Association<br>Botswana Technology Centre Gaborone<br>National Food Technology Research Centre, Kanye<br>Higher Educational Institutions: Botswana College of Agriculture, Gaborone; University of Botswana, Gaborone |
| Cameroon | Higher Educational Institutions: Centre Univérsitaire de Ngaoundere |
| Cote d'lvoire | Higher Educational Institutions: Institute National Supérieur del'Enseignement Technique, Yamoussoukro |
| DR Congo | Higher Educational Institutions: University of Kinshasha; Universite Catholique du Graben |
| Ethiopia | Food Research and Development Centre, Ethiopian Food Corporation, Addis Ababa<br>Higher Educational Institutions: Hawassa University; Addis Ababa University; Awasa Junior Agricultural College of Addis Ababa University; Kotebe College of Teacher Education |
| Gabon | Institute de Recherche Technologique,libreville-Akebe |
| Gambia | Gambia Food and Nutrition Association, Banjul |
| Ghana. | Technology Consultancy Centre, University of Science and Technology, Kumasi.<br>Ghana Regional Appropriate Technology Industrial Service, Tema<br>Ghana Nutrition Association (GNA)<br>Higher Educational Institutions: University of Ghanalegon; Kwame Nkrumah University of Science and Technology, Kumasi |
| Kenya | Higher Educational Institutions: University of Nairobi; Egerton University; Jomo Kenyatta University of Agric \& Technology, Nairobi; Technical University of Kenya; Moi University; Dairy Training School Naivasha; Egerton University |
| Lesotho | Higher Educational Institutions: National University oflesotho Maseru |
| Malawi | TCC/PHN Women in Development Project, Namadzi.<br>Malawi Enterprise Development Institute, Mpnela<br>Small Enterprise Development Organisation of Malawi, Blantyre<br>Higher Educational Institutions: The Malawi Polytechnic; University of Malawi |
| Mauritius | Higher Educational Institutions: University of Mauritius, Reduit |
| Namibia | Development Centre for Research Information Action in Africa, Windhoek<br>Higher Educational Institutions: University of Namibia |

| Country | Institutions/ Societies |
|---|---|
| Nigeria | Federal Institute of Industrial Research, Oshodi (FIIRO)<br>International Institute of Tropical Agriculture, Ibaban<br>Nutrition Society of Nigeria (NSN)<br>Nigerian Institute of Food Science and Technology (NIFST)<br>National Agency for Food and Drug Administration and Control (NAFDAC)<br>Higher Educational Institutions: Ahmadu Bello University; University of Calabar; Michael Okpara (Federal) University of Agriculture, Umuahia; Federal University of Agriculture, Abeokuta; Federal University of Technology, Owerri; University of Agriculture, Makurdi; University of Ibadan; Obafemi Awolowo University; University of Nigeria Nsukka; University of Maiduguri; Michael Okpara University of Agriculture, Umudike;ladoke Akintola University of Technology, Ogbomoso; Rivers State University of Science and Technology, Port Harcourt; Federal University of Technology, Akure; Federal University of Technology, Yola; Federal University of Technology, Minna; University of Uyo, Uyo; Kogi State University, Anyigba, Bowen University, Iwo; Imo State University, Owerri, Bells University of Technology, Ota; Ebonyi State University, Abakaliki; University of Ilorin, Ilorin; Anambra State University of Technology; Bendel State University; Enugu State University of Science and Technology; Yaba College of Technology; Idah Polytechnic, Idah; |
| Rwanda | Higher Educational Institutions: National University of Rwanda |
| Sierraleone | Higher Educational Institutions: University of Sierraleone |
| South Africa | Food Science Institute for Africa, Human Nutrition Institute, Stellenbosch<br>Medical Research Council, Nutritional Intervention Research Unit, Tygerberg, Cape Town<br>Council for Scientific and Industrial Research, Pretoria<br>Agricultural Research Council - Animal Production, Institute. Human Nutrition and Sensory Science, Irene<br>South African Association for Food Science and Technology(SAAFoST)<br>Nutrition Society of South Africa (NSSA)<br>Higher Educational Institutions: University of Pretoria, Stellenbosch University; University of Venda; Central University of Technology, Bloemfontein; University of the Free State, Bloemfontein; Cape Peninsula University of Technology, Bellville; University of Johannesburg; Tshwane University of Technology, Pretoria; Durban University of Technology; Monash University |
| Sudan | Food Processing Research Centre, Khartoum<br>Higher Educational Institutions: Ahfad University for Women, Omurdman; School of Hygiene, Khartoum |
| Swaziland | Higher Educational Institutions: University of Swaziland |
| Tanzania | Small Industries Development Organisation, Dar-es-Salaam<br>Ministry of Agriculture Training Institute, Mwanza and Kilosa<br>National Food Control Commission, Dar-es-Salaam<br>Food and Nutrition Association of Tanzania (FONATA)<br>Higher Educational Institutions: Sokoine University of Agriculture, Morogoro; University of Dar-es-salaam; University of Dodoma |

| Country | Institutions/ Societies |
|---|---|
| Uganda | Uganda Manufacturers Association, PO Box 6966, Kampala<br>Uganda Small Scale Industries Association, Mbale<br>National Agricultural Research Organisation, Kampala<br>Nile Vocational Institute, Jinja<br>Higher Educational Institutions: Makerere University, Kampala; Gulu University; Mbarara University of Science and Technology |
| Zambia | Higher Educational Institutions: University of Zambia; Natural Resources Development Collegelusaka |
| Zimbabwe | Ranche House College, Harare<br>Higher Educational Institutions: Chinhoyi University of Technology, Zimbabwe; Midlands State University, Zimbabwe; University of Zimbabwe, Harare |

being of the people, particularly of the most vulnerable groups. However, with 115 people dying every hour in SSA from diseases linked to poor sanitation, poor hygiene and contaminated water, there is need to halve by 2015, the proportion of people without sustainable access to safe drinking water and basic sanitation (UN, 2012). Akinyele (2009) outlined that such policies should aim at:

1. promoting the establishment of a viable system for guiding and coordinating food and nutrition activities;

2. incorporating food and nutrition considerations in development plans and allocating adequate resources toward solving the problems pertaining to food and nutrition at all levels;

3. promoting habits and activities that will reduce the level of malnutrition and improve the nutritional status of the population; and

4. promoting good indigenous food cultures and dietary habits for healthy living and development.

Some other challenges common to all SSA countries on food and nutrition security include:

1. lack of government policies on innovation sensitive to the African context in optimizing abundant resources;

2. translation of outputs from R&D into usable and accessible solutions; and

3. lack of nutritional guidelines to help consumers and chefs.

Indicators of food insecurity in SSA include very low levels of average food consumption, large fluctuations of food consumption and the large population of absolute poor (Aworh & Egounlety, 2011).

## 8 Financial and other resources

The impact of the world economic and financial crisis, combined with food and fuel crises, has undermined the development and progress of many developing economies. Lack of financial resources is one of the biggest constraints to both LDCs and other countries in achieving sustainable development and progress (UN, 2012). Increased access to financial services especially for SMEs, increased government spending on productive capacity-building, and support development to science and technology by all stakeholders will increase agricultural output and impact positively on food and nutrition security. Most countries in SSA have put in place financial resource mechanisms contributing to objectives such as:

1. internationalising the research platform,

2. enhancing networking within the global science system,

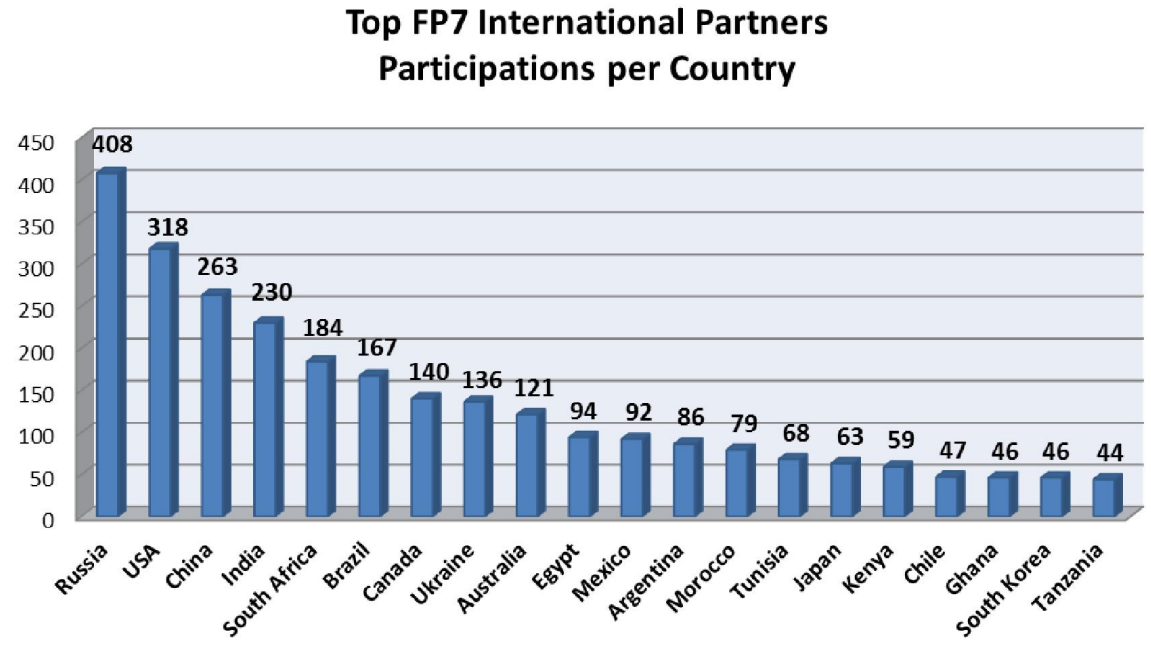


Figure 2: International participation in European Union FP7 (Hogan, 2013)

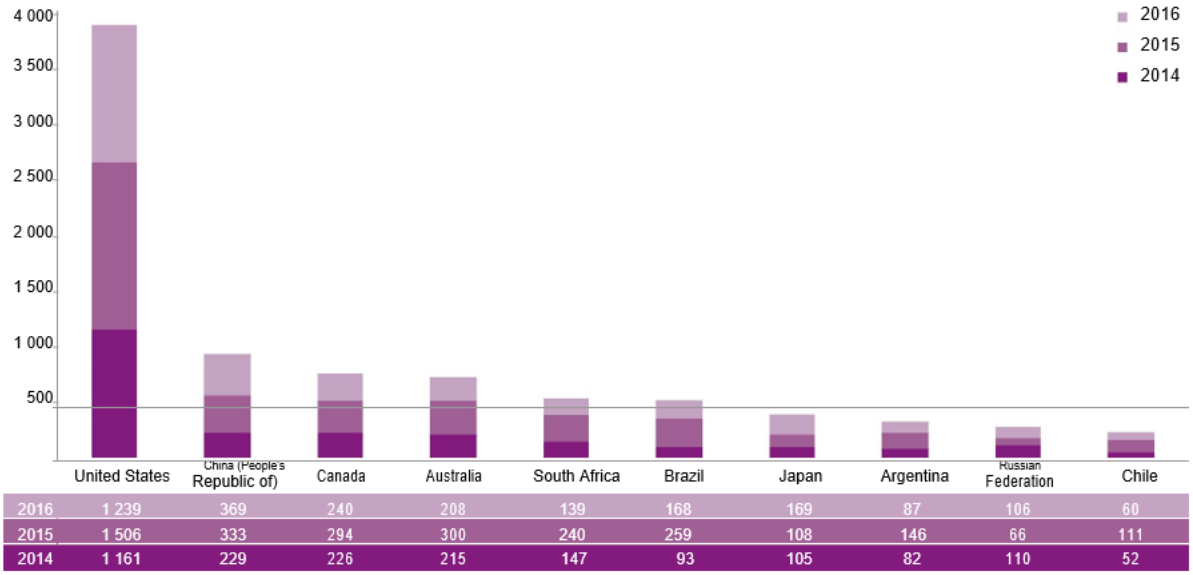


Figure 3: Number of Horizon 2020 applications from the 10 most active third countries, 2014-2016 (European Commission, 2018)

3. fostering collaboration in order to improve the quality of research outputs by researchers,
4. promoting research into how climate change can alter agricultural strategies to support food security and nutrition in Africa,
5. identifying research priorities for Food Security and Nutrition Adequacy,
6. tackling land issues that impact on Food and Nutrition Security,
7. investigating ways to meet the food and nutrition needs of the Africa's growing population without damaging the soils,
8. collecting and disseminating national statistics for policy, planning and research
9. developing knowledge and approaches through applied research, and
10. developing irrigation and water harvesting systems to increase production.

Research organisations in African countries directly or indirectly benefit from financial support administered by developed economies like the EU-Horizon 2020 (n. d.), which is the European Union's biggest (2014-2020) ever research and innovation framework programme. However, few countries in the continent were among the top participants in the EU-Framework Programme 7 (2007 - 2014) grant as shown in Figure 2. The strategic programme (focus areas) of most developed economies include: Personalising health and care for quality of life, sustainable food security, and water innovation (Hogan, 2013). In addition to bilateral and multilateral financing instruments, Africa has put in place several domestic financing mechanisms. A good example is the National Research Foundation (NRF) of South Africa. The internationalisation of research is an intrinsic part of funding instruments, built into research grants awarded through programmes such as Competitive Funding for Rated Researchers, the South African Research Chairs Initiatives (SARChI), and the Centres of Excellence (CoE) Programme (NRF, 2014). The number of Horizon 2020 applications from the 10 most active third countries, 2014-2016 is shown in Figure 3) (European Commission, 2018).

## 9 Conclusion

Strengthening research, development and capacity building is critical for food and nutrition insecurity in Africa. This will require among others actions, the

1. acceptance of the application of biotechnology in food production by all countries,
2. effective collaboration within and outside sub-Saharan Africa,
3. provision, by the government of each country in the continent, of enhanced financial and technical support to research and innovation, science and technology, including strengthening national and regional institutions,
4. ensuring that science and technology are mainstreamed into national development and sectoral policies as this will ensure better information dissemination typical of digitalisation age,
5. broadening access to secondary, tertiary and vocational education and skill development training,
6. eliminating gender disparities in education and training,
7. increasing the quality of education and training at all levels,
8. helping African countries go beyond MDG education targets, especially in increased enrolment and decreased drop-out rates,
9. strengthen the sharing of knowledge, applied research and extension as well as transfer of technology under mutually agreed terms to African countries and support them in strengthening their capacity to manage their natural resources, and
10. good governance for greater efficiency and better delivery of goods and services.

Good governance and rule of law are essential for sustained, inclusive and equitable economic growth, sustainable development and the eradication of poverty and hunger (UN, 2012). Also, attention is needed in reducing food losses as it has immediate and significant impact on livelihoods and wellness, tackling poor infrastructure (access to farm gate, processing facilities/consumer), developing irrigation, water harvesting systems, use of renewable energy, effective use of resource wealth - land resources for food crops, and nutrition education and communication - helping people to improve their diet through discussion, demonstration and practice (FAO, 2011a; FAO and WEP, 2010).

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# Assessment and Evaluation of Student Learning Through a Project-Based Assignment on Note by Note Cooking

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**Abstract**

Many innovative teaching and learning methods are used in higher level education including project-based learning (PBL). Since 2012 a PBL assignment project has been undertaken by master students of the Advanced Molecular Gastronomy module at Technological University Dublin (TU Dublin). The aim is to stimulate student learning and creativity by using Note by Note cooking in a PBL assignment while at the same time complying with the requirements of the annual International Note by Note contest which is held in Paris, France. Direct and indirect assessment methods were used to assign individual grades and to gather student feedback about the module. The direct methods were both formative and summative. The indirect method used was a student feedback questionnaire. Results to date (2012-2019) showed that 92% of learners successfully passed the module. However, further evaluation of individual assessment results revealed that most students achieved higher scores for finding and using resources, asking further questions and developing their own answers than for analysing, synthesising and evaluating information ($P \leq 0.01$). Overall students were happy with the module content and said that they learnt about Note by Note cooking, chemical compounds, researching, independent-thinking and perseverance. In future students should carry out a more in-depth analysis, synthesis and evaluation of information.

***Keywords:*** Molecular Gastronomy; Project-based learning; Note by Note Cooking

## 1 Introduction

Food science education has undergone a paradigm shift from a delivery of knowledge in a traditional lecture and laboratory system to a more inquiry-based and discovery process (Iwaoka, Britten & Dong, 1996 cited in Shewfelt (2012)). Traditional style laboratory practicals often leave little room for creativity or contextualisation, and are usually a verification of a known quantity or a testing of a theory that has been presented in lectures (Mc Donnell, O'Connor & Seery, 2007). Innovations such as the use of journals, team-based learning, simulations, problem-based studies, and other techniques engage students more actively in the learning process (Shewfelt, 2012).

Many terms are used for learning through inquiry, including 'inquiry-based learning', 'guided-inquiry', 'problem-based learning' and 'research-based teaching' (Spronken-Smith & Walker, 2010). Project-based learning (PBL) uses instructional strategies that are intended to engage students in authentic, "real world" tasks

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to enhance learning. It can be an individual or group activity that goes on over a period of time, resulting in a product, presentation, or performance (Donnelly & Fitzmaurice, 2005). These authors explain that PBL typically has a timeline and milestones, and other aspects of formative evaluation as the project proceeds. Students engage in deeper learning, high-level reading and increased motivation (Bell, 2010). One study, carried out over the course of three years in Britain, showed that three times as many PBL students achieved the highest possible grade in mathematics in the national exam than the students at a traditional school. Students at the PBL school were equally able to answer procedural questions that used formulas, but they were superior in answering applied and conceptual problems (Boaler, 1999). In the United States of America, Thomas (2000) noted that studies conducted on the effectiveness of using PBL over three years in a school in Iowa showed that reading gains "ranged from 15% in one school to over 90% in two other schools while the district average remained the same", and in Boston, eight graders exhibited the second highest scores in the district on the Stanford 9 open ended reading assessment. Similar findings in Portland, Maine, led to a conclusion that a middle school using a PBL approach showed significant increases in all achievement areas on the Maine educational assessment test for cognitive development after only one year of using the PBL approach. The gains made by this school were three to ten times higher than the state average. Similar improvements were reported for schools in Colorado, Illinois, Georgia, Cincinnati, Ohio, Memphis, Tennessee, and New York City. Doppelt (2003) states that students who took part in PBL were motivated to learn their discipline and willing to work on their projects for a longer time. In another study, attendance was found to be higher in PBL schools (Thomas, 2000).

The process used in PBL can replicate the commonly used systemic approach to resolving problems or meeting challenges that are encountered in life and career. Historically the project-based approach has been widely used in the science classroom (Krajcik & Shin, 2014) and as a result, the findings from evaluations of science orientated PBL has helped to build evidence for the efficacy of the design principles (Condliffe, Visher, Bangser, Drohojowska & Saco, 2016).

Molecular gastronomy, a sub-discipline of food science, has since 2009 been established as a subject discipline at the Technological University Dublin (abbreviated as TU Dublin and formerly known as the Dublin Institute of Technology) in Ireland (Burke & Danaher, 2018; Burke, Danaher & Traynor, 2012). The culinary activity called "Note by Note" cooking is an application of molecular gastronomy (MG) and it makes an important contribution to the fight against spoilage, while sparing water, energy, foodstuffs, and taking care of the environment (This, 2017). In this type of cooking, traditional food ingredients are not used to make dishes but pure compounds or mixtures of pure compounds are used (Burke & Danaher, 2016; This, 2014). They are assembled by the chef to design the shapes, colours, tastes, odours, temperatures, trigeminal stimulation, consistencies, nutritional aspects, and more, of the desired dish (This, 2017). The project-based principles for the Note by Note assignment are shown in Figure 1.

This paper provides results and discussion on the assessment of individual students and an evaluation of the achievement of the module learning outcomes.

## 2 Methodology

### 2.1 Student Group

The student group were TU Dublin students from the taught M.Sc. programme in Culinary Innovation and Food Product Development who took the postgraduate module in Advanced Molecular Gastronomy (approximately 13 in each academic year since 2012/2013 until the present time, 2018/2019).

### 2.2 Curriculum

The module ran for three consecutive hours, each week, over twelve weeks in one semester of the academic year (36 hours class contact). It was delivered by a teaching team of a culinary sci-


Figure 1: Stages in the Note by Note project-based learning assignment

ence lecturer (theory and practicals) and a culinary arts lecturer (practicals).

The main features of the postgraduate module were theoretical lectures, practical kitchen classes, and a PBL assignment that was conducted during the last five classes (5×3 hours = 15 hours). The curriculum included theory lectures about the chemical reactivity of pure compounds such as sodium alginate and calcium chloride. Toxicological and nutritional facts relating to chemical compounds used for Note by Note cooking were presented and discussed. Students were asked to check information from the Food Safety Authority of Ireland and the European Union regarding the maximum permitted daily intake levels of compounds that they were using in their Note by Note dishes. Lectures and practicals were focused on the chemical and physical properties of pure compounds such as gelling agents, liquid nitrogen and dry ice, scientific testing of culinary precisions, demonstration of how a Note by Note dish is made and the creation of an entirely new food using Note by Note cooking. Culinary precisions are all the technical additions that are not part of the operating protocol of a recipe; e.g. the operating protocol for an orange jam (marmalade) recipe involves slices of oranges plus sugar plus heat and the culinary precision is that it is sometimes 'said' that you have to cook until a drop of the liquid forms a gel on a cold plate (Burke, This & Kelly, 2016).

## 2.3 Assessment and Evaluation of the assignment

Breslow (2007) emphasises that best practice in educational research dictates triangulating data. If several different sources of data are used, it increases the probability that the findings present an accurate picture. The essential elements of the assignment were identified as (1) finding and using resources, asking further questions, developing answers and (2) analysing, synthesising and evaluating information. These essential elements were then matched against the learning outcomes of the module and assessment methods. Direct and indirect assessment methods were used in this study and are outlined in Table 1.

### Direct assessment

Summative assessment is product-oriented and assesses the final product, whereas formative assessment focuses on the process toward com-

Table 1: Learning outcomes matched to essential elements of the PBL exercise and corresponding assessment methods

| Essential element | Learning Outcome | Assessment | |
|---|---|---|---|
| | | Formative | Summative |
| 1. Finding and using resources, asking further questions, developing their own answers (Dahm, 2014) | 2. Produce a novel and innovative dish/cocktail using ingredients and techniques associated with applications of Molecular Gastronomy. | Informal observation | Academic report |
| | 3. Develop new skills to a high level through using novel techniques such as Note by Note cooking. | In-class activities | Sections 1,2,7: Literature search relating to Molecular Gastronomy, Note by Note cooking and the specific theme for the assignment. Aim(s) of assignment.<br>3,8: Materials, Equipment, Methods/Recipes. Recording kitchen trials and improvements to be made.<br>International competition |
| 2. Analysis, synthesis and evaluation of information (Dahm, 2014) | 1. Critically evaluate the fundamental scientific theories of Molecular Gastronomy. | In formal observation | Academic report |
| | | In-class activities | Sections 4,5,6,7: Results, discussion and conclusion(s) and related supporting literature. |

pleting the product (Northern Illinois University, 2012). Both were used to assess the students.

### Formative

- Informal observation
  The teaching team (culinary science lecturer and culinary arts lecturer) were both present during the kitchen classes each week. They were able to informally observe visually, each individual student's approach to planning and development of the Note by Note dishes over the five weeks of kitchen trials.

- In-class activities comprised four experimental kitchen trials which included physical and chemical tests, informal sensory analysis, and photographing of their dish(es) and, in the last class, a presentation of the final dish.

### Summative

- The academic report
  An academic report on the work which was carried out during five assignment classes accounted for 100 % of the total mark for the module. The sections in the report were an (1) introduction, (2) the aim of the assignment; (3) final materials and methods; (4) results; (5) discussion; (6) conclusions, (7) references and (8) a log book for each of the 5 weeks of the assignment (Table 2). To pass the module, students must have obtained a final mark of 40 %, as calculated by formula 1, in their Note by Note cooking project report.

Formulae

1) Final % awarded to student
   The academic report was marked out of a maximum total of 100
   Introduction (10 marks) + aim (5 marks) + final materials and methods (20 marks) + results (20 marks) + discussion (30 marks) + conclusion(s) (5 marks) + references (5 marks) + log book (5 marks) = a final % (awarded to the individual student).
   Formulae 2 and 3 below were used by the teaching team to evaluate achievement of the learning outcomes of the module.

2) Calculation of Essential Element 1
   Introduction (10 marks) + aim (5 marks) + final materials and methods (20 marks) + references (5 marks) + log book (5 marks) = Total number/45 X 100.

3) Calculation for Essential Element 2

Results (20 marks) + discussion (30 marks) +conclusion(s) (5 marks) + references (5 marks) = Total number/60 x 100.

- The International Note by Note contest The dish that most closely matched the entry requirements of the Note by Note International Contest was selected by the teaching team and entered. If chosen for the finals it was further evaluated by an international jury of scientific, culinary and industry experts. Each year the theme is different, for example in 2018 Hervé This (co-founder of Molecular Gastronomy) decided on the theme 'But the crackling is superb' in remembrance of Professor Nicholas Kurti, his fellow molecular gastronomy co-founder. The aim was to create a dish to include the consistencies of crispiness, crunchiness and crackling by following the principles of Note by Note cooking and the originality of the use of compounds would be evaluated (This, 2017). Each proposed dish would be described in a Word file by a recipe giving (1) the ingredients, including quantities, (2) the process and photographs were to be included. The candidates would have to accept that their recipes and pictures could be used (with their name) by the organizers and the partners of the contest.

Evaluation criteria included:

- Feasibility, reproducibility
- Making crisp, crunchy, crackling products
- Originality of the work.
- The use of pure compounds rather than fractions.
- The ingredients and completed dishes should not be toxic.

The complexity of flavour: dishes should have a shape, consistency, odour, taste, trigeminal sensation, and the effect of temperature should be considered.

## 2.4 Indirect assessment

### Questionnaire

The 2016/17 student cohort (n = 13) was asked to answer a series of open-ended questions relating to the MG module (theory lectures, practicals and Note by Note cooking assignment) that they undertook. By using an open-ended questionnaire, emerging data was collected with the primary intent of developing themes from the data (Creswell, 2003). The initial questions were general relating to prior qualifications and any work experience. The following qualitative questions were more detailed, so that participants opinions and observations could be uncovered. Twelve of the thirteen M.Sc. Students (92 % response rate) answered the questionnaire.

### Statistical analysis

In order to determine if there was a significant difference between the results of essential element 1 (formula 2) and essential element 2 (formula 3) a t-test was carried out on the assessment results for students who had an overall standard of fair (40-49%); good (50-59%); very good (60-69%) or excellent (70-100%) using Excel (Microsoft Office 365 ProPlus). The t-test was used to compare means and show whether they were different from each other and how significant those differences were. A correlation coefficient was also calculated using Excel (Microsoft Office 365 ProPlus) to determine the strength of relationships between the individual student grade and the project-based essential elements 1 and 2.

# 3 Results and Discussion

## 3.1 The task and project brief

At the beginning of the twelve week module, the task and project brief was outlined and discussed with the students in the introductory class of the Advanced Molecular Gastronomy module. As Grant (2002) outlines, the task, guiding question, or driving question explicates what has to be accomplished and embeds the content to be studied. The tasks should be engaging, challenging, and do-able.
By using PBL, students can explore the driving questions by participating in scientific practices and they learn and apply important ideas in the discipline (Krajcik & Shin, 2014). Through this learning approach the students in TU Dublin were able to critically evaluate questions about food structure, sensory properties, nutrition and toxicity. This approach was underpinned by the fact that Note by Note cooking had a real-world context in that it was contributing to the development of a sustainable food system to feed the expanding world population.

## 3.2 Research and Planning

Students then started researching their dish or dishes. They accessed information from library resources, developed a theme and created drawings and designs of what their dish would look like. They could use the information from the theory lectures and practical classes which were given in weeks 1-7 of the module. It was important when designing the dish to ensure that all sensory attributes would be acceptable. Appropriate sensory tests needed to be identified. The students followed the PBL approach as described by Larmer and Mergendoller (2015) which involved an active, in-depth process over time, generating questions, finding and using resources, asking further questions, and developing their own answers.

Table 2: Evaluation criteria for the academic report

| | Marking Scheme | | | | |
|---|---|---|---|---|---|
| Report sections | Excellent (> 70%) | Very good (60-69%) | Good (50-59%) | Fair (40-49%) | Fail (< 40%) |
| 1. Introduction (10 Marks) | Consideration of a wide range of relevant literature sources relating to molecular gastronomy, Note by Note cooking and the specific assignment topic. These sources were considered critically and analysed thoroughly. Accurate referencing | Able to critically appraise the relevant literature and theory gained from a variety of sources. Referencing is mainly accurate. | Clear evidence and application of readings which are relevant to the subject. Referencing is mainly accurate. | Literature is presented uncritically, in a purely descriptive way and indicates limitations of understanding. | Published documents summarised, but not linked in any effective way to the aim(s) of the assignment. |
| 2. Aim of the assignment (5 Marks) | Clear concise and coherent statement of what is to be achieved. | Clear statement that indicates achievability. | Aim(s) stated, but either too many or is not concise enough. | Aim(s) stated but unclear. | The aim(s) does not link to the work carried out. |
| 3. Final Materials and Methods (20 Marks) | Materials and methods clearly described. Details of makes and models of all equipment provided. Accurate and clear recipes for all elements of a dish and/or cocktail. Accurate referencing. | Good description of materials and methods. Recipes included. Referencing mainly accurate. | Adequate description of the materials and methods used. Referencing is mainly accurate | Vague and with some gaps in the materials. Methods are not presented in a logical order but are partially related to the project aim(s). Some attempt at referencing. | Incomplete or no list of materials used. Methods, handled incompletely, with little evidence of link to aim(s). Inaccurate or no recipes included. |
| 4. Results (20 Marks) | Relevant results clearly set out. All figures, charts (sensory and spider plots), tables, photos etc. are correctly and uniformly labelled. Photos (300 dpi). | Results are well presented. All relevant figures, charts, tables, photos etc. are included. | Results are adequately presented. All relevant figures, charts, tables, photos etc. are included. | Results are not presented in a logical order. Inaccuracies in labelling of figures, charts, tables, photos etc. | Incomplete set of results with inaccurate or no labelling of figures, charts, tables, photos etc. No clear link between results and assignment aim(s). |
| 5. Discussion (30 Marks) | The results are compellingly supported by appropriate evidence. In depth discussion and exploration of the driving questions. | The results are clearly supported by appropriate evidence. | The application of analysis and validity of results and evidence are indicated | Results are repeated but very little attempt of a discussion of their relevance is presented. | Findings bear little or no relation to evidence. |

Table 2: Evaluation criteria for the academic report (cont.)

| | Marking Scheme | | | | |
|---|---|---|---|---|---|
| Report sections | Excellent (> 70%) | Very good (60-69%) | Good (50-59%) | Fair (40-49%) | Fail (< 40%) |
| 6. Conclusion(s) (5 Marks) | Conclusions are clearly and succinctly stated and are relevant to the aim(s). They are linked to results and discussion. | Conclusions stated which are relevant to the aim(s) and linked to results and discussion. | Attempts to draw conclusions from results are not entirely convincing. | Conclusions are weak and do not really follow from the results and discussion. | No detectable conclusions |
| 7. References (5 Marks) | Complete and accurate referencing in Harvard format | Referencing is mainly accurate. | Referencing is mainly accurate. | Some attempt at referencing. | Incomplete, unsystematic or no reference list. Referencing errors. |
| 8. Log book (5 Marks) | All 5 weeks work to be included. Written in correct format. | All 5 weeks work is included and it is written in the correct format but more detail is required | All 5 weeks work is included and it is written in the correct format but a lot more detail is required | Not all work is included. Not always in a logical sequence. Lack of detail. | Incomplete and not written in the correct format. |

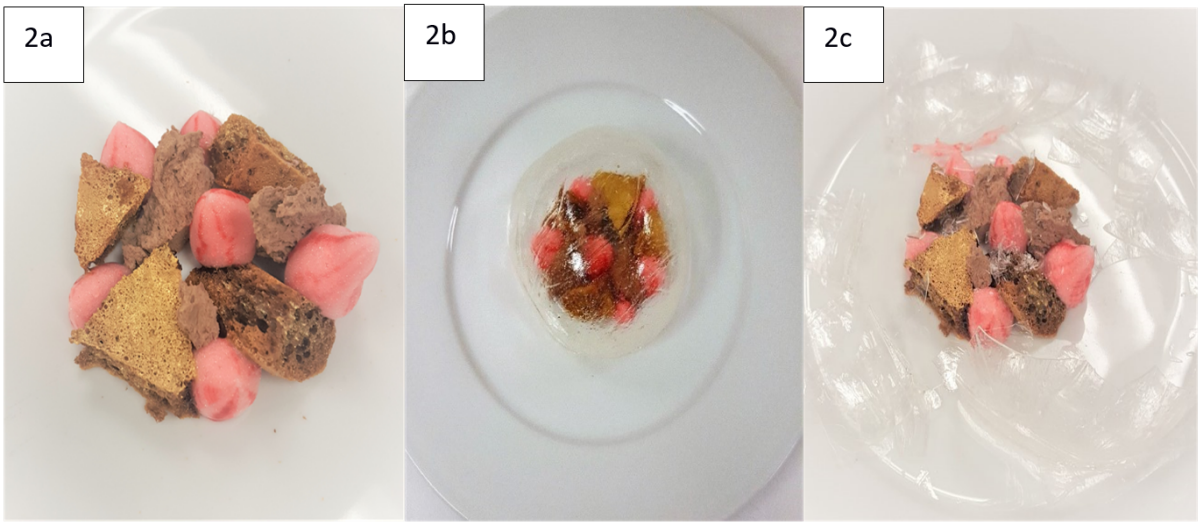


Figure 2: '*A Reminisence of a Black Forest Gateaux*' (2018). Dish by Ruth Kelly. Theme: Crispiness, Crackling, Crunchiness. Figure 2a shows the dish without the isomalt dome. Figure 2b shows the dish covered with the intact isomalt dome. In Figure 2c the diner shatters the dome with a spoon and flavoured smoke is released, which gives the impression of a misty forest atmosphere


Figure 3: Gold colouring being applied to cut honeycomb - Image: Ruth Kelly (2018).

### 3.3 Kitchen trials and improving recipes

In the last five weeks of the twelve week module, the students carried out three hour kitchen classes once a week. The time planning for these classes was done during the first seven weeks of the module. Ingredients and equipment needed to be ordered a minimum of two weeks in advance of starting the kitchen work. During the kitchen trials, the students kept a log book and recorded information about ingredients and equipment, their progress and recommendations for the next week. Grant (2002) notes that the process and investigation stage of project-based learning includes the steps necessary to complete the task or answer the guiding or driving question. The process should include activities that require higher-level and critical thinking skills, such as analysis, synthesis, and evaluation of information. For example in 2018, it was necessary for the students to explore what was meant by crispiness, crunchiness and crackling and to design a dish with food structures that would give the sensation of these consistencies. This was not an easy task and the student who best met the challenge developed these elements in her dish. An isomalt ((3R,4R,5R)-6-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyhexane-1,2,3,4,5-pentol) dome was created following testing of thicknesses, temperatures, concentrations of ingredients and moisture content. The goal was to create a crisp and brittle texture (Figures 2a, 2b and 2c). For the crunchy and crackling element, she developed a honeycomb which was made by producing $CO_2$ gas from sodium hydrogen carbonate (Figure 3).

The gas bubbles evaporated during heating and formed pockets, of approximately 1 to 1.5 mm diameter on average, which were set in place by sugar. The structure collapsed in the mouth when broken with the teeth. This resulted in crunching and crackling sounds while eating. In this project, scientific practices included physical and chemical testing and sensory analysis testing (informal) each week with fellow classmates. Students discussed their ongoing project with lecturing staff (culinary science and culinary arts) and gained formative feedback to help them improve their recipes both scientifically and artistically.

### 3.4 Presentation of the dish(es)

In the final class the students presented their dish(es) for sensorial valuation by the lecturing staff. They took photographs to include in their TU Dublin project and also for the Note by Note competition. An example of a recent winning dish (joint first place in the student category) is shown in Figures 2a, 2b and 2c.

### 3.5 Assessment and evaluation of the project-based learning assignment

The aim of the Advanced Molecular Gastronomy module was to allow the learner to gain an in-depth understanding of the principles and applications of molecular gastronomy at an advanced level. The learning outcomes of the module are outlined in Table 1. According to Nusche (2008), learning outcomes refer to the personal changes or benefits that follow as a result of learning. Such changes or benefits can be measured in terms of abilities or achievements. Since 2012 until the present time, 92 % of all students passed the module. Results ranged from 40 % (pass standard) to 76 % (excellent standard). However, the individual student percentage mark did not provide information on the level of attainment for each of the two essential elements of the project-based assignment. Dahm (2014) crafted detailed rubrics for each of the essential elements in his courses and mapped student outcomes to project elements. He then compiled the data in Excel which allowed a summary of student performance with respect to each of the programme's student outcomes to be automatically generated. A similar rubric with defined evaluation criteria was used (Table 2) to provide a detailed insight into the level of achievement of the essential elements and corresponding learning outcomes.
Figure 4 shows the results for overall totals and for corresponding essential elements from a representative sample of individual marks in the fair, good, very good and excellent categories. It was carried out for recent student cohorts (2016/17, 2017/18 and 2018/19) and revealed that overall students achieved a significantly higher percentage for essential element 1 compared to essential element 2 ($P \leq 0.01$). The exceptions were results in the excellent category (>70%) where essential element 2 scored higher than 1. Correlation coefficients for the project-based essential elements and the individual student grades were 0.92 and 0.97 for essential elements 1 and 2 respectively, showing that there was a good relationship between the individual grade and each of the essential elements.

### 3.6 International assessment

The student who was selected by the teaching team to represent their class at the international Note by Note contest had not always achieved the highest individual grade in the class but did create a dish that best matched the criteria for the contest. In doing so they demonstrated what they had learnt by creating a product that was presented to people beyond the classroom (Larmer & Mergendoller, 2015; Thomas, 2000). All of the representative dishes which were entered by TU Dublin into each of the six international Note by Note contests (2013-2018) were awarded first place in the student category of the contest.

### 3.7 Questionnaire

The students were asked if they had studied science subjects before and if so at what level. There is no entry requirement for applicants to have previously studied science. However, the

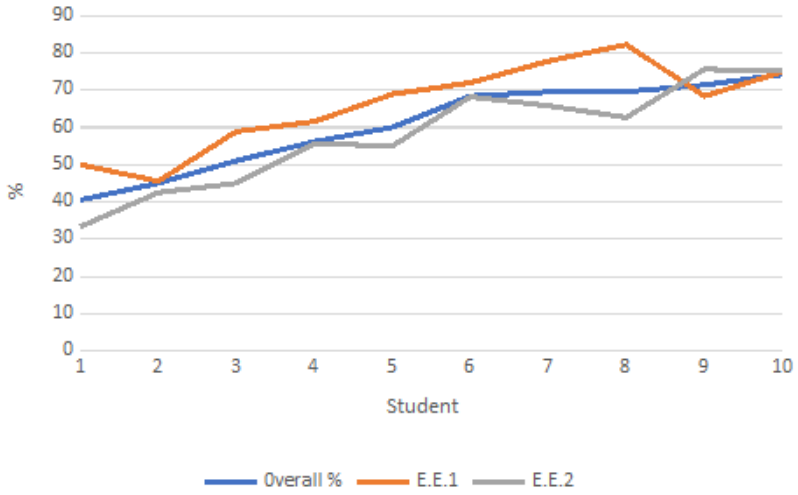


Figure 4: Individual overall marks and individual essential element (E.E.) 1 and 2 marks.

Table 3: Student Evaluation of the Module

| **Question to students** | **Collation of responses** |
|---|---|
| Highlights of the MG module | The main highlights of the postgraduate module were the kitchen practicals including the demonstration of Note by Note cooking and the use of liquid nitrogen. They liked that they had the freedom to experiment. The students also enjoyed learning about Note by Note cooking and Note by Note cuisine as well as new culinary concepts |
| Improvements that can be made to the modules | Many of the postgraduate students would have liked more time in the kitchen and more time allocated to experimenting with Note by Note cooking in the kitchen as well as more time allocated to the Note by Note project. |
| Theory lectures before the practical kitchen classes | The most effective teaching strategy was to have theory lectures followed by the application of knowledge in practical kitchen classes. The M.Sc. students found the theory classes very beneficial and important in helping them to understand the properties and chemical reactions of the compounds that they would use in Note by Note cooking. A couple of students suggested condensing the lectures and giving more time in the kitchen. |
| Team teaching | All the postgraduate respondents were unanimous in their comments that the team teaching was very important and helpful. Half of the postgraduate students, those who had not previously worked in industrial kitchens, commented that they learnt from the other students about working in a kitchen environment and food presentation skills. |
| Project-based learning assignment | The postgraduate students got a better understanding of the compounds and e-numbers and their functions and culinary uses. They also learnt about Note by Note cooking, as well as researching, independent thinking and perseverance. |

responses from the participants of the MG module showed that some had studied science subjects albeit at various levels. These included final year school biology, and culinary science, microbiology, biochemistry, food safety, biology, chemistry, physics and nutrition at higher education level (see Table 3).

## 4 Conclusion

By using a project-based Note by Note cooking exercise, 92% of students achieved the learning outcomes of the module. A detailed evaluation of the individual assessment grades however revealed that most of the students were better at finding and using resources, asking further questions and developing their own answers than analysing, synthesising and evaluating information. In future students should carry out a more in-depth analysis, synthesis and evaluation of information.

Students who were chosen to represent their class at the international contest in Paris did not always have the highest grade in their class but were deemed to have produced the dish which matched the requirements of the competition the closest. This was endorsed by their success, winning first place in the student category each year since the contest started six years earlier.

## Acknowledgements

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# Modelling Relationships Between Raw Milk Quality Parameters and Climatic Conditions – The Case Study of a 3-years Survey in Serbia

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**Abstract**

This work examined the relationships between quality characteristics of raw milk and climatic conditions. Over a period of three years, a total of 5,065 samples were collected encompassing two types of farms. The quality characteristics analysed were titratable acidity (TA), total plate count (TPC) and somatic cells count (SCC). Climatic conditions were evaluated in respect to the outdoor air temperature, pressure, humidity and precipitation.
Big farms showed a stronger correlation between TA and climatic conditions as opposed to SCC and climatic conditions. TPC was out of limit in big farms when the outdoor air temperature was higher than 19.8 °C ($p<0.05$) and during periods with accumulated precipitation over 4.2 mm ($p>0.05$). Small farms showed a stronger correlation between SCC and climatic conditions as opposed to TA. In these farms, occurrence of acidity out of limit was detected in less than 7.2% of samples. Samples with TA out of limit were observed when air temperature was higher than 18.4 °C ($p<0.05$) and accumulated precipitation was below 3.1 mm ($p>0.05$). These results can be used to improve good agricultural practices in respect to climatic conditions and size of farms.

***Keywords:*** Raw milk; Quality characteristics; Climatic conditions; Farm size

## 1 Introduction

Dairy products are considered as very important in human diet due to their uniqueness and nutritional value (Djekic, Miocinovic, Tomasevic, Smigic, & Tomic, 2014). The quality and safety of these products are of highest importance and represent one of the main goals throughout the milk chain (Djekic, Miocinovic, Pisinov, Ivanovic, & Smigic, 2013). Therefore, production and distribution of high quality raw milk is necessary for achieving high quality dairy products (Smigic, Djekic, Tomasevic, Miocinovic, & Gvozdenovic, 2012). Due to its specific composition and characteristics, milk is a good environment

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for the growth of both spoilage and pathogenic microorganisms (Nsofor & Frank, 2013). Although milk is sterile in the mammary gland, different bacteria might contaminate raw milk as a result of direct contact with soil, air, workers hands, faeces, grass and excretion from the udder of an infected animal (Lejeune & Rajala-Schultz, 2009), but also with contaminated surfaces during storage and transport of raw milk (Millogo, Sjaunja, Ouedraogo, & Agenas, 2010) and occasionally by milking of mastitic cows (Hayes et al., 2001; Pantoja, Reinemann, & Ruegg, 2009).

Microbiological quality of raw milk is assessed by total plate count (TPC) and somatic cell count (SCC), and these parameters are used for the comparison and estimation of milk quality (Piepers, Zrimsek, Passchyn, & De Vliegher, 2014; Smigic et al., 2012). SCC is an important measure of milk quality, reflecting the health status of the mammary gland, the increased risk of non-physiological changes to milk composition and reduced milk yield (More, Clegg, Lynch, & O'Grady, 2013). Titratable acidity (TA) is a quality parameter that is normally used to estimate the freshness of milk and to monitor the production of lactic acid during fermentation (McCarthy & Singh, 2009).

The microbiological quality indicators of raw milk may depend on the climate conditions, as increased outdoor air temperature allows faster increase in environmental bacterial population (Elmoslemany et al., 2010), better survival, proliferation and increase of total bacterial load in animal reservoirs. On the other hand, higher precipitation might allow development of environmentally mediated bacteria transmission pathways (Lal, Hales, French, & Baker, 2012) and consequently greater TPC.

Relationship between various hygienic indicators and climatic conditions, mostly temperature and precipitation, have been confirmed in various studies (Djekic et al., 2016). It has been shown that climatic conditions have an impact on food safety as well as on the prevalence of foodborne diseases under certain circumstances (Bezirtzoglou, Dekas, & Charvalos, 2011; Jacxsens et al., 2010). The majority of research on farms has focused on the contamination pathway of pathogens. Parker, McIntyre, and Noble (2010) investigated the effects between animals on farms and climatic conditions by analyzing pathways from manure at livestock farms and from grazing pastures. Effects of climate change on agriculture include variations in the seasons, modifications of the areas suitable for growing crops, grazing of livestock, production efficiency of livestock and changes in plant pests (Miraglia et al., 2009). Consequently, they can have an influence on the raw milk quality and indirectly on the quality and safety of dairy products. This brings to the attention the importance of managing certain decisions related to agricultural practices on farms caused by seasonal climatic variability as suggested by (McCown, Carberry, Dalgliesh, Foale, & Hochman, 2012).

Studies analyzing the influence of seasonal variation on the raw milk quality are usually of small scale, below 1,000 samples and over a short period of time, mostly covering a one-year period (Auldist, Walsh, & Thomson, 1998; Heck, van Valenberg, Dijkstra, & van Hooijdonk, 2009; Jahreis, Fritsche, & Steinhart, 1996; Lock & Garnsworthy, 2003). Studies investigating long term relations between climatic conditions and quality of raw milk have not been in the focus of research, and this was identified as a research gap by the authors of this paper. The objective of this study was to examine possible correlations between selected quality characteristics of raw milk and climatic conditions, primarily outdoor air temperature and precipitation during a period of three years in respect to the size of farms.

## 2 Materials and Methods

### 2.1 Sampling

A total of 5,065 raw milk samples were analyzed on a daily basis at the reception of a dairy plant during a period of three years (from 2012 until 2014). Raw milk was transported to the plant from two different sources: (i) big farms and (ii) centres for collecting raw milk from small farms and households. All farms were situated in the vicinity of Belgrade, capital of Serbia, and have good agricultural practices in place. Transportation of raw milk from big and small farms was organized in vehicles equipped with temperature control units to provide an adequate cold-chain

and to satisfy high hygienic criteria.

## 2.2 Analytical methods

Samples of raw milk were analyzed for titratable acidity (TA), total plate count (TPC) and somatic cells count (SCC). TPC was determined according to ISO 4833:2003. TA of milk was analyzed by titratable method and expressed in Soxhlet-Henkel degrees ($^o$SH). SCC was determined using a Fossomatic Minor dairy analyser (Foss, Denmark).

### Data processing and statistical methods

Based on TPC, raw milk was classified in three classes (E, I and II) in line with current legislation in Serbia (Serbia, 2009). Extra class (E) is classified as raw milk with TPC not exceeding 100,000 CFU/ml ($\leq$5 $\log_{10}$), while I and II classes are defined as groups with TPC between 100,001 (5 $\log_{10}$) and 400,000 (5.6 $\log_{10}$) CFU/ml and TPC $\geq$ 400,000 ($\geq$5.6 $\log_{10}$) CFU/ml, respectively. Samples with TPC greater than 1,000,000 ($\geq$ 6 $\log_{10}$) CFU/ml were considered as nonconforming raw milk according to the internal specification of the dairy plant. Requirements for the somatic cell count were always the same: less than 400,000 cells/ml. The percentage of raw milk which belonged to a specific category was calculated as the amount in the total quantity of received raw milk.
The limits for TA were set between 5.5 and 7.0 $^o$SH. Samples that had a TA out of this range were considered as nonconforming milk. The percentage of these samples was calculated as the quantity of non-conforming raw milk in the total quantity of received raw milk.
The climatic parameters used in statistical analysis during the three year period were the mean outdoor air temperature, the mean pressure, the mean humidity and accumulative precipitation calculated from the daily data as reported from the nearest local weather station (RHSS, 2013, 2014, 2015).
Classes of raw milk were expressed as percentages. The Chi-Square test for association was used in analyzing possible relationships between raw milk classes (based on TPC) and types of farms.
Pearson's rank order correlation coefficients were calculated for selected raw milk quality parameters, namely TPC, SCC and TA and the climatic parameters temperature, pressure, humidity and precipitation. This was performed separately for the big and the small farms.
Binomial logistic regression was employed to determine the probability of the occurrence of out of limit raw milk quality parameters with respect to climatic parameters. If normality or equality of variance could not be assumed, the Mann Whitney U test was used to determine the difference between the climate parameters and raw milk quality parameters.
The level of statistical significance was set at 0.05. Statistical processing was performed using Microsoft Excel 2010 and SPSS Statistics 17.0.

# 3 Results and discussion

## 3.1 Raw milk quality and type of farms

The results indicated that there was a statistically significant association between the raw milk classes based on the TPC and types of farms, Table 1 ($\chi^2$ = 3,074,385; p < 0.05). It was shown that small scale farms delivered inferior quality of raw milk compared to big farms. The production of raw milk with low bacterial counts is influenced by many factors related to good agricultural practices on dairy farms (Elmoslemany et al., 2010). Several studies confirm that milking, equipment hygiene, sanitizing procedure (Elmoslemany et al., 2010), and milk storage conditions, are the crucial factors influencing the variability in bacterial counts and overall microbial load. Also, herd health management, transition and feeding management, or housing, which are known to affect udder health, are also identified as important for achieving good quality of raw milk (Piepers et al., 2014).
Big farms included in this study were characterized as intensive production system with clearly defined management procedures at all levels compared to small scale farmers. Educational level of farmers and milking methods differed between

the two farm types but both had good agricultural practices in place. During the last few years, there has been a higher demand in terms of quality and microbiological integrity of raw milk in Serbia and hence small farmers have shifted to commercial family farms with increased production and improved agricultural practices to enable more profitability and economically sustainable production (Bogdanovic & Petrovic, 2015).

## 3.2 Big farms

Pearson's rank correlation was conducted using all data gathered during the survey. Regarding the subset of raw milk samples from big farms (Table 2), two quality characteristics, TA and SCC were significantly correlated with each other ($p < 0.05$) showing a negative correlation (-0.113). Strong significant correlations were observed between SCC and outdoor air temperature, atmospheric pressure and humidity ($p < 0.05$) while TA positively correlated only with precipitation (below 0.10). Results from Brazil confirm a positive and significant correlation between the outdoor air temperature and SCC, while rainfall and humidity showed no correlation (Vargas et al., 2014).

Seasonal effects on the occurrence of TPC and TA out of limit obtained from big farms are presented in Figures 1a and 1b. Most frequently TPC was out of limit in July, which is the time of the year when the outdoor air temperature was the highest. This was in agreement with other reports, which noted a positive association between summer temperature and bacterial levels in raw milk (Elmoslemany et al., 2010; Van Schaik, Lotem, & Schukken, 2002). On the contrary, Piepers et al. (2014) found an opposite trend. The exact reasons were not found but results might be related to the temperature and precipitation in different regions. Climate condition in some regions influences the animal housing and exposure of udder and teats and humid weather conditions can contribute to the high bacterial levels, increasing the risk for contamination (Piepers et al., 2014). Occurrence of TPC out of limit ranged from 0.0% (January, March, September, October and November) up to 1.7% (July). It is of note that the highest temperatures occurred in July (Fig. 1c).

Comparing TPC results and outdoor air temperature, it was noted that the average temperature (19.8 $^{o}$C) was significantly higher than the temperature (14.0 $^{o}$C) in the subset of samples showing result out of and within limits respectively (Mann–Whitney U test, $p < 0.05$), Figure 2a.

A higher, but not significant, accumulated precipitation (4.3 mm) was noted when TPC was out of limit compared to the value (4.2 mm) when TPC was within limits. In the rainy period, it is more difficult to perform good agricultural practices, as more dirt and mudis present in the environment and consequently may be present on the udder and different contact surfaces. In contrast, TPC results were out of limit in periods with lower humidity (55.0% compared to 65.5%), Figure 2a.

The greatest number of raw milk samples with TA out of limit was detected in August (1.8%), July (1.7%) and December (1.7%). During the observation period no samples with TA out of limit were detected in January, September, October and November. Samples with TA out of limit were reported when temperature was significantly higher than in the cases when TA was within limits (17.0 $^{o}$C compared to 14.0 $^{o}$C). A higher (not significant) accumulated precipitation (4.9 mm) was noted when TA was out of limit compared to 4.2 mm when TA was within limits. In contrast, TA was out of limit in periods with lower (not significant) humidity (60.3% compared to 65.6%), Figure 2b.

Regression modelling was performed to ascertain the effects of outdoor air temperature, pressure, precipitation, humidity and SCC on the likelihood that TPC or TA were out of limit. Results obtained for raw milk samples delivered from big farms, were not statistically significant.

## 3.3 Small scale farms

Regarding the samples of raw milk obtained from small scale farms (Table 3), TPC and SCC were significantly and positively correlated with each other ($p < 0.05$,) 0.406). In some studies, correlations between high SCC and high TPC have also been reported (D'Amico & Donnelly, 2010).

Table 1: Quantity and classes of received raw milk based on TPC by type of farm

| Quantity (L) | 2012 | 2013 | 2014 | Total (L) |
|---|---|---|---|---|
| Farms „A“ | 10,085,155 | 9,849,975 | 8,999,927 | 28,935,057 |
| Farms „B“ | 14,866,760 | 10,561,929 | 8,468,923 | 33,897,612 |
| Total (L) | 24,951,915 | 20,411,904 | 17,468,850 | 62,832,669 |
| n (%) | Class E | Class I | Class II | Total |
| Farms „A“ | 1,796 (65,43%) | 860 (31,33%) | 89 (3,24%) | 2,745 (100%) |
| Farms „B“ | 84 (3,62%) | 533 (22,98%) | 1,702 (73,39%) | 2,319 (100%) |
| $\chi^2 = 3074{,}385$; $p < 0.05$ | | | | |

(n) represents the number of samples of raw milk during the observed period; (%) represents their share in the sample of that group of farms

Note: Items denoted with different letters are significantly different at the level of 5%.

Legend: Class E ($\leq 5$ $\log_{10}$ CFU/ml); Class I (results between 5 $\log_{10}$ CFU/ml and 5.6 $\log_{10}$ CFU/ml); Class II (results $\geq 5.6$ $\log_{10}$ CFU/ml)

Table 2: Pearson's Rho correlation coefficient between quality parameters of raw milk samples from big farms and climatic conditions

| | | Pressure | Temperature | Humidity | Precipitation | TA | TPC | SCC |
|---|---|---|---|---|---|---|---|---|
| Pressure | Coefficient | | -0.232 | -0.049 | -0.107 | | | -0.055 |
| | N | | 2,745 | 2,745 | 1,317 | | | 2,745 |
| Temperature | Coefficient | -0.232 | | -0.619 | | | | 0.240 |
| | N | 2,745 | | 2,745 | | | | 2,745 |
| Humidity | Coefficient | -0.049 | -0.619 | | 0.240 | | | -0.071 |
| | N | 2,745 | 2,745 | | 1,317 | | | 2,745 |
| Precipitation | Coefficient | -0.107 | | 0.240 | | 0.063 | | |
| | N | 1,317 | | 1,317 | | 1,317 | | |
| TA | Coefficient | | | | 0.063 | | | -0.113 |
| | N | | | | 1,317 | | | 2,745 |
| TPC | Coefficient | | | | | | | |
| | N | | | | | | | |
| SCC | Coefficient | -0.055 | 0.240 | -0.071 | | -0.113 | | |
| | N | 2,745 | 2,745 | 2,745 | | 2,745 | | |

[a] TA - titratable acidity

[b] TPC – total plate counts

[c] SCC – somatic cells count

[d] N: amount of samples

Results in tables present the combinations which showed significant correlations ($p < 0.05$)

Table 3: Pearson's Rho correlation coefficient between quality parameters of raw milk samples from small scale farms and climatic conditions

| | | Pressure | Temperature | Humidity | Precipitation | TA | TPC | SCC |
|---|---|---|---|---|---|---|---|---|
| Pressure | Coefficient | | -0.237 | | -0.109 | | -0.068 | -0.132 |
| | N | | 2,319 | | 1,091 | | 2,319 | 2,319 |
| Temperature | Coefficient | -0.237 | | -0.626 | | | -0.050 | 0.236 |
| | N | 2,319 | | 2,319 | | | 2,319 | 2,319 |
| Humidity | Coefficient | | -0.626 | | 0.240 | 0.044 | | -0.076 |
| | N | | 2,319 | | 1,091 | 2,319 | | 2,319 |
| Precipitation | Coefficient | -0.109 | | 0.240 | | | | |
| | N | 1,091 | | 1,091 | | | | |
| TA | Coefficient | | | 0.044 | | | | |
| | N | | | 2,319 | | | | |
| TPC | Coefficient | -0.068 | -0.050 | | | | | 0.406 |
| | N | 2,319 | 2,319 | | | | | 2,319 |
| SCC | Coefficient | -0.132 | 0.236 | -0.076 | | | 0.406 | |
| | N | 2,319 | 2,319 | 2,319 | | | 2,319 | |

[a]TA - titratable acidity

[b]TPC – total plate counts

[c]SCC – somatic cells count

[d]N: amount of samples

Results in tables present the combinations which showed significant correlations ($p < 0.05$)

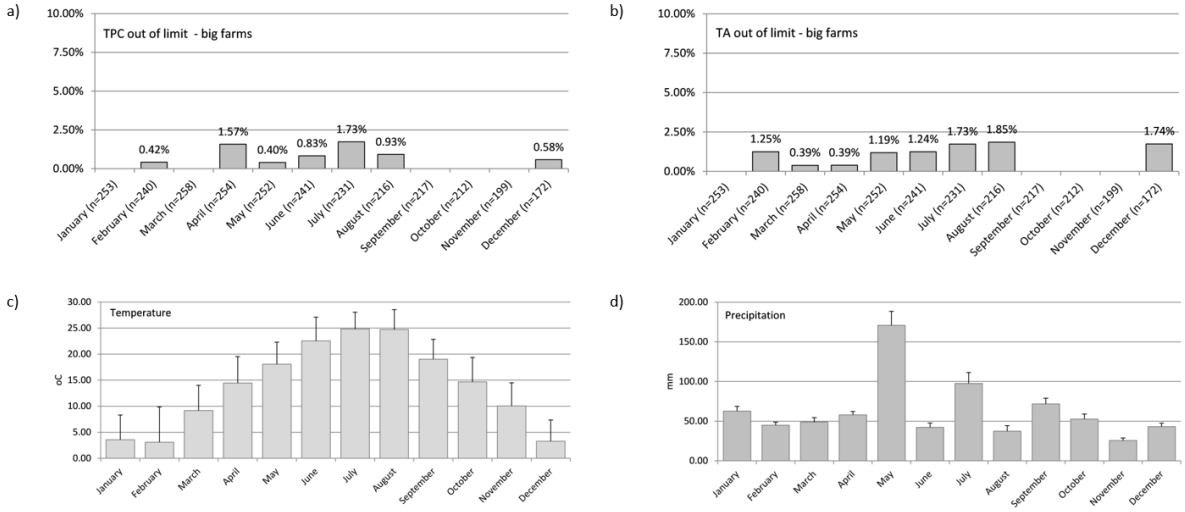


Figure 1: Seasonality of raw milk results out of limits and characteristics of climatic conditions by month. a: TPC out of limit (n = 15); b: TA out of limit (n = 22); c: Outdoor air temperature (3 years); and d: Precipitation (3 years). Bars are the 95% confidence intervals and n = the amount of samples. The outdoor air temperature and precipitation included is the mean temperature and accumulative precipitation calculated from the daily data of temperature and precipitation collected from the national hydrometeorological service

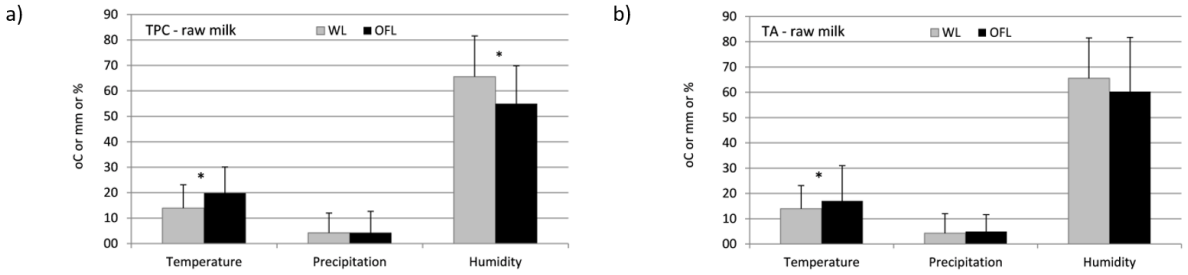


Figure 2: The potential impact of the climatic parameters (outdoor air temperature, precipitation and humidity) on the occurrence of samples out of limits in the raw milk samples from big farms a: TPC (Temperature WL n = 2,730 and OFL n = 15; Precipitation WL n = 1,311 and OFL n = 6; Humidity WL n = 2,730 and OFL n = 15); b: TA (Temperature WL n = 2,733 and OFL n = 22; Precipitation WL n = 1,309 and OFL n = 8; Humidity WL n = 2,733 and OFL n = 22). WL – samples within limits; OFL – samples out of limits Mann–Whitney U test performed to indicate significant difference ($p < 0.05$). Bars are 95% confidence interval; (*) significant difference

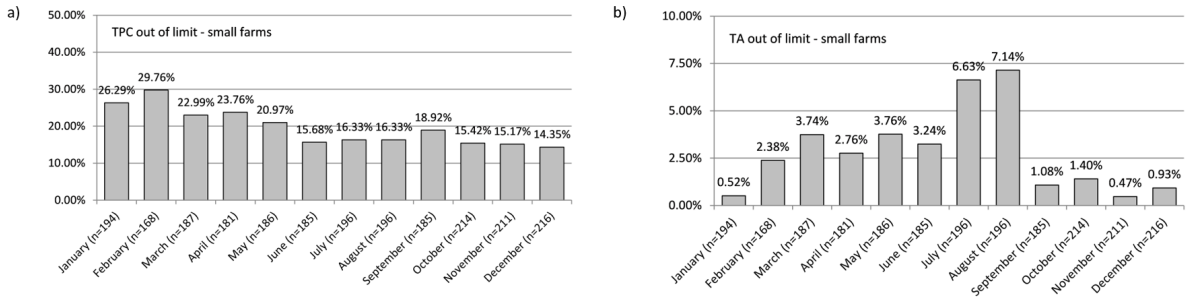


Figure 3: Seasonality of raw milk results out of limits and characteristics of climatic conditions iby month. a: TPC out of limit (n = 450); b: TA out of limit (n = 65); Bars are the 95% confidence intervals and n = the amount of samples

In contrast to this, Rysanek and Babak (2005) considered that SCC data do not sufficiently reflect the hygiene status of herds because of low correlation coefficients between bulk tank milk somatic cell score and log bulk tank total bacterial count. Microbiological quality of raw milk is more influenced by hygiene and environmental conditions than the mastitis frequency in dairy herds (Souto et al., 2008). The positive correlation between TPC and SCC may have indicated that producers were effectively controlling good agricultural practice (reflected in low TPC) and have also implemented good herd health management practices (reflected in low SCC) (Borneman & Ingham, 2014).

The strongest positive correlation between quality characteristics and climatic parameters was observed between SCC and outdoor air temperature (0.236). SCC was correlated with pressure, temperature and humidity, TPC with pressure and temperature while TA was correlated with humidity. There was no significant correlation between precipitation and raw milk quality parameters.

A study in the USA indicated that during hot and humid summer dairy farms produce less milk

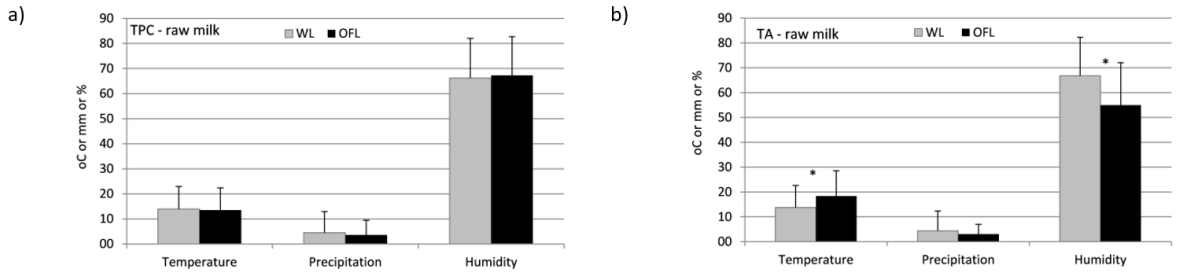


Figure 4: The potential impact of the climatic parameters (outdoor air temperature, precipitation and humidity) on the occurrence of samples out of limits in the raw milk samples from small farms a: TPC (Temperature WL n = 1,869 and OFL n = 450; Precipitation WL n = 840 and OFL n = 251; Humidity WL n = 1,869 and OFL n = 450); b: TA (Temperature WL n = 2,254 and OFL n = 65; Precipitation WL n = 1,066 and OFL n = 25; Humidity WL n = 2,254 and OFL n = 65). WL – samples within limits; OFL – samples out of limits Mann–Whitney U test performed to indicate significant difference ($p < 0.05$). Bars are 95% confidence interval; (*) significant difference

and milk with higher SCC (Ferreira & De Vries, 2015). The lowest level of SCC was observed in February, March, and April, while the highest was reported during August, September, and October. In these months it is necessary to introduce programmes for improving milk quality. Similar data were presented by Shock et al. (2015) analyzing data on farms in Ontario, Canada . Since the elevation of SCC is a response to an insult to the mammary gland and is modulated by inflammatory mediators, Harmon (1994) points that the major factor influencing SCC is infection status and that few factors other than infection status may have a significant impact on milk SCC. Several authors emphasize seasonal variation of SCC (Bernabucci et al., 2015; Paula, Ribas, Monardes, Arce, & Andrade, 2004; Roma Júnior, Montoya, T. Martins, Cassoli, & Machado, 2009; Simioni et al., 2014).

Results obtained for small scale farms were complemented by the binary logistic regression (odds ratios) between TPC, TA, SCC and climatic conditions (data not shown). A logistic regression was performed to ascertain the effects of outdoor air temperature, precipitation, pressure, humidity, TA and SCC on the likelihood that TPC was out of limit. The logistic regression model was statistically significant, $\chi^2 = 192{,}306$; $p < 0.005$. Results suggested a significant association between TPC and climatic parameters (odds ratio >0.99). The model explained 24.5% of the variance in TPC and correctly classified 80.0% of cases. It is 2.7 times more likely to exhibit TPC out of limit when TA is out of limit. Increasing temperature, pressure, humidity and accumulated precipitation as well as TA were associated with an increased likelihood of exhibiting TPC out of limit, but increasing SCC was associated with a reduction in the likelihood of exhibiting TPC out of limit. The results indicated that outdoor air temperature, pressure, humidity, TA and SCC were significant predictor variables in the regression model.

Seasonal effects of occurrence of TPC and TA out of limit in raw milk samples obtained from small scale farms are presented in Figures 3a and 3b. Occurrence of TPC out of limit ranged from 14.3% (December) up to 29.8% (February). Raw milk samples containing TPC within limit was found when the outdoor air temperature (13.6 $^oC$) was lower than the outdoor air temperature (14.0 $^oC$) in the subset of samples showing result out of limit (Mann–Whitney U test, $p < 0.05$), Figure 4a. Lower accumulated precipitation (3.6 mm) was noted when TPC was out of limit compared to 4.5 mm when it was within limits. In contrast, TPC was out of limit in periods with higher humidity (67.3% compared to 66.2%), Figure 4a. However, none of the results

were statistically significant (p>0.05).
The significant presence of raw milk samples showing TA out of limit was detected in August (7.1%), and July (6.6%), while in January, November and December they were below 1%. Samples with TA out of limit were detected when average outdoor air temperature was significantly higher than in the cases of samples with TA within limits (18.4 $^{o}$C compared to 13.8 $^{o}$C). A lower (not significant) accumulated precipitation (3.1 mm) was noted when TA was out of limit compared to 4.4 mm when TA was within limits. In contrast, TA was out of limit in periods with lower humidity (54.9% compared to 66.2%), Figure 4b. Research from Iran stresses that milk obtained in the winter and spring seasons has the lowest (although not necessarily significant) acidity levels compared to those collected in the summer and autumn seasons (Najafi, Mortazavi, Koocheki, Khorami, & Rekik, 2009).
Same regression model on the likelihood that TA was out of limit was performed to ascertain the effects of outdoor air temperature, pressure, humidity, precipitation, TPC and SCC (data no shown). The logistic regression model was not statistically significant, $\chi^2 = 10{,}038$, $p > 0.005$.

## 4 Conclusions

This study contributes to the literature by providing another perspective into the possible nature of raw milk quality parameters out of limit originating from different farm types and affected by climate parameters. It brings to the attention the necessity of analysing various climatic conditions influencing the raw milk quality.
In big farms, a negative correlation was observed between TA and SCC. A stronger correlation was observed between TA and climatic conditions opposed to SCC. The occurrence of TPC out of limit was below 2.0%, with the highest share during the hottest period of the year. In contrast, TPC was out of limit in periods with lower humidity. Samples with TA out of limit were detected when temperature was significantly higher than in the cases of samples with TA within limits.
In small farms, a positive correlation was observed between TPC and SCC. A stronger correlation was observed between SCC and climatic conditions opposed to TA. Logistic regression confirmed that increasing temperature, pressure, humidity and accumulated precipitation were associated with an increased likelihood of exhibiting TPC out of limit. Occurrence of TPC ranged from 14.3% up to 29.8%. TA out of limit was detected in less than 7.2% of all samples during periods when temperature was significantly higher and when humidity was lower.
Our results provide practical implications for both food technologists and farmers. This bottom-up approach in analyzing raw milk samples from a climate perspective provides an added value regarding analysis of the current practices in farms. The scientific value of this approach is that results confirmed that the temperature and precipitation are two climatic conditions that have an effect on the quality of raw milk.
A limitation of this research is the fact that the authors did not include knowledge of the employees working on farms. Also, good veterinary practices at farms, namely animal health and adequate usage of medicine for treating the animals, animal welfare and animal feeding were not analysed.
These results can be used as a basis for discussion in order to improve good agricultural practices in respect to climatic conditions and size of farms. Application of the similar method to the results of raw milk in other regions could offer a better insight into effects of climatic conditions globally in order to enhance milk quality along the chain.

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# Sensory Perception and Psychological Aspects of Eating Behaviour: Factors Influencing Fat Hedonics in Malaysia

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**Abstract**

Understanding the causes of obesity epidemic requires examination of what contributes to preference of palatable foods. Using a sensorial-consumer approach, this research examined the relationship between the sensation of the hedonic liking of fat with psychological and weight profiles. The study began with preliminary testing of the hedonic ratings of 24 food items (12 low fat (LF), 12 high fat (HF)) and completion of the Three Factor Eating Questionnaire (TFEQ-R18) investigating cognitive restraint, uncontrolled eating and emotional eating aspects. Eight (8) out of the twelve (12) food pairs that had discriminating characteristics were selected, for inclusion in the study, by 347 panellists. Results showed that overweight individuals had significantly lower liking towards LF sensation ($4.27 \pm 2.13$, $p = 0.001$) but significantly higher liking towards HF sensation ($5.26 \pm 2.33$, $p = 0.001$), compared to normal BMI individuals who had a significantly higher liking towards LF sensation ($5.69 \pm 2.35$, $p = 0.001$) but significantly lower liking towards HF sensation ($4.17 \pm 2.40$, $p = 0.001$). The Pearson product-moment correlation revealed similar trends on the association between liking of fat sensation and eating behaviour regardless of weight statuses. Specifically, cognitive restrainers were found to prefer the LF sensation while HF sensation were more favoured among uncontrolled and emotional eaters. This highlights the importance of investigating the combined effect of psychological aspects of eating behaviour and weight profiles towards liking of fat sensation.

***Keywords:*** Fat sensation; Cognitive restraint; Uncontrolled eating; Emotional eating

## 1 Introduction

Fat sensation enhances the palatability and hedonic appeal of food products, therefore preference for fat sensation often leads to overconsumption. According to Coccurello and Maccarrone (2018), most palatable foods have a unique fat "taste" that contributes to food hedonic characteristics. The foods consumed are usually high in both calories and fat content, in comparison with foods that are less energy-dense such as fruits and vegetables. Through its contribution to food palatability and hedonic characteristics, the presence of fat in food leads to overconsumption. This food intake is often beyond human basic physiological need of essential fats as source of energy, regulation of hormone functions, and protection of internal organs (Folken-

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berg & Martens, 2003). Since diets high in fat are detrimental and lead to multiple health complications such as obesity (Hurt, Kulisek, A Buchanan & A McClave, 2010), it is therefore important to measure the preference for fat sensation and influence of hedonics to tackle the issue of overconsumption as cause of obesity.

Many factors affect human's food selection, taste remains as one of the major contributing factors of food choice and food consumption; with preference for foods that are high in fats, sugars and salts (Shepherd, 2001). Apart from taste, emotion and mood affect food choices in various ways. A person chooses food according to his/her state of emotion and makes food choices to enhance their state of emotion (Gutjar et al., 2015). When bored, one tends to indulge in variety of foods in order to kill time; state of mind or feeling tend to affect appetite and satiety. Stressful events disturb mental equilibrium such that people tend to choose foods that are higher in sugars and fats (Kavitha, Souji & Prabh, 2011), hence food consumption might differ from the usual settings. This suggests the importance of psychological effects on involuntary food choice and food intake that affect body weight status. Overall, psychology has a huge effect on food consumption and therefore needs exploration from consumer's point of view (Jansson-Boyd, 2010; Koster, 2009; Shepherd, 2001).

Studying consumer behaviour involves understanding the why, what, how, where and when consumer purchase products (Kavitha et al., 2011). It leads us to the understanding of complex consumers'decision-making procedures. TFEQ is one of the most frequently used questionnaires that measure consumer eating behaviour, it was originally developed by Stunkard and Messick (1985), with a total of 51 items that measures scales of cognitive inhibition, disinhibition of eating and hunger behaviour mainly targeted at obese population. Modified from the previous version, TFEQ-R18 consists of three subscales which are known as cognitive restraint (CR), uncontrolled eating (UE) (grouping of disinhibition and hunger) and emotional eating (EE) (Karlsson, Persson, Sjostrom & Sullivan, 2000). Validated across age, gender and BMI variations, several studies have proven that TFEQ-R18 can also be used to evaluate eating behaviour in populations other than those who are obese (de Lauzon et al., 2004; Elfhag & Linne, 2005; Loeffler et al., 2015).

Studies on sensory evaluation were either based on single stimuli such as sweet/sour/salty taste or using solutions such as sucrose/citric acid/sodium chloride (Baharuddin & Sharifudin, 2015; Balan, Chua, Choong, Chang & Say, 2013; Sia et al., 2013; Thai et al., 2011). In this study, a wider dimension of fat sensation was investigated by using real food stimuli to imitate a real food eating situation, to obtain a better representative of an eating event. This study aims to fill the knowledge gap by proposing explanations based on how CR, UE, and EE lead to heightened hedonics for fat sensation in a more holistic approach.

## 2 Materials and Methods

This study was divided into two stages, first, a preliminary test was conducted to explore the appropriateness of the questionnaires and to ensure that the panellists were able to distinguish between foods of low fat (LF) sensation from those of high fat (HF) sensation. This preliminary study helped to refine the questions and identify additional key factors from panellists' opinion that would either be included or excluded from the full-blown study (second stage). Such modifications rendered the questionnaires more suitable to the food items in use or represented in the panels. In the second stage, study panellists completed the modified questionnaires and had a better understanding of the phrases used. Furthermore, the food items selected from preliminary study were more representative of LF and HF sensations, which helped to achieve the research objective (Table 1). A written informed consent was obtained from all panellists prior to their participation. This study protocol has been approved by the Human Ethics Committee at Taylor's University (Ethics reference no: HEC/2016/SBS/005).

### 2.1 Preliminary Study

Panellists were recruited via a mailing list of Taylor's University Lakeside Campus (Selangor,

Malaysia) staff and students aged between 18-59 years old. Panellists were eligible, if they met the following inclusion criteria: have a habitual breakfast eating routine, in good overall physical and mental condition, and not lactating or pregnant. Exclusion criteria were: having food allergies, intolerances, dislike of the food items to be evaluated and with a smoking history/current smoker including the electronic cigarette, as smoking prejudices sensory acuity (Tamime et al., 2011). Eligible panellists subsequently completed a short questionnaire that dealt with their usual consumption of the food items to be evaluated (Table 1). Those who consumed any of the food items less than 1 to 3 times a month were disqualified from the study. Finally, panellists performed hedonic evaluation of the food items representing LF and HF sensations and a set of consumer behaviour TFEQ-R18 questionnaires (Karlsson et al., 2000).

Twelve food items with varying physical properties, food matrix, serving temperature and representations of food groups were selected from a list of 165 food items in the Food Frequency Questionnaire (FFQ) used in the 2014 Malaysian Adult Nutrition Survey (MANS) (Institute for Public Health, 2014). Among the selected food items, 6 food items were chosen to represent the fatty-sweet sensation and another 6 food items represented the fatty-salty sensation (Table 1). Each food item was further divided into 2 categories representing low fatty-sweet/salty and high fatty-sweet/salty sensations, resulting in a total of 24 food items.

Each panellist attended one session, each morning, for 3 days. Protocols for each session were as follows: The first session consisted of, briefing and sensory evaluation of 3 food pairs (1 h); the second session, involved hedonic evaluation of 6 food pairs (1 h); The third session, involved completion of TFEQ-R18 questionnaire and 3 food pairs (1 h). A short briefing was presented before the start of each session. Panellists first recorded basic demographic information such as age, race, gender, and smoking status. The panellists' digital height and weight, and Body Mass Index (BMI) were measured and calculated. The WHO reference scale for BMI was used as a reference.: normal weight (BMI 18.5-24.9 kg/m$^2$) and overweight (BMI 25.0-29.9 kg/m$^2$) (WHO, 1998). All food items were prepared 3 h before each sensory evaluation session. To standardize appetite, all panellists were advised to consume breakfast as usual and refrain from consuming foods or beverages other than water 2 h before the start of each session. For sensory evaluation, panellists were presented with food pairs similar in nutrient contents but representing LF and HF sensations, respectively. Each sample was presented in a tasting cup or bowl labelled with a randomized 3-digit numbers in single-blinded balanced order. To allow sensation discrimination, appropriate portion size and serving temperature were monitored throughout the evaluation sessions. A ballot sheet was presented to the panellists to rate hedonic liking on a 9-point hedonic scale, from dislike extremely (1) on the left end towards like extremely (9) on the right end. Panellists rinsed their mouth with distilled water until no aftertaste remained and tested the next sample. In the final session, TFEQ-R18 was administered to panellist after the completion of the hedonic evaluation of food items.

## 2.2 Application Stage of the Study

Final selection of food items was based on samples that received mean ratings of 3 to 7 on a 9-point hedonic scale from preliminary study (Prescott et al., 1998). There were 8 final food pairs for application study (Table 2 and 3). The nutrient contents of the food items were derived from the Malaysian Food Composition Database (Malaysian Food Composition Database, 2017) and nutrition labelling of respective commercial food products. Presentation order, procedures, directions and rules for hedonic evaluation were similar to that used in the preliminary study. TFEQ-R18 was administered after the panellists completed hedonic evaluation of food items.

## 2.3 Statistical Analysis

Descriptive statistics were used to describe frequency and percentages (n, %) for categorical data, mean and standard deviation for continuous variables. Kolmogorov-Smirnov normality test was carried out on preliminary data to

Table 1: Food items served in preliminary test

| Sensation | Food Item[a] | | Prevalence (%)[a] | Serving Temperature[b] | Portion Size (g)[c] | Food Group Category[a] |
|---|---|---|---|---|---|---|
| Fatty-sweet | *Teh Tarik* (Milk tea) | | 70.35 | Warm | 20 | Beverages |
| | Seri kaya toast | : Bread | 78.25 | Warm | 25 | Cereal and cereal products; Spreads |
| | | : Seri kaya | 35.31 | | | |
| | | : Butter | 22.20 | | | |
| | Cereals | : Milk | 29.57 | Cold | 20 | Milk and milk products; Cereal and cereal products |
| | | : Cereal | 12.89 | | | |
| | *Kuih Keria* (Sweet potato doughnuts) | | 79.94 | Room | 15 | Confectioneries |
| | Malted milk | | 59.09 | Warm | 20 | Beverages |
| | Ice cream | | 38.45 | Cold | 15 | Confectioneries |
| Fatty-salty | French fries | | 36.58 | Warm | 20 | Starchy vegetables |
| | Egg mayonnaise sandwich | : Bread | 78.25 | Room | 20 | Cereal and cereal products; Eggs; Condiments and misc. |
| | | : Egg | 95.17 | | | |
| | | : Dressing | 17.56 | | | |
| | Coleslaw | | 32.74 | Cold | 20 | Vegetables; Condiments and misc. |
| | Chicken hamburger | | 39.99 | Warm | 25 | Meat and meat products |
| | Mashed potato | | 31.15 | Warm | 20 | Starchy vegetables |
| | Curry puff | | 79.94 | Room | 25 | Confectioneries |

[a]Selected food items commonly consumed among Malaysian population, their prevalence and food group categories according to MANS 2014 (Institute for Public Health, 2014)
[b]Serving temperature of each food item according to suggestion by respective manufacturer and that of habitual consumption condition
[c]Serving portion of each food item in order to evoke fatty-sweet or fatty-salty sensation

Table 2: Nutrient contents of low and high fatty-sweet sensation food items

| Sensation | Food Item | Description | Fat Content | Sugar Content |
|---|---|---|---|---|
| Low fatty-sweet | *Teh Tarik* | BOH *Teh Tarik* modified[c] | 1.4g/100ml | 2.9g/100ml |
| | Cereal | Dutch Lady Pure Farm Low Fat High Calcium Milk[a] | 1.3g/100ml | 4.6g CHO/100ml |
| | | Nestle Cornflakes[a] | 2.0g/100g | 10.0g/100g |
| | *Kuih Keria* | *Kuih Keria* modified[c] | 1.8g/100g | 23.9g CHO/100g |
| | Ice Cream | Bulla Real Dairy 98% Fat Free Light Vanilla[a] | 1.6 g/100g | 17.1g/100g |
| High fatty-sweet | *Teh Tarik* | BOH *Teh Tarik* original[a] | 2.8g/100ml | 5.8g/100ml |
| | Cereal | Dutch Lady Pure Farm Full Cream Milk[a] | 3.3g/100ml | 4.8g CHO/100ml |
| | | Nestle Honey Gold Cornflakes[a] | 2.1g/100g | 33.8g/100g |
| | *Kuih Keria* | *Kuih Keria* original[b] | 3.9g/100g | 47.7g CHO/100g |
| | Ice Cream | Bulla Real Dairy Vanilla[a] | 6g/100g | 21g/100g |

[a]Nutrition labelling of respective commercial food product
[b]Malaysian Food Composition Database
[c]Reduced nutrient level compared to the original food item

Table 3: Nutrient contents of low and high fatty-salty sensation food items

| Sensation | Food Item | Description | Fat Content | Sodium Content |
|---|---|---|---|---|
| Low fatty-salty | French fries | Kawan Shoestring French Fries[a] | 4.00/100g | 0.04g/100g |
| | Egg mayonnaise sandwich | Gardenia Original Classic[a] | 2.60g/100g | 0.44g/100g |
| | | Whole hen egg[b] | 12.80g/100g | 0.01g/100g |
| | | Praise traditional 99% Fat-free creamy mayonnaise[a] | 0.80g/100g | 0.73g/100g |
| | Chicken hamburger | Chicken burger patty[b] | 11.50g/100g | 0.24g/100g |
| | | Praise traditional 99% Fat-free creamy mayonnaise[a] | 0.80g/100g | 0.73g/100g |
| | Curry puff | Wheat flour-based curry puff modified[c] | 7.05g/100g | 0.09g/100g |
| High fatty-salty | French fries | Simplot Shoestring French Fries[a] | 6.00g/100g | 0.04g/100g |
| | Egg mayonnaise sandwich | Gardenia Original Classic[a] | 2.60g/100g | 0.44g/100g |
| | | Whole hen egg[a] | 12.80g/100g | 0.01g/100g |
| | | Praise traditional mayonnaise[a] | 66.00g/100g | 0.52g/100g |
| | Chicken hamburger | Chicken burger patty[b] | 11.50g/100g | 0.24g/100g |
| | | Praise traditional mayonnaise[a] | 66.00g/100g | 0.52g/100g |
| | Curry puff | Wheat flour based curry puff original[b] | 14.10g/100g | 0.17g/100g |

[a]Nutrition labelling of respective commercial food product
[b]Malaysian Food Composition Database
[c]Reduced nutrient level compared to the original food item

identify parametric or non-parametric properties, followed by Skewness and Kurtosis analyses. A non-normal distribution was observed among food pairs; therefore, Mann-Whitney U test was carried out to compare difference of the independent groups of each food pair. Food pairs that had significant differences, $p < 0.05$, were selected to represent each sensation for the application stage. Data from the application stage yielded normal distribution, hence, independent t-test was carried out to compare liking for fat sensation between normal and overweight individuals with $p < 0.05$ considered significant. The Pearson correlation (r) coefficient was calculated to measure association between fat sensation and variables of the TFEQ-R18 questionnaire. To measure internal consistency of (responses to) the questionnaire, Cronbach's alpha value was calculated, consistency of items within each LF and HF sensations, as well as within and between subscales of the TFEQ-R18 questionnaire were measured accordingly (Gliem & Gliem, 2003). Cronbach's alpha was 0.965 for 8 items of LF sensation and 0.968 for 8 items of HF sensation. Cronbach's alpha for 6 items of CR was 0.937, 0.913 for 9 items of UE and 0.854 for 3 items of EE. Cronbach's alpha of items within each subscale of TFEQ-R18 also showed good reliability

and were highly acceptable. All statistical analyses were carried out with IBM SPSS statistics version 20 (IBM Corporation, Armonk, NY, USA).

## 3 Results and Discussion

### 3.1 The Preliminary Study

There were 41 responses of which 11 were invalid (responses), therefore 30 sets of results were usable in the preliminary stage of the study. There were significant differences ($p < 0.05$) in hedonic ratings between LF and HF content level for each food item and 4 food pairs did not show significant differences between liking scores, coleslaw ($p = 0.107$), mashed potatoes ($p = 0.467$), *Seri kaya* toast ($p = 0.625$) and malted milk ($p = 0.222$) and were not considered further, during application study.

### 3.2 The Study

#### Hedonic Liking of Fat Sensation in Relation to Weight Status

A total of 379 panellists participated in the application stage of the study, there were 32 sets of unusable data which included incomplete questionnaires and inappropriate data such as blurred or stained handwriting, resulting in final 347 sets of usable data. The majority of panellists were under the age of 25 years old (73.2%), with 46.1% male and 53.9% female. Up to 64.0% of panellists belong to normal BMI (18.5-24.9 kg/m2) whereas 36.0% panellists belong to overweight BMI (25.0-29.9 kg/m$^2$) (Table 4).

In our study, we found that overweight individuals had statistically significant ($p = 0.001$) lower mean liking towards LF sensation foods compared to normal BMI individuals (Table 5). Overweight individuals were found to show enhanced preference for fat and tended to consume more energy-dense diets (Drewnowski & Almiron-Roig, 2010). Similarly, in a five-year longitudinal study on the relationship between obesity risk and liking for fat sensation, Lampure et al. (2016) also found that fat liking was prospectively linked with an increased risk of obesity and diet appeared to greatly explain this relationship.

People with high liking with regards to HF sensation have proportionally higher intake of energy dense foods, fats, butter, sweet pastry and desserts which makes them more prone towards weight gain. With respect to specific food types, HF sensation likers consume more high fat foods along with less fruits and vegetables. HF sensation likers also have a higher consumption of sodium, and they preferred more savoury tastes than bland tastes (Mejean et al., 2014). As most palatable foods are high in energy density and calories, HF sensation likers therefore have a higher risk for weight gain problems. While there were studies supporting positive relation between weight and likings for HF sensation (De Graaf, 2005; Rissanen et al., 2002), other studies reported that liking towards fat sensation does not differ between different weight status (Matsushita et al., 2009; Salbe, DelParigi, Pratley, Drewnowski & Tataranni, 2004).

Hedonic liking of fat sensation is not solely dependent on sensory cue, instead a complete or partial interaction of taste, odour and texture mediate liking towards fat sensation (Mattes, 2005; Proserpio et al., 2016; Slocombe, Carmichael & Simner, 2016). Individuals exhibiting higher oral sensitivity towards fat sensation have a lower total energy and fat intake, which leads to lower BMI. Similarly, those with overweight BMI might have reduced oral sensitivity which leads to reduced capability to detect fat taste, and a higher consumption of milk products, butter, and meat products (Stewart, Newman & Keast, 2011). In this study, high BMI individuals responded to higher taste intensity and therefore experienced less taste sensation compared to normal weight responders, in other words they tend to select food that are higher in fat that are more palatable and hence lead to excess energy and calories consumption. Taste and olfactory functions are correlated negatively with BMI, according to Carlos Fernandez-Garcia et al. (2017) those that are normal weight showed a higher score of taste and olfactory function measurements compared to overweight individuals. Human's basic senses also has a collaborative effect on liking of fat sensation, individuals evaluating similar taste sensation might exhibit vari-

Table 4: Panellists characteristics for the study

| Characteristic | Male | | Female | | Total | |
|---|---|---|---|---|---|---|
| | Frequency (n) | Percentage (%) | Frequency (n) | Percentage (%) | Frequency (n) | Percentage (%) |
| Age | | | | | | |
| ≤25 years old | 111 | 31.99 | 143 | 41.21 | 254 | 73.20 |
| >25 years old | 49 | 14.12 | 44 | 12.68 | 93 | 26.80 |
| Race | | | | | | |
| Malay | 76 | 21.90 | 50 | 14.41 | 126 | 36.31 |
| Chinese | 58 | 16.71 | 99 | 28.53 | 157 | 45.24 |
| Indian | 26 | 7.50 | 38 | 10.95 | 64 | 18.44 |
| BMI | | | | | | |
| Normal | 103 | 29.68 | 119 | 34.29 | 222 | 64.00 |
| Overweight | 57 | 16.43 | 68 | 19.59 | 125 | 36.00 |

Table 5: Mean and standard deviation (SD) of subscale scores of TFEQ-R18[a]

| TFEQ-R18 subscales | Normal BMI (n=222) | Overweight (n=125) | Independent value t-test $p$ value |
|---|---|---|---|
| LF sensation | 5.69 ± 2.35 | 4.27 ± 2.13 | 0.001 |
| HF sensation | 4.17 ± 2.40 | 5.26 ± 2.33 | 0.001 |
| CR (score 1-6) | 3.94 ± 1.43 | 3.37 ± 1.42 | 0.001 |
| UE (score 1-6) | 2.53 ± 0.78 | 3.49 ± 1.28 | 0.001 |
| EE (score 1-6) | 3.47 ± 2.06 | 4.13 ± 1.92 | 0.003 |

[a]Subscale scores of TFEQ-R18
CR = Cognitive restraint behaviour subscale of TFEQ-R18
UE = Uncontrolled eating behaviour subscale of TFEQ-R18
EE = Emotional eating behaviour subscale of TFEQ-R18

ation on degree of sensorial liking due to different physiological characteristics such as tongue shape, temperature of oral cavity and sensitivity of oral receptors (Engelen & Van Der Bilt, 2008).

## Eating Behaviour and Weight Status Relationship

Effectiveness of weight management is dependable on one's self-control and psychological well-being, which is also correlated with the variable CR (Lazzeretti, Rotella, Pala & Maria Rotella, 2015). Although promotion of weight loss by means of physical activity has been long executed by government and private health interventions, it has led to disappointing outcomes due to different levels of unsupervised exercise adherence (Colley et al., 2008). Besides, food choices have a huge influence on weight status. Restraint eaters tend to be extra stringent on food selection by conscientious calorie counting and calculating energy density in order to limit or control daily energy intake. They also have a better adherence to strict diet, coupled with determination to live a healthy lifestyle and intensified physical activity. Restraint eaters who control their diet tend to reduce weight on a long-term basis (Keranen et al., 2009) and may create an overall improved self-control over food intake (Elfhag & Morey, 2008) hence having healthy BMI and not developing eating disorders such as uncontrolled eating or binging activities. In our study, results showed that CR level was significantly ($p$ = 0.001) higher among normal BMI compared to those with overweight BMI (Table 5). There-

Table 6: Pearson correlation matrix between fat sensation and TFEQ-R18 variables among panellists with normal BMI and overweight BMI

| | CR | UE | EE | LF sensation | HF sensation |
|---|---|---|---|---|---|
| Normal BMI (n=222) | | | | | |
| CR | *1* | | | | |
| UE | -0.318** | *1* | | | |
| EE | -0.827** | 0.328** | 1 | | |
| LF sensation | 0.926** | -0.344** | -0.825** | 1 | |
| HF sensation | -0.928** | 0.300** | 0.837** | -0.950** | 1 |
| Overweight (n=125) | | | | | |
| CR | *1* | | | | |
| UE | -0.691** | *1* | | | |
| EE | -0.462** | 0.403** | 1 | | |
| LF sensation | 0.850** | -0.698** | -0.462** | 1 | |
| HF sensation | -0.907** | 0.739** | 0.496** | -0.936** | 1 |

** Correlation is significant at $p < 0.01$
CR = Cognitive restraint behaviour subscale of TFEQ-R18
UE = Uncontrolled eating behaviour subscale of TFEQ-R18
EE = Emotional eating behaviour subscale of TFEQ-R18

fore, high CR scorers tend to achieve a higher success rate in weight management compared to those without restraint eating behaviour. High CR scorers proved better adherence, motivation to exercise and were more adapted to change in healthy lifestyle and diet (Bryant, Caudwell, Hopkins, King & Blundell, 2012).

On the other hand, UE and EE levels were significantly ($p = 0.001$) higher among those with overweight BMI compared to those with normal BMI (Table 5). These results are in line with the results of Loeffler et al. (2015). Highest mean BMI was recorded for individuals rated high on both UE and EE, while lowest mean BMI was recorded for those who rated low on all three subscales of TFEQ-R18. A review on relationship between BMI and eating behaviour reported consistent linkage between UE and eating disinhibition with BMI (French, Epstein, Jeffery, Blundell & Wardle, 2012). These findings are also similar to those by Koenders and van Strien (2011) who also found consistent link with EE and weight gain, whereas the reverse was true for CR eating. Those who are physically active may reduce effects of EE on BMI but not completely solving the problem, instead, psychological factors such as mindful eating, emotion regulation and positive body image might be more effective in reducing weight gain issues (Frayn, Livshits & Knauper, 2018).

Our research also corroborates the findings of Geliebter and Aversa (2003) that concluded that overweight subjects consumed more during negative emotional states and bad moods, whereas those with underweight BMI consumed lesser than usual during bad emotion states and were more correlated with restrictive eating behaviour. With the use of Dutch eating behaviour questionnaires, another study also demonstrated that EE scores were higher among those morbidly obese than obese patients whom had underwent gastric restrictive operation (Horchner, Tuinebreijer & Kelder, 2002). Negative emotions and state of despair resulted in an increase in appetite and consumption of foods that are high in fats such as sweet foods and junk foods, coupled with reduced consumption of leafy vegetables leading to negative energy balance and weight gain problems (Konttinen, Mannisto, Sarlio-Lahteenkorva, Silventoinen & Haukkala, 2010). During critical emotional states, inabilities to control emotions disrupt body's psycho-

logical regulatory processes, which lead to failure in appetite suppression and disruption of normal eating behaviour (Macht, 2008).

### Eating Behaviour Association with Hedonics of Fat Sensation

A Pearson product-moment correlation analysis was carried out to determine the relationship between eating behaviour and liking for LF and HF sensations (Table 6). Our results found that for those who are overweight, liking for LF sensation was statistically positively strong correlated ($r = 0.850$, $n = 125$, $p = 0.001$) with CR. This is in accordance with research that compared hedonic rating among restraint and non-restraint eaters on regular and zero fat fudge, wherein authors concluded that that those with high restraint preferred zero fat or fat-free sensation (Tuorila, M Kramer & Engell, 2001). Individuals with low CR have higher liking for palatable food and consume more than 43% of energy from fat in daily diet (Blundell et al., 2005). In addition, they also experience less satiety compared to restraint eaters, which explains their excess energy intake with high preference for HF food items. Restraint eaters tend to restrict intake of fats or foods that are high in fats, however, blinded sensory fat test showed restraint eaters have no increased tastes aversion towards HF sensation (Schebendach et al., 2014). This shows that psychological control rather than actual taste preference has an effect on eating behaviour. We speculate that restrictive eaters do not discriminate between LF and HF sensations but rather rely more on cognitive and psychological control on food selection. For instance, a recent low-fat diet may have led to frustration due to high CR, thus Lampure et al. (2014) found that women who were currently dieting were more likely to prefer fat.

## 4 Conclusions

Eating behaviours are often driven by psychological factors other than sensory properties of a food. Evidences from the present study suggest that overweight individuals favoured HF sensation as opposed to LF sensation foods. Nevertheless, similar trends were observed on the association between liking of fat sensation and eating behaviour regardless of weight statuses. Specifically, cognitive restrainers had a heightened liking for LF sensation whereas HF sensation was more preferred among uncontrolled and emotional eaters. Hence, understanding consumer eating behaviour that contributes towards liking of fat sensation and obesity consequences deserve more attention to tackle effectively the underlying cause of overconsumption. This would assist food authorities and organizations to implement impactful health policies and educational strategies that guide consumers on proper food selection with emphasis on maintenance of healthy body weight in order to improve health status of the population.

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# Cooking and Functional Properties of Parboiled Milled Local Rice Marketed in the South-East Zone of Nigeria

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**Abstract**

Imported rice is *perceived* to have better cooking properties than locally grown rice in Nigeria and it has increased its market share while reducing patronage for local rice. Rice in Nigeria has many applications, including consumption as whole cooked grain or dumpling or use as an adjunct in making beverages. Eighteen varieties of parboiled milled local rice and three imported rice varieties, coded Ip1, Ip2 and Ip3, were studied for their cooking and functional properties using standard methods. There was a significant ($p< 0.05$) increase in the dimensions of all the rice varieties when cooked. There was a 25 g increase in the grain weight and an elongation ratio of more than 1.26 in all the rice varieties. Ghesua had the highest cooked grain weight (68.67 g) while Omor-Mas (6.00) and R-Bus (6.00) had the highest volume expansion ratio (VER). The VER was more than 3.00 for all the rice varieties. All the local rice varieties imbibed less water (17.67-25.33 ml) compared to the imported rice varieties (26.00-27.67ml) before they reached their optimum cooking time. The imported rice varieties were of soft gel consistency (89.67-73.50 mm) and intermediate amylose content (20.71-23.14 %) while the local rice varied in amylose and gel consistency. Abakiliki-Mas (27.00 mm) and R-8 (33.67 mm) were of hard gel-consistency, intermediate (21.11 %) and high amylose (27.21 %) content respectively and have not been exploited although they would be appropriate for making canned rice, dry mixes and rice-noodles.

***Keywords:*** *Oryza sativa* L; Dimensions; Elongation ratio; Volume expansion ratio; Gel consistency; Amylose

## 1 Introduction

Rice is the staple food for over 3 billion people, constituting over half of the world's population (Abiona, 2011; Anonymous, 2009; Danbaba et al., 2014; Imolehin & Wada, 2000; Muthayya, Sugimoto, Montgomery & Maberly, 2014; Oko & Ugwu, 2011; Sanusi, Akinoso & Danbaba, 2017) especially in India, China, other parts of Asia, and Africa (Akaeze, 2010). Nigeria has been the largest rice producing country in West Africa since 1980 and the third largest in Africa, after Egypt and Madagascar (Imolehin & Wada, 2000). Since the 1960s when rice was served mainly at banquets and celebrations in Nigeria, it has become one of the basic foods in the Nigerians' diet, with consumption rising from 8 kg per person in 1960 to 27 kg per person in

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2007 (Diagne, Bamba, Manful & Ajayi, 2011). This led to importation of rice from Thailand and USA as the quantity produced in Nigeria was not enough for the masses. Today, importation is sustained and imported rice is preferred over local rice in Nigeria because of its cleaner general appearance and *perceived* better cooking properties than locally grown rice. Rice quality is partially determined by the genetic makeup of a variety but mainly by a variety of subjective and objective factors (Bida, 2013; Mutters & Thompson, 2009). Among the qualities determined objectively are the cooking and functional properties of the rice grain (Webb, 1972).

Cooking properties' evaluation is essential in order to know the way in which each rice variety will react when heated in boiling water at a temperature of 100±2$^o$C. Rice exhibits different characteristics when cooked including tendency to cling together, ability to become fluffy and lie separate, expansion in volume and length, increase in weight and loss of rice solids. Food vendors prefer rice with high volume expansion ratio (VER) in order to increase profit from sales. For increased satiety, rice with higher weight gain is prescribed. In Chinese cuisine, short-grain rice is preferred because the grains are sticky or mushy and are easier to eat with chopsticks (Webb, 1972). In the US, the preference is for long grain rice that becomes fluffy and lies separately when cooked.

Rice is consumed as whole grain cooked rice along with sauce, Jollof or fried rice, local beverages and dumpling in Nigeria. Some of the most important uses and processing applications of rice include boiled or steamed whole-grain rice for consumer use; dry breakfast and baby-food cereals; brewers rice; canned rice such as in soups, puddings and mixes; quick cooking, other convenience and specialty food products; rice flour as a thickener in sauces, gravies and puddings; rice starch for industrial and other processes; and preparations for certain types of fermented foods (Webb, 1972). Amylose content and gel consistency can highly influence processing qualities of rice, which can vary based on the varieties as evaluated by Thomas, Wan-Nadiah and Bhat (2013) for Malaysian rice and Bhonsle and Krishnan (2010) for aromatic rice varieties in Goa India.

There are limited studies and paucity of information on the cooking and functional characteristics of rice milled and consumed in Nigeria (Akaeze, 2010) which makes Nigerians limit their use of local rice and perceive that imported rice has better cooking properties than local rice. It is imperative to evaluate the cooking and functional properties of the rice varieties as this will influence their post-harvest handling (Sanusi et al., 2017). Parameters determined as indices of the cooking properties of rice were physical dimensions, elongation ratio (ER), weight increase (WI), volume expansion ratio (VER), water uptake ratio (WUR), minimum and optimum cooking time, solid loss in water (SLIW) and volume of water absorbed (VWA). Parameters determined as indices of the functional properties were amylose content and gel consistency. The objective of this study was to determine the cooking and functional properties of local rice varieties sold in South-East Nigeria and also establish how they correlate.

## 2 Materials and Methods

### 2.1 Materials

Eighteen varieties of parboiled milled domestic rice were obtained from different rice processing units and markets in Enugu (FARO 44, Fadama, Fortin 16 and Fortin 16, old variety), Anambra (Omor-Mas, R-Bus, FARO 40, Igboukwu rice, Aguleri rice, Taraba rice and B-G) and Ebonyi (Akpujie, kpurukpuru, Afikpo-Mas, Abakiliki Mas, R-8, 306 and Geshua) states as represented in Tables 1-4. Three imported parboiled rice varieties were purchased from Ogige market in the Nsukka Local Government Area of Enugu State, Nigeria. The imported rice varieties served as a control and were coded as Ip1, Ip2, and Ip3. The imported rice varieties were procured based on cost (Ip1), commonly consumed rice variety (Ip2) and difference in length size (Ip3). The samples collected were manually cleaned using plastic trays in order to remove contaminants such as husk, shrivelled kernels (defectives), stones and seeds. All samples were stored at 25±2$^o$C, in a moisture free environment, until needed.

## 2.2 Methods

The cooking method adopted was as described by Odenigbo, Ngadi, Ejebe, Woin and Ndindeng (2014). Four grams of each rice variety were placed in a beaker containing 60 ml ($V_1$) of distilled water. The rice grains were cooked in a water bath at 100±1 $^oC$. Measurements were taken after 10 minutes of cooking and every minute thereafter. The measurements involved collection of 5 grains from the cooking vessel and pressing between two glass slides. The time when a minimum of 95% of the collected boiled grains no longer displayed an opaque core or un-gelatinized centers was recorded as the Minimum Cooking Time (MCT). The rice was allowed to simmer for another 2 min to ensure that the core of all grains had been gelatinized. This additional 2 min after the MCT was referred to as Optimum Cooking Time (OCT).

### Cooked grain dimensions

Cooked grain dimensions were determined by the method described by Bida (2013).

#### Cooked grain length

Ten cooked whole rice grain samples of each variety were randomly selected and the length of the grains measured using a digital calliper (0.1-100mm A&D Company Limited). The mean value of each variable was determined and noted as $L_2$. The value obtained was recorded as each sample's cooked grain size.

#### Cooked grain shape

Ten optimum cooked whole rice grain samples of each variety were randomly selected and the width of the cooked grains were determined using a digital calliper (0.1-100mm A&D Company Limited) and noted as $Wd_2$. The length/ width ratio of the samples were calculated using Equation (1). The mean value of each variable was obtained and the value obtained was recorded as the grain shape for each sample.

$$\frac{L}{W} = \frac{L_2}{Wd_2} \tag{1}$$

#### Thickness of cooked rice grain

Ten cooked whole rice grain samples of each variety were randomly selected and the thickness of the grains measured using a digital calliper (0.1-100mm A&D Company Limited). The mean value was obtained and recorded as each sample's thickness.

#### One-thousand (1000) cooked grain weight

One hundred optimum cooked kernel representative sample (triplicates) for each variety were randomly selected. The weight of each sample was determined using a 500g capacity weighing scale (Electronic Pocket Scale Model EHA251). The value obtained was multiplied by 10. The cooked 1000 kernel mean weight of the samples were obtained and noted as $W_2$.

#### Volume of cooked grains (VCG)

Volume of cooked grains ($V_2$ ml) was determined by a displacement method as described by Gariboldi (1979). The optimum cooked rice grains were removed from the water bath and allowed to drain off excess water. The wet cooked grains were placed on filter paper to blot out excess water. One hundred cooked grains were counted and put in a measuring cylinder containing 20 ml of water ($V_3$). The new volume of water after the cooked grains were added to the measuring cylinder was noted as ($V_4$). The volume of cooked grains was obtained by subtracting the Volume of water containing cooked-rice grains from the initial volume of water contained in the measuring cylinder (Equation 2).

$$V_2(ml) = V_4 - V_3 \tag{2}$$

Where, $V_2$ = volume of cooked rice grain (ml), $V_4$ = New volume of water after the cooked grains were added to the measuring cylinder (ml) $V_3$ = volume of water used in determining volume of cooked grain (ml).

#### Density of cooked rice grain (DCG)

Density of a cooked rice grain was obtained by dividing one-thousand grain weight of cooked rice

grain with its volume (Equation 3)

$$Density(g/ml) = \frac{W_2}{V_2} \quad (3)$$

## Determination of minimum and optimum cooking time

The minimum and optimum cooking times were determined according to the method described by Odenigbo et al. (2014).

## Quantity of water absorbed

Quantity of water $V_6$ (ml) absorbed by rice samples was determined as described by Gariboldi (1979). The remaining distilled water, after the rice reached its optimum cooking time, was measured in a volumetric flask and its volume recorded ($V_5$). Quantity of water absorbed was obtained by subtracting the volume of remaining distilled water from the initial volume of water used in cooking rice samples (Equation 4).

$$V_6(ml) = V_1 - V_5 \quad (4)$$

Where, $V_6$ = volume of water absorbed by rice sample (ml);
$V_1$ = initial volume of water used in cooking rice sample (ml)
$V_5$ = volume of water remaining after cooking rice (ml)

## Volume expansion ratio (VER)

Volume expansion ratio was determined according to the method described by Gariboldi (1979). The volume expansion ratio ($V_8$) was calculated using Equation (5)

$$V_8 = \frac{V_2}{V_7} \quad (5)$$

Where, $V_2$ = volume of cooked rice (ml) and $V_7$ = volume of raw rice (ml).

## Weight increase (WI)

The increase in weight of rice samples was obtained as described by Gariboldi (1979). The cooked rice grains were removed from the water bath and allowed to drain off excess water. One hundred (100) cooked rice grains were weighed ($W_2$) and multiplied by 10. The increase in weight was obtained by subtracting the weight of cooked rice grains from the uncooked rice grains W1 (Equation 6).

$$W_8(g) = W_2 - W_1 \quad (6)$$

Where, $W_3$ = weight increase of cooked rice (g);
$W_2$ = weight of cooked rice (g)
$W_1$ = weight of raw rice (g)

## Elongation ratio (ER)

Elongation ratio of rice grain samples was determined according to the method of Odenigbo et al. (2014). Ten optimum cooked grains of each sample were measured using a digital calliper (0.1-100mm A&D Company Limited). Average length of the cooked grains ($L_2$) was divided by average length of 10 uncooked grains sample ($L_I$) for each variety and Equation (7) used to evaluate their Elongation ratio.

$$ER = \frac{L_2}{L_1} \quad (7)$$

## Water uptake ratio (WUR)

Water Uptake Ratio was determined according to the method described by Bida (2013). The optimum cooked grains were strained and placed on filter paper to remove excess water and weighed. The WUR was determined as shown in equation 8

$$WUR = \frac{W_2}{W_1} \quad (8)$$

## Degree of agglutination (solid loss in water)

The Resistance of kernels to become sticky on boiling (degree of agglutination) was determined according to the method described by Gariboldi (1979). All the water used for boiling rice was drained off and afterwards evaporated. The amount of solid matter lost while cooking rice samples was obtained and weighed. The weight obtained is the degree of agglutination expressed in grams $W_3$.

## 2.3 Functional properties

### Gel consistency (GC) determination

Gel Consistency (GC) of the rice samples was determined according to the method of Cagampang, Perez and Juliano (1973). 100 mg rice flour of 12% moisture was placed in 13 x 100mm culture tubes. The powder was wet with 0.2ml 95% ethanol containing 0.025% thymol blue. The tube was shaken and 2.0ml of 0.2N KOH added immediately and the mixture dispersed. The tubes were covered with glass marbles and placed for 8 minutes in a boiling water bath. Afterwards, the samples were removed and kept at room temperature for 5 minutes, and then cooled in ice cold water for 15 minutes. The tubes were removed afterwards from ice water and laid horizontally over a ruled paper graduated in millimetres and the length of the gel from the bottom of the test tube was measured after 30-60 minutes. The rice was classified based on these categories: Soft = 61-100 mm, Medium= 41-60 mm, Hard= 26-40 mm

### Amylose content determination

Amylose Content (AC) was determined according to the method of Juliano (1971). 100mg of rice flour was poured into a 100 ml volumetric flask. 1ml of 95% ethanol and 9 ml of 1 N NaOH was added to the rice flour and the mixture heated for 10 minutes in a boiling water bath. After heating, the mixture was cooled and made up to 100 ml volume with distilled water. 5 ml of the 100 ml solution was taken and put into another 100 ml volumetric flask. 1 ml of 1 N acetic acid was added and 2 ml Iodine-potassium iodide solution added subsequently. The volume of the mixture was made up to 100 ml with distilled water. The sample was shaken and allowed to stand for 20 minutes and the per cent Transmittance determined at 620 nm using a colorimeter. Amylose content of the samples was determined in reference to a standard curve (graph) and expressed on percent basis.

$$Amylose(\%) = [Amylose]_{Std} \cdot \frac{A_{sample}}{A_{std}} \quad (9)$$

where $[Amylose]_{Std}$ is the Amylose content of standard in

$$Amylopectin(\%) = 100 - \%Amylose \quad (10)$$

Based on amylose content, milled rice is classified in "amylose groups", as follows: waxy (1-2% amylose), very low amylose content (2-9% amylose), low amylose content (10-20% amylose), intermediate amylose content (20-25% amylose) and high amylose content (25-33% amylose) (Bida, 2013).

## 2.4 Statistical Analysis

The study adopted a completely randomized design (CRD). The data generated were subjected to one way analysis of variance (ANOVA) using SPSS version 20.0. Means were separated using Duncan's Multiple Range Test (DMRT) and significance was accepted at 0.05 level of probability (Akande, Onyegbula, Salawu, K Oladipo & Adetunji, 2017).

# 3 Results and Discussion

## 3.1 Cooked grain dimensions

Table 1 shows the length, width, thickness and length-width ratio of raw and cooked domestic rice varieties marketed in South-East Nigeria. There was a significant ($p < 0.05$) increase in the length, width and thickness of all the rice varieties when cooked. All of the raw medium and long grain rice varieties became extra-long grains when cooked as described by the FAO (Foot note Table 1). Akpujie had the highest cooked grain length (10.19 mm) compared to Ip2 an imported rice variety (9.95 mm). Ip1 had the highest length-width ratio (3.52 mm) compared to Abakiliki Mas a local rice variety (3.20 mm). Table 2 shows the weight, volume and density of raw and cooked domestic rice varieties sold in South-East Nigeria. There was a significant ($p < 0.05$) increase in the volume and weight of all the rice grains when cooked. There was an increase of more than 25 g in weight of all the rice varieties when cooked. Geshua had the highest cooked grain weight (68.67 g) compared to Ip3 (59.33 g). FARO 40 had the highest cooked grain

Table 1: Length, Width, Thickness and Length-width ratio of Raw and Cooked Domestic Rice Varieties Sold in the South-East Nigeria

| Rice variety | LRG (mm) | LCG (mm) | WRG(mm) | WCG (mm) | TRG(mm) | TCG (mm) | L/WRG | L/WCG |
|---|---|---|---|---|---|---|---|---|
| **Imported Rice Variety** | | | | | | | | |
| Ip1 | $7.19^{ab}\pm0.35$ | $9.48^{bc}\pm0.64$ | $2.03^{ij}\pm0.15$ | $2.71^{g}\pm0.19$ | $1.69^{e}\pm0.12$ | $2.33^{ef}\pm0.25$ | $3.57^{a}\pm0.34$ | $3.52^{a}\pm0.19$ |
| Ip2 | $7.07^{abc}\pm0.27$ | $9.95^{ab}\pm0.57$ | $2.08^{hij}\pm0.12$ | $2.87^{fg}\pm0.16$ | $1.77^{abcde}\pm0.11$ | $2.30^{f}\pm0.09$ | $3.40^{ab}\pm0.19$ | $3.47^{a}\pm0.27$ |
| Ip3 | $6.11^{ij}\pm0.47$ | $9.02^{cde}\pm0.42$ | $2.00^{j}\pm0.12$ | $3.01^{def}\pm0.26$ | $1.68^{e}\pm0.11$ | $2.45^{def}\pm0.18$ | $3.08^{cde}\pm0.20$ | $3.03^{bcde}\pm0.33$ |
| **Enugu State** | | | | | | | | |
| Faro 44 | $6.86^{bcdef}\pm0.31$ | $9.20^{cd}\pm0.43$ | $2.24^{defgh}\pm0.13$ | $3.13^{cdef}\pm0.22$ | $1.83^{abc}\pm0.12$ | $2.44^{def}\pm0.18$ | $3.08^{cde}\pm0.20$ | $2.95^{bcdefg}\pm0.17$ |
| Fadama | $6.12^{ij}\pm0.56$ | $8.09^{gh}\pm0.39$ | $2.21^{efgh}\pm0.20$ | $2.94^{efg}\pm0.18$ | $1.86^{ab}\pm0.16$ | $2.46^{def}\pm0.21$ | $2.79^{fgh}\pm0.21$ | $2.78^{efgh}\pm0.19$ |
| Fortin 16 | $6.41^{ghij}\pm0.27$ | $8.35^{fgh}\pm0.98$ | $2.26^{cdefg}\pm0.17$ | $2.90^{fg}\pm0.35$ | $1.88^{a}\pm0.11$ | $2.42^{def}\pm0.16$ | $2.84^{efg}\pm0.27$ | $2.90^{cdefg}\pm0.38$ |
| Fortin 16 (old variety) | $6.45^{fghij}\pm0.54$ | $8.60^{defg}\pm0.26$ | $2.19^{efghi}\pm0.21$ | $2.95^{efg}\pm0.36$ | $1.85^{ab}\pm0.14$ | $2.31^{f}\pm0.23$ | $2.99^{defg}\pm0.43$ | $2.98^{bcdef}\pm0.41$ |
| **Anambra State** | | | | | | | | |
| Omor-Mas | $6.25^{hij}\pm0.30$ | $8.66^{defg}\pm0.60$ | $2.29^{cdef}\pm0.09$ | $3.25^{bcd}\pm0.31$ | $1.73^{bcde}\pm0.08$ | $2.62^{bcd}\pm0.23$ | $2.74^{gh}\pm0.10$ | $2.69^{gh}\pm0.30$ |
| R-Bus | $6.60^{defgh}\pm0.51$ | $9.48^{bc}\pm0.90$ | $2.17^{efghij}\pm0.22$ | $3.10^{cdef}\pm0.37$ | $1.70^{de}\pm0.16$ | $2.43^{def}\pm0.24$ | $3.07^{de}\pm0.35$ | $3.07^{bcd}\pm0.21$ |
| Faro 40 | $6.55^{efghi}\pm0.41$ | $8.97^{cdef}\pm0.57$ | $2.17^{efghij}\pm0.12$ | $3.01^{def}\pm0.17$ | $1.82^{abcd}\pm0.06$ | $2.52^{cdef}\pm0.15$ | $3.03^{def}\pm0.19$ | $2.98^{bcdef}\pm0.19$ |
| Igboukwu rice | $6.07^{jk}\pm0.44$ | $8.46^{efgh}\pm0.61$ | $2.40^{bcd}\pm0.13$ | $3.25^{bcd}\pm0.15$ | $1.82^{abcd}\pm0.10$ | $2.63^{bcd}\pm0.21$ | $2.54^{hi}\pm0.22$ | $2.60^{h}\pm0.22$ |
| Aguleri rice | $6.33^{ghij}\pm0.56$ | $8.62^{defg}\pm0.47$ | $2.57^{a}\pm0.13$ | $3.40^{b}\pm0.25$ | $1.86^{ab}\pm0.10$ | $2.59^{bcd}\pm0.19$ | $2.45^{i}\pm0.22$ | $2.53^{hi}\pm0.19$ |
| Taraba rice | $6.71^{cdefg}\pm0.36$ | $8.87^{cdef}\pm0.87$ | $2.14^{fghij}\pm0.21$ | $3.21^{bcde}\pm0.30$ | $1.68^{e}\pm0.15$ | $2.53^{bcdef}\pm0.25$ | $3.17^{bcd}\pm0.36$ | $2.79^{defgh}\pm0.31$ |
| B-G | $6.50^{efghij}\pm0.39$ | $8.96^{cdef}\pm0.44$ | $2.41^{bc}\pm0.24$ | $3.31^{bc}\pm0.31$ | $1.84^{abc}\pm0.11$ | $2.73^{abc}\pm0.21$ | $2.73^{gh}\pm0.26$ | $2.73^{fgh}\pm0.32$ |
| **Ebonyi State** | | | | | | | | |
| Akpujie | $7.47^{a}\pm0.76$ | $10.19^{a}\pm0.62$ | $2.33^{bcde}\pm0.17$ | $3.45^{b}\pm0.25$ | $1.78^{abcde}\pm0.10$ | $2.91^{a}\pm0.26$ | $3.22^{bcd}\pm0.24$ | $2.95^{bcdefg}\pm0.17$ |
| Kpurukpuru | $5.69^{kL}\pm0.15$ | $7.89^{h}\pm0.72$ | $2.47^{ab}\pm0.12$ | $3.42^{b}\pm0.37$ | $1.83^{abc}\pm0.11$ | $2.76^{ab}\pm0.16$ | $2.30^{ij}\pm0.14$ | $2.34^{ij}\pm0.30$ |
| Afikpo-Mas | $6.72^{cdefg}\pm0.35$ | $9.23^{cd}\pm0.43$ | $2.11^{ghij}\pm0.13$ | $2.99^{def}\pm0.22$ | $1.70^{de}\pm0.22$ | $2.55^{bcde}\pm0.33$ | $3.20^{bcd}\pm0.21$ | $3.12^{bc}\pm0.33$ |
| Abakiliki Mas | $7.00^{bcd}\pm0.30$ | $9.11^{cd}\pm0.51$ | $2.10^{ghij}\pm0.12$ | $2.87^{fg}\pm0.26$ | $1.66^{e}\pm0.13$ | $2.49^{def}\pm0.23$ | $3.35^{abc}\pm0.29$ | $3.20^{b}\pm0.29$ |
| R-8 | $6.63^{defgh}\pm0.38$ | $9.22^{cd}\pm0.35$ | $2.22^{efgh}\pm0.18$ | $3.06^{cdef}\pm0.15$ | $1.72^{cde}\pm0.08$ | $2.59^{bcd}\pm0.16$ | $3.02^{def}\pm0.40$ | $3.01^{bcdef}\pm0.19$ |
| 306 | $6.93^{bcde}\pm0.61$ | $8.72^{def}\pm0.84$ | $2.18^{efghi}\pm0.12$ | $3.13^{cdef}\pm0.19$ | $1.77^{abcde}\pm0.07$ | $2.63^{bcd}\pm0.36$ | $3.18^{bcd}\pm0.28$ | $2.79^{defgh}\pm0.32$ |
| Geshua | $5.55^{L}\pm0.31$ | $8.09^{gh}\pm0.47$ | $2.57^{a}\pm0.27$ | $3.70^{a}\pm0.30$ | $1.74^{bcde}\pm0.13$ | $2.74^{abc}\pm0.29$ | $2.18^{j}\pm0.19$ | $2.21^{j}\pm0.17$ |
| Mean | 6.53±0.62 | 8.91±0.81 | 2.24±0.22 | 3.13±0.34 | 1.77±0.14 | 2.54±0.26 | 2.95±0.44 | 2.89±0.40 |
| LSD0.05 | 0.38 | 0.54 | 0.15 | 0.23 | 0.11 | 0.20 | 0.24 | 0.24 |
| CV (%) | 6.6 | 6.9 | 7.4 | 8.5 | 6.9 | 8.8 | 9.1 | 9.4 |

* Values are means± standard deviation of replicate determination. Means in the same column carrying similar superscript are not significantly (P>0.05) different. Extra-long grain rice = 7.00 mm and above, Long grain rice =6.00 to 6.99 mm, medium grain rice = 5.00 to 5.99 mm, short grain rice = less than 5.00 mm. LRG= Length of raw grain, LCG= Length of cooked grain WRG= Width of raw grain, WCG= Width of cooked grain, TRG= thickness of raw grain, TCG= thickness of cooked grain, L/WRG=Length-width ratio of raw grain, L/WCG=Length-width ratio of cooked grain

volume (80.00 ml) compared to Ip1 (73.33 ml) while Ip3 had the highest cooked grain density (1.93 g/ml$^3$). The cooked grains were less dense than the raw grains as a result of the increase in volume of the rice grains when cooked (expansion of the starch granules) and a non-variation or change in the molecular mass of the cooked grains.

## 3.2 Other cooking properties of domestic rice varieties

Table 3 shows other cooking properties of domestic rice varieties sold in South-East Nigeria. Ip3 had the highest ER (1.50) compared to a local rice variety (1.46) though there was no significant ($p > 0.05$) difference between them. The elongation ratios of all the local rice varieties were more than 1.26 of its raw grain length when cooked. The values of ER (1.27-1.50) were within those (1.08-3.20) reported by Oko, Ubi and Nahemiah (2012) for selected local and newly introduced rice varieties in Ebonyi State, Nigeria. Thomas et al. (2013) reported ER values of 1.37-1.77 for six different rice cultivars in Penang, Malaysia. Yadav, Khatkar and Yadav (2007) had earlier reported ER values of 1.52-1.89 for some Indian rice (*oryza sativa L.*) cultivars which were similar to those (1.29-1.74) reported by Singh, Kaur, Sodhi and Sekhon (2005) for 23 milled varieties of Indian rice. Geshua had the highest weight increase (49.67 g) compared to an imported rice variety (41.33 g).

Omor-mas and R-Bus had the highest VER (6.00) compared to an imported rice variety (5.00). There was an expansion ratio of more than 3.00 for the rice varieties' raw grain volume (3.03-6.00) when cooked (Table 3) except for Fadama (2.0). These rice varieties with high VER would produce rice with higher volume than those with less VER, of the same quantity, when cooked and this is desirable. According to Akaeze (2010), imported rice swelling capacity is mostly preferred by restaurants and fast food

Table 2: Weight, Volume and Density of Raw and Cooked Domestic Rice Varieties Sold in South-East Nigeria

| Rice variety | RGW(g) | CGW (g) | VRG (ml) | VCG (ml$^3$) | DRG(g/ml) | DCG (g/ml$^3$) |
|---|---|---|---|---|---|---|
| **Imported rice variety** | | | | | | |
| Ip1 | $21.00^{cdefg}$±0.00 | $51.33^{gh}$±4.16 | $16.67^{ab}$±5.77 | $73.33^{ab}$±11.55 | $1.33^{ab}$±0.58 | $0.70^{f}$±0.10 |
| Ip2 | $19.33^{ghi}$±0.58 | $57.33^{cdefg}$±3.05 | $13.33^{ab}$±5.77 | $53.33^{cde}$±11.55 | $1.67^{ab}$±0.55 | $1.13^{bcdef}$±0.23 |
| Ip3 | $18.00^{i}$±1.00 | $59.33^{bcdef}$±4.16 | $10.00^{b}$±0.00 | $33.33^{f}$±11.55 | $2.00^{a}$±0.10 | $1.97^{a}$±0.72 |
| **Enugu State** | | | | | | |
| Faro 44 | $23.00^{ab}$±0.00 | $50.00^{gh}$±0.00 | $13.33^{ab}$±5.77 | $40.00^{ef}$±0.00 | $1.67^{ab}$±0.64 | $1.30^{bcd}$±0.00 |
| Fadama | $20.67^{defg}$±0.58 | $50.00^{gh}$±0.00 | $20.00^{a}$±0.00 | $40.00^{ef}$±0.00 | $1.03^{b}$±0.06 | $1.30^{bcd}$±0.00 |
| Fortin 16 | $20.67^{defg}$±0.58 | $46.67^{h}$±3.05 | $13.33^{ab}$±5.77 | $60.00^{bcd}$±0.00 | $1.67^{ab}$±0.64 | $0.77^{ef}$±0.06 |
| Fortin 16 (old variety) | $21.33^{bcdef}$±1.15 | $52.67^{efgh}$±1.15 | $13.33^{ab}$±5.77 | $60.00^{bcd}$±0.00 | $1.73^{ab}$±0.59 | $0.90^{def}$±0.00 |
| **Anambra State** | | | | | | |
| Omor-Mas | $20.67^{defg}$±0.58 | $62.67^{abc}$±13.31 | $13.33^{ab}$±5.77 | $73.33^{ab}$±11.55 | $1.73^{ab}$±0.55 | $0.87^{def}$±0.31 |
| R-Bus | $21.67^{bcd}$±1.53 | $53.33^{defgh}$±1.15 | $13.33^{ab}$±5.77 | $73.33^{ab}$±11.55 | $1.67^{ab}$±0.62 | $0.77^{ef}$±0.12 |
| Faro 40 | $22.67^{abc}$±0.58 | $56.00^{cdefg}$±4.00 | $20.00^{a}$±0.00 | $80.00^{a}$±0.00 | $1.53^{ab}$±0.06 | $0.73^{f}$±0.06 |
| Igboukwu rice | $22.33^{bcd}$±0.58 | $52.00^{fgh}$±2.00 | $18.33^{ab}$±2.89 | $60.00^{bcd}$±20.00 | $1.77^{ab}$±0.21 | $0.97^{cdef}$±0.31 |
| Aguleri rice | $24.33^{a}$±1.53 | $60.67^{bcd}$±1.15 | $18.33^{ab}$±2.89 | $73.33^{ab}$±11.55 | $1.47^{ab}$±0.15 | $0.87^{def}$±0.12 |
| Taraba rice | $19.67^{fghi}$±0.58 | $54.00^{defgh}$±0.00 | $13.33^{ab}$±2.89 | $73.33^{ab}$±11.55 | $1.40^{ab}$±0.40 | $0.77^{ef}$±0.12 |
| **Ebonyi State** | | | | | | |
| B-G | $22.67^{abc}$±1.53 | $60.00^{bcde}$±2.00 | $16.67^{ab}$±2.89 | $50.00^{def}$±0.00 | $2.07^{a}$±0.15 | $1.20^{bcde}$±0.00 |
| Akpujie | $22.33^{bcd}$±1.15 | $66.00^{ab}$±2.00 | $16.67^{ab}$±5.77 | 70.00a$^{bc}$±10.00 | $1.50^{ab}$±0.67 | $0.97^{cdef}$±0.12 |
| Kpurukpuru | $20.00^{efg}$±0.00 | $66.67^{ab}$±3.06 | $10.00^{b}$±0.00 | $50.00^{def}$±10.00 | $1.43^{ab}$±0.00 | $1.40^{bc}$±0.36 |
| Afikpo-Mas | $19.33^{ghi}$±0.58 | $52.67^{efgh}$±3.06 | $11.67^{ab}$±2.89 | $55.00^{cde}$±5.00 | $1.27^{ab}$±0.38 | $0.93^{def}$±0.06 |
| Abakiliki Mas | $20.33^{efg}$±0.58 | $55.33^{cdefg}$±4.16 | $15.00^{ab}$±5.00 | $60.00^{bcd}$±0.00 | $1.30^{ab}$±0.57 | $0.93^{def}$±0.06 |
| R-8 | $19.33^{ghi}$±1.53 | $56.00^{cdefg}$±6.00 | $16.67^{ab}$±2.89 | $65.00^{abcd}$±5.00 | $1.30^{ab}$±0.26 | $0.87^{def}$±0.06 |
| 306 | $20.67^{defg}$±0.58 | $60.00^{bcde}$±3.46 | $13.33^{ab}$±5.77 | $55.00^{cde}$±5.00 | $1.53^{ab}$±0.64 | $1.07^{bcdef}$±0.06 |
| Geshua | $19.00^{hi}$±1.73 | $68.67^{a}$±1.15 | $10.00^{b}$±0.00 | $50.00^{def}$±10.00 | $1.63^{ab}$±0.17 | $1.43^{b}$±0.35 |
| Mean | 20.90±1.74 | 56.73±6.73 | 14.60±4.61 | 59.44±14.68 | 1.58±0.45 | 1.04±0.36 |
| SE | 0.54 | 2.35 | 2.42 | 5.21 | 0.28 | 0.13 |
| $LSD_{0.05}$ | 1.55 | 6.71 | 6.91 | 14.89 | 0.81 | 0.37 |
| CV (%) | 4.5 | 7.2 | 28.7 | 15.2 | 31.6 | 21.8 |

* Values are means± standard deviation of triplicate determination. Means in the same column carrying similar superscript are not significantly (P>0.05) different
RGW= Raw grain weight, CGW= cooked grain weight, VRG= volume of raw grain VCG= volume of cooked grain, DRG= density of raw grain DCG= density of cooked grain

outlets, which is one of the reasons why imported rice consumption in Nigeria has expanded at the expense of local rice market development. It is clear that the above statement is not absolutely correct as Omor-Mas and R-Bus, which are local rice varieties, had the highest volume expansion ratio among all the rice varieties. Food vendors should use rice varieties with high VER in order to increase profit from sales.

From Table 4, the water uptake ratio of Geshua (3.63) was higher compared to Ip3 an imported rice variety (3.30). WUR is a measure of the rate at which the rice grains take up water and increase in weight. WUR had a high positive correlation (0.95) with weight increase. Geshua and Kpurukpuru had the highest water uptake ratio of 3.63 and 3.33 respectively, and thus had higher weight. Geshua which had the least raw grain weight (Table 1) among the local rice varieties had the highest cooked grain weight of 68.67 because of its WUR. Grains with high WUR are desirable as they will cause increase in weight and increased satiety when consumed.

Kpurukpuru and Geshua had the highest optimum cooking time (OCT) of 45.33 and 40.00 minutes respectively compared to the imported rice varieties (22.67-31.33 mins). However, a significant percentage (33.33%) of the local rice varieties had a lower OCT (17.00-21.33 mins) than the imported rice varieties while a large percentage (83.33%) of the local rice varieties were within the range of OCT (17.00-29.33 mins)

Table 3: Some Cooking Properties of Domestic Rice Varieties Sold in South-East Nigeria

| Rice variety | ER | WI (g) | VER |
|---|---|---|---|
| **Imported rice variety** | | | |
| Ip1 | $1.32^{bcd}\pm0.13$ | $30.33^{ghij}\pm4.16$ | $5.00^{ab}\pm2.65$ |
| Ip2 | $1.41^{abcd}\pm0.07$ | $38.00^{cdefg}\pm2.65$ | $4.33^{abc}\pm1.53$ |
| Ip3 | $1.50^{a}\pm0.17$ | $41.33^{bcde}\pm5.13$ | $3.33^{abc}\pm1.15$ |
| **Enugu State** | | | |
| Faro 44 | $1.35^{bcd}\pm0.11$ | $27.00^{ij}\pm0.00$ | $3.33^{abc}\pm1.15$ |
| Fadama | $1.33^{bcd}\pm0.14$ | $29.33^{hij}\pm0.58$ | $2.0^{c}\pm0.00$ |
| Fortin 16 | $1.30^{d}\pm0.13$ | $26.00^{j}\pm2.65$ | $5.00^{ab}\pm1.73$ |
| Fortin 16 (old variety) | $1.34^{bcd}\pm0.10$ | $31.33^{fghij}\pm1.15$ | $5.00^{ab}\pm1.73$ |
| **Anambra State** | | | |
| Omor-Mas | $1.40^{abcd}\pm0.12$ | $42.00^{bcd}\pm13.74$ | $6.00^{a}\pm2.00$ |
| R-Bus | $1.46^{ab}\pm0.17$ | $31.67^{fghij}\pm2.08$ | $6.00^{a}\pm2.00$ |
| Faro 40 | $1.38^{abcd}\pm0.10$ | $33.33^{efghij}\pm4.04$ | $4.00^{abc}\pm0.00$ |
| Igboukwu rice | $1.39^{abcd}\pm0.15$ | $29.67^{ghij}\pm2.08$ | $3.23^{abc}\pm0.68$ |
| Aguleri rice | $1.38^{abcd}\pm0.18$ | $36.33^{cdefgh}\pm2.08$ | $4.00^{abc}\pm0.00$ |
| Taraba rice | $1.32^{bcd}\pm0.15$ | $34.33^{defghi}\pm0.58$ | $5.77^{ab}\pm2.04$ |
| B-G | $1.37^{abcd}\pm0.13$ | $37.33^{cdefgh}\pm2.08$ | $3.03^{bc}\pm0.46$ |
| **Ebonyi State** | | | |
| Akpujie | $1.38^{abcd}\pm0.18$ | $43.67^{ab}\pm3.06$ | $4.50^{abc}\pm1.32$ |
| Kpurukpuru | $1.40^{abcd}\pm0.16$ | $46.67^{ab}\pm3.06$ | $5.00^{ab}\pm1.00$ |
| Afikpo-Mas | $1.38^{abcd}\pm0.10$ | $33.33^{efghij}\pm3.21$ | $4.93^{ab}\pm1.44$ |
| Abakiliki Mas | $1.31^{cd}\pm0.06$ | $35.00^{defghi}\pm3.61$ | $4.33^{abc}\pm1.53$ |
| R-8 | $1.39^{abcd}\pm0.10$ | $36.67^{cdefgh}\pm6.66$ | $3.93^{abc}\pm0.40$ |
| 306 | $1.27^{d}\pm0.15$ | $39.33^{bcdef}\pm3.79$ | $4.60^{abc}\pm1.64$ |
| Geshua | $1.45^{abc}\pm0.12$ | $49.67^{a}\pm1.73$ | $5.00^{ab}\pm1.00$ |
| Mean | 1.37±0.14 | 35.83±7.14 | 4.40±1.54 |
| SE | 0.04 | 2.44 | 0.83 |
| LSD0.05 | 0.12 | 6.98 | 2.37 |
| CV (%) | 9.9 | 11.8 | 32.7 |

* Values are means ± standard deviation of triplicate determination.
Means in the same column carrying similar superscript are not significantly (P>0.05) different. ER=Elongation ratio, WI=weight increase, VER=volume expansion ratio

Table 4: Some Cooking Properties of Domestic Rice Varieties Sold in South-East Nigeria (Continued)

| Rice variety | WUR | MCT (mins) | OCT (mins) | SLIW (g) | VWA (ml) |
|---|---|---|---|---|---|
| **Imported rice variety** | | | | | |
| Ip1 | $2.47^{defg}\pm0.21$ | $20.67^{fghi}\pm3.79$ | $22.67^{fghi}\pm3.79$ | $0.03^{bc}\pm0.06$ | $26.33^{ab}\pm1.15$ |
| Ip2 | $2.97^{bcd}\pm0.15$ | $20.00^{fghi}\pm7.81$ | $22.00^{fghi}\pm7.81$ | $0.17^{abc}\pm0.12$ | $26.00^{ab}\pm4.00$ |
| Ip3 | $3.30^{ab}\pm0.46$ | $29.33^{c}\pm0.58$ | $31.33^{c}\pm0.58$ | $0.20^{ab}\pm0.20$ | $27.67^{a}\pm2.52$ |
| **Enugu state** | | | | | |
| Faro 44 | $2.20^{g}\pm0.00$ | $20.67^{fghi}\pm3.21$ | $22.67^{fghi}\pm3.21$ | $0.10^{abc}\pm0.10$ | $25.00^{abcd}\pm3.46$ |
| Fadama | $2.43^{efg}\pm0.06$ | $15.00^{j}\pm0.00$ | $17.00^{j}\pm0.00$ | $0.17^{abc}\pm0.15$ | $24.67^{abcde}\pm2.08$ |
| Fortin 16 | $2.27^{fg}\pm0.12$ | $17.67^{ghij}\pm2.08$ | $19.67^{ghij}\pm2.08$ | $0.07^{bc}\pm0.06$ | $24.33^{abcde}\pm2.52$ |
| Fortin 16 (old variety) | $2.50^{defg}\pm0.10$ | $23.67^{def}\pm0.58$ | $25.67^{def}\pm0.58$ | $0.10^{abc}\pm0.00$ | $17.67^{h}\pm2.31$ |
| **Anambra state** | | | | | |
| Omor-Mas | $3.03^{bc}\pm0.70$ | $36.67^{b}\pm0.58$ | $38.67^{b}\pm0.58$ | $0.10^{abc}\pm0.00$ | $22.00^{bcdefgh}\pm2.65$ |
| R-Bus | $2.47^{defg}\pm0.21$ | $19.33^{fghij}\pm4.04$ | $21.33^{fghij}\pm4.04$ | $0.13^{abc}\pm0.15$ | $22.00^{bcdefgh}\pm3.61$ |
| Faro 40 | $2.47^{defg}\pm0.15$ | $17.00^{hij}\pm1.73$ | $19.00^{hij}\pm1.73$ | $0.13^{abc}\pm0.06$ | $23.67^{abcde}\pm0.58$ |
| Igboukwu rice | $2.37^{fg}\pm0.12$ | $19.33^{fghij}\pm0.58$ | $21.33^{fghij}\pm0.58$ | $0.07^{bc}\pm0.12$ | $22.67^{bcdefg}\pm2.08$ |
| Aguleri rice | $2.50^{defg}\pm0.17$ | $26.33^{cde}\pm0.58$ | $28.33^{cde}\pm0.58$ | $0.07^{bc}\pm0.06$ | $25.33^{abc}\pm2.08$ |
| Taraba rice | $2.73^{cdef}\pm0.06$ | $27.33^{cd}\pm0.58$ | $29.33^{cd}\pm0.58$ | $0.07^{bc}\pm0.06$ | $23.00^{abcdef}\pm3.00$ |
| B-G | $2.67^{cdefg}\pm0.21$ | $26.00^{cde}\pm3.61$ | $28.00^{cde}\pm3.61$ | $0.27^{a}\pm0.06$ | $18.67^{fgh}\pm1.53$ |
| **Ebonyi state** | | | | | |
| Akpujie | $2.97^{bcd}\pm0.21$ | $21.67^{efgh}\pm0.58$ | $23.67^{efgh}\pm0.58$ | $0.07^{bc}\pm0.06$ | $18.67^{fgh}\pm2.08$ |
| Kpurukpuru | $3.33^{ab}\pm0.15$ | $43.33^{a}\pm0.58$ | $45.33^{a}\pm0.58$ | $0.13^{abc}\pm0.12$ | $20.00^{efgh}\pm3.46$ |
| Afikpo-Mas | $2.70^{cdefg}\pm0.17$ | $16.33^{ij}\pm2.52$ | $18.33^{ij}\pm2.52$ | $0.13^{abc}\pm0.06$ | $22.67^{bcdefg}\pm0.58$ |
| Abakiliki Mas | $2.73^{cdef}\pm0.15$ | $20.00^{fghi}\pm1.73$ | $22.00^{fghi}\pm1.73$ | $0.13^{abc}\pm0.06$ | $20.33^{defgh}\pm2.52$ |
| R-8 | $2.90^{bcde}\pm0.44$ | $22.67^{defg}\pm2.89$ | $24.67^{defg}\pm2.89$ | $0.00^{c}\pm0.00$ | $21.00^{cdefgh}\pm2.65$ |
| 306 | $2.93^{bcde}\pm0.21$ | $26.33^{cde}\pm0.58$ | $28.33^{cde}\pm0.58$ | $0.07^{bc}\pm0.06$ | $18.00^{gh}\pm1.73$ |
| Geshua | $3.63^{a}\pm0.32$ | $38.00^{b}\pm0.00$ | $40.00^{b}\pm0.00$ | $0.07^{bc}\pm0.06$ | $17.67^{h}\pm2.52$ |
| | | | | | |
| Mean | 2.74±0.43 | 24.16±7.66 | 26.16±7.66 | 0.11±0.10 | 22.25±3.64 |
| SE | 0.15 | 1.53 | 1.53 | 0.05 | 1.45 |
| LSD0.05 | 0.42 | 4.38 | 4.38 | 0.15 | 4.15 |
| CV | 9.2 | 11.0 | 10.2 | 82.3 | 11.3 |

* Values are means± standard deviation of triplicate determination. Means in the same column carrying similar superscript are not significantly (P>0.05) different.
WUR= water uptake ratio, MCT= minimum cooking time, OCT= optimum cooking time,
SLIW= solid loss in water, VWA= volume of water absorbed

Table 5: Functional properties of Milled Domestic and Some Imported Rice Varieties Sold in South-East Nigeria

| Rice variety | Gelconsistency (mm) | Gelconsistency Behaviour | Amylose (%) | Amylopectin (%) | Classification based on Amylose Content |
|---|---|---|---|---|---|
| Imported rice variety | | | | | |
| Ip1 | $80.00^{abc}\pm10.00$ | Soft | $20.71^{gh}\pm1.11$ | $79.29^{cd}\pm1.11$ | Intermediate |
| Ip2 | $73.50^{bcd}\pm27.50$ | Soft | $23.14^{def}\pm0.41$ | $76.86^{efg}\pm0.41$ | Intermediate |
| Ip3 | $89.67^{ab}\pm2.52$ | Soft | $21.63^{fg}\pm1.54$ | $78.37^{de}\pm1.54$ | Intermediate |
| Enugu state | | | | | |
| FARO 44 | $88.00^{ab}\pm10.00$ | Soft | $25.00^{abc}\pm0.80$ | $75.00^{hij}\pm0.80$ | High |
| Fadama | $91.00^{a}\pm0.00$ | Soft | $25.44^{ab}\pm0.58$ | $74.56^{ij}\pm0.58$ | High |
| Fortin 16 | $97.00^{a}\pm2.00$ | Soft | $24.20^{bcd}\pm0.97$ | $75.80^{ghi}\pm0.97$ | Intermediate |
| Fortin 16 (old variety) | $89.50^{ab}\pm0.50$ | Soft | $26.07^{fg}\pm1.54$ | $73.93^{de}\pm1.54$ | High |
| Anambra state | | | | | |
| Omor-Mas | $91.67^{a}\pm4.51$ | Soft | $26.08^{a}\pm0.22$ | $73.92^{j}\pm0.22$ | High |
| R-Bus | $41.67^{e}\pm16.50$ | Medium | $26.28^{a}\pm1.05$ | $73.72^{j}\pm1.05$ | intermediate |
| FARO 40 | $97.00^{a}\pm3.00$ | Soft | $22.43^{ef}\pm0.63$ | $77.57^{ef}\pm0.63$ | Intermediate |
| Igboukwu rice | $94.25^{a}\pm5.25$ | Soft | $20.11^{gh}\pm0.94$ | $79.89^{cd}\pm0.94$ | Low |
| Aguleri rice | $93.67^{a}\pm7.51$ | Soft | $19.20^{hi}\pm0.38$ | $80.80^{bc}\pm0.38$ | High |
| Taraba rice | $93.00^{a}\pm11.00$ | Soft | $26.04^{a}\pm0.15$ | $73.96^{j}\pm0.15$ | intermediate |
| B-G | $97.00^{a}\pm3.00$ | Soft | $23.74^{gh}\pm0.95$ | $76.26^{cd}\pm0.95$ | Intermediate |
| Ebonyi state | | | | | |
| Akpujie | $69.27^{cd}\pm9.25$ | Soft | $18.31^{cde}\pm0.38$ | $81.69^{fgh}\pm0.38$ | Low |
| kpurukpuru | $63.67^{cd}\pm2.52$ | Soft | $20.07^{ij}\pm0.65$ | $80.72^{ab}\pm0.65$ | Intermediate |
| Afikpo-Mas | $92.83^{a}\pm4.25$ | Soft | $25.30^{hi}\pm0.63$ | $74.70^{bc}\pm0.63$ | High |
| Abakiliki Mas | $27.00^{e}\pm2.00$ | Hard | $21.11^{ef}\pm1.25$ | $78.89^{ef}\pm1.25$ | Intermediate |
| R-8 | $33.67^{e}\pm0.58$ | Hard | $27.21^{j}\pm0.64$ | $79.29^{a}\pm0.64$ | High |
| 306 | $60.25^{d}\pm4.25$ | Medium | $23.14^{gh}\pm1.11$ | $76.86^{cd}\pm1.11$ | Intermediate |
| Geshua | $89.00^{ab}\pm12.00$ | Soft | $25.47^{def}\pm0.41$ | $74.53^{efg}\pm0.41$ | High |
| Mean | 78.70±22.60 | | 22.26±2.71 | 77.67±2.71 | |
| SE | 5.36 | | 0.50 | 0.50 | |
| $LSD_{0.05}$ | 15.32 | | 1.42 | 1.42 | |
| CV | 11.8 | | 3.9 | 1.1 | |

* Values are means± standard deviation of triplicate determination. Means in the same column carrying similar superscript are not significantly ($P > 0.05$) different. Soft = 61 -100 mm; medium = 41 -60 mm; hard =26
-40 mm Waxy Rice (1-2% amylose), Very low amylose content (2-9% amylose), Low amylose content (10-20% amylose), Intermediate amylose content (20-25% amylose), High amylose content (25-33% amylose).

for imported rice varieties. There is a positive correlation (0.76) between OCT and WUR which shows that an increase in WUR will increase cooking time. A reduced cooking time is desirable because less energy and fuel would be consumed during cooking. The minimum cooking time for all the rice varieties ranged from 15-43.33 minutes and the optimum cooking time ranged from 17-45.33 minutes. This is within the ranges reported by Thomas et al. (2013) and Oko et al. (2012). The extra-long and long grains cooked for a shorter time (17-38.67 minutes) compared to the medium grains, Kpurukpuru and Ghesua, that cooked for 40 and 45.33 minutes respectively which were also of intermediate and high amylose content respectively. Nigeria's medium grain rice can be described as harder cooked rice than its extra-long and long grain rice. This is in contrast to domestic rice in the United States where their extra-long and long grain rice are harder cooked rice than the short and medium grain rice (Webb, 1972).
Ip3 had the highest SLIW (0.20 g) compared to Fadama a local rice variety (0.17 g) though there was no significant ($p > 0.05$) difference amongst them. The SLIW of a large percentage (88.89 %) of the domestic rice varieties were minimal (0.03-0.13 %). This result was similar to values

reported by Yadav et al. (2007) for gruel solid losses of 0.025-0.52 g. Oko et al. (2012) also reported solids in cooking water of 0.01-0.95 g while Thomas et al. (2013) reported gruel solid loss of 0.03-0.06 g. The minimal values obtained for SLIW showed that there is less tendency of the grains to cling together when cooked.
The imported rice varieties had the highest VWA (26.00-27.67 ml) compared to a local rice (25.33 ml). All the domestic rice samples imbibed less water when cooked than the imported rice varieties. This is desirable and showed that local rice varieties conserve water better than the imported rice varieties. From the results of the VWA (Table 4), using the optimum cooking time the quantity of water required to cook the grains to its optimum level can be deduced.

### 3.3 Functional properties of domestic rice varieties

The functional properties of domestic rice varieties sold in South-East Nigeria are shown in Table 5. Rice varieties of the same amylose content are usually differentiated by their degree of gel consistency. Those of high amylose-soft gel consistency are more-tender than those of high amylose-hard gel consistency. Large percentages (77.78 %) of the domestic rice varieties were of soft gel consistency (63.67-97.00 mm) and did not differ significantly ($p > 0.05$) from the imported rice varieties (89.67-73.50 mm). Akpujie and Igboukwu-rice were of low amylose-soft gel consistency and will be suitable for use in baby foods and breakfast cereals because of their ability to produce relatively stable gels which tend to harden slowly during storage. Varieties with softer gel consistency are preferred for baby foods and cooked whole grains because of their higher degree of tenderness. Low amylose-soft gel consistency rice can also be used for popped rice and puffed rice as a result of its greater expansion volume (Juliano, 1971). In making fermented rice cakes, varieties with intermediate amylose-soft gel consistency are used such as Fortin 16, FARO 40, Taraba rice, B-G and Kpurukpuru because of their optimum volume expansion on steaming and their soft texture. R-Bus and 306 were of intermediate amylose-medium gel consistency and can find use in breakfast cereals as long as the water requirement is adjusted. Grains with high amylose-hard gel consistency find application in canned rice products and manufactured noodles. Abakiliki-Mas and R-8 should be used for canning purposes, rice-noodle manufacture and other high-temperature treatment processes because of their greater resistance to disintegration during cooking.

## 4 Conclusion

Akpujie had the highest cooked grain length (10.19 mm) among all the rice varieties. Geshua had the highest cooked grain width (3.70 mm), Akpujie had the highest cooked grain thickness (2.91 mm) and Ip1 had the highest cooked grain length-width ratio (3.52 mm). Geshua had the highest cooked grain weight (68.67 g). FARO 40 had the highest cooked grain volume (80.00 ml), Ip3 had the highest cooked grain density (1.93 g/ml$^3$) and Ip3 had the highest ER (1.50). Omor-mas and R-Bus had the highest VER (6.00) and Geshua had the highest WUR (3.63). Kpurukpuru and Geshua had the highest OCT of 45.33 and 40.00 minutes respectively while 83.33% of the local rice varieties had OCT of 17.00-29.33 mins. Ip3 had the highest SLIW (0.20 g) while the imported rice varieties had the highest VWA (26.00-27.67 ml). Procurement and consumption of local rice varieties is highly encouraged for individuals, restaurants and fast food joints as a better increased weight, high volume expansion ratio, increased grain dimensions and conservation of water during cooking is obtained when compared with rice imported into Nigeria from Thailand and USA. The domestic rice varieties also have an elongation ratio comparable to imported rice, therefore, the statement that imported rice varieties had better cooking quality than the domestic rice varieties is not absolutely true. Most of the domestic rice varieties were of soft gel consistency but varied in amylose content, and therefore are excellent not only for consumption as cooked whole grain but in their diversification for making breakfast cereals, baby foods, puffed rice, popped rice, rice cakes, canned soups, dry mixes and rice noodles which will eliminate food bore-

dom and stereotyped consumption. Abakiliki-Mas and R-8 should be used in making rice-noodles and subjected to other high-temperature processes which will limit over dependence on Durum wheat grain.

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# Estimation of the Dietary Exposure of Polycyclic Aromatic Hydrocarbons in Syria and Their Health Risks Assessment

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**Abstract**

In this work, the exposure of people, through their diet, to polycyclic aromatic hydrocarbons (PAHs) has been assessed for the urban, rural, and general populations in Syria. The food categories consumed have been divided into major groups, and the health risk assessment on dietary exposure of PAHs determined in each food category. For this purpose, two approaches were used: incremental lifetime cancer risk (ILCR) and margin of exposure approach (MOE). The results showed that each of the following food categories: oils and fats, meat and meat products, vegetables, and cereals dominantly contribute in the dietary exposure of PAHs. Also their MOE values are the lowest. Additionally, they have higher ILCR values. Therefore, these groups are a main risk source to health. On the other hand, the dietary exposure of PAHs in each of urban, rural and general populations was of low health concern, whereas their ILCR values reached to 10E-05 in total food categories, nevertheless it remains lower than serious risk level (ILCR > 10E-04). This work is the first study that is dealing with dietary exposure of PAHs and their health risk assessment in Syria.

***Keywords:*** Polycyclic aromatic hydrocarbons; Benzo[a]pyrene; Dietary exposure; Health risks assessment; Incremental lifetime cancer risk; Margin of exposure

## 1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are well-known ubiquitous organic pollutants that belong to the group of persistent organic pollutants (Halfadji, Touabet, Portet-Koltalo, Le Derf & Machour, 2017). They consist of carbon and hydrogen with two or more fused aromatic rings. PAHs are primarily formed and released from the incomplete combustion or pyrolysis of organic matter, during industrial, geochemical processes and other human activities (Yebra-Pimentel, Fernandez-Gonzalez, Martinez-Carballo & Simal-Gandara, 2015). Most PAHs have lipophilic and hydrophobic characteristics with low water solubility (Domingo & Nadal, 2015) and are generally found throughout the environment in air, water, soils, and sediments in the form of complex mixtures (Falco et al., 2003).

In toxicological studies, several PAHs have been demonstrated to be genotoxic and carcinogenic to humans. On the other hand, other PAHs that have not been found to be carcinogenic may act as synergists (Poster, Schantz, Sander & Wise, 2006). PAHs classification is based on their toxicity and a list of 16 PAHs issued by the U.S. Environmental Protection Agency (EPA) have been described as priority pollutants. The International Agency for Research on Cancer (IARC)

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has classified some of these PAHs as human carcinogens (International Agency for Research on Cancer, 2016). In the EU, a list of 15+1 EU priority PAHs was recently established. The Scientific Committee on Food (SCF) prioritized 15 PAHs and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (EFSA, 2008) identified one additional PAH.

Food consumption is by far the major source of exposure of humans to PAHs (Phillips, 1999). This contamination by PAHs is due to environmental pollution and/or as result of certain food processing methods (Gomez-Ruiz, Cordeiro, Lopez & Wenzl, 2009). On the other hand, the dietary intake of PAHs depends on both the contaminant concentration in food and the nutritional habits of the examined population. The presence of PAHs has been reported extensively in various food samples including: vegetable oils, fish and seafood, meats, bread, cereals, sweets, tea, coffee, chesses, milk, fruits and vegetables (Bansal & Kim, 2015; Phillips, 1999; Plaza-Bolanos, Garrido Frenich & Martinez Vidal, 2010; Zelinkova & Wenzl, 2015). However, studies concerning health risk assessment on dietary exposure of PAHs are quite limited (Bansal & Kim, 2015; Domingo & Nadal, 2015; Yebra-Pimentel et al., 2015). Two approaches were used to determine the health risk assessment on dietary exposure of PAHs. The first is the incremental lifetime cancer risk (ILCR). This classification was developed by the EPA and provides the cancer risk estimate for PAH mixtures relative to benzo[a]pyrene (BaP). The second approach is the margin of exposure (MOE), adopted by European Food Safety Authority (EFSA), which is the ratio between the no-observed-adverse-effect level or benchmark dose lower confidence limit (BMDL) for the critical effect to the theoretical, predicted, or estimated dose or concentration of human intake. Dietary exposure to PAHs and the corresponding health risk assessment have been reported in some countries. Among them and for this purpose, in Spain, series of surveys have been carried out in 2000, 2006, 2010, and 2012 (Falco et al., 2003; Marti-Cid, Llobet, Castell & Domingo, 2008; Martorell et al., 2012; Martorell et al., 2010). In the frame of the second French Total Diet Study (TDS), the 15+1 EU PAHs were analysed in 725 foodstuffs habitually consumed by the French population (Veyrand et al., 2013). Recently, the content of PAHs in most common consumed foodstuffs was determined in a market basket study made at the National Food Agency in Sweden (Abramsson-Zetterberg, Darnerud & Wretling, 2014). Furthermore in Korea, 27 different food commodities frequently consumed were analysed for the profile of 14 PAH congeners (Yoon, Park, Lee, Yang & Lee, 2007). While in China, 25 kinds of seven categories of foods were analysed for determination of the concentrations of 16 PAHs (Xia et al., 2010). In another study, 16 PAHs in 24 duplicate-diet samples were also determined (Nie et al., 2014). In Azerbaijan, due to lack of PAHs concentration data from middle-Eastern countries, only data from European countries were adopted (Nwaneshiudu et al., 2007). Finally, in recent study, cancer risks of long-term exposure to PAHs through consumption of major food categories in India for eight societal groups were evaluated (Singh & Agarwal, 2018). Notable absence of such data from big countries such as Australia, Canada, Germany, or Japan (for example), among many others has been highlighted (Domingo & Nadal, 2015). On the other hand, in previously studies, the levels of 16 EPA PAHs in medicinal plants from Syria and in Syrian olive oils have been reported (Krajian & Odeh, 2013, 2014). In addition, the levels of 15+1 EU PAHs in different edible oils, available on the Syrian market, were determined (Krajian & Odeh, 2018). However, there is no study dealing with health risk assessment on dietary exposure of PAHs in Syria. The current work will investigate the health risk assessment on dietary exposure of PAHs in Syria. The dietary exposure of PAHs was estimated, for both urban and rural populations and the incremental lifetime cancer risks were calculated. The margin of exposure was evaluated to observe whether the local levels posed any potential health problem for Syrian consumers.

## 2 Materials and Methods

### 2.1 Dietary exposure estimates

Target population was divided into three main groups (urban, rural, and general). Food

categories included: meat and meat products, fish and shellfish, vegetables, tubers, fruits, eggs, milk, dairy products, cereals, pulses, oils and fats, and industrial bakery. The PAHs studies were the 16 EPA PAHs namely: naphthalene (NAP), acenaphthylene (ACY), acenaphthene (ACP), fluorene (FLR), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), benz[a]anthracene (BaA), chrysene (CHR), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), BaP, dibenz[a,h]anthracene (DahA), benzo[ghi]perylene (BgP), and indeno[1,2,3-cd]pyrene (IcP). Data on food consumption, for the year of 2009, was obtained from the Syrian State Central Bureau of Statistics. PAHs concentration data for each food category were obtained from other study (Martorell et al., 2010) due the lack of such data from Syria. The body weight for a typical Syrian individual (67.4 kg) was obtained from the study by Walpole et al. (2012). Dietary exposures to PAHs (the sum of 16 EPA PAHs), PAH8 (the sum of eight genotoxic PAHs), PAH4 (the sum of the four PAHs namely: CHR, BaA, BaP, and BbF), PAH2 (the sum of CHR and BaP), and BaP were estimated for each food category. The exposure levels were obtained by multiplying the corresponding concentration of each PAHs by the amount of food consumption by individuals per day (expressed in units of mass per unit time, or mass per unit time normalized to body weight).

## 2.2 Cancer risk estimates

The BaP equivalent value for individual PAHs ($BaP_{eqi}$) was calculated for each PAH from its concentration in each food category multiplied by its toxic equivalency factor as proposed by Nisbet and Lagoy (1992). The BaP equivalent value for the mixture of 16 EPA PAHs ($BaP_{eq}$) was calculated as the sum of the $BaP_{eqi}$ values in each food category. Dietary exposures of $BaP_{eq}$ for each food category (Ei) were calculated in the same way as described previously. The incremental lifetime cancer risk (ILCR) of each population groups in Syria were estimated to express the risk caused by PAHs dietary exposure using the following equation (1):

$$ILCR = \frac{Ei \times ED \times EF \times SF}{(BW \times AT)} \quad (1)$$

where: ED is the exposure duration (43 year), EF is the exposure frequency (365 days $year^{-1}$), SF is the oral cancer slope factor of BaP (4.5, 5.9, 9.0, and 11.7, with a geometric mean of 7.3 mg $kg^{-1}$ $day^{-1}$), BW is the body weight (kg), and AT is the average lifespan for carcinogens (25550 days) (Xia et al., 2010).

## 2.3 Margin of exposure

The risk associated with the dietary exposure of PAHs was evaluated using the approach based on the margin of exposure (MOE). MOE values of BaP, PAH2, PAH4, and PAH8 were calculated using the ratio between the EFSA $BMDL_{10}$ corresponding values (benchmark dose lower confidence limit for a 10 % increase in the background incidence of tumors in bearing animals) and dietary exposure of BaP, PAH2, PAH4, and PAH8. The $BMDL_{10}$ values for BaP, PAH2, PAH4 and PAH8 are 0.07, 0.17, 0.34 and 0.49 mg $kg^{-1}$ bw $day^{-1}$, respectively (EFSA, 2008).

## 2.4 Statistical analysis

Statistical analyses were performed using OriginPro 9.2 software. The non-parametric Kruskal-Wallis test was applied to assess statistically significant differences in ILCR's results relative to exposure for different food categories among urban, rural, or general populations. A $p$-value of $< 0.05$ was considered statistically significant.

# 3 Results and Discussion

The food categories consumed have been divided into major groups as shown in Table 1, where we also derived the average amount of food categories consumption per capita for the urban, rural, and general populations of Syria. Table 2 shows the concentration values of PAHs (the sum of 16 EPA PAHs), PAH8 (the sum of eight genotoxic PAHs), PAH4 (the sum of the four PAHs namely: CHR, BaA, BaP, and BbF), PAH2 (the sum of

Table 1: Food categories and their average consumption per capita for urban, rural, and general populations of Syria

| Food Category | Average consumption per capita (g day$^{-1}$) | | |
|---|---|---|---|
| | Urban | Rural | General |
| Meat and meat products | 71 | 65 | 68 |
| Fish and shellfish | 22 | 24 | 23 |
| Vegetables | 326 | 350 | 338 |
| Tubers | 87 | 111 | 99 |
| Fruits | 108 | 91 | 100 |
| Eggs | 24 | 22 | 23 |
| Milk | 40 | 53 | 47 |
| Dairy products | 107 | 94 | 101 |
| Cereals | 470 | 564 | 517 |
| Pulses | 30 | 33 | 31 |
| Oils and fats | 66 | 76 | 71 |
| Industrial bakery | 10 | 8 | 9 |
| Total | 1361 | 1491 | 1427 |

Table 2: Concentrations values of studied PAHs in the studied food categories

| Food Category | PAHs Concentrations (ng g$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | BaP | PAH2 | PAH4 | PAH8 | PAHs | $BaP_{eq}$ |
| Meat and meat products | 0.14 | 0.69 | 1.45 | 1.70 | 39.0 | 0.33 |
| Fish and shellfish | 0.07 | 0.26 | 0.57 | 0.86 | 2.85 | 0.16 |
| Vegetables | 0.07 | 0.12 | 0.23 | 0.36 | 1.19 | 0.11 |
| Tubers | 0.02 | 0.04 | 0.08 | 0.16 | 0.75 | 0.05 |
| Fruits | 0.02 | 0.04 | 0.08 | 0.16 | 0.78 | 0.05 |
| Eggs | 0.10 | 0.20 | 0.40 | 0.80 | 3.68 | 0.25 |
| Milk | 0.01 | 0.02 | 0.04 | 0.08 | 0.45 | 0.02 |
| Dairy products | 0.05 | 0.10 | 0.20 | 0.40 | 7.61 | 0.13 |
| Cereals | 0.03 | 0.06 | 0.12 | 0.24 | 1.24 | 0.07 |
| Pulses | 0.04 | 0.08 | 0.16 | 0.32 | 1.52 | 0.1 |
| Oils and fats | 0.49 | 0.98 | 1.96 | 3.92 | 18.8 | 1.21 |
| Industrial bakery | 0.03 | 0.06 | 0.13 | 0.25 | 1.38 | 0.08 |
| Total | 1.07 | 2.65 | 5.42 | 9.25 | 79.3 | 2.56 |

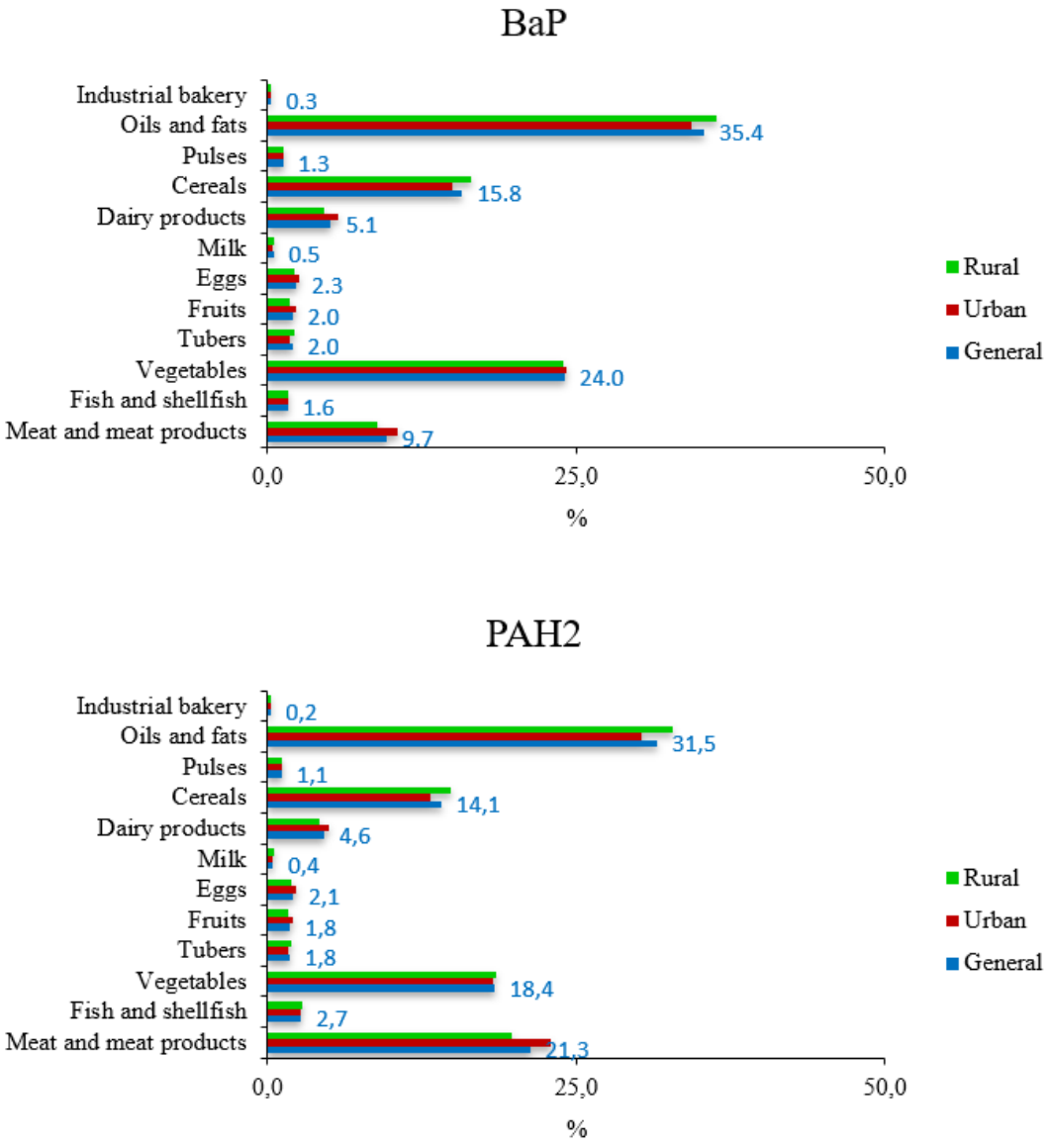


Figure 1: Contribution ratios of dietary exposures to studied BaP and PAH2 in the food categories for urban, rural, and general populations in Syria

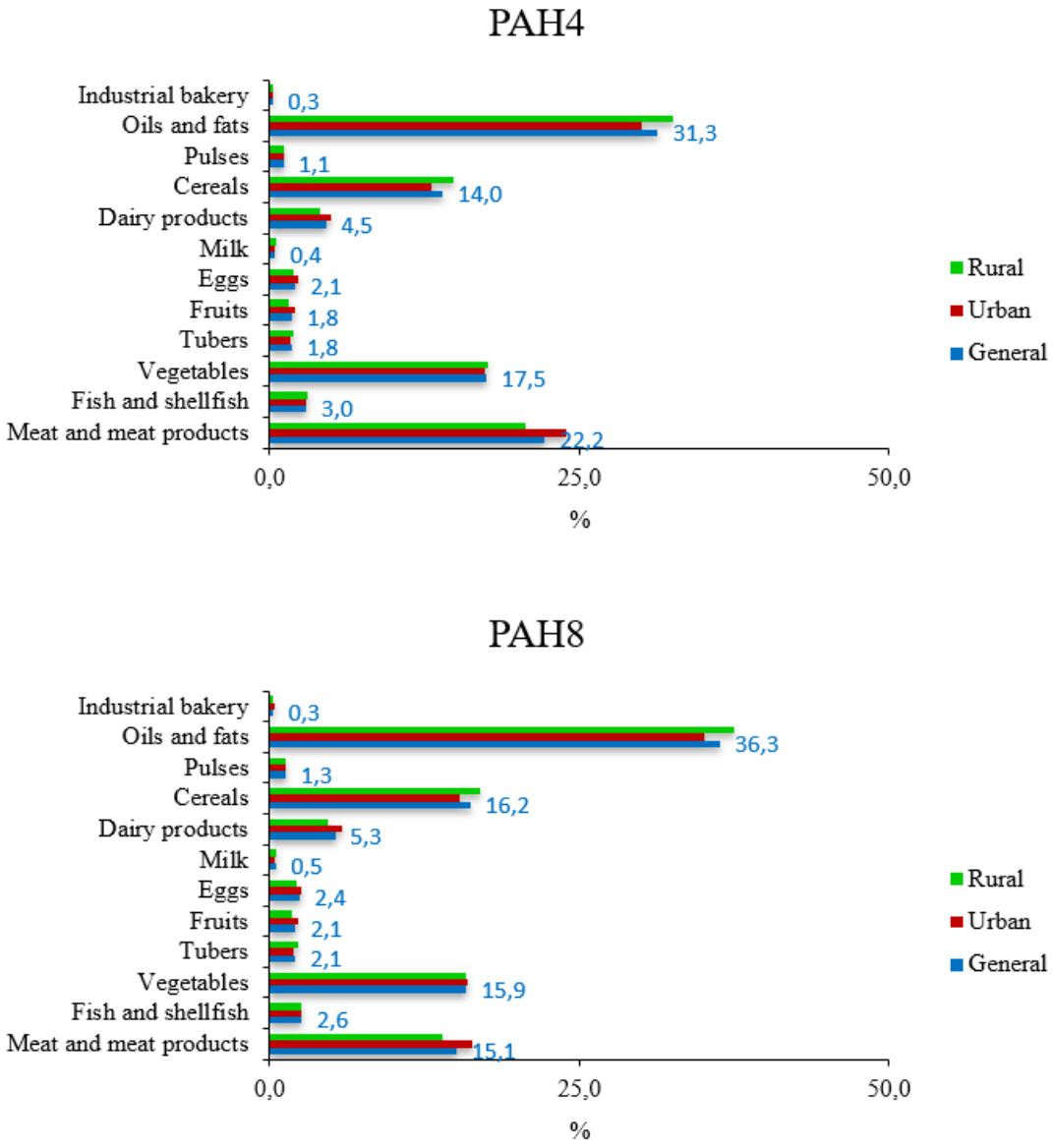


Figure 2: Contribution ratios of dietary exposures to studied PAH4 and PAH8 in the food categories for urban, rural, and general populations in Syria

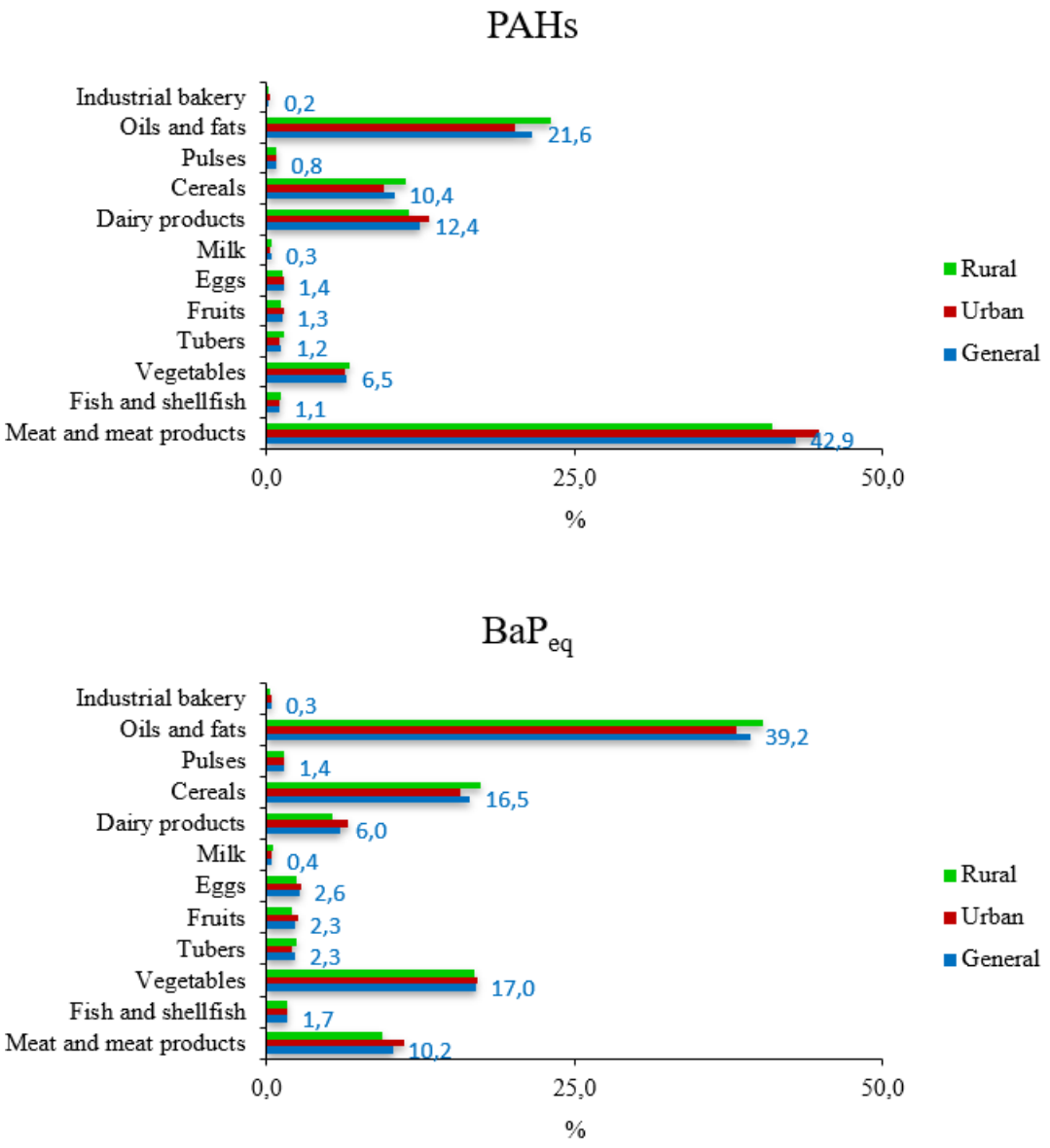


Figure 3: Contribution ratios of dietary exposures to studied PAHs and $BaP_{eq}$ in the food categories for urban, rural, and general populations in Syria

Table 3: Dietary exposure to studied PAHs for urban population in Syria

| Food Category | Dietary exposure of PAHs ng day$^{-1}$ (ng kg$^{-1}$ bw day$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | BaP | PAH2 | PAH4 | PAH8 | PAHs | BaPeq |
| Meat and meat products | 10 (0.15) | 49 (0.73) | 103 (1.53) | 121 (1.79) | 2767 (41.1) | 23 (0.35) |
| Fish and shellfish | 2 (0.02) | 6 (0.08) | 13 (0.19) | 19 (0.28) | 63 (0.93) | 4 (0.05) |
| Vegetables | 23 (0.34) | 39 (0.58) | 75 (1.11) | 117 (1.74) | 388 (5.76) | 36 (0.53) |
| Tubers | 2 (0.03) | 3 (0.05) | 7 (0.10) | 14 (0.21) | 65 (0.97) | 4 (0.06) |
| Fruits | 2 (0.03) | 4 (0.06) | 9 (0.13) | 17 (0.26) | 84 (1.25) | 5 (0.08) |
| Eggs | 2 (0.04) | 5 (0.07) | 10 (0.14) | 19 (0.28) | 88 (1.31) | 6 (0.09) |
| Milk | <1 (0.01) | 1 (0.01) | 2 (0.02) | 3 (0.05) | 18 (0.27) | 1 (0.01) |
| Dairy products | 5 (0.08) | 11 (0.16) | 21 (0.32) | 43 (0.64) | 814 (12.1) | 14 (0.21) |
| Cereals | 14 (0.21) | 28 (0.42) | 56 (0.84) | 113 (1.67) | 583 (8.65) | 33 (0.49) |
| Pulses | 1 (0.02) | 2 (0.04) | 5 (0.07) | 10 (0.14) | 46 (0.68) | 3 (0.04) |
| Oils and fats | 32 (0.48) | 65 (0.96) | 129 (1.92) | 259 (3.84) | 1239 (18.4) | 80 (1.18) |
| Industrial bakery | <1 (<0.01) | 1 (0.01) | 1 (0.02) | 3 (0.04) | 14 (0.20) | 1 (0.01) |
| Total | 94 (1.40) | 214 (3.17) | 431 (6.39) | 737 (10.93) | 6169 (91.5) | 210 (3.11) |

CHR and BaP), BaP, and $BaP_{eq}$ (BaP equivalent value for the mixture of 16 EPA PAHs) in the studied food categories. These values were calculated based on the data of PAHs in the study of Martorell et al. (2010). According to the food categories (Table 2) the oils and fats have the highest concentration values of PAH8, PAH4, PAH2, BaP, and $BaP_{eq}$. The only exception noticed is for PAHs, where the meat and meat products category has the highest concentration values followed by the oils and fats food categories. The contributions of the oils and fats in total studied food categories ranged between 23.7 % for PAHs to 47.3 % for $BaP_{eq}$. On the other hand, the milk category has the lowest concentration values with average contribution of 0.8 %. The high concentrations of PAHs in oils and fats food category might be due to the lipophilic nature of PAHs and consequently, this food category can be easily contaminated by PAHs either directly or indirectly as an ingredient in food (Dennis et al., 1991; Krajian & Odeh, 2018). Tables 3, 4, and 5 report the dietary exposures to BaP, PAH2, PAH4, PAH8, BaP, PAHs, and $BaP_{eq}$ for urban, rural, and general populations in Syria, respectively. It was noticed that there are some comparable values of PAHs dietary exposures between urban and rural populations. Indeed, the total PAHs dietary exposures for the rural population are relatively higher than for urban population, taking into account that the differences in the values of

Table 4: Dietary exposure to studied PAHs for rural population in Syria

| Food Category | Dietary exposure of PAHs ng day$^{-1}$ (ng kg$^{-1}$ bw day$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | BaP | PAH2 | PAH4 | PAH8 | PAHs | BaPeq |
| Meat and meat products | 9 (0.14) | 45 (0.67) | 94 (1.40) | 111 (1.64) | 2533 (37.6) | 21 (0.32) |
| Fish and shellfish | 2 (0.02) | 6 (0.09) | 14 (0.20) | 21 (0.31) | 68 (1.01) | 4 (0.06) |
| Vegetables | 25 (0.36) | 42 (0.62) | 81 (1.19) | 126 (1.87) | 417 (6.18) | 39 (0.57) |
| Tubers | 2 (0.03) | 4 (0.07) | 9 (0.13) | 18 (0.26) | 83 (1.24) | 6 (0.08) |
| Fruits | 2 (0.03) | 4 (0.05) | 7 (0.11) | 15 (0.22) | 71 (1.05) | 5 (0.07) |
| Eggs | 2 (0.03) | 4 (0.07) | 9 (0.13) | 18 (0.26) | 81 (1.20) | 6 (0.08) |
| Milk | 1 (0.01) | 1 (0.02) | 2 (0.03) | 4 (0.06) | 24 (0.35) | 1 (0.02) |
| Dairy products | 5 (0.07) | 9 (0.14) | 19 (0.28) | 38 (0.56) | 715 (10.6) | 12 (0.18) |
| Cereals | 17 (0.25) | 34 (0.50) | 68 (1.00) | 135 (2.01) | 699 (10.4) | 39 (0.59) |
| Pulses | 1 (0.02) | 3 (0.04) | 5 (0.08) | 11 (0.16) | 50 (0.74) | 3 (0.05) |
| Oils and fats | 37 (0.55) | 74 (1.11) | 149 (2.21) | 298 (4.42) | 1427 (21.2) | 92 (1.36) |
| Industrial bakery | <1 (<0.01) | <1 (0.01) | 1 (0.02) | 2 (0.03) | 11 (0.16) | 1 (0.01) |
| Total | 102 (1.52) | 227 (3.37) | 457 (6.78) | 795 (11.8) | 6180 (91.7) | 228 (3.38) |

dietary exposures between urban and rural populations in this study were caused by variations in the average amount of food categories consumption per capita. These values could change if the concentrations of PAHs in food categories for urban and rural were taken into account. Generally, high values of dietary exposure to PAHs in Syria were observed (6178 ng day$^{-1}$). For comparison, in UK it was equal to 3700 ng day$^{-1}$ (Phillips, 1999), while in China it was equal to 1830 ng day$^{-1}$ (Yu et al., 2015), whereas in Korea it was equal to 198 ng day$^{-1}$ (Yoon et al., 2007). Moreover, the dietary exposure to BaP were equal to 76, 13, and 2 ng day$^{-1}$ in Spain (Martorell et al., 2010), France (Veyrand et al., 2013), and China (Nie et al., 2014), respectively, against 98 ng day$^{-1}$ in this study (Syria). Concerning PAH4, it was equal to 104 ng day$^{-1}$ in France (Veyrand et al., 2013) against 444 ng day$^{-1}$ in this study (Syria). Finally, for BaP$_{eq}$, it was equal to 572 ng day$^{-1}$ in China (Xia et al., 2010), while in this study (Syria) it was equal to 219 ng day$^{-1}$ . The contribution ratios of dietary exposures to studied PAHs in the food categories for urban, rural, and general populations in Syria are highlighted in Figure 1, 2 and 3. We found that the oils and fats category has the highest contribution in dietary exposures to BaP, PAH2, PAH4, PAH8, BaP, and BaP$_{eq}$ but not for PAHs (the meat and meat products 42.9 %), followed by the meat and meat products, the vegetables, and cereals categories. The sum of these food

Table 5: Dietary exposure to studied PAHs for general population in Syria

| Food Category | Dietary exposure of PAHs ng $day^{-1}$ (ng $kg^{-1}$ bw $day^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | BaP | PAH2 | PAH4 | PAH8 | PAHs | $BaP_{eq}$ |
| Meat and meat products | 10 (0.14) | 47 (0.70) | 99 (1.46) | 116 (1.72) | 2650 (39.3) | 22 (0.33) |
| Fish and shellfish | 2 (0.02) | 6 (0.09) | 13 (0.19) | 20 (0.29) | 66 (0.97) | 4 (0.05) |
| Vegetables | 24 (0.35) | 41 (0.60) | 78 (1.15) | 122 (1.81) | 402 (5.97) | 37 (0.55) |
| Tubers | 2 (0.03) | 4 (0.06) | 8 (0.12) | 16 (0.24) | 74 (1.10) | 5 (0.07) |
| Fruits | 2 (0.03) | 4 (0.06) | 8 (0.12) | 16 (0.24) | 78 (1.16) | 5 (0.07) |
| Eggs | 2 (0.03) | 5 (0.07) | 9 (0.14) | 18 (0.27) | 85 (1.26) | 6 (0.09) |
| Milk | <1 (<0.01) | 1 (0.01) | 2 (0.03) | 4 (0.06) | 21 (0.31) | 1 (0.01) |
| Dairy products | 5 (0.07) | 10 (0.15) | 20 (0.30) | 40 (0.60) | 769 (11.4) | 13 (0.19) |
| Cereals | 16 (0.23) | 31 (0.46) | 62 (0.92) | 124 (1.84) | 641 (9.51) | 36 (0.54) |
| Pulses | 1 (0.02) | 2 (0.04) | 5 (0.07) | 10 (0.15) | 47 (0.70) | 3 (0.05) |
| Oils and fats | 35 (0.52) | 70 (1.03) | 139 (2.06) | 278 (4.13) | 1333 (19.8) | 86 (1.27) |
| Industrial bakery | <1 (<0.01) | 1 (0.01) | 1 (0.02) | 2 (0.03) | 12 (0.18) | 1 (0.01) |
| Total | 98 (1.46) | 221 (3.27) | 444 (6.59) | 766 (11.4) | 6178 (91.7) | 219 (3.25) |

categories contributes to more than 80 % of the total dietary exposures. Whereas, the proportion of each milk and the industrial bakery categories do not exceed 0.5 %. Similar results have been obtained in other studies (Martorell et al., 2010; Phillips, 1999; Veyrand et al., 2013). The incremental lifetime cancer risk (ILCR) of each population groups in Syria through consumption of each studied food categories were calculated (Table 6) in order to study the risk caused by PAHs dietary exposure. According to the EPA, a one in a million chance of additional human cancer over a 70 year lifetime (ILCR = 10E-06) is the level of risk considered acceptable or inconsequential, whereas additional lifetime cancer risk of one in ten thousand or greater (ILCR = 10E-04) is considered serious. We noticed that the ILCR values for all studied food categories for all population groups fell within the range 10E-06 to 10E-08, where they exceeded the acceptable or inconsequential risk level (ILCR > 10E-06) in each of the oils and fats, the cereals, the vegetables, and the meat and meat products categories. Whereas, it reached 10E-05 in total food categories. However, it remained lower than serious risk level (ILCR > 10E-04). On the other hand, there are no statistically significant differences in ILCR's results relatively to exposure for different food categories among urban, rural, or general populations with relatively higher risk values for rural population. Comparing the risk values in this study with

Table 6: Incremental lifetime cancer risk (ILCR) in each studied food categories through dietary exposures to PAHs for urban, rural, and general populations in Syria

| Food Category | Incremental lifetime cancer risk (ILCR)[a] | | |
|---|---|---|---|
| | Urban | Rural | General |
| Meat and meat products | 1.58x10E-06 | 1.45x10E-06 | 1.52x10E-06 |
| Fish and shellfish | 2.38x10E-07 | 2.60x10E-07 | 2.49x10E-07 |
| Vegetables | 2.33x10E-06 | 2.50x10E-06 | 2.42x10E-06 |
| Tubers | 2.86x10E-07 | 3.65x10E-07 | 3.26x10E-07 |
| Fruits | 3.55x10E-07 | 3.00x10E-07 | 3.29x10E-07 |
| Eggs | 3.94x10E-07 | 3.62x10E-07 | 3.78x10E-07 |
| Milk | 6.61x10E-08 | 8.76x10E-08 | 7.77x10E-08 |
| Dairy products | 9.21x10E-07 | 8.09x10E-07 | 8.69x10E-07 |
| Cereals | 2.32x10E-06 | 2.79x10E-06 | 2.56x10E-06 |
| Pulses | 1.97x10E-07 | 2.17x10E-07 | 2.04x10E-07 |
| Oils and fats | 5.32x10E-06 | 6.13x10E-06 | 5.72x10E-06 |
| Industrial bakery | 5.02x10E-08 | 4.02x10E-08 | 4.52x10E-08 |
| Total | 1.41x10E-05 | 1.53x10E-05 | 1.47x10E-05 |

[a]The ILCRs relatively to exposure for different food categories among urban, rural, or general populations are not significantly different ($p$>0.05)

other studies in different parts of the world, similar trends were observed. The risk of PAHs exposure from ingested food in Azerbaijan ranged between 9.34x10E-05 to 3.67x10E-04 (Nwaneshiudu et al., 2007). The cancer risk faced by Indian population through their complete diet ranged from 7.63x10E-10 to 5.05 (Singh & Agarwal, 2018). The duplicate-diet study in China has estimated cancer risk values of 9.07x10E-04 to 1.12x10E-04 (Nie et al., 2014). Whereas, in Spain the cancer risk of 4.5x10E-06 was calculated for male adults (Martorell et al., 2010). Finally in Korea, the cancer risk posed by food ingestion was reported as 2.3x10E-05 (Yoon et al., 2007). The risk associated with the dietary exposure of PAHs was evaluated following the approach based on the margin of exposure (MOE). The EFSA used the MOE as a new approach to risk assessment for genotoxic and carcinogenic PAHs (EFSA, 2008). The values of MOEs that are close to or less than 10000 indicate a potential concern for consumer health and a possible need for risk management action. For total dietary exposures, the MOE values of BaP, PAH2, PAH4, and PAH8 were equal to (50000, 53600, 53200, and 44800) for urban population, (46000, 50400, 50100, and 41600) for rural population, and (47900, 51900, 51600, and 43100) for general population, respectively. The lowest MOE values of BaP, PAH2, PAH4, and PAH8 for all population groups were observed for oils and fats category followed by the vegetables, the cereals, and the meat and meat products categories. On the other hand, the MOE values for urban population were higher than those for rural population. However, the results of MOE values in this study (> 10000) indicated that the dietary exposure of PAHs for urban, rural, and general populations was considered a low concern for consumer health. Again, similar trends were observed when comparing the values of MOEs with the results from other studies in different parts of the world. The MOE values for PAH4 calculated for exposure of the French population to PAHs through the whole diet were 150000 for children and 230000 for adults (Veyrand et al., 2013). In a market basket study carried out at the National Food Agency in Sweden, the MOE value of BaP was about 100000 (Abramsson-Zetterberg et al., 2014).

The results obtained from both approaches (ILCR and MOE) highlighted that the following categories namely: oils and fats, cereals, vegetables, and the meat and meat products have the major contribution in the dietary exposure of PAHs. The reason is due to the high concentrations of PAHs in the oils and fats and the meat and meat products categories, while in the cereals and the vegetables categories are resulting from high amount of consumption. Consequently, these categories have the highest ILCR values and the lowest MOE values, indicating that they represent the main risk source to health.

## 4 Conclusions

This is the first study dealing with dietary exposure of PAHs and their health risk assessment in Syria. The results indicated that the dietary exposure of PAHs for urban, rural, and general populations was of low concern for consumer health. The cancer risk values reached 10E-05 in total food categories remaining lower than serious risk level (ILCR > 10E-04). However, future studies should be carried out to monitor the PAHs levels in food product samples from local markets, which are absent in Syria. Furthermore, the dietary exposure of PAHs and the associated risk values for children and adults are needed to establish maximum limits or guideline levels for PAHs in food products. These values should be included in the national standards, to reduce health risks posed by dietary exposure of PAHs in the Syrian population.

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# Physicochemical and Sensory Characteristics of Green Coconut (*Cocos nucifera* L.) Water Kefir

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**Abstract**

This research aims to examine the effects of fermentation time on the physicochemical and sensory characteristics of green coconut water kefir in order to determine the optimal fermentation time based on the resulting sensory attributes. There were four fermentation time treatments (12, 24, 36, and 48 hours), each with five replications. The materials used were green coconut water and 5 % kefir grains. Physical analyses included pH and viscosity, while the chemical analyses included total dissolved solids (TDS), alcohol content, water content, protein content and fat content. Sensory attributes included sourness, soda sensation, sour aroma, viscosity and turbidity. The results showed that fermentation time had significant effects on pH, TDS, alcohol content, water content, protein content and the sensory attributes of green coconut water kefir. Viscosity and fat content were not affected by fermentation time. The ideal fermentation time was 12 hours resulting in a pH level of 4.6, viscosity of 0.09, TDS of 3.8$^o$ Brix, alcohol content of 1.16%, water content of 97.14 %, protein content of 6.64 % and fat content of 1.17%. Sensory evaluation found a low level of sourness, low soda sensation, high sour aroma, high viscosity and low turbidity.

***Keywords:*** Fermentation; Green coconut; Physicochemical; Sensory; Water kefir

## 1 Introduction

Coconut (*Cocos nucifera*) is one of the palm tree species which is widely cultivated in tropical regions, especially in areas near beaches (Chidambaram, Singaraja, Prasanna, Ganesan & Sundararajan, 2013). There are many varieties of coconut such as green dwarf, yellow dwarf and red dwarf. Green dwarf or green coconut (*C. nucifera* L.) is the most utilized variety of coconut due to its high content of total phenols and ascorbic acid (Santos et al., 2013). Indonesia is the largest producer of green coconut in the world, with the highest diversity (Kailaku, Syah, Risfaheri, Setiawan & Sulaeman, 2015; Pandin, 2015). The edible part of the fruit consists of coconut meat and coconut water (Yong, Ge, Ng & Tan, 2009). Recently, the green coconut water market has grown rapidly in the functional beverages category due to its hydration qualities (Marsh, Hill, Ross & Cotter, 2014). Furthermore, green coconut water contains micronutrients such as inorganic ions and vitamins that are beneficial in promoting the human body's antioxidant system (Evans & Halliwell, 2001; Yong et al., 2009), antimicrobial peptides (Mandal et al., 2009), catechins and epicatechins (Chang & Wu, 2011). Green coconut water is also a rich

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source of cytokinin that has antiaging properties in human skin cells (Ge et al., 2006).
Green coconut water is widely consumed in its natural form (Franco, Yamamoto, Tadini & Gut, 2015) or in a processed, ready-to-drink beverage form (Santana, Ribeiro & Iguti, 2011). Heat treatment is used in commercial coconut water manufacture so as to prevent microbial spoilage and oxidative enzymatic (Tan, Cheng, Bhat, Rusul & Easa, 2014). However, heat treatment often leads to changes in the product's organoleptic and nutritional quality (Cappelletti et al., 2015). Therefore, an innovative approach is required to develop new products based on green coconut water.
Fermentation is one of food preservation methods which can improve the nutritional value of food (Marsh et al., 2014). Green coconut water contains sugars, proteins, free amino acids and minerals (Flávera et al., 2015) thus it is possible to process it into a fermented non-dairy beverage such as water kefir. Water kefir is obtained by fermenting water sucrose with kefir grains containing lactic acid bacteria and yeast (Marsh, O'Sullivan, Hill, Ross & Cotter, 2013), resulting in a beverage with effervescent characteristics (Ismaiel, Ghaly & El-Naggar, 2011; Liu & Lin, 2000).
The characteristics of a fermented product are influenced by fermentation time. As there was no reported work, it was necessary to analyze the effect of fermentation time on the physicochemical and sensory properties of green coconut water kefir. Therefore, this research aims to establish the optimal fermentation time for green coconut water kefir by assessing the physicochemical and sensory characteristics.

## 2 Materials and Methods

### 2.1 Materials

Five liters of commercially available coconut water was obtained at a Mulawarman Street store in Semarang, Central Java. Kefir grains were obtained from the beadsnik online shop located in Denpasar. Selenium, sulfuric acid, 4 % $H_3BO_3$, Methyl Red (MR) and Methyl Blue (MB), Aquadest, 45 % NaOH, 0.1N HCl, 91 % $H_2SO_4$, and amyl alcohol were used. Porcelain dishes, an oven (Memmert, Germany), Kjeldahl flasks (Pyrex, Japan) were also used.

### 2.2 Kefir preparation from green coconut water

The method used to produce green coconut water kefir was adapted from Lestari, Bintoro and Rizqiati (2018). The green coconut water was pasteurized for 30 seconds at 60 $^o$C, poured into jars and then cooled to 28 $^o$C. Kefir grains were added to the jars at 5 % (w/v) to begin the fermentation process. The kefir samples were treated with different fermentation times, which were 12 hours (T1), 24 hours (T2), 36 hours (T3) and 48 hours (T4). The coconut kefir was then filtered to separate the grains from the coconut water before the specified testing.

### 2.3 Physicochemical properties

#### pH analysis

The sample pH was measured using a pH meter (AOAC, 2013). The pH meter was calibrated with standard buffers (pH 4.0 and pH 7.0) just prior to use.

#### Viscosity analysis

The Ostwald viscometer was calibrated with deionized water. The mass of the pycnometer was weighed with an analytical balance, then again containing 10 mL of water and finally containing 10 mL of each sample. The time taken for each sample to drain by gravity between two etched marks of the Ostwald viscometer was measured. The viscosity of each sample was calculated according to the equation below (Fathima, Devi, Rekha & Dhathathreyan, 2009):

$$\eta_s = \eta_w \cdot \frac{t_s}{t_w} \cdot \frac{m_{p+s} - m_p}{m_{p+w} - m_p} \quad (1)$$

$\mathbf{m}_p$ : Mass of pycnometer (g)

$\mathbf{m}_{p+s}$ : mass of pycnometer + filled volume of the sample (g)

$\mathbf{m}_{p+w}$ : mass of pycnometer + filled volume of water (g)

$\eta_w$ : water viscosity (cP)

$\eta_s$ : sample viscosity (cP)

$\mathbf{t}_s$ : drain time for sample (s)

$\mathbf{t}_w$ : drain time for water (s)

## Alcohol content

The alcohol content was measured by distillation and a pycnometer (AOAC, 2013). The samples (50 mL) were placed in a Kjeldahl flask and 100 mL aquadest was then added. The distillation process occurred at 80 $^o$C and the distillate was collected in an Erlenmeyer flask. Fifty mL of the distillate was transferred to a pycnometer. Excessive distillate was removed from the top of the capillary tube of the pycnometer. The distillate-filled pycnometer was then weighed. The same procedure was repeated for aquadest. The density of alcohol was calculated using the formula below:

$$\rho = \frac{m_{p+d} - m_p}{m_{p+a} - m_p} \quad (2)$$

$\rho$ ; Alcohol density (g/cm$^3$)

$\mathbf{m}_p$ : Mass of pycnometer (g)

$\mathbf{m}_{p+d}$ : mass of pycnometer + filled volume of the distillate (g)

$\mathbf{m}_{p+a}$ : mass of pycnometer + filled volume of aquadest (g)

The alcohol content was then obtained using the conversion table for alcohol.

## Total Dissolved Solids (TDS)

Total Dissolved Solid (TDS) was measured by a hand-held refractometer (AOAC, 1995). Three drops of aquadest were added to the prism of the refractometer and then wiped off with tissue paper. Three drops of a sample were then added to the cleaned prism, and the lid shut properly. The scale was read at a bright room condition. It showed the percentage of Total Dissolved Solids according to the International Sugar Scale of 1936 in $^o$Brix unit. The prism of the refractometer was rinsed off again with tissue paper, before the next sample was measured.

## Water content

Water content was measured by oven-drying. Empty porcelain dishes were dried in the oven at 105 $^o$C for 4 hours, and then weighed using an analytical balance. Two grams of each sample was weighed out onto each dish, which were placed in the oven and dried at 105 $^o$C for 4 hours. The dishes were then transferred, with partially covered lids, to the desiccator to cool down. The dishes and their dried samples were reweighed. The water content was calculated using the following formula based on AOAC (2005):

$$\text{Water content}(\%) = \frac{B - (C - A)}{B} \times 100 \quad (3)$$

**A** : container's weight (g)

**B** : sample's initial weight (g)

**C** : container's and sample's weight after drying (g)

## Fat content

Fat content was measured using the Gerber method (AOAC, 2002). A butyrometer was filled with 10 mL of sulfuric acid. Eleven mL of a sample and 1 mL of amyl alcohol were placed into the butyrometer. The tube was sealed with a rubber stopper and shaken until the sample was dissolved. The solution was then centrifuged for 15 minutes at 1200 rpm and transferred from the butyrometer into a water bath at 60-63 $^o$C. The

solution was immersed, leaving only the small bulb exposed. The fat column was equilibrated for 5 minutes or longer. The scale on the tube of the butyrometer was read to indicate the fat content of the sample.

### Protein content

The protein content of a sample was determined based on the total nitrogen content using the Kjeldahl method. A half gram of sample was weighed and placed into the Kjeldahl flask. A half mL of selenium and 10 mL of sulfuric acid were then added to the flask. The resulting solution was digested until a clear-green color was achieved. The digested sample was then distilled. The trap which contains 5 mL 4 % $H_3BO_4$, two drops of MR and two drops of MB was placed below the distiller. The sample along with 100 mL aquadest and 40 ml 45 % NaOH were added sequentially into the distillation flask. The stove was switched on and the distillation process was allowed to proceed until the trap changed its color from purple to green. Forty mL of distillate was obtained. For the blank control, the same procedure was repeated using 200 mL of aquadest. The distillate was titrated using 0.1 N HCl until the color turned to purple. The protein content was calculated using the following formula based on AOAC (2000):

$$Protein(\%) = \frac{(titrant - blank) \cdot NHCL \cdot 14.008 \cdot 6.25}{\text{Mass of the samples} \cdot 1000} \cdot 100\% \quad (4)$$

## 2.4 Sensory evaluation test

Sensory quality was evaluated by the rank test (Lawless & Heymann, 1999). Twenty-five semi-trained panelists (fifteen women and ten men) were used in this study. The age of the panelists were between 22 and 25 years. Panelists were given questionnaires containing name, test date, the names of the test samples and instructions. The sensory attributes assessed in this test were the level of sourness, sour aroma, soda sensation, turbidity and viscosity. Panelists evaluated five samples and ranked each attribute on a 1-4 scale. They were also instructed to cleanse their palate with mineral water between evaluating each sample.

## 2.5 Statistical analysis

The parameters of pH, viscosity, Total Dissolved Solids, alcohol content, protein content, fat content and water content were analysed statistically by Analysis of Variance (ANOVA) using SPSS V22.0. Duncan's multiple range test was then used to determine significant differences amongst the results. Non-parametric data arising from sensory evaluation was analysed by the Kruskal-Wallis test. The significant results obtained by sensory evaluation were investigated using the Mann Whitney u-test to determine significant differences from each treatment.

# 3 Results and Discussion

## 3.1 Physicochemical properties

### pH analysis

Acidity level, denoted by pH, is commonly used to determine the quality of fermented products as it influences the texture and flavour of the product. As shown in Table 1, pH was affected by the fermentation time. There was a significant difference in the fermentation duration of 12 hours, while in other treatments the difference was not significant. The pH after 12 hours of fermentation time was 4.6; and was 3.4 after 24 hours, 3.6 after 36 hours, and 3.68 after 48 hours. Generally, the pH of water kefir ranges between 3.5 and 4 (Randazzo et al., 2016).

The decrease in pH that occurred in kefir green coconut water after 24 hours of fermentation was due to the growth of bacteria that will convert sugar into lactic acid and acetic acid, thereby decreasing the pH of the product. This was consistent with the findings of Delgado-Fernandez, Corzo, Olano, Hernandez-Hernandez and Javier Moreno (2019) which stated that the longer the fermentation time, the more active the bacteria and the greater the accumulation of organic acids resulting in increased sourness. The presence of too many free hydrogen ions ($H^+$) may affect the survival of the bacteria after 36 and 48 hours of

fermentation. A longer fermentation time will lead to the death of microorganisms present in kefir due to increasing alcohol levels and decreasing nutrients available for growth (Laureys & De Vuyst, 2014). This result showed that pH can be used as a reference to determine the optimal time to end the fermentation process.

## Viscosity analysis

As shown in Table 1, the viscosity of green coconut water kefir throughout the fermentation period ranged from 0.08 to 0.1 cP and was not affected by fermentation time. According to Zannini, Waters, Coffey and Arendt (2016), the viscosity is low if it is less than 2 cP for a 5% w/w solution in water. Viscosity in a fermented beverage was affected by the nutrient content of the raw material and the production process.
Green coconut water contains about 0.72 g/100 g of protein which is a low amount (Yong et al., 2009). Protein content in raw materials is one of the most important factors in determining kefir viscosity. A low protein content in raw materials results in a low viscosity of water kefir since there is insufficient energy for the growth of microbes (Dimitreli, Petridis, Akakiadou & Chrysalidou, 2014). Sabokbar, Moosavi-Nasab and Khodaiyan (2015) also reported that the viscosity values of kefir are related to exopolysaccharide (EPS) or kefiran production by the kefir grain during the fermentation. Therefore, sufficient nutrient content and optimal fermentation conditions are needed to obtain the desired viscosity.
Gul, Atalar, Mortas and Dervisoglu (2018) also observed that kefir viscosity increases with higher fat content as the interaction of fat globule membranes in the protein network improves water holding capacity (WHC) and results in the formation of a more stable gel. Green coconut water kefir contains 0.33 g/100 mL of fat which a low amount (Prades, Dornier, Diop & Pain, 2012). Other factors that may affect the viscosity of kefir are the state of the protein in the main ingredients, total solids and the ability of microbes to produce acid during fermentation (Yoo, Seong & Yoon, 2013).

## Total Dissolved Solids (TDS)

In the present study, there were significant differences in TDS across different fermentation times ($P < 0.05$). Significant differences were found in the fermentation periods of 12, 24 and 48 hours; but 36 hours was not significantly different from 24 hours of fermentation. The TDS values of the treatments were 3.8 $^{o}$Brix, 2.16 $^{o}$Brix, 2.04 $^{o}$Brix and 1.04 $^{o}$Brix, respectively.
The TDS values reduced with increasing fermentation time. TDS indicates the amount of sugar dissolved in coconut water which mostly consists of glucose, fructose and sucrose. According to Yong et al. (2009), the amount of TDS in green coconut water is 21.68 mg/mL, consisting of 9.18 mg/mL sucrose, 7.25 mg/mL glucose and 5.25 mg/mL fructose. The TDS values of T1 to T4 decreased due to the fermentation process. Yeast in kefir grain can hydrolyze sucrose into monosaccharides, namely glucose and fructose through the action of invertase enzymes. Glucose that is produced from this activity is subsequently transformed into organic acids. This is consistent with Gulitz, Stadie, Wenning, Ehrmann and Vogel (2011) who stated that generally all species of yeast contained in water kefir along with lactic acid bacteria (LAB) produce organic acids from glucose. The accumulation of acid as a product of LAB activity can also trigger a decrease in sugar content, as shown by the results of T4. The process of breaking down sugar by microbes from water kefir grain continuously reduces the availability of sugar and increases the acids. Jeong, Lee, Jung, Choi and Jeon (2013) reported that a decrease in nutrient availability and the accumulation of organic acids produced by LAB occurs with the increasing fermentation duration. Furthermore, these nutrients will deplete and cause an increase in alcohol accumulation which results in microbes entering the death phase.

## Alcohol content

The observed results showed that fermentation time significantly affected the alcohol content of green coconut water kefir, where alcohol content increased with longer fermentation periods. The average alcohol content produced in samples fermented for 12, 24, 36 and 48 hours

Table 1: Physical Characteristics of Green Coconut Water Kefir

| Parameters | Treatments (hours) | | | |
|---|---|---|---|---|
| | 12 | 24 | 36 | 48 |
| pH | $4.6 \pm 0.27^{a}$ | $3.4 \pm 0.07^{b}$ | $3.6 \pm 0.27^{b}$ | $3.68 \pm 0.07^{b}$ |
| Viscosity (cP) | $0.09 \pm 0.03^{ns}$ | $0.1 \pm 0.01^{ns}$ | $0.1 \pm 0.04^{ns}$ | $0.08 \pm 0.01^{ns}$ |

Data shown as the mean of repetitions ± standard deviation (SD). Different superscript letters on the same horizontal line show significant differences ($p<0.05$).

Table 2: Chemical Characteristics of Green Coconut Water Kefir

| Parameters | Treatments (hours) | | | |
|---|---|---|---|---|
| | 12 | 24 | 36 | 48 |
| Total Dissolved Solids ($^{o}$Brix) | $3.8 \pm 0.14^{a}$ | $2.04 \pm 0.09^{b}$ | $2.16 \pm 0.48^{b}$ | $1.04 \pm 0.09^{c}$ |
| Alcohol content (%) | $1.16 \pm 0.16^{a}$ | $1.96 \pm 0.18^{b}$ | $2.80 \pm 0.93^{c}$ | $4.1\ 4\pm 0.87^{d}$ |
| Water Content (%) | $97.14 \pm 0.09^{a}$ | $97.19 \pm 0.07^{a}$ | $97.1 \pm 0.06^{a}$ | $97.35 \pm 0.12^{b}$ |
| Protein Content (%) | $6.04 \pm 0.94^{a}$ | $5.46 \pm 0.39^{a}$ | $5.05 \pm 0.92^{ab}$ | $4.05 \pm 0.84^{b}$ |
| Fat Content (%) | $1.7 \pm 0.38^{ns}$ | $1.95 \pm 0.31^{ns}$ | $1.75 \pm 0.27^{ns}$ | $1.67 \pm 0.39^{ns}$ |

Data shown as the mean of repetitions ± standard deviation (SD). Different superscript letters on the same horizontal line show significant differences ($p<0.05$).

Table 3: Sensory Test of Green Coconut Water Kefir

| Sensory Attributes | Treatments (hours) | | | |
|---|---|---|---|---|
| | 12 | 24 | 36 | 48 |
| Level of Sourness | $3.52\pm0.96^{a}$ | $2.28\pm0.68^{b}$ | $1.60\pm1.04^{c}$ | $2.60\pm0.87^{b}$ |
| Soda Sensation | $2.40\pm1.50^{ns}$ | $2.40\pm0.76^{ns}$ | $2.28\pm0.94^{ns}$ | $2.92\pm1.12^{ns}$ |
| Sour Aroma | $3.52\pm1.00^{a}$ | $2.56\pm0.87^{b}$ | $2.24\pm0.72^{b}$ | $1.68\pm1.03^{c}$ |
| Viscosity | $2.52\pm1.26^{ns}$ | $2.76\pm1.05^{ns}$ | $2.00\pm1.12^{ns}$ | $2.76\pm0.97^{ns}$ |
| Turbidity | $3.12\pm1.30^{a}$ | $2.16\pm1.07^{b}$ | $2.48\pm0.92^{b}$ | $2.24\pm0.97^{b}$ |

Data shown as the mean of repetitions ± standard deviation (SD). Different superscript letters on the same horizontal line show significant differences ($p<0.05$). Sensory test scores from 1 to 4 represent: very high, high, low, very low

were 1.16 %, 1.96 %, 2.80 % and 4.14 %, respectively. Longer fermentation periods were associated with the higher activity of yeast and alcohol-producing microbes. The microbes that are primarily responsible for producing alcohol in kefir grain is yeast (*Saccharomyces cerevisiae*) (de Melo Pereira, Ramos, Galvao, Souza Dias & Schwan, 2010). Some *Lactobacillus* strains also have the ability to produce alcohol because they have alcohol-dehydrogenase that can convert substrates into ethanol (Magalhães-Guedes, Pereira, Campos, Dragone & Schwan, 2011).
A study on pomegranate and orange juice kefir by Kazakos et al. (2016) found that the alcohol level was below 1 %. Similar results were also found in brown sugar (Magalhães-Guedes, Pereira, Dias & Schwan, 2010) and cow's milk kefir (Zajsek & Gorsek, 2010). In general, the alcohol content of kefir usually ranges from 0.5 to 2% depending on the substrate used (Setyawardani, Rahardjo, Sulistyowati & Wasito, 2014). The higher alcohol content observed in green coconut was possibly due to the higher sugar content of 21 mg/mL, consisting of sucrose, glucose and fructose, in green coconut water (Yong et al., 2009). These sugar matrices stimulate the metabolism of kefir yeast, resulting in increased concentrations of ethanol, glycerol and esters in the final product. These metabolites provide the distinct sensory characteristics of kefir such as refreshing flavor, fruity aroma and texture (Fiorda et al., 2017). In conclusion, fermentation for 12 hours gave the best alcohol contentamong the treatments.

## Water content

It was found that the average water contents of green coconut kefir, with a fermentation time of 12, 24, 36 and 48 hours were 97.14 %, 97.19 %, 97.1 % and 97.35 %, respectively. Fermentation time had a significant effect on water content but there was no significant difference between green coconut water kefir of treatments T1, T2 and T3. Magalhães-Guedes et al. (2010) and Rocha-Gomes et al. (2018) also found similar results in Brazilian sugary water kefir and brown sugar water kefir within a range of 95-98%. The high water content of kefir in the current study was also caused by the largest component of the medium which consists of 95% water (Yong et al., 2009), hence the name water kefir.
The water content tends to increase with longer fermentation period. Currently, research on physicochemical properties of water kefir, especially water content, is still limited. However, the increase of water content that occurred after 48 hours of fermentation was allegedly due to the decreasing ability of kefir grains to retain moisture. Kefir grain is a matrix of exopolysaccharide (EPS) which is capable of binding with water in aqueous solution (Wang, Zhao, Tian, Yang & Yang, 2015). The reduction of EPS or kefiran might also give rise to this finding, where more moisture is available in the product. As reported by Kok-Tas, Seydim, Ozer and Guzel-Seydim (2013), enzymatic degradation of EPS occurs during fermentation and the storage period of kefir, and leads to a decrease in EPS content.

## Protein content

Based on the results, fermentation time affected the protein content in green coconut water kefir. Protein levels significantly decreased from T1 to T4, although T2 was not significantly different to T3. This is consistent with the results of Mechmeche, Kachouri, Ksontini, Setti and Hamdi (2018) who found that kefir can reduce protein levels and increase antioxidant activity during fermentation through the production of bioactive peptides. Protein content of samples throughout the fermentation period ranged between 4.05% and 6.04%. These results were higher than the protein content of Brazilian sugary water kefir fermented for 24 hours which was 0.4% (Magalhães-Guedes et al., 2010) and brown sugar water kefir which was 0.27% after 48 hours fermentation (Rocha-Gomes et al., 2018).
The protein content of a fermented product usually increases with fermentation time due to the increase of microbial biomass and secretion of protein molecules (Magalhães-Guedes et al., 2011). A different result was obtained in this study where the protein content decreased with increasing fermentation time. This is supposedly due to an inadequate supply of nutrients after 24 hours or a medium acidity level that is incompatible with the microbes' survival and leads to their death. The type and amount of protein con-

tained in the main ingredient may also affect the quality of kefir where the protein may coagulate during fermentation due to lactic acid accumulation and produce different functional peptides (Shi, Chen, Li, Huang & He, 2018). Overall, T1 (with 12 hours of fermentation time) had the best result among the treatments.

### Fat content

As shown in Table 2, there was no significant differences in the fat content of water kefir with different fermentation times ($P > 0.05$). The fat content produced at 12, 24, 36 and 48 hours of fermentation were 1.7 %, 1.95 %, 1.75 % and 1.67 %, respectively. These findings suggest the duration of fermentation did not affect fat content of water kefir. However, there was a decrease if fat content from 24 hours to 48 hours of fermentation. This is possibly due to the lipases produced by the kefir grain (Gonzalez-Sanchez, Azaola, Gutierrez-Lopez & Hernandez-Sanchez, 2010). Another explanation is the production of invertase enzyme by microbes in kefir grain, which hydrolyze sucrose into glucose and fructose that are subsequently transformed into organic acids by yeast and LAB (Fiorda et al., 2017).
Fat is a minor component of green coconut water kefir, with a fat content lower than that in milk kefir (2.34%) fermented for 24 hours (Magalhães-Guedes et al., 2011). This is in accordance with the observations of Prades et al. (2012) who reported the fat content of coconut water to be around 0.33 g/100 mL. A low fat content makes water kefir a good alternative for those with cholesterol issues who seek a low calories beverage with similar health benefits as milk kefir. Fat content affects the texture of kefir. A higher fat content will increase water holding capacity (WHC) of the product and cause a firmer consistency and higher viscosity (Gul et al., 2018).

## 3.2 Sensory evaluation

Sensory test results for green coconut water kefir included level of sourness, soda sensation, sour aroma, viscosity and turbidity (Table 3).

### Level of sourness of green coconut water kefir

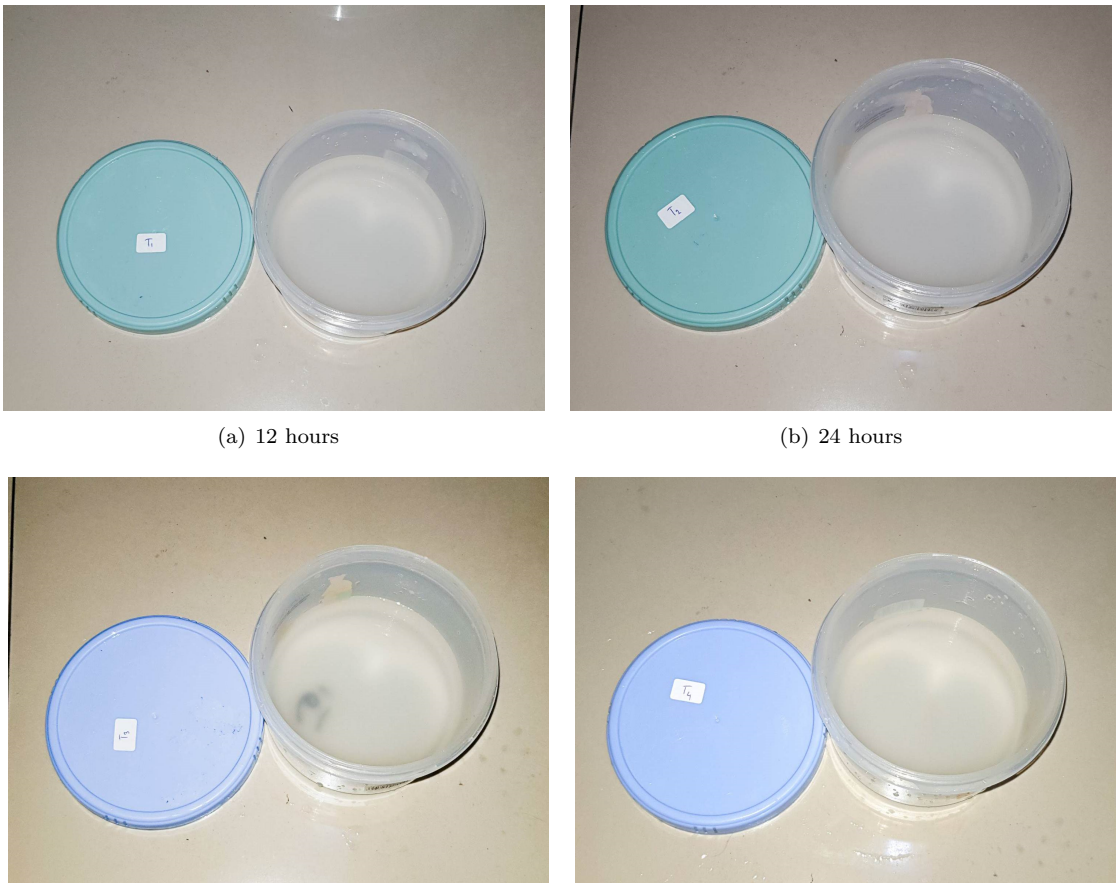
Sensory test results on green coconut water kefir showed that differences in fermentation time had significant effects ($P < 0.05$) on the level of sourness. As shown in Table 3, panelists could distinguish differences in the level of sourness of treatments. However, the level of sourness for the T2 (24 hours) and T4 (48 hours) treatments tended to be indistinguishable. The T3 (36 hours) treatment was known to have the highest sourness caused by the fermentation process. Fermentation by kefir grain produces lactic acid as the main metabolite. Acetic acid, glycerol and mannitol were also produced in low concentrations (Laureys & De Vuyst, 2014). The level of sourness should increase with increasing fermentation time. However, this did not occur in T4 where kefir grain cells entered the death phase due to an excessive fermentation process. Longer fermentation times can cause the accumulation of metabolites (lactic acid and carbon dioxide) which can then inhibit the growth of cells and result in a non-optimal fermentation process (Yuliana, 2012).

### Soda sensation of green coconut water kefir

The soda sensation is the impression of numbing, burning or biting when consuming food products containing carbon dioxide (Kappes, Schmidt & Lee, 2007). Sensory tests on green coconut water kefir showed that the panelists could not distinguish the soda sensation between different treatments. The bursting of carbon dioxide bubbles is a metabolite result of sugar conversion by microorganisms (Wu et al., 2010). A limited sugar content in green coconut water could only produce a small amount of carbon dioxide through yeast fermentation thus the inability to distinguish between treatments.

### Sour Aroma of Green Coconut Water Kefir

The differences in fermentation time had significant effects ($P < 0.05$) on the sour aroma of green coconut water kefir. The intensity of



(a) 12 hours (b) 24 hours

(c) 36 hours (d) 48 hours

Figure 1: Green coconut water kefir fermentation

sour aroma increased with fermentation time. This is shown in Table 3. The average value of sour aroma was the lowest in T4 (48 hours). Sour aroma increased due to the presence of volatile compounds during the fermentation process. Kefir grains produced aroma-forming compounds due to the presence of volatile compounds such as acetaldehyde, acetone, ethyl acetate, 2-butanone, diacetyl and ethanol (Cheng, 2010). The longer the fermentation time, the more volatile compounds that are produced, increasing the intensity of the sour aroma (Beshkova, Simova, Frengova, Simov & Dimitrov, 2003).

## Viscosity of Green Coconut Water Kefir

Sensory tests showed that panelists could not distinguish the viscosity of the green coconut water kefir across the different treatments. This is also in accordance with the quantitative analysis of viscosity that showed there were no significant differences in viscosity with fermentation time. The viscosity of a solution tends to increase with the addition of ingredients such as sweeteners or fibers (Mattes & Rothacker, 2001). However, there was no addition of those ingredients in the manufacturing process of green coconut water kefir, thus viscosity was not affected. Furthermore, as green coconut water contains only a low amount of protein the resulting viscosity may

not change significantly as fermentation time increases. In a fermented beverage, protein denaturation could lead to texture thickening of a finished product (Novelina, Sayuti & Rahmadani, 2013).

### Turbidity of Green Coconut Water Kefir

Sensory tests of green coconut water kefir showed that the differences in fermentation time had a significant effect ($P < 0.05$) on turbidity. As shown in Table 3, panelists were able to distinguish the turbidity of green coconut water kefir between T1 (12 hours) and the other treatments. However, T2 (24 hours), T3 (36 hours) and T4 (48 hours) tended to be indistinguishable. As shown in Figure 1(a), T1 (12 hours) produced a clearer solution than other treatments. While T2 (24 hours), T3 (36 hours) and T4 (48 hours), as presented in Figures 1(b)-1(d), were apparently similar. Panelists considered that T1 produced a lower turbidity intensity than the other treatments due to a shorter fermentation time. Under a short fermentation time, cells in the kefir grain were still in the adaptation phase, whilst in the other treatments the cells were already in the growth phase and thus increasing in number. This is in accordance with Parhusip and Kusuma (2003) who stated that the greater the number of microbes, the higher the turbidity of solutions.

## 4 Conclusions

This study showed that longer fermentation times were associated with less favorable physical and chemical characteristics in green coconut water kefir. The ideal fermentation time for producing green coconut water kefir was 12 hours, resulting in a pH of 4.6, viscosity of 0.09 cP, TDS of $3.8^o$ Brix, alcohol content of 1.16 %, water content of 97.14 %, protein content of 6.64 %, fat content of 1.17 % and a lower level of sourness that was considered more acceptable by the panelists.

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# Biodegradable Film Development by Nisin Z Addition into Hydroxypropylmethylcellulose Matrix for Mozzarella Cheese Preservation

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**Abstract**

Currently, improvement of food preservation has been a substantial challenge for industries to increase shelf-life of products and to maintain food quality during storage. These goals are often tied to the sustainable tendency for use of eco-friendly packaging to store these products without loss of the packaging features. Therefore, the aim of this study was to produce biodegradable antimicrobial films by the incorporation of nisin Z peptide under different concentrations (0 %, 5 %, 10 %, 15 % and 20 % wt.) into hydroxypropylmethylcellulose (HPMC) matrices. The active film properties were evaluated in terms of their antimicrobial capacity in vitro, mechanical performance and microscopic characteristics. Hence, active films containing 10 % (wt.) of nisin Z and control films were placed in contact with sliced mozzarella cheese for eight days, and microbiological growth was monitored during storage. Nisin Z's antimicrobial effects were observed against the Gram-positive microorganisms such as *Staphylococcus aureus* and *Listeria innocua*, regardless if the compound was free as a suspension or incorporated into HPMC matrices. However, the expected low action of nisin Z against Gram-negative bacteria, as reported in literature, was not observed since *Salmonella enterica* Choleraesuis's growth was inhibited. Moreover, active films with added nisin Z (10 % wt.) were more effective than the control film to inhibit mesophilic microorganisms in mozzarella cheese during 8 days of storage. The mechanical properties of the films were not influenced by nisin Z incorporation, since the addition of the compound enhanced the active function without the loss of mechanical properties required for a good food packaging. These results suggest that biodegradable films produced by nisin Z addition into HPMC matrix are an excellent biomaterial for mozzarella cheese preservation.

***Keywords:*** HPMC; Antimicrobial packaging; Mozzarella cheese; Active packaging; Food preservation

## 1 Introduction

Food packaging is a great strategy to differentiate products in the market under consumers' attention. The use of a suitable packaging for food quality maintenance, coupled with the demands of consumers, is one of the most important factors for success in a competitive market. The packaging material must meet the criteria for preserving the food and its nutrients, and protect against environmental factors such as light, moisture, oxygen and microorganisms, in order to prevent or hinder contact between the external environment and the contents inside the pack-

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## Nomenclature

| | | | |
|---|---|---|---|
| HPMC | hydroxypropymethylcellulose | SS | solution stock |
| MIC | minimum inhibitory concentration | EB | elongation at break |
| CFU | colony-forming unit | ME | modulus of elasticity |
| HCl | hydrochloric acid | ML | maximum load |
| SEM | scanning electron microscopy | | |

aging (Bradley, Castle & Chaudhry, 2011; Jorge, 2013).

Although traditional food packaging has many properties to ensure the integrity of foods during the marketing, distribution and storage, they are not sufficient to satisfy new consumers' requirements about food safety, which is, packaging that promotes food quality as well as fulfilling its basic packaging function (Dainelli, Gontard, Spyropoulos, Zondervan-van den Beuken & Tobback, 2008; Yam, Takhistov & Miltz, 2005). Active packaging is defined as systems that interact and change food conditions in order to extend product shelf life, as well as improve its safety or alter its sensory properties, thus maintaining or improving the food quality (Azeredo, 2012). How active packaging functions can be classified according to O2-scavenging, antioxidants, flavourings, absorbers and antimicrobials (Soares et al., 2009; Vermeiren, Devlieghere, van Beest, de Kruijf & Debevere, 1999). Antimicrobial packaging is a promising type of active packaging that confers antimicrobial compounds incorporated into the polymer matrices to eliminate or inhibit microorganisms present in the food (Cowan, 1999; Cox et al., 2000; Holley & Patel, 2005; Lambert, Skandamis, Coote & Nychas, 2001). In recent years, there has been a noticeable increase in research on antimicrobial packaging to assure or improve the food quality. These researchers have tended to study natural antimicrobials, such as bioactive compounds of vegetables and fruits; essential oils; and bacteriocins that are not adverse to consumers' health (Mlalila, Hilonga, Swai, Devlieghere & Ragaert, 2018; Mulla et al., 2017; Niu, Liu, Song, Han & Pan, 2018; Pola et al., 2016; Sarwar, Niazi, Jahan, Ahmad & Hussain, 2018; Woraprayote et al., 2018).

Bacteriocins are peptides, produced by Gram-positive and Gram-negative bacteria, which exhibit antimicrobial activity against other bacteria in medium (Arena et al., 2016; Karpinski & Szkaradkiewicz, 2013). One of the most studied and applied bacteriocins in the food industry is nisin, which is considered non-toxic and safe for human health (Karpinski & Szkaradkiewicz, 2013). Nisin is a peptide composed by 34 amino acid units, presenting a cationic and hydrophobic character, and it is considered a lantibiotic. This bacteriocin is produced by species of *Lactococcus lactis* subsp. *Lactis* and has been studied since its discovery in 1928 (Nascimento, Moreno & Kuaye, 2008; Siamansouri, Mozaffari & Alikhani, 2013). There are five natural variants of nisin: nisin A, nisin Z, nisin Q, nisin U and nisin F (De Kwaadsteniet, Fraser, Van Reenen & Dicks, 2006). The bacteriocins most used in industry and food research are nisin A and nisin Z, which structurally differ in only one amino acid at position 27, and both are produced by strains of *Lactococcus lactis*. The differences between them are few, however, nisin Z is slightly more diffusible in agar and more soluble at neutral pH than nisin A (Mulders, Boerrigter, Rollema, Siezen & Devos, 1991).

According to the active packaging concept, this work sets out to develop active films, incorporated with peptide nisin Z into HPMC matrix, to come up with a novel material that provides anti-

microbial activity, in order to improve mozzarella cheese preservation during storage.

## 2 Materials and Methods

### 2.1 Material

Nisin Z was purchased from Handary (Belgium). Hydroxypropylmethylcellulose (HPMC) was obtained from Sigma-Aldrich (United States). Glycerol was purchased from Labsynth (Brazil). Broth TSB, Mueller-Hinton agar and PCA agar were purchased from DIFCO® (USA). Hydrochloric acid (HCl) was obtained from SPLabor (Brazil). Strains of *Salmonella enterica* serotype Choleraesuis (ATCC 10708), *Staphylococcus aureus* (ATCC 6538), and *Listeria innocua* (ATCC 33090) were acquired from Food Packaging Laboratory (UFV, Brazil). Deionized water (Millipore Inc.) was used at electrical resistivity of 18.2 MΩ.cm.

### 2.2 Determination of Minimum Inhibitory Concentration of nisin Z (MIC)

The minimum inhibitory concentration (MIC) of nisin Z was performed in accordance with the broth macro dilution method standardized by the Clinical and Laboratory Standards Institute (CLSI, 2012), with modifications. The microorganisms evaluated were *S. aureus*, *L. innocua* and *S. enterica* Choleraesuis. Initially, a stock solution (SS) containing deionized water pH 4.6 (adjusted with 0.1 mol.L$^{-1}$ HCl solution) and nisin Z (concentration determined according previous test) was produced. SS solution was diluted in Muller-Hinton broth according to Table 1. Then 2.0 mL of each dilution (Table 1) and 2.0 $\mu$L of the microorganism suspension (turbidity based on McFarland 0.5 standard solution which corresponds to a concentration of $10^8$ CFU.mL−1) were added to tubes, which were incubated at 35 $^o$C for 18 h. To confirm the result, after the incubation time, Mueller-Hinton agar plates were inoculated, in duplicate, with 0.1 mL of each dilution tube, and incubated at 35 $^o$C for 18 h. The nisin's MIC was the lowest concentration of the antimicrobial agent capable to inhibit the visible microorganism growth. The experiment was performed in three repetitions, in duplicate, for each microorganism.

### 2.3 Preparation of antimicrobial films

The antimicrobial films were prepared according to the method of Sanchez-Gonzalez, Vargas, Gonzalez-Martinez, Chiralt and Chafer (2009), with modifications (Figure 1). 150 mL of deionized water pH 4.6 (adjusted with 0.1 mol.L$^{-1}$ HCl solution) was heated to 80 $^o$C. Afterwards, 2 % (w/v) of hydroxypropylmethylcellulose (HPMC) and 20 % (wt.) of glycerol were added to the water and then, the polymer dispersion was mixed on a magnetic stirrer at 700 rpm for 5 min. This was followed by nisin Z addition at 0 %, 5 %, 10 %, 15 % and 20 % (wt.) concentrations, wherein the percentage added was relative to HPMC mass. The polymeric material was poured into bordered glass plate and left for 72 h at 25 ± 2.0 $^o$C.

### 2.4 Characterization of films

#### *In vitro* antimicrobial activity of films

The *in vitro* antimicrobial activity of films was measured according to the agar diffusion method. Films discs ($\phi$ = 0.8 cm), containing nisin Z (0 %, 5 %, 10 %, 15 % and 20 % (wt.)), were placed in contact with inoculated Mueller-Hinton agar, that was obtained by the friction of a swab soaked in the microorganism suspension. The microorganism suspension had the same turbidity as the standard 0.5 McFarland solution (corresponding to a bacterial concentration of $10^8$ CFU.mL$^{-1}$). The antimicrobial effectiveness of the films was determined by comparing the inhibition zone formed after the Petri dishes were incubated for 24 h at 35 $^o$C. The microorganisms evaluated were *S. aureus*, *L. innocua* and *S. enterica* Choleraesuis.

Table 1: Dilutions of nisin Z dispersion to determinate nisin Z's MIC

| Solution | Aliquot (mL) | Volume of Muller-Hinton broth (mL) |
|---|---|---|
| S1 | 2.0 mL of SS | 0.0 |
| S2 | 1.0 mL of SS | 1.0 |
| S3 | 1.0 mL of SS | 3.0 |
| S4 | 1.0 mL of SS | 7.0 |
| S5 | 1.0 mL of S4 | 1.0 |
| S6 | 1.0 mL of S4 | 3.0 |
| S7 | 1.0 mL of S4 | 7.0 |
| S8 | 1.0 mL of S7 | 1.0 |
| S9 | 1.0 mL of S7 | 3.0 |
| S10 | 1.0 mL of S7 | 7.0 |

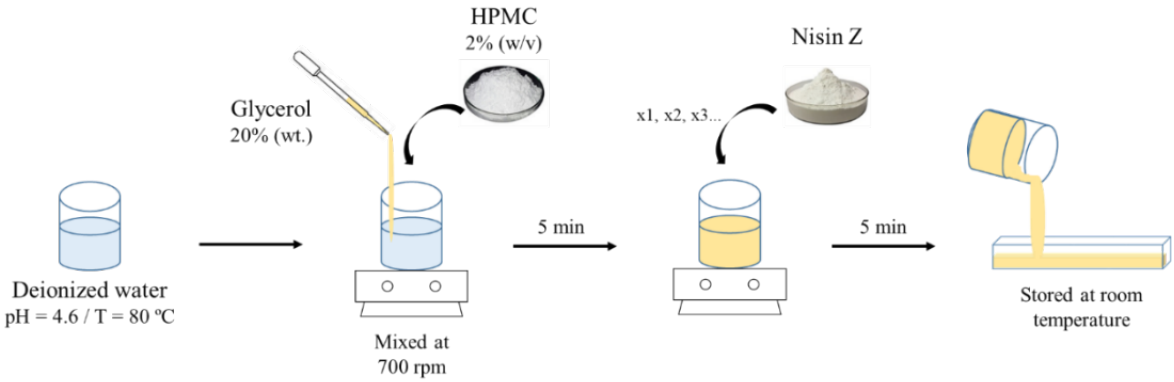


Figure 1: Schematic illustration of antimicrobial and control films' preparation

## Mechanical properties of antimicrobial films

Maximum load (N), elongation at break (%) and modulus of elasticity (MPa) of films with added nisin Z or not (control film) were measured using the Universal Machine of Mechanical Testing (Instron Corporation, Norwood, MA, USA), according to ASTM (2012) method. The specimens (17.5 x 2.5 cm) were grabbed by two grips initially separated by 125 mm, and stretched at a crosshead speed of 12.5 mm.min$^{-1}$. Each treatment was performed in three repetitions, and each repetition in quintuplicate.

## Scanning electron microscopy (SEM)

The microscopic structure of films was recorded by scanning electron microscope (Hitachi, model TM3000, Japan). Film samples (0.5 cm$^2$) were fixed in stubs covered with a carbon layer (Camilloto, 2009). The acceleration voltage was in automatic mode. The images were taken at 7000x magnification.

## Antimicrobial activity of the HPMC-based active film in cheese

The potential *in situ* antimicrobial ability of the films containing nisin Z was evaluated in mozzarella cheese slices. The active film with the best antimicrobial properties *in vitro*, 10 % (wt.)

of nisin Z, was chosen to conduct the application test according to the methodology of Soares and Hotchkiss (1998), with modifications. Cheese slices were intercalated with active films, containing enough area to cover the sample surface. Then, they were placed in expanded polystyrene trays, wrapped with polyethylene-nylon and stored at 5 ± 2 $^{o}$C during 8 days (Figure 4a). 25 g of cheese samples were taken, aseptically, on 0, 2, 4, 6 and 8 days of storage and then, they were homogenized with 225 mL of 0.1 % (w/v) peptone water in sterile bags by a shake stomacher. 1 mL of this suspension was transferred to a tube containing 9 mL of sterile 0.1 % (w/v) peptone water, obtaining a dilution of $10^{-2}$. From this, subsequent dilutions were obtained and for each dilution, 0.1 mL was spread on the non-selective PCA agar, in duplicate, and incubated at 37 $^{o}$C for 24 h. Plates containing 25 to 250 colonies were selected to determine the amounts of microorganism capable of growth (Log CFU.mL$^{-1}$). Equations describing the growth behavior of mesophilic microorganisms in cheese over the time were adjusted with 95 % significance level.

### 2.5 Statistical analysis

The data were analyzed by Analysis of Variance (ANOVA), and when it was possible, a regression equation (at 5 % probability level) was adjusted, for each response variable, considering as a factor the nisin Z concentrations incorporated in the films. All statistical analysis was performed with Minitab statistical program, version 17.

## 3 Results and Discussion

### 3.1 Minimum inhibitory concentration (MIC) of nisin Z

The MIC for *S. aureus*, *L. innocua* and *S. enterica* Choleraesuis were determined to verify the antimicrobial potential of the nisin Z. According to the Table 2, nisin Z was more effective in inhibiting microbial growth of Gram-positive *S. aureus* and *L. innocua* than Gram-negative bacteria *S. enterica* Choleraesuis. Unlike Gram-positive bacteria, which have a peptidoglycan layer, Gram-negative bacteria have an outer lipopolysaccharide layer that acts as a barrier and prevents the diffusion of bacteriocins into the cell (Gyawali & Ibrahim, 2014). One of the most accepted mechanisms of nisin activity, as an antimicrobial compound, is its interaction with the anionic lipids present in the cytoplasmic membrane that promote a disturbance, forming pores that cause alteration in the vital ions gradient, leading to cell death (Breukink & de Kruijff, 2006; Tong, Ni & Ling, 2014).

The MIC for the bacteria *L. innocua* and *S. aureus* were in agreement with inhibition concentrations reported by other authors (Niaz et al., 2018; Ramos et al., 2012; Sadiq et al., 2016; Yoneyama, Fukao, Zendo, Nakayama & Sonomoto, 2008). In addition, the MIC determined for *S. enterica* Choleraesuis was above the values found in other studies, however, with slight changes of microbial species and types of nisin (Field et al., 2012; Kim, Jung, Kim & Shin, 2006). The divergent responses are justified by the variation in the fluidity of the bacteria cytoplasmic membrane as a function of several factors such as temperature, pH and the presence of chelators (Prudencio, Mantovani, Cecon, Prieto & Dantas Vanetti, 2016).

### 3.2 Antimicrobial activity of films *in vitro*

Equations ($P<0.05$) and behaviors that described the antimicrobial activity of the films against *L. innocua* and *S. aureus* are shown in Table 3 and Figure 2, respectively. There was not inhibitory action of the films in *S. enterica* Choleraesuis presence, regardless of the nisin Z concentration added into the HPMC film, so no equation was adjusted ($P>0.05$).

The treatments evaluated were not able to inhibit *S. enterica* Choleraesuis due to the low action of nisin Z against Gram-negative microorganisms, corroborating with MIC values (item 3.1). Furthermore, the nisin concentrations added in the films may not have been enough to diffuse in an amount capable of *S. enterica* Choleraesuis inhibition. The antimicrobial activity against *S.*

Table 2: MIC values for the microorganisms evaluated.

| Microorganism | MIC ($mg.mL^{-1}$) |
|---|---|
| *S. aureus* | 0.19 |
| *L. innocua* | 0.19 |
| *S. enterica* Choleraesuis | 2.00 |

*aureus* and *L. innocua* (Figure 2c) showed a maximum inhibition zone at 10 % (wt.) of nisin Z addition into HPCM. Above 10 % wt. of nisin Z concentration into the polymer matrices, the halos formed maintained almost the same size, and the differences observed were not so prominent. Therefore, from an economic point of view, the same antimicrobial effect can be obtained using less nisin Z, reducing costs, and from the health point of view, the intake of this additive by the consumer is decreased. Similar behavior was observed in other polymer matrices, such as studies carried out by Cao-Hoang, Chaine, Gregoire and Wache (2010) that produced films based on sodium caseinate, with added nisin. More studies on antimicrobial activity *in vitro* of active packaging are required because these packages may better inhibit microorganisms than their direct addition in the food or in the culture medium. The contact of nisin with the food surface, promoted by the direct contact between the food and the packaging, can facilitate its diffusion whilst diminishing the complexation with food components or headspace (de Barros, Kunigk & Jurkiewicz, 2010). In addition, the peptide nisin Z added into the polymer matrix can be used to control release and to promote antimicrobial activity over time, from the production until the food consumption (Sadiq et al., 2016; Salmieri et al., 2014; Shahbazi, Shavisi & Mohebi, 2016).

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The treatments evaluated were not able to inhibit *S. enterica* Choleraesuis due to the low action of nisin Z against Gram-negative microorganisms, corroborating with MIC values (item 3.1). Furthermore, the nisin concentrations added in the films may not have been enough to diffuse in an amount capable of *S. enterica* Choleraesuis inhibition. The antimicrobial activity against *S. aureus* and *L. inoccua* (Figure 2c) showed a maximum inhibition zone at 10 % (wt.) of nisin Z addition into HPCM. Above 10 % wt. of nisin Z concentration into the polymer matrices, the halos formed maintained almost the same size, and the differences observed were not so prominent. Therefore, from an economic point of view, the same antimicrobial effect can be obtained using less nisin Z, reducing costs, and from the health point of view, the intake of this additive by the consumer is decreased. Similar behavior was observed in other polymer matrices, such as studies carried out by Cao-Hoang et al. (2010) that produced films based on sodium caseinate, with added nisin.

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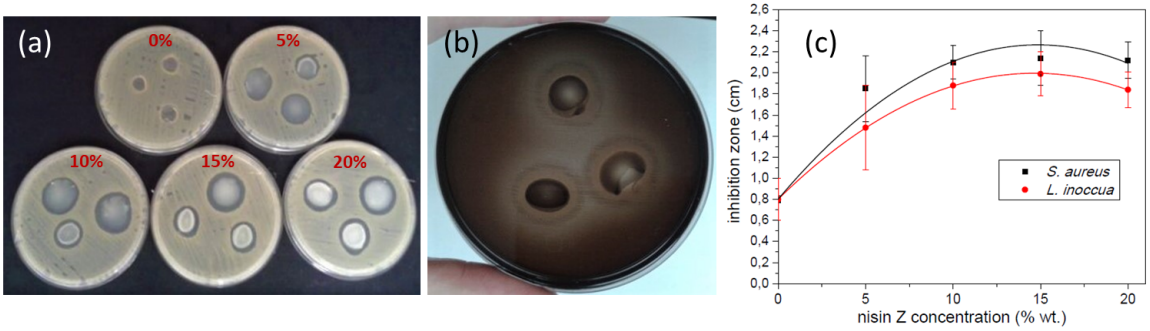


Figure 2: Inhibition images of films containing different concentrations of nisin Z against the microorganisms *S. aureus* (a) and *L. innocua* (b) and their inhibition behaviors for each microorganism (c).

Table 3: Equations obtained by regression analysis for the "halo test" in *S. aureus*, *L. innocua* and *S. enterica* Choleraesuis presence

| **Microorganism** | **Equation**[a] | **$R^2$** | **Faj**[b] |
|---|---|---|---|
| *S. aureus* | $Y_1 = 0.086 + 3.099*x - 0.140*x^2$ | 0.789 | 0.543 |
| *L. innocua* | $Y_2 = -0.150 + 3.180*x - 0.157*x^2$ | 0.794 | 0.273 |
| *S. enterica* Choleraesuis | $Y_3 = 0.800$ | - | - |

[a] Was used the Quadratic model ($\alpha$ =0.05).
[*] Significant by t-Test (P<0.05).
[b] Faj: P-value for the lack of fit (not significant for P>0.05).

bazi et al., 2016).

## 3.4 Scanning electron microscopy (SEM)

According to the micrographs taken of the film surfaces (Figure 3), it was possible to observe the presence of HPMC clusters in all films, regardless of nisin Z concentrations, represented by white agglomerates in different formats and sizes. The clusters are originated by hydrophobic interactions between the methyl replacement groups present on the HPMC structure. The presence of these aggregates showed the heterogeneity of the film at the microscope level, which were also found by other authors such as Sanchez-Gonzalez et al. (2009).
Several unformed and agglomerated points were visualized in the micrographs of the films containing nisin Z. These points increased with increasing nisin Z concentration into the polymer matrix (Figure 3b, c, d, e), which was also reported by other authors (Meister Meira et al., 2014; Scaffaro, Botta, Marineo & Puglia, 2011). This result agrees with the antimicrobial behavior *in vitro* of the films (item 3.2). There were no prominent differences in the film surfaces when nisin concentration varied from 10 %, 15 % and 20 % wt., which may have promoted similar rates of nisin diffusion into the medium among these treatments. The presence of pores in all films was due to the formation of bubbles from the film-forming process (Bastarrachea et al., 2010). Changes observed in the film's microstructure such as aggregates, bubbles, degree of compaction and cohesion can influence several characteristics of materials like intermolecular, thermal and barrier properties.

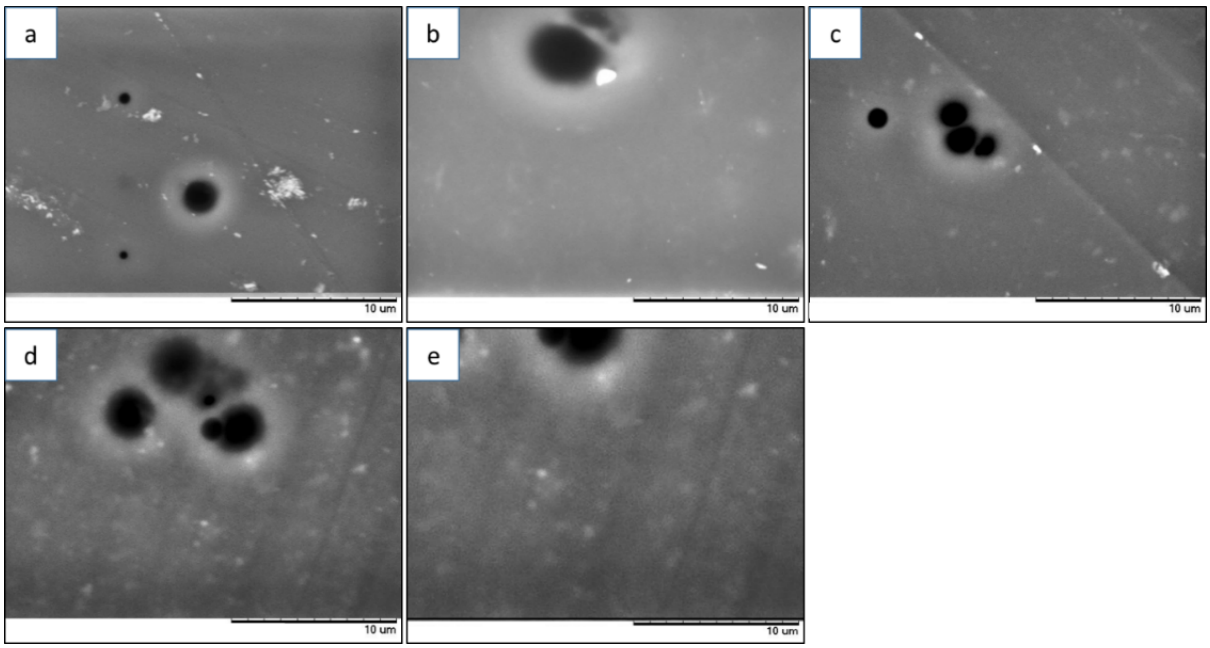


Figure 3: SEM micrographs of the antimicrobial films added by nisn Z at 0 % (a), 5 % (b), 10 % (c), 15 % (d) and 20 % (wt.) (e) (magnification of 7000x and the acceleration voltage in automatic mode)

### 3.5 Mechanical properties of antimicrobial films

In order to evaluate the film's mechanical properties caused by addition of nisin Z into the polymer matrix, the samples were submitted to stress tests and the maximum load (ML), the elongation at break (EB) and the modulus of elasticity (ME) were determined (Table 4).

None of the mechanical properties had significant equation adjusts ($P>0.05$) as nisin Z concentration increased (Table 4). The mechanical properties were constant regardless of antimicrobial peptide addition into the matrices and this behavior can be related to the lower interaction between nisin Z and the polymer chains. According to Ko, Janes, Hettiarachchy and Johnson (2001), no changes in the mechanical characteristics of the films occurred due to the hydrophilicity features of the HPMC polymer matrix, which was responsible for the lower interaction with hydrophobic nisin. In addition, the mechanical performance of polymer films involves other factors such as matrix interface filling and irregular dispersion of additives in matrices that can be visualized by microscopic analysis (item 3.3). These results are similar to those reported by other authors (Basch, Jagus & Karina Flores, 2013; Meister Meira et al., 2014). Therefore, the mechanical studies suggest no difference between films with added nisin Z and the control films, indicating its mechanical potential to be applied as food packaging.

### 3.6 Antimicrobial activity of HPMC-based film applied in cheese

A significant effect ($P<0.05$) in microbial growth, caused by time, can be observed during storage for both treatments evaluated (Figure 4b). This behavior can be explained by the refrigerator temperature used (5 $^{o}$C), which caused initial inhibition due thermal shock of mesophilic bacteria in the mozzarella cheese. Only after the fourth day of storage, it was observed that the resistant microbial population returned to growth. Al-

Table 4: Mechanical properties of films with added nisin Z or not

| Nisin Z (% wt.) | ML* (N) | EB* (%) | ME* (MPa) |
|---|---|---|---|
| 0 | $153.6^{a} \pm 29.9$ | $14.1^{b} \pm 0.8$ | $2841.0^{c} \pm 317.9$ |
| 5 | $143.2^{a} \pm 26.7$ | $14.0^{b} \pm 5.3$ | $2409.6^{c} \pm 400.0$ |
| 10 | $162.6^{a} \pm 26.5$ | $16.8^{b} \pm 3.7$ | $2565.8^{c} \pm 770.0$ |
| 15 | $145.3^{a} \pm 24.7$ | $17.5^{b} \pm 4.0$ | $1862.0^{c} \pm 674.3$ |
| 20 | $150.2^{a} \pm 23.2$ | $16.2^{b} \pm 2.7$ | $1956.3^{c} \pm 752.0$ |

* Equal letters in the same column indicate not significant by the Tukey test.

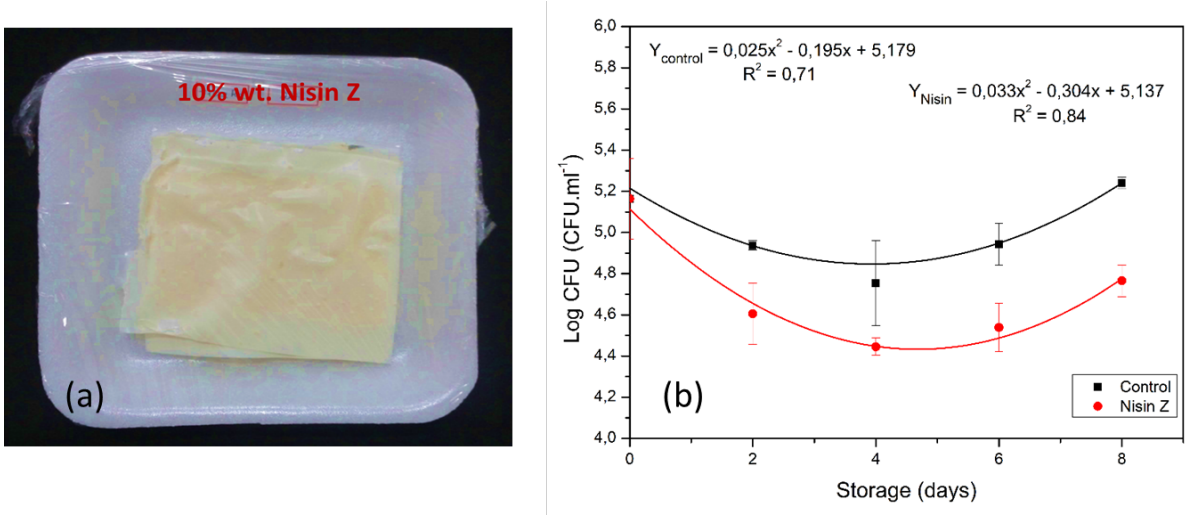


Figure 4: Image of cheese slices intercalated with active film (10 % wt. of nisin Z) placed in expanded polystyrene trays (a); behavior and adjusted equations (P<0.05) for the growth of aerobic mesophilic in the mozzarella packed with the control film and the antimicrobial film (y) during storage (x) (b). Each treatment was performed in three repetitions, and each repetition in triplicate.

though low temperatures inhibit some mesophilic microorganisms, cold-adapted bacteria can survive and proliferate under low temperature conditions (Remenant, Jaffres, Dousset, Pilet & Zagorec, 2015). Other authors, even at higher storage temperatures (Conte, Scrocco, Sinigaglia & Del Nobile, 2007; Gorrasi et al., 2016), found similar growth behavior in packed mozzarella cheese.

The incorporation of nisin Z into the HPMC film influenced (P<0.05) the microbial growth when compared with the control film, gives lower microbial counts at all times, as can be observed by regression analysis (Figure 4b). This difference was highest on the eighth day of storage, where the film with added nisin Z was approximately 0.5 logarithmic cycle lower than the control film. This result indicates that there was diffusion over time of the nisin Z added into the polymer matrix to the cheese, influencing the cheese microbiota and inhibiting microorganisms. The inhibition behavior agrees with other studies that applied different polymer matrices and different antimicrobial agents to control microorganisms in mozzarella cheese (Dannenberg et al., 2017; Gorrasi et al., 2016; Lucera et al., 2014).

Nisin activity is attributed to its interaction with the anionic lipids in the cytoplasmic membrane of

bacterial cells, resulting in a plasma membrane disturbance. This peptide increases the membrane permeability by formation of pores, resulting in an efflux of intracellular material, essential components, adenosine triphosphate (ATP), amino acids, potassium ions, and promotes several changes that end in cell death (Breukink et al., 2003). The effectiveness of HPMC-based films presented in this work agrees with many studies where nisin has been incorporated in packaging to inhibit microorganisms for application in dairy products (Cui, Wu, Li & Lin, 2016; Martins, Cerqueira & Vicente, 2012).

## 4 Conclusion

Compared to petroleum-based plastics, biopolymers can be a less environmentally aggressive solution. HPMC-based film can be developed for several applications, where one of them is to act as an antimicrobial packaging by nisin Z incorporation. Nisin Z is a bioactive peptide that presents antimicrobial action mainly for *L. innocua* and *S. aureus*. The mechanical properties of the HMPC films are not altered by the nisin Z incorporation which is an interesting result when it comes to the mechanical strength of the food packaging. From the SEM images, it was possible to observe the presence of nisin Z on the film surfaces, which indicates direct contact with the medium and explains the antimicrobial activity *in vitro* of films in contact with mozzarella cheese. The results found in this work showed a great potential to apply these films to increase the shelf life of dairy foods, ensuring the quality and safety of the products.

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# Quality of Postharvest Strawberries: Comparative Effect of Fungal Chitosan Gel, Nanoparticles and Gel Enriched with Edible Nanoparticle Coatings

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**Abstract**

This study compared, for the first time, the postharvest conservative action of edible fungal chitosan coatings (gel, nanoparticles and gel-nanoparticle) on the physico-chemical, sensorial and microbiological characteristics of strawberries. The nanoparticles were prepared by an ionic gelation method and characterized by dynamic light scattering and scanning electron microscopy. The antioxidant (DPPH* and ABTS*) activity of the edible coatings and the antimicrobial (macrodilution method) action against phytopathogenic fungi were verified. The nanoparticles had a size of 331.1 nm and a zeta potential of + 34 mV. The gel, nanoparticles and gel+nanoparticles exhibited minimum inhibitory concentration values ranging from 4 to 5, 1.5 to 2.5 and 1.0 + 0.5 to 2.0 + 1.5 $g.L^{-1}$, respectively. All the edible coatings exhibited antifungal action. All the coatings had high scavenging activity, especially the gel edible coating. The coatings, especially the gel+nanoparticles, decreased the weight loss, microbiological growth, soluble solids, maturity index and moisture loss of the strawberry and preserved the pH values, anthocyanin content, titratable acidity and sensory characteristics. Therefore, the use of chitosan edible coating containing nanoparticles can be a promising strategy to improve the post-harvest quality of strawberries.

***Keywords:*** Antifungal activity; Antioxidant activity; Biopolymer; Nanotechnology; Shelf-life

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## 1 Introduction

Strawberry (*Fragaria* × *ananassa Duch.*) is a non-climateric fruit cultivated worldwide and characterized by an attractive taste, flavour and important nutritional compounds. However, due to its high respiration rate, excessive soft texture, water loss and microbiological decay, strawberries are considered highly perishable fruit (postharvest life approx. 3-4 d at 20 $^{o}$C) (Shahbazi, 2018). So, various technologies have been developed for strawberry preservation in accordance with the principles of green chemistry (Dhital et al., 2017; Oregel-Zamudio, Angoa-Pérez, Oyoque-Salcedo, Aguilar-González, & Mena-Violante, 2017).

The application of edible coating has been considered as an alternative method to prevent fruit postharvest decay and to extend the storage life, while retaining the overall quality of different fresh commodities (Yousuf, Qadri, & Srivastava, 2018). Different biological materials have been used to prepare packaging materials, especially chitosan. This polymer is traditionally obtained from crustacean shells. However, fungi biomass is a promising eco-friendly alternative for obtaining chitosan because it is not affected by seasonal factors, can be produced on a large scale without changing the physicochemical characteristics of the polymer and does not contain any of the proteins that induce allergic reactions to crustaceans (Berger et al., 2018).

Chitosan has received much interest for its application in food preservation because of its excellent film-forming ability, antimicrobial and antioxidant activities, biocompatibility, biodegradability and non-toxicity (Muzzarelli et al., 2012). Nevertheless, it is believed that the characteristics of edible coatings can be improved by incorporating nanostructures into the polymer matrix (Pilon et al., 2015). According to Eshghi et al. (2014), nanotechnology could facilitate the development of novel packaging materials and non-polluting, cheaper, and more efficient packaging techniques.

Unique physicochemical characteristics are consequences of a reduction in particle size and increasing the surface to particle size ratio in nanoparticles. Due to the interaction between nanoparticles and chitosan gel, the chitosan edible composite coatings are supposed to show enhanced antimicrobial activity and barrier properties to the internal gas atmosphere of the fruit, which gives better maintenance of the physicochemical and microbiological characteristics of fresh strawberry (Eshghi et al., 2014).

However, until now, no research has demonstrated that the use of chitosan nanoparticle alone in the edible coating is actually a better fruit preservative than the use of chitosan gel edible coating or coating composed with gel enriched with chitosan nanoparticles. Therefore, the aim of this research was to compare, for the first time, the preservative action of three different fungal chitosan edible coatings (gel, nanoparticles and gel enriched with nanoparticles) on the quality of postharvest strawberries during storage time.

## 2 Materials and Methods

### 2.1 Materials

Strawberries were purchased from Supplies and Service Company of Pernambuco (Recife, Brazil) and selected according to size and colour; they showed no signs of deterioration or mechanical damage. The chitosan (KiOnutrime®) was provided by the Kitozyme® Company (Herstal, Belgium) and it is a fungal chitosan (deacetylation degree of 86 %, molecular weight of 4 x $10^3$ Da), extracted from *Aspergillus niger* mycelium. The other substances used were obtained from commercial sources.

### 2.2 Preparation and characterization of chitosan nanoparticles

The fungal chitosan nanoparticles (FCN) were prepared by an ionic gelation method with some modification (Calvo, RemunanLopez, VilaJato, & Alonso, 1997). The chitosan (CS) solution, at a concentration of 2 g.L$^{-1}$, was prepared by dissolving the polymer in 24 mL of pH 4 solution (1 % acetic acid + 1 % NaOH) and stirred for 30 min. The sodium polyphosphate (TPP) solution was dissolved in deionized water at concentration of 1 g.L$^{-1}$. Then, 6 mL of TPP solution

was gradually dropped (0.2 mL.min$^{-1}$) into 24 mL of chitosan solution using a peristaltic pump (Atlas Syringe Pump) under vigorous magnetic stirring at room temperature. The final ratio of CS:TPP was 4:1. Once the dropwise addition was completed, the nanoparticle suspension was stirred for an additional 1 h.

The morphology and particle size of the FCN were characterized by scanning electron microscopy (SEM) (SEM Quanta 200 FEI). The freeze-dried nanoparticles were mounted on a specimen stub (with carbon ribbon and silver ink) (Taab, Berkshire, UK) and the sample was coated with a 10 nm thick gold film using a sputter coater. Nanoparticle morphology was observed using an electron acceleration voltage of 20 kV. The zeta potential of the nanoparticles was measured using a Malvern Zetasizer (model Nano ZS90, Malvern, UK). The analysis was performed at a scattering angle of 90$^o$ at 25 $^o$C and 633 nm.

The size of the FCN was analyzed by Dynamic Light Scattering system (Zetasizer Nano ZS90, Malvern Instruments, UK) (633 nm, 90$^o$, 25 $^o$C) (Tsai, Chen, Bai, & Chen, 2011).

## 2.3 Determination of the antifungal activity

The three forms of fungal chitosan (nanoparticles, gel and gel enriched with nanoparticles) were used in the broth macrodilution technique to determine the minimum inhibitory concentration (MIC) against strawberry phytopathogenic fungi (*Botrytis cinerea* URM 2802, *Rhizopus stolonifera* URM 3728 and *Aspergillus niger* URM 7282). For this assay, phytopathogenic fungi stock cultures were subcultured in Sabouraud agar at 28 $^o$C for 7 days to allow sufficient sporulation. The fungal spores were collected in a sterile saline solution (0.85 g.(100 mL)$^{-1}$ NaCl) in fungal growth medium, and the suspension was filtered through a sterile triple-gauze layer to retain hyphal fragments. The number of spores present in the suspension was determined with a haemocytometer. The spore concentration obtained was adjusted with sterile saline solution to provide a fungal inoculum of approximately $10^6$ spores.mL$^{-1}$ (Vasconcelos de Oliveira et al., 2014a).

The broth macrodilution technique was performed in triplicate. Initially, 0.5 mL of a suspension of the phytopathogenic fungi was inoculated, separately, into 2 mL of Sabouraud broth (with the concentration adjusted to 5 mL), and 2.5 mL of the solutions containing different concentrations of test substances were added. The mixture was incubated at 25 $^o$C for 7 days, and at the end of the incubation period, the lowest concentration of test substances that exhibited no visible fungal growth was considered to be the MIC (Sharma & Tripathi, 2008). This assay was performed separately for each test substance and fungi strains.

For this assay, the freeze-dried FCN and the CS were diluted in a solution of 1 % acetic acid and pH was adjusted to 5.8 with 1 % NaOH. The solution of 1 % acetic acid with pH adjusted to 5.8 was tested, separately, as a control and exhibited no inhibitory effects against the phytopathogenic fungi.

## 2.4 Comparative antioxidant activity

The antioxidant activity of the fungal CS gel, FCN and CS gel enriched with FCN was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) assay.

### DPPH radical scavenging ability

The comparative scavenging effect of test substances on DPPH radicals was measured according to the method described by Chen et al. (2015). 2.5 mL of the solutions of the test substances at different concentrations (MIC/2, MIC and 2xMIC) were added into 2.5 mL of freshly prepared DPPH solution in ethanol (50 g.L$^{-1}$). The mixture was shaken thoroughly with a vortex mixer and incubated for 30 min at 33 $^o$C. Then, the absorbance was measured at 517 nm using a UV-vis spectrophotometer (Agilent, USA). The percentage of DPPH radical scav-

enged was calculated as follows:

$$DPPH(\%) = \left(1 - \left(Aa - \frac{Ab}{Ac}\right)\right) \times 100 \quad (1)$$

where $Aa$ = absorbance of the sample mixed with DPPH solution, $Ab$ = absorbance of the sample without DPPH solution and $Ac$ = absorbance of blank control without sample.

### ABTS radical scavenging ability

The analysis of ABTS scavenging activity was determined according to the method described by Larrauri, Ruperez, and SauraCalixto (1997). In this method, ABTS was prepared by mixing 5 mL of ABTS solution (7.9 mM) with 88 $\mu$L of potassium persulfate ($K_2S_2O_8$) solution (140 mM). This mixture was allowed to stand for 16 h at room temperature in the dark until reaching a stable oxidative level. Then, 1 mL of ABTS solution was diluted in ethanol to given an absorbance of 0.700 ± 0.05 nm at 734 nm. In a dark place, 3 mL of ABTS solution was added to 30 $\mu$L of test substances at different concentrations (MIC/2, MIC and 2xMIC). After 6 min, the absorbance was taken at 734 nm using the spectrophotometer (Agilent, USA). The scavenging capability of test substances was calculated using following equation:

$$ABTS(\%) = \left(1 - \left(Aa - \frac{Ab}{Ac}\right)\right) \times 100 \quad (2)$$

where $Ac$=absorbance of a control (blank) lacking any radical scavenger and $Aa$=absorbance of the remaining ABTS in the presence of scavenger, $Ab$=absorbance of the sample without ABTS.

## 2.5 Preparation and application of edible coatings on strawberries

The strawberries were immersed in sodium hypochlorite (1 %) for 15 min, washed with potable water and left to dry (2 h). Then, the strawberries were immersed (1 min) in the coating solutions and left to dry (30 min) on a nylon filter to drain the excess liquid (Vasconcelos de Oliveira et al., 2014a).

The strawberries were divided into five groups. One group was the negative control (without edible coating), the other group was the positive control (fruit coated with glycerol – 2.5 mL.(100 mL)$^{-1}$) and the other three groups were coated with CS gel, FCN and CS gel enriched with FCN. For the formation of the different fungal chitosan edible coatings, we used the highest MIC value determined for the strawberries phytopathogenic fungi. The pure chitosan or lyophilized nanoparticles were diluted in 1 % acetic acid and the pH was adjusted to 5.8 (Vasconcelos de Oliveira et al., 2014a).

Each treatment included 40 strawberries that were stored for 6 days at room temperature (25 ± 2 $^o$C) and 12 days at cold temperature (10 ± 2 $^o$C) (Shahbazi, 2018). These temperatures were chosen based on the methodologies used by Dantas Guerra et al. (2015), dos Santos et al. (2012), Gol, Patel, and Rao (2013), Vasconcelos de Oliveira et al. (2014a, 2014b) and Castelo Branco Melo et al. (2018).

## 2.6 Physicochemical analysis on strawberries

The strawberries were evaluated every 3 days for general quality parameters such as moisture content, total soluble solids (TSS), titratable acidity (TA), maturity index (MI), pH and anthocyanin content. The day of the application of the edible coatings was considered time zero (day zero) in the storage period.

The TSS content was determined with a refractometer (Model AUS JENA, Germany), and the results were expressed as % TSS (AOAC, 932.12). The AT was determined by titrating with 0.1 mol.L$^{-1}$ NaOH to pH 8.2, and the results were expressed as a percentage of citric acid (AOAC 920.149). The MI was calculated as the quotient of soluble solids and acidity. The pH of the fruit samples was assessed using a digital pH meter (Model: Micronal B474) according to the standard method described in AOAC (2012) (AOAC 981.12). The moisture content was determined by dehydration of the fruit (70 $^o$C / 24 h) until the dry weight was obtained, and the weight losses were expressed as a percentage of

the intitial weight (AOAC 934.06). The anthocyanin content was estimated by the method described by Lees and Francis (1972). The results were expressed in $g.L^{-1}$.

## 2.7 Weight loss percentage

The weight loss was considered the difference between the initial and final weight of coated and uncoated strawberries. The results were expressed as the percentage loss from the initial weight, according to the method described by Gol et al. (2013).

## 2.8 Decay rate of strawberries

The strawberries were examined for any microorganism infection during storage. The decay percentage of coated and uncoated fruit was calculated as the number of decayed fruit divided by the initial number of all strawberries multiplied by 100 (Castelo Branco Melo et al., 2018).

## 2.9 Sensory evaluation

The sensory analyses were performed after approval by an Ethics Research Committee - Certificate Number: 58937016.3.0000.5208. Uncoated and coated strawberries at the same concentration used in the physicochemical analysis, were stored at a cold temperature. Sensory attributes of the strawberries were analysed 3 days after the coating application in a standardized testing room in the sensory laboratory of the Department of Nutrition located in Federal University of Pernambuco (Brazil).
Each sample was presented simultaneously in dishes coded with 3-digit random numbers to each panellist for evaluation three days after the coating application. The panellists were asked to drink water and eat a salty biscuit between samples. In the preference test, the tasters were asked to choose the most and least appreciated samples based on their overall evaluation. The intent to purchase was assessed on a five-point structure hedonic scale ranging from one (certainly would not by) to five (certainly would buy). For the acceptability test, a nine-point structured hedonic scale was used, ranging from one (dislike very much) to nine (like very much) (Castelo Branco Melo et al., 2018).

## 2.10 Sensory evaluation

All data were analysed by analysis of variance (ANOVA) using Origin 8.0 software. ANOVA was followed by a post hoc Tukey's test. This test was performed to determine differences ($p < 0.05$) between the results.

# 3 Results and discussion

## 3.1 Characterization of FCN

The Figure 1 shows that FCN presented 331.1 ± 7.21 nm. Based on previous studies, this particle size contributes to the performance of FCN as antifungal agent. This effect can be seen in the research of Saharan et al. (2015) and Xing et al. (2016), which produced particles with an average size of 374.3 nm and 296.96 nm, respectively, for use as an antimicrobial agent against phytopathogenic fungi. According to MubarakAli et al. (2018), chitosan nanoparticles present antifungal activity due to their large surface area. It allows them to fix on a large number of fungi, which contributes to their use as an edible coating.
FCN also had a narrow size distribution with a polydispersity index of 0.377. Generally, the use of low molecular weight chitosan produces small and uniform nanoparticles (Paomephan et al., 2018), as was seen in our FCN. According to Saharan et al. (2015), this size uniformity also contributes to the antifungal activity of chitosan nanoparticles. Therefore, the particles produced in the present research exhibited an average size which contributed to the antifungal activity and consequently to the ability of these particles to preserve the quality of postharvest fruit as an edible coating. Furthermore, the FCN produced, according to Severino et al. (2012), will not be absorbed by the human intestinal epithelium when people eat a fruit with an edible coating containing FCN. These authors reported that particles > 300 nm are not absorbed by the human intestinal epithelium.
The morphological characterization of FCN by

the SEM confirmed the uniform spherical shape of the particles (Fig. 1). However, SEM analysis showed the formation of aggregates between the particles. Fan, Yan, Xu, and Ni (2012) reported that the formation of these aggregates is caused by the hydrogen bonds between the particles formed during the drying process of the sample for SEM analysis.

The FCN observed by SEM exhibited a smaller size than the size of FCN analyzed by dynamic light scattering. It could have occurred because the DLS technique observes the state of the sample in the presence of solvent associated with the particles, while SEM gives the size of the particles in dry form (Kiilll et al., 2017).

The zeta potential of FCN was + 34 mV, which meant that these nanoparticles could be stable for a long time. This is in agreement with the results of Sullivan et al. (2018) who reported that when chitosan and TPP are mixed with each other, they spontaneously formed complexes with an overall positive surface charge. According to Yien, Zin, Sarwar, and Katas (2012), nanoparticles with a surface charge greater than + 30 mV are more stable. Furthermore, Saharan et al. (2015) reported that particles with a positive surface charge exhibit a higher affinity towards biological membranes in an aqueous environment and, therefore, more antifungal activity.

## 3.2 Determination of the antifungal activity

The MIC results are shown in Table 1. These results were in agreement with Verlee, Mincke, and Stevens (2017) who showed that the MIC values of chitosan against fungi ranges from 0.01 $g.L^{-1}$ to 7.75 $g.L^{-1}$. Furthermore, the MIC values found in our research were lower than the values found by dos Santos et al. (2012) and Dantas Guerra et al. (2015) when they analysed the MIC values of chitosan from shrimp against *A. niger, B. cinerea* and *R. stolonifera*. However, the different MIC values found in our research could not be related to microbiological origin of the chitosan used. Vasconcelos de Oliveira et al. (2014a) and Vasconcelos de Oliveira et al. (2014b) also used fungal chitosan against phytopathogenic fungi and found MIC = 15 $g.L^{-1}$ for *B. cinerea* and MIC = 7.5 $g.L^{-1}$ for *A. niger* and *R. stolonifera*. It was suggested that the difference between the chitosan MIC values against the same species of fungi could be related to the physicochemical characteristics of the polymer, such as the degree of deacetylation and molecular weight (Verlee et al., 2017).

According to Badawy and Rabea (2011), low molecular weight chitosan presents a better antifungal activity. This occurs due to the ability of this polymer to penetrate in the fungal cell wall. This suggests that the main target of action of the low molecular weight chitosan is the plasma membrane of the fungi (Park et al., 2008). The fungal chitosan used in the present research had a low molecular weight, which could have contributed to the high antifungal activity and low MIC values.

In addition, Kong, Chen, Xing, and Park (2010) reported that the chitosan presents a polycationic structure due to the presence of amino groups in the polymer chain. These groups give the polymer a high density of positive charges that could be measured by the degree of deacetylation. Therefore, a chitosan with a high degree of deacetylation exhibited better electrostatic interaction with the fungal cell wall. In general, the antifungal activity increases when the degree of deacetylation is higher and the molecular weight is lower (Verlee et al., 2017).

Despite the discrepancies between the physicochemical characteristics of chitosan, it is known that chitosan can affect the fungal cell membrane via electrostatic interactions with the negatively charged phospholipids. Once the cell membrane is disrupted, chitosan is capable of entering the cell. This could lead to inhibition of DNA/RNA synthesis and disruption of protein synthesis (Ma, Garrido-Maestu, & Jeong, 2017). FCN improved the antifungal activity when compared to CS gel. According to Kheiri, Jorf, Malihipour, Saremi, and Nikkhah (2016), chitosan nanoparticles exhibit higher antifungal activity than CS gel on account of the special characteristics (size and surface charge) of these particles. The negatively charged surface of the fungal cell is the target site of the polycation. Therefore, the polycationic FCN with higher surface charge density interacts with the fungus to a greater de-

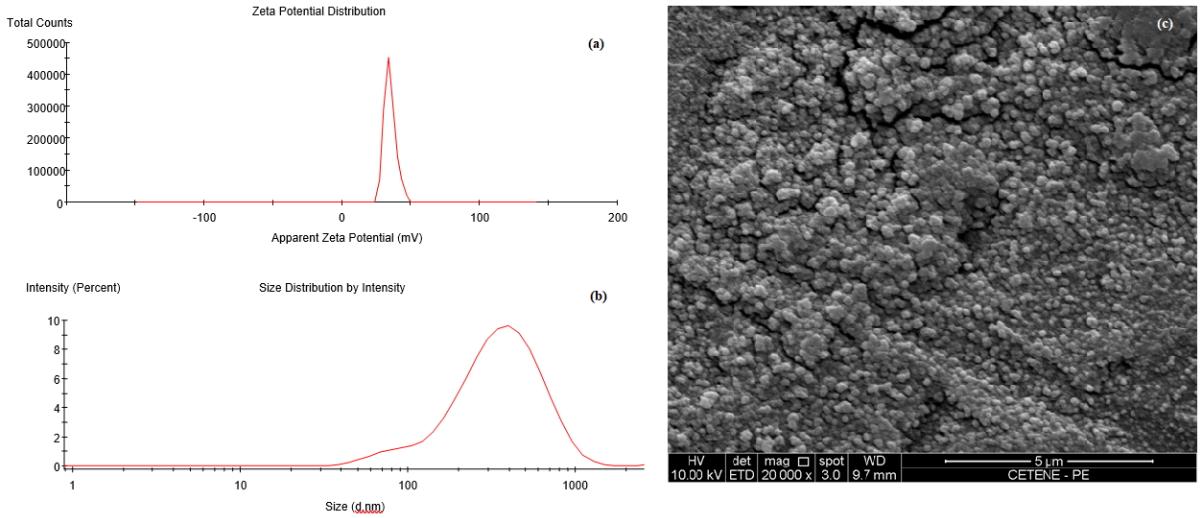


Figure 1: Zeta potential (a), average size distribution (b) and SEM image (c) of fungal chitosan nanoparticles

gree than CS itself (Kheiri et al., 2016). Due to the larger surface area of the nanoparticles, FCN could be tightly adsorbed onto the surface of the fungal cells so as to disrupt the membrane, which would lead to the leakage of cellular contents, thus killing the fungal cells (Garcia-Rincon et al., 2010). Chitosan nanoparticles penetrate into fungal cells and tightly bind nucleic acids via electrostatic interaction between cationic FCN and anionic DNA. This suggests that they may cause a variety of damage and selective inhibition such as inactivation of the synthesis of essential mRNA encoded by various genes required for important metabolic and infectious processes of the microorganism (Yien et al., 2012).

The MIC values for *A. niger* were higher than the values found for the other fungi for all test substances. Yien et al. (2012) found a similar result. This fungus, in particular, was found to be highly resistant to chitosan, because *A. niger* contains 10 % of chitin in the cell wall, and fungi that have chitosan as one of their cell wall components are more resistant to externally applied chitosan and consequently more resistant to chitosan nanoparticles (Allan, Allan, & Hadwiger, 1979).

When the CS gel was enriched with FCN an improvement was observed in the antifungal activity. The mixture of the gel with FCN presented the best inhibitory effect against the strawberry phytopathogenic fungi. This suggests that when the gel and nanoparticles were together, they improved their individual antifungal activity. According to Lee, Song, and Lee (2010), when chitosan nanoparticles are well-dispersed in the solution they are more efficiently transported to the cell wall, which causes metabolic disorders and leads to increased antimicrobial activity.

### 3.3 Antioxidant activity

As is shown in Figure 2a, the test substances showed strong activity to scavenging the DPPH free radicals in a dose-dependent manner. Similar results were found by Chen et al. (2015) and MubarakAli et al. (2018). The same behaviour was found in the ABTS assay (Fig. 2b). DPPH radicals are less reactive than ABTS radicals. Unlike the reactions with DPPH radicals, which involve H-ion transfer, the reactions with ABTS radicals involve an electron-transfer process (Chen et al., 2015).

In DPPH assay, any test substance at the concentration of MIC/2 showed no scavenging effect. The scavenging effect occurred only when

Table 1: Antifungal activity of chitosan gel (CS gel), fungal chitosan nanoparticles (FCN) and gel enriched with nanoparticles (CS Gel + FCN) against strawberry phytopathogenic fungi.

| Test Substance | Phytopathogenic fungi | | |
|---|---|---|---|
| | *R. stolonifer* (URM 3728) $g.L^{-1}$ | *B. cinerea*(URM 2802) $g.L^{-1}$ | *A. niger* (URM 7282) $g.L^{-1}$ |
| CS gel | 4.0 | 4.0 | 5.0 |
| FCN | 1.5 | 1.5 | 2.5 |
| CS gel + FCN | 1.0 + 0.5 | 1.0 + 0.5 | 2.0 + 1.5 |

Note: Values represent the mean of three replicates.
The standard deviation was zero for all mean values.

the test substances were used at the MIC and 2MIC concentrations. However, at a low concentration (CIM/2), the scavenging ability of the test substances on ABTS was stronger than with DPPH radicals.

The scavenging ability of CS gel on DPPH and ABTS was stronger than FCN and CS gel enriched with FCN. It may have been due to the presence of the amino groups in the CS chain (Siripatrawan & Harte, 2010). According to Yen, Yang, and Mau (2008), the scavenging mechanism of chitosan is related to the fact that free radicals can react with the residual free amino ($NH_2$) groups to form a stable macromolecule radical, and the $NH_2$ can form ammonium ($NH_3^+$) groups by absorbing a hydrogen ion from the solution. Therefore, chitosan with higher degree of deacetylation will probably exhibit a strong antioxidant activity.

The FCN presented the lowest antioxidant activity for both tests. This could be due to the lower number of free amino groups present in the nanoparticles. During the ionic gelation method, the process of the nanoparticles synthesis, the amino groups of the chitosan chain bind to TPP to form the FCN (Bugnicourt, Alcouffe, & Ladavière, 2014). Therefore, the fungal chitosan nanoparticles have fewer free NH2 groups than CS gel. It contributed to the lower FCN antioxidant activity, because these free amino groups are responsible for binding to free radicals.

It is important to note that the MIC concentrations of FCN (2.5 $g.L^{-1}$) is half of the MIC concentration of CS gel (5 $g.L^{-1}$). This suggests that the FCN had a lower antioxidant activity than the CS gel not only due to the smaller number of free amino groups, but also to its lower MIC value that were used in the DPPH and ABTS assays.

However, when FCN was dispersed in the CS gel an improvement in their scavenging ability occured. It could be seen in the ABTS assay when the CS gel and CS gel enriched with FCN was tested in 2MIC concentration. These substances exhibited similar scavenging effect at this concentration ($p > 0.05$). This result suggested that when CS gel was mixed with FCN was an increase not only their antifungal activity, but also an improvment in the antioxidant activity of this mixture.

## 3.4 Effects on physicochemical characteristics of strawberries

The physicochemical changes in uncoated and coated strawberries were evaluated throughout storage at room temperature and under refrigeration for 6 and 12 days, respectively (Tables 2 and 3). According to Oregel-Zamudio et al. (2017), the soluble solids content during the ripening of strawberries should range from 4.6 % to 11.9 %. In the present research, all fruit samples presented soluble solids content in the range recommended. However, at the end of the storage period at both temperatures, the control sample exhibited significantly higher soluble solids content ($p < 0.05$) than the chitosan-based edible coatings. This result suggested that the control fruit continued to have active metabolism, which contributed to the conversion of the starch to acid

Table 2: Mean values of physicochemical quality parameters in strawberries stored at room temperature for 6 days in the absence of edible coating (control) and with glycerol, chitosan gel (CS Gel), fungal chitosan nanoparticles (FCN) and chitosan gel enriched with fungal chitosan nanoparticles (Gel + FCN).

| | Days of storage | | |
|---|---|---|---|
| Treatments | 0 | 3 | 6 |
| Soluble solids (%) | | | |
| Control | $8.00(\pm0.02)^{Aa}$ | $8.27(\pm0.23)^{Aa}$ | $8.27(\pm0.23)^{Aa}$ |
| Glycerol | $8.20(\pm0.02)^{Aa}$ | $7.20(\pm0.00)^{Bb}$ | $8.10(\pm0.00)^{Aa}$ |
| CS Gel | $8.46(\pm0.58)^{Aa}$ | $7.17(\pm0.06)^{Bb}$ | $7.20(\pm0.00)^{Bb}$ |
| FCN | $8.13(\pm0.12)^{Aa}$ | $7.20(\pm0.00)^{Bb}$ | $6.93(\pm0.46)^{Bb}$ |
| Gel + FCN | $8.03(\pm0.06)^{Aa}$ | $7.20(\pm0.00)^{Bb}$ | $7.20(\pm0.00)^{Bb}$ |
| Titratable acidity (% citric acid) | | | |
| Control | $1.15(\pm0.01)^{Aa}$ | $0.88(\pm1.13)^{Ab}$ | $1.04(\pm0.02)^{Aa}$ |
| Glycerol | $1.07(\pm0.08)^{Aab}$ | $1.16(\pm0.05)^{Aa}$ | $1.18(\pm0.02)^{Aa}$ |
| CS Gel | $1.00(\pm0.01)^{Bab}$ | $1.06(\pm0.01)^{Aab}$ | $1.10(\pm0.01)^{Aa}$ |
| FCN | $1.06(\pm0.00)^{Bab}$ | $1.18(\pm0.03)^{Aa}$ | $1.12(\pm0.04)^{ABa}$ |
| Gel + FCN | $0.98(\pm0.01)^{Ab}$ | $1.11(\pm0.03)^{Aab}$ | $1.18(\pm.11)^{Aa}$ |
| Maturity index | | | |
| Control | $7.04(\pm0.04)^{Aa}$ | $9.38(\pm1.04)^{Aa}$ | $8.12(\pm0.16)^{Aa}$ |
| Glycerol | $7.59(\pm0.74)^{Aa}$ | $6.24(\pm0.26)^{Ab}$ | $6.89(\pm0.13)^{Aab}$ |
| CS Gel | $8.29(\pm0.59)^{Aa}$ | $6.82(\pm0.04)^{Bb}$ | $6.54(\pm0.08)^{Bb}$ |
| FCN | $7.64(\pm0.13)^{Aa}$ | $6.10(\pm0.14)^{Bb}$ | $6.03(\pm0.32)^{Bb}$ |
| Gel + FCN | $8.20(\pm0.06)^{Aa}$ | $6.48(\pm0.16)^{Bb}$ | $6.12(\pm0.59)^{Bb}$ |
| pH | | | |
| Control | $3.58(\pm0.02)^{Aa}$ | $3.49(\pm0.02)^{Bb}$ | $3.05(\pm0.00)^{Cb}$ |
| Glycerol | $3.41(\pm0.01)^{Ac}$ | $3.42(\pm0.02)^{Ac}$ | $3.06(\pm0.08)^{Bb}$ |
| CS Gel | $3.49(\pm0.01)^{Ab}$ | $3.52(\pm0.02)^{Ab}$ | $3.45(\pm0.01)^{Bab}$ |
| FCN | $3.51(\pm0.01)^{Ab}$ | $3.41(\pm0;01)^{Ac}$ | $3.76(\pm0.57)^{Aa}$ |
| Gel + FCN | $3.50(\pm0.01)^{Bb}$ | $3.58(\pm0.01)^{Aa}$ | $3.39(\pm0.02)^{Cab}$ |
| Moisture (%) | | | |
| Control | $94.69(\pm0.42)^{Aa}$ | $91.69(\pm0.00)^{Aa}$ | $88.22(\pm4.76)^{Aa}$ |
| Glycerol | $94.34(\pm0.42)^{Aa}$ | $92.62(\pm0.30)^{Ba}$ | $91.66(\pm0.15)^{Ca}$ |
| CS Gel | $93.94(\pm0.09)^{Aa}$ | $92.55(\pm0.14)^{Ba}$ | $92.12(\pm0.08)^{Ba}$ |
| FCN | $94.58(\pm0.21)^{Aa}$ | $92.14(\pm0.43)^{Ba}$ | $90.54(\pm0.23)^{Ca}$ |
| Gel + FCN | $94.31(\pm0.13)^{Aa}$ | $92.00(\pm0.47)^{Ba}$ | $91.40(\pm0.30)^{Ba}$ |
| Anthocyanin ($g.L^{-1}$) | | | |
| Control | $29.13(\pm0.02)^{Ac}$ | $25.97(\pm0.01)^{Cc}$ | $26.30(\pm0.03)^{Ba}$ |
| Glycerol | $37.04(\pm0.02)^{Aa}$ | $27.71(\pm0.08)^{Bb}$ | $25.02(\pm0.04)^{Cb}$ |
| CS Gel | $28.41(\pm0.05)^{Ad}$ | $23.98(\pm0.06)^{Be}$ | $23.51(\pm0.01)^{Cc}$ |
| FCN | $32.49(\pm0.01)^{Ab}$ | $25.66(\pm0.08)^{Bd}$ | $21.60(\pm0.01)^{Cd}$ |
| Gel + FCN | $26.00(\pm0.04)^{Be}$ | $28.72(\pm0.17)^{Aa}$ | $21.14(\pm0.05)^{Ce}$ |

Note: The storage time Day 0 represents the day of application of the edible coatings. Values represent the mean of three replicates with their standard error (±SD). $^{A-C}$For each trial, different superscript lowercase letters within a row denote significant differences ($p < 0.05$) between the mean values according to Tukey's test. $^{a-e}$ Different superscript capital letters in the same column denote significant differences ($p < 0.05$) between the mean values according to Tukey's test

Table 3: Mean values of physicochemical quality parameters in strawberries stored at cold temperature for 12 days in the absence of edible coating (control) and with glycerol, chitosan gel (CS Gel), fungal chitosan nanoparticles (FCN) and chitosan gel enriched with fungal chitosan nanoparticles (Gel+FCN).

| Treatments | Days of storage | | | | |
|---|---|---|---|---|---|
| | 0 | 3 | 6 | 9 | 12 |
| Soluble solids (%) | | | | | |
| Control | $8.07(\pm0.12)^{Bb}$ | $8.13(\pm0.06)^{Ba}$ | $8.27(\pm0.23)^{Ba}$ | $8.53(\pm0.46)^{Ba}$ | $9.60(\pm0.00)^{Aa}$ |
| Glycerol | $8.47(\pm0.23)^{Aa}$ | $8.03(\pm0.06)^{Aab}$ | $8.13(\pm0.23)^{Aa}$ | $8.53(\pm0.46)^{Aa}$ | $8.67(\pm0.23)^{Ab}$ |
| CS Gel | $8.00(\pm0.00)^{Ab}$ | $8.00(\pm0.00)^{Ab}$ | $8.00(\pm0.00)^{Aa}$ | $8.27(\pm0.23)^{Aa}$ | $8.40(\pm0.04)^{Ab}$ |
| FCN | $8.07(\pm0.12)^{Bb}$ | $8.03(\pm0.06)^{Bab}$ | $8.00(\pm0.00)^{Ba}$ | $8.00(\pm0.00)^{Ba}$ | $8.40(\pm0.00)^{Ab}$ |
| Gel + FCN | $8.00(\pm0.00)^{Ab}$ | $8.00(\pm0.00)^{Ab}$ | $8.00(\pm0.00)^{Aa}$ | $8.00(\pm0.00)^{Aa}$ | $8.27(\pm0.23)^{Ab}$ |
| Titratable acidity (% citric acid) | | | | | |
| Control | $1.06(\pm0.01)^{Aa}$ | $1.04(\pm0.02)^{Aa}$ | $1.11(\pm0.08)^{Aa}$ | $1.06(\pm0.11)^{Aa}$ | $1.14(\pm0.02)^{Aa}$ |
| Glycerol | $1.18(\pm0.01)^{Aa}$ | $0.89(\pm0.42)^{Ba}$ | $1.02(\pm0.06)^{ABab}$ | $0.94(\pm0.01)^{Ba}$ | $1.14(\pm0.05)^{Aa}$ |
| CS Gel | $1.10(\pm0.01)^{Aa}$ | $1.00(\pm0.01)^{Aba}$ | $0.92(\pm0.01)^{Bb}$ | $0.93(\pm0.04)^{Ba}$ | $1.08(\pm0.02)^{Aab}$ |
| FCN | $0.97(\pm0.00)^{Aa}$ | $0.78(\pm0.23)^{Aa}$ | $1.02(\pm0.02)^{Aab}$ | $1.10(\pm0.06)^{Aa}$ | $1.08(\pm0.01)^{Aab}$ |
| Gel + FCN | $1.02(\pm0.15)^{Aa}$ | $1.00(\pm0.00)^{Aa}$ | $1.03(\pm0.00)^{Aab}$ | $1.05(\pm0.03)^{Aa}$ | $1.01(\pm0.00)^{Ab}$ |
| Maturity index | | | | | |
| Control | $7.58(\pm0.05)^{Aa}$ | $7.69(\pm0.24)^{Aa}$ | $7.39(\pm0.31)^{Ab}$ | $8.34(\pm0.89)^{Aa}$ | $8.38(\pm0.16)^{Aa}$ |
| Glycerol | $7.14(\pm0.20)^{Ba}$ | $9.00(\pm0.43)^{Aa}$ | $8.00(\pm0.22)^{ABab}$ | $8.98(\pm0.67)^{Aa}$ | $7.76(\pm0.34)^{ABa}$ |
| CS Gel | $7.30(\pm0.04)^{Ba}$ | $8.02(\pm0.57)^{ABa}$ | $8.74(\pm0.07)^{Aa}$ | $8.78(\pm0.04)^{Aa}$ | $7.55(\pm0.11)^{Ba}$ |
| FCN | $8.24(\pm0.00)^{Aa}$ | $8.08(\pm0.76)^{Aa}$ | $7.80(\pm0.16)^{Ab}$ | $7.28(\pm0.37)^{Aa}$ | $7.74(\pm0.05)^{Aa}$ |
| Gel + FCN | $7.96(\pm1.17)^{Aa}$ | $8.00(\pm0.00)^{Aa}$ | $7.76(\pm0.00)^{Ab}$ | $7.62(\pm0.20)^{Aa}$ | $8.12(\pm0.28)^{Aa}$ |
| pH | | | | | |
| Control | $3.45(\pm0.01)^{Ab}$ | $3.47(\pm0.01)^{Ab}$ | $3.30(\pm0.07)^{Bc}$ | $3.32(\pm0.00)^{Bc}$ | $3.33(\pm0.00)^{Bd}$ |
| Glycerol | $3.41(\pm0.01)^{Cc}$ | $3.50(\pm0.01)^{Aa}$ | $3.36(\pm0.00)^{Bbc}$ | $3.37(\pm0.01)^{Bc}$ | $3.37(\pm0.01)^{Bcd}$ |
| CS Gel | $3.46(\pm0.01)^{Cb}$ | $3.50(\pm0.00)^{BCa}$ | $3.52(\pm0.01)^{Ba}$ | $3.57(\pm0.00)^{Aa}$ | $3.54(\pm0.00)^{ABa}$ |
| FCN | $3.51(\pm0.00)^{Aa}$ | $3.41(\pm0.00)^{Bc}$ | $3.38(\pm0.00)^{CDbc}$ | $3.37(\pm0.00)^{Dc}$ | $3.39(\pm0.01)^{Cc}$ |
| Gel + FCN | $3.44(\pm0.01)^{Ab}$ | $3.46(\pm0.01)^{Ab}$ | $3.43(\pm0.02)^{Aab}$ | $3.45(\pm0.04)^{Ab}$ | $3.44(\pm0.01)^{Ab}$ |
| Moisture (%) | | | | | |
| Control | $91.46(\pm0.06)^{Ba}$ | $91.27(\pm0.47)^{Ba}$ | $91.48(\pm0.09)^{Bb}$ | $91.88(\pm0.13)^{Ba}$ | $92.86(\pm0.17)^{Ab}$ |
| Glycerol | $90.94(\pm0.85)^{Aa}$ | $92.38(\pm0.48)^{Aa}$ | $92.80(\pm0.12)^{Aa}$ | $91.65(\pm0.74)^{Aa}$ | $92.91(\pm0.11)^{Ab}$ |
| CS Gel | $91.06(\pm0.12)^{Ba}$ | $91.48(\pm0.11)^{Ba}$ | $92.65(\pm0.17)^{Aa}$ | $92.49(\pm0.21)^{Aa}$ | $92.91(\pm0.08)^{Ab}$ |
| FCN | $90.78(\pm0.18)^{Da}$ | $91.31(\pm0.08)^{CDa}$ | $92.13(\pm0.28)^{ABab}$ | $91.60(\pm0.18)^{BCa}$ | $92.66(\pm0.15)^{Ab}$ |
| Gel + FCN | $92.08(\pm0.01)^{Ca}$ | $92.44(\pm0.04)^{BCa}$ | $92.98(\pm0.37)^{ABa}$ | $92.42(\pm0.01)^{BCa}$ | $93.42(\pm0.11)^{Aa}$ |
| Anthocyanin ($g.L^{-1}$) | | | | | |
| Control | $28.60(\pm0.00)^{Ac}$ | $28.23(\pm0.03)^{Ab}$ | $25.98(\pm0.01)^{Bab}$ | $23.62(\pm0.01)^{Cc}$ | $22.71(\pm0.31)^{Db}$ |
| Glycerol | $39.88(\pm0.03)^{Aa}$ | $31.69(\pm0.58)^{Ba}$ | $24.87(\pm1.17)^{Cb}$ | $19.97(\pm0.04)^{De}$ | $21.05(\pm0.06)^{Dc}$ |
| CS Gel | $30.01(\pm0.16)^{Ab}$ | $24.54(\pm0.02)^{Ed}$ | $26.43(\pm0.02)^{Da}$ | $28.11(\pm0.03)^{Ba}$ | $27.64(\pm0.00)^{Ca}$ |
| FCN | $27.53(\pm0.00)^{Ad}$ | $26.68(\pm0.20)^{Bc}$ | $22.52(\pm0.00)^{Cc}$ | $21.89(\pm0.04)^{Dd}$ | $22.50(\pm0.02)^{Cb}$ |
| Gel + FCN | $22.40(\pm0.01)^{De}$ | $25.31(\pm0.24)^{Bd}$ | $25.68(\pm0.01)^{Aab}$ | $24.51(\pm0.01)^{Cb}$ | $22.51(\pm0.01)^{Db}$ |

Note: The storage time Day 0 represents the day of application of the edible coatings. Values represent the mean of three replicates with their standard error (±SD). $^{A-E}$For each trial, different superscript lowercase letters within a row denote significant differences ($p < 0.05$) between the mean values according to Tukey's test. $^{a-e}$Different superscript capital letters in the same column denote significant differences ($p < 0.05$) between the mean values according to Tukey's test.

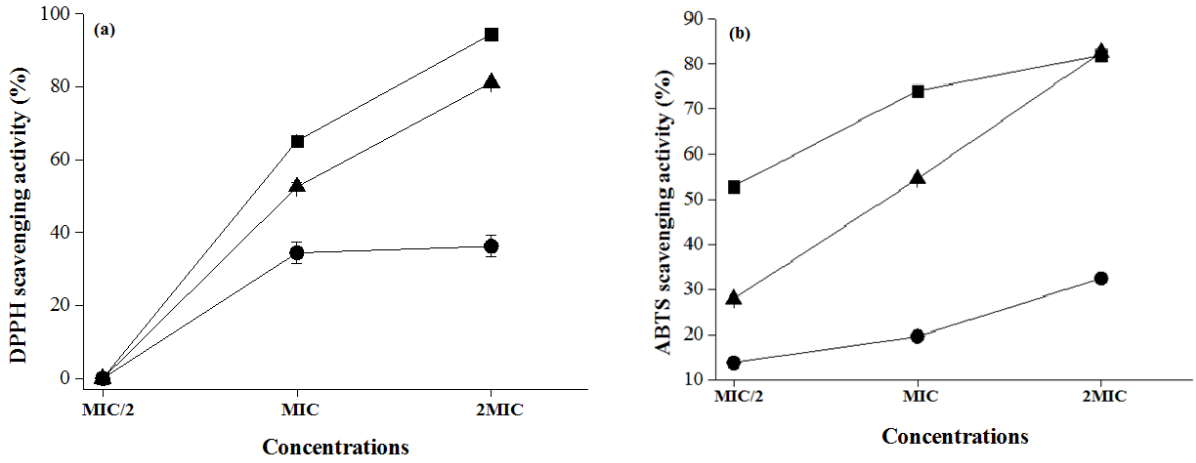


Figure 2: Scavenging effect of CS gel (■), FCN (●) and CS gel + FCN (▲) towards DPPH (a) and ABTS (b) radicals.

and sugar, thereby increasing the soluble solids content (Cao, Guan, Dai, Li, & Zhang, 2015).
Coated strawberries presented lower values of soluble solids at the end of storage because chitosan modified the internal atmosphere of the fruit, reducing the $O_2$ and increasing $CO_2$. It could have reduced the respiration rate and the metabolic activity of the fruit, which also reduced the conversion of sugars into $CO_2$ and water (Ghasemnezhad, Nezhad, & Gerailoo, 2011). There was no significant difference ($p > 0.05$) in the soluble solids content of the samples coated with edible chitosan-based coatings. Therefore, this result suggested that the capacity of chitosan to maintain the soluble solids content of strawberries was independent of the form of the polymer that was applied (gel or nanoparticles).
The titratable acidity values of the strawberries with and without edible coatings were maintained during the storage period at room temperature. According to Gol et al. (2013), the titratable acidity is directly related to the amount of organic acids present in the fruit and in the strawberry the main acids are citric and malic. A reduction in the fruit acidity could occur due to changes in fruit metabolism or the use of organic acids in the respiratory process (Hajji, Younes, Affes, Boufi, & Nasri, 2018). The edible coatings reduce the fruit respiration rate that delays the use of organic acids which maintains titratable acid values during storage (Yaman & Bayoindirli, 2002).
The strawberries stored at the cold temperature also maintained titratable acidity values in relation to day 0. However, on day 12, the strawberries coated with the gel+FCN differed significantly ($p < 0.05$) from the control sample and presented the lowest titratable acidity value. This could be related to the low values of titratable acidity that this strawberry sample already had from the beginning of the analysis. In this case, the edible coating only maintained these values, which showed that the gel+FCN was able to retard the metabolism of the fruit, and consequently decreased the consumption of the organic acids, so maintaining the titratable acidity.
The strawberries with the edible coatings and stored at room temperature decreased their maturation index from the third day of storage. In addition, at the same temperature, the coated samples differed significantly ($p < 0.05$) at the end of the storage period when compared to the control sample.
The increase in maturity index during storage is

associated with the progression of the fruit ripening process (Perdones, Sanchez-Gonzalez, Chiralt, & Vargas, 2012). The low values of the maturity index in the coated samples showed the capacity of the edible chitosan based coatings to delay the metabolic activity of the strawberries. However, in cold storage, at in the end of the storage period, the maturity index of the strawberries with and without coatings did not differ significantly ($p > 0.05$). According to Valenzuela et al. (2015), this increase in the maturity index during the final storage period is a result of the fruit senescence process.

Strawberries coated with FCN and gel + FCN kept the maturity index constant, which reflected the delay of fruit senescence. Otherwise, strawberries coated with glycerol and CS gel showed an increase in their maturation index.

The anthocyanin content can also reflect the delay of fruit senescence. At the end of storage, all samples showed a decrease ($p < 0.05$) in the anthocyanin content. However, on the last day of storage, at room temperature the control sample had the highest anthocyanin content ($p < 0.05$). The edible coatings formed a barrier between the fruit and the environment, which modified the concentrations of $CO_2$ and $O_2$ in the fruit affecting the synthesis and degradation of anthocyanin. The greatest accumulation of anthocyanin in the control sample may have been related to its advanced maturation, which includes the synthesis of this pigment from glucose (Meng, Li, Liu, & Tian, 2008).

However, anthocyanin, the pigment responsible for the red colour of strawberries, affects the sensory quality and health benefits of this fruit (Velde, Tarola, Güemes, & Pirovani, 2013). Therefore, low values of this pigment can affect the sensorial acceptability of the fruit. At cold temperatures, the strawberries coated with gel+FCN maintained their anthocyanin content (first and last day of storage) and indirectly the sensorial quality of the fruit.

Furthermore, there was a decrease in the pH of the samples. According to Famiani, Battistelli, Moscatello, Cruz-Castillo, and Walker (2015), in the pulp of many fruits during maturation there is an increase of organic acids. It has been shown that the main organic acid accumulated in ripe strawberries is citric acid (Famiani et al., 2005), which may be involved in the decrease of the pH observed in the samples analyzed.

The strawberries coated with FCN and gel+FCN and stored at room temperature and at cold temperatures, respectively, were the only ones that did not present a significant difference ($p > 0.05$) in the pH values during all the evaluated days, suggesting a delay in the senescence of these samples. It is important to note that only the coatings that had chitosan nanoparticles in their composition (alone or mixed with the gel) were able to maintain pH values and delay the senescence of fruits. Therefore, when chitosan is in nanoparticle form, it is possible that it potentiates its protective effects contributing to the conservation of the physical-chemical parameters of the fruits (Castelo Branco Melo et al., 2018).

The percentage of moisture in the strawberries showed that, at the end of the storage period at room temperature, the moisture content of the samples did not differ significantly ($p > 0.05$). The same behaviour was found in the fruit stored at the cold temperature. The only exception was the strawberries coated with gel+FCN, which presented the highest moisture content on the $12^{th}$ day that differed significantly ($p < 0.05$) from all other samples. Therefore, the storage temperature did not influence in the moisture percentage of the strawberries. Castelo Branco Melo et al. (2018) suggests that the longer storage time of the fruits at refrigeration temperature (12 days) in relation to fruits stored at room temperature (6 days) may also favour an advance in fruit metabolism which contributes to a higher loss of moisture.

The gel + FCN coating applied to strawberries stored in refrigeration showed that the association of these two forms of chitosan could significantly improve the barrier properties of an edible coating. According to Amarante, Banks, and Ganesh (2001), gas exchanges between the fruit and the environment occur through open pores and the high permeability of the skin fruit. The chitosan coatings act as a barrier between the fruit and the environment, thus avoiding the loss of moisture (Guerreiro, Gago, Faleiro, Miguel, & Antunes, 2015). When we added nanoparticles to this type of coating, we created a double blocking effect - the barrier formed by the CS gel and FCN blocked the pores present in the straw-

berries (Castelo Branco Melo et al., 2018).
In addition, it is important to note that the concentration of the gel and the nanoparticles (2 $g.L^{-1}$ + 1 $g.L^{-1}$) in the coating made by the mixture was much lower than the concentration used in the coating made only with the CS gel (5 $g.L^{-1}$) and only with FCN (2.5 $g.L^{-1}$). It showed that the two forms of chitosan presented a potentiation of their barrier properties when together, even in low concentrations.

## 3.5 Weight loss

The samples stored at room temperature had high weight loss and there was no statistically significant difference ($p > 0.05$) between the results of these samples (Fig. 3a). According to Garcia, Medina, and Olias (1998), the maximum commercially acceptable limit for strawberries weight loss is 6 %. In our research, all samples stored at room temperature exhibited weight loss percentages higher than the acceptable limit. Any edible coating was able to prevent significant weight loss at room temperature. This could have occurred due to the storage temperature, since the cold temperature decreased the weight loss of the strawberries by about 15 times when compared to the room temperature. Ventura-Aguilar, Bautista-Banos, Flores-Garcia, and Zavaleta-Avejar (2018) also found a similar decrease when they applied chitosan coatings and cinnamon extract on strawberries stored at cold temperatures.
Fruit weight loss is associated with the fruit respiration and evaporation processes. Therefore, at high temperatures, there is an increase in the respiratory rate of the fruit, associated with a loss of moisture and weight (Ali, Noh, & Mustafa, 2015). So, the weight loss was more intense in samples stored at room temperature than at the cold temperature. A similar result was found by Castelo Branco Melo et al. (2018), who used CS gel enriched with FCN edible coatings to preserve table grapes.
According to Khalifa, Barakat, El-Mansy, and Soliman (2016), chitosan is able to decrease weight loss in fruit due to its filmogenic property. This polymer acts as a barrier to water vapour and the gaseous exchanges between the fruit and the external environment. At cold temperatures, the strawberries coated with CS gel did not present a statistically significant difference ($p > 0.05$) when compared to the fruit coated with FCN or CS gel enriched with FCN. Kaewklin, Siripatrawan, Suwanagul, and Lee (2018), analyzed the weight loss of tomatoes coated with CS film and chitosan-titanium dioxide nanocomposite film and also reported that there was no statistically significant difference ($p > 0.05$) in the weight loss between the tomatoes coated with the CS nanocomposite and CS alone.
Therefore, the prevention of weight loss is more related to the formation of the external barrier produced by the edible coating than the form of the polymer present in this coating. However, all strawberries with the edible coatings produced in the present research and stored at cold temperatures had a commercially acceptable weight loss according to the limit defined by Garcia et al. (1998).

## 3.6 Decay rate

The lowest percentage of decay was found in strawberries coated with FCN alone or FCN associated with CS gel (Fig. 3b). These strawberry samples differed statistically ($p < 0.05$) from the control samples at both temperatures. Otherwise, the fruit coated with CS gel did not statistically differ ($p > 0.05$) from the control fruit at any temperature analyzed. This suggested that there was an improvement in the conservative action of chitosan when this polymer is in the nanoparticle form.
According to Hajji et al. (2018), chitosan plays a dual function by interfering directly in the fungal growth through the hydrolysis of chitin (important cellular component of the fungus) and activating many biological processes in plant tissues. Furthermore, the antimicrobial activity of this polymer is related to its ability to induce severe cellular damage to the molds and to interfere in the polygalacturonases secretion of fruit (Velickova, Winkelhausen, Kuzmanova, Alves, & Moldao-Martins, 2013).
The edible coating itself is another factor that contributes to a lower percentage of visible infection in fruits, since it acts as a barrier that pre-

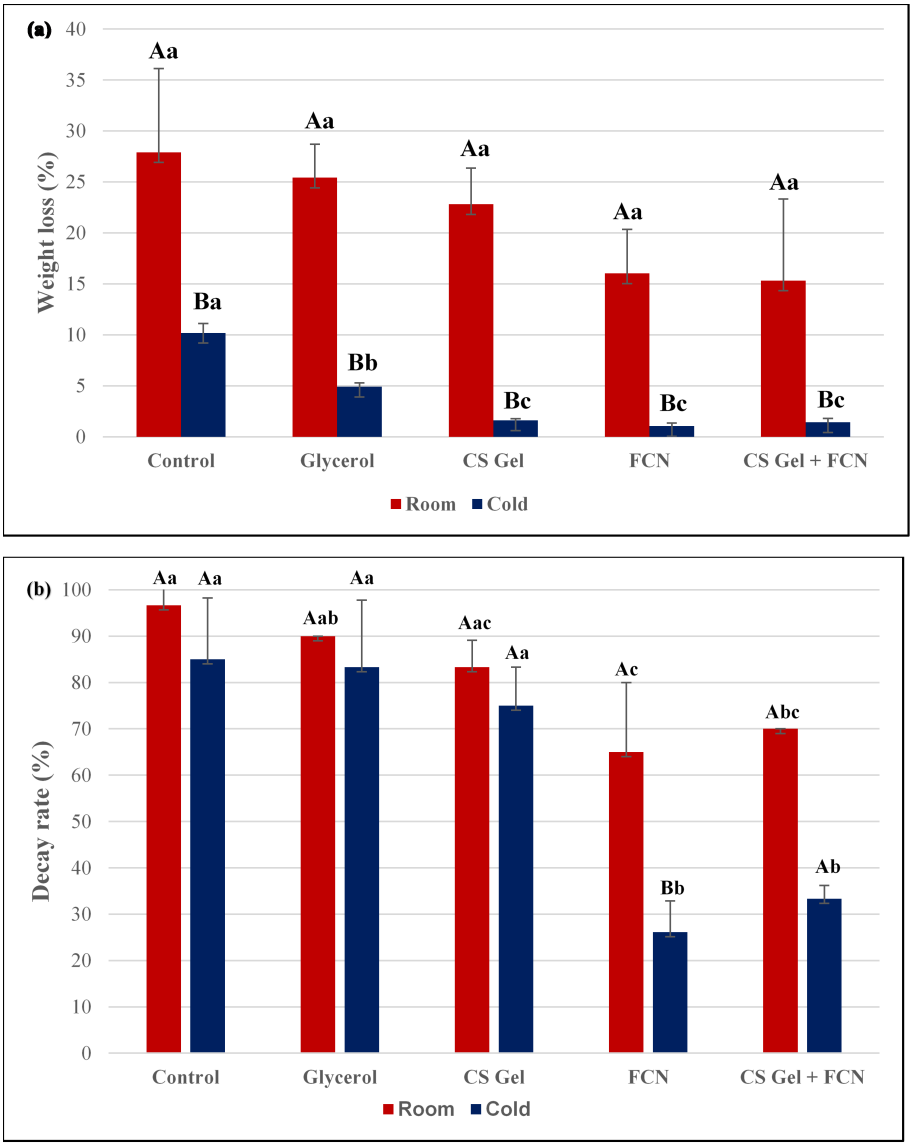


Figure 3: The weight loss (a) and the decay rate (b) of strawberries after 6 and 12 days of room and cold stored temperatures, respectively.

Note: Values represent the mean of three replicates with their standard error (±SD). $^{a-c}$ Different superscript lowercase letters at the same temperature denote significant differences ($p < 0.05$) between the mean values according to Tukey's test. $^{A-B}$ Different superscript capital letters between the temperatures denote significant differences ($p < 0.05$) between the mean values according to Tukey's test.

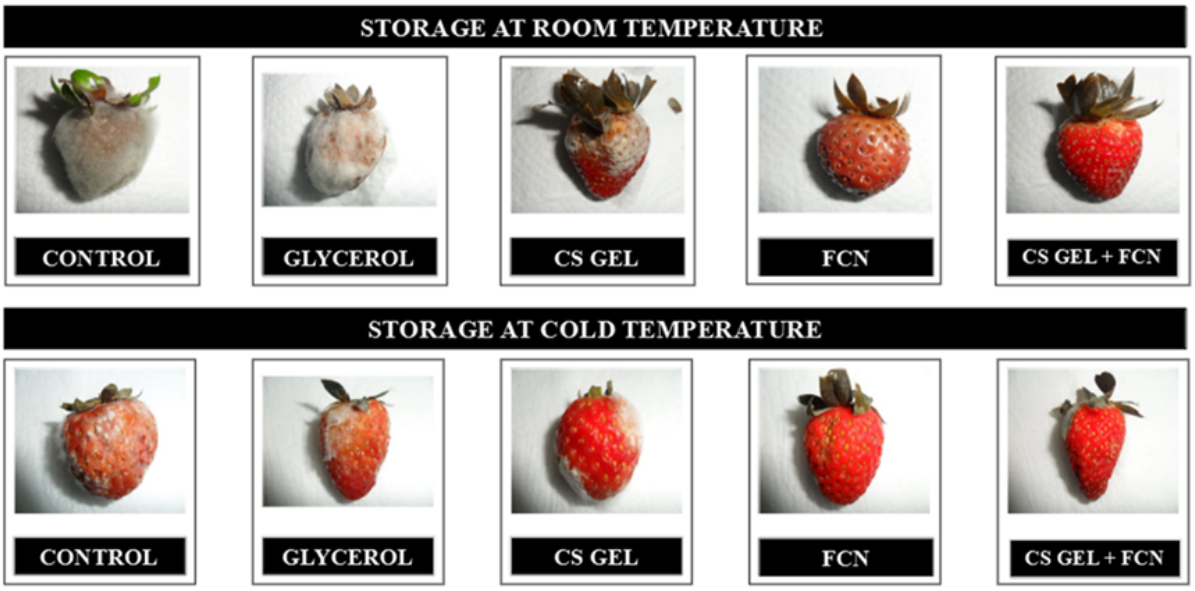


Figure 4: The appearance of uncoated and coated strawberries after 6 and 12 days of room and cold stored temperatures, respectively

vents the contact of the strawberry with external microorganisms (Hajji et al., 2018). The edible coatings also act by retarding the senescence of fruits and the resistance to fungal infections can be improved by the delay in senescence (Gol et al., 2013).

A significant decrease ($p < 0.05$) in the decay rate was observed when FCN coating was applied to the strawberries stored at cold temperatures in relation to strawberries with this same type of coating, but stored at room temperatures (Fig. 4). Similar to the results found in the present research, Castelo Branco Melo et al. (2018) also observed that at cold temperatures the conservative action of the chitosan nanoparticles edible coating is improved. Low temperature storage slows the physiological processes in fruit and pathogens have weaker pathogenicity, and this reduces the incidence of decay compared to fruit stored at room temperature, which decay rapidly (Meng et al., 2008). Therefore, the present research confirms the idea that the use of chitosan with nanoparticles enhances its conservative action on fruits, especially on strawberries. This action could be more effective when the fruit is stored at low temperatures.

### 3.7 Sensory evaluation

Changes in sensory attributes are presented in Table 4. Uncoated fruit received the highest scores for all analyzed attributes when compared to coated fruit. However, there were no significant differences ($p > 0.05$) between the control, CS gel and FCN samples in relation to the strawberry colour, taste, flavour, firmness and overall evaluation. It could be suggested that the form of the chitosan polymer (gel or nanoparticles) did not influence in the sensory quality of the strawberries. According to Vargas, Albors, Chiralt, and Gonzalez-Martinez (2006), chitosan could show an astringent taste due to its dissolution in an acidic medium, but this was not observed in the strawberries coated with CS gel and FCN, since the sensory analysis was performed on the third day of storage. This result is in agreement with the results reported by Velickova et al. (2013) and Castelo Branco Melo et al. (2018).

It is important to note that the edible coating

Table 4: Mean sensory scores for uncoated (control) fruit and fruit coated with glycerol, chitosan gel (CS gel), fungal chitosan nanoparticles (FCN), and gel enriched with fungal chitosan nanoparticles (Gel + FCN) stored at cold temperature.

| **Attributes** | **Control** | **Glycerol** | **CS Gel** | **FCN** | **Gel + FCN** |
|---|---|---|---|---|---|
| Appearance | $7.81(\pm1.51)^{a}$ | $6.61(\pm1.79)^{c}$ | $6.94(\pm1.91)^{bc}$ | $7.52(\pm1.44)^{ab}$ | $6.94(\pm1.75)^{bc}$ |
| Colour | $7.87(\pm1.46)^{a}$ | $6.95(\pm1.66)^{c}$ | $7.30(\pm1.71)^{abc}$ | $7.64(\pm1.44)^{ab}$ | $7.07(\pm1.75)^{bc}$ |
| Flavour | $7.50(\pm1.55)^{a}$ | $6.95(\pm1.70)^{ab}$ | $7.22(\pm1.55)^{ab}$ | $7.20(\pm1.56)^{ab}$ | $6.78(\pm1.66)^{b}$ |
| Firmness | $8.04(\pm1.04)^{a}$ | $7.64(\pm1.43)^{a}$ | $7.52(\pm1.41)^{a}$ | $7.70(\pm1.49)^{a}$ | $7.59(\pm1.37)^{a}$ |
| Taste | $7.61(\pm1.80)^{a}$ | $6.86(\pm1.75)^{b}$ | $7.51(\pm1.53)^{ab}$ | $7.58(\pm1.70)^{a}$ | $6.85(\pm1.94)^{b}$ |
| Overall evaluation | $7.77(\pm1.46)^{a}$ | $7.02(\pm1.48)^{b}$ | $7.43(\pm1.42)^{ab}$ | $7.68(\pm1.47)^{a}$ | $6.85(\pm1.71)^{b}$ |

Note: Sensory attributes were evaluated by one hundred untrained tasters after 3 days from the application of the edible coatings. The strawberries were stored at cold temperature before sensory evaluation. Values represent the mean with their standard error (±SD). $^{a-c}$For each trial, different superscript lowercase letters within a row denote significant differences ($p<0.05$) between the mean values according to Tukey's test.

made by the mixture of CS gel and FCN differed significantly ($p < 0.05$) from the control group for all attributes, except for the firmness. As previously described in the present research, the gel+FCN coating applied to strawberries stored in refrigeration showed that the association of these two forms of chitosan (gel and nanoparticles) could significantly improve the barrier properties of an edible coating. The strawberries coated with gel+FCN kept the maturity index and pH constant and presented the highest humidity percentage. The application of this type of coating could have delayed the ripening process, which contributed to the lower acceptance of this sample.

When asked to report about the intent to purchase, the panellists reported differences between uncoated and coated fruit. The control strawberries did not present significant differences ($p > 0.05$) in relation to fruit coated with FCN. These samples exhibited scores around four (control: $4.34 \pm 1.03$ and FCN: $4.09 \pm 1.10$) and were the most accepted, followed by CS gel ($3.84 \pm 1.10$), which did not differ ($p > 0.05$) from FCN.

No difference ($p > 0.05$) in preference was observed between fruit coated with glycerol ($3.61 \pm 1.10$) and gel + FCN ($3.64 \pm 1.14$). These groups were the least appreciated sample, which is in agreement with the acceptability test. However all samples exhibited scores around four which shows that the tasters would "possibly buy" the fruit. The same scores were found by our previous study when we applied an edible coating made by CS gel and FCN to grapes (Castelo Branco Melo et al., 2018). These scores made the use of the edible coatings produced in the present study feasable on a commercial level based on the analysis of the sensory aspects.

## 4 Conclusions

All chitosan based edible coatings were effective in maintaining the physicochemical, sensory and microbiological qualities of the postharvest strawberries. However, the coating made with FCN+CS gel was the most effective in maintaining these quality parameters of the strawberries and presented the best antifungal activity against the phytopathogenic fungi tested. The sensory evaluation of the strawberries showed that fruit coated with FCN received similar scores to control group, which suggest that the FCN did not modify the sensory quality of the fruit.

Based on these results, edible coatings made with different forms of chitosan, especially the mixture of FCN and CS gel, could be used to improve the post-harvest quality of strawberries. In addition, the application of nanotechnology could be a good alternative to improve the properties

of chitosan, as well as increase the cost benefit of the use of edible coatings based on this polymer.

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# Food, Fish and Campylobacteriosis

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**Abstract**

Food is a necessity of human beings, and the consumption of food is aimed at obtaining energy and nutrients necessary for the growth and proper functioning of the body. However, food can also be a vehicle for various diseases, and the causal agents can have physical, chemical or biological origin with relevance to health due to their incidence, mortality and negative consequences in the population. Bacteria are the main agents of biological origin associated with foodborne diseases. Among these microorganisms are species of the genus *Campylobacter*, which cause a zoonosis with one of the highest incidences globally, known as Campylobacteriosis. This document provides an overview of foodborne diseases, specifically the causal agents of Campylobacteriosis, including the different measures of control and prevention for this disease in different foods such as poultry, milk, meat, and fish, among others. It also covers the phenomenon of resistance to antimicrobials by these pathogens and the health implications to consumers. The above can generate and maintain safety practices in food production for the protection of public health in different regions around the world.

***Keywords:*** *Campylobacter*; Fish; Foodborne disease; Food safety

## 1 Introduction

Food is a necessity for human beings, and food consumption is aimed at obtaining energy and nutrients necessary for the growth and proper functioning of the body. However, food can also be contaminated by various agents harmful to the consumer's health, resulting in different diseases. Food safety, together with nutrition, organoleptic qualities and commercial processes, are the characteristics that make up the integral qualities of foods that do not cause illness to the person who consumes them (Fuente-Salcido & Corona, 2010; Jorquera, Galarce & Borie, 2015). Food security, nutritional value and food safety are concepts currently considered a global priority and are related to the different phases of the food chain, such as procedures in agricultural production, livestock management, aquaculture and fishing, product manipulation, processing, conservation, transport, and distribution (Gentile, 2010; Palomino & Muñoz, 2014; WHO, 2018a). Contaminated food generates a vicious cycle of illness and malnutrition, which affects the general population, specifically children and the elderly; therefore, access to safe and nutritious food in quantity becomes essential to maintain life and promote good health (WHO, 2018a). This document is an overview of foodborne diseases, specifically the causal agents of Campylobacteriosis, and includes the different measures of control and prevention of this disease for different foods such as poultry, milk, meat, and fish, among others. It also covers the phenomenon of

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resistance to antimicrobials by these pathogens and the health implications for consumers. The above can generate and maintain safety practices in food production for the protection of public health in different regions around the world.

## 2 Foodborne diseases

Foodborne diseases (FD) are considered a serious public health problem worldwide due to high levels of morbidity, mortality and economic costs in health services (Alerte et al., 2012; Garcia-Huidobro, Carreno, Alcayaga & Ulloa, 2012; Jorquera et al., 2015; Palomino & Muñoz, 2014). Approximately 250 FD-causing agents have been described, including chemical, physical and biological agents; the latter being specifically bacteria such as Salmonella spp., *Listeria monocytogenes, Vibrio cholerae, Staphylococcus aureus, Campylobacter* spp., *Shigella* spp., and *Escherichia coli*, which are frequently related to outbreaks (Garcia-Huidobro et al., 2012; Jorquera et al., 2015; WHO, 2018a).
Factors such as globalization, the development of new products and manufacturing processes, changes in food habits, the growing demand for ready-to-eat foods, new forms of transmission, the emergence of vulnerable population groups, and the increase in resistance to antimicrobial compounds by pathogenic microorganisms have contributed to the increase of these diseases (Jorquera et al., 2015; Palomino & Muñoz, 2014). It is estimated that around the world, 600 million people get sick from eating contaminated food and 420,000 deaths occur from this same cause every year. The availability of safe and nutritious food is essential for the maintenance of life and promotion of good health. Currently, food production and supply chains have an international presence; therefore, collaboration among governments, producers, industry and consumers is a fundamental part of actions related to food safety and disease prevention (WHO, 2018a).

## 3 *Campylobacter* generalities

The genus *Campylobacter* consists of several different species of clinical and economic importance, including *Campylobacter fetus, Campylobacter coli, Campylobacter concisus, Campylobacter curvus, Campylobacter gracilis, Campylobacter helveticus, Campylobacter hominis, Campylobacter hyointestinalis, Campylobacter jejuni, Campylobacter lanienae, Campylobacter lari, Campylobacter mucosalis, Campylobacter rectus, Campylobacter showae, Campylobacter sputorum, Campylobacter upsaliensis, Campylobacter insulaenigrae, Campylobacter pyloridis, Campylobacter avium, Campylobacter subantarticus, Campylobacter canadensis, Campylobacter cunicolurum, Campylobacter volucris, and Campylobacter ureolyticus* (Cabello, 2007; Cecilia, Arreola & Graciela, 2013; Lapierre, 2013). The characteristics presented by these microorganisms are as follows: spherical bacilli form, curved, comma or coccoid shape of 0.2 $\mu$m to 0.5 $\mu$m in old cultures or 0.2 $\mu$m to 5 $\mu$m in fresh bacterial cultures, Gram negative, mobile, nonsporulated, noncapsule-forming, and microaerophilic. They present surface immunogens as "O" antigens that distinguish the 23 serotypes and are considered a pathogenicity factor. The antigen "H" is present in flagella protein. They are metabolically oxidase-positive and can reduce nitrates, produce hydrogen sulfide, and hydrolyze hippurate. They do not ferment or oxidize carbohydrates, presenting a negative reaction to methyl red, Voges-Proskauer and gelatin hydrolysis; therefore, energy is obtained from amino acids or cycle intermediaries of tricarboxylic acids. Most strains are resistant to cephalothin and may be urea-negative, except for some strains of *Campylobacter lari*. Prolonged exposure to air and water causes them to take the shape of cocci, which are difficult to grow and may even be uncultivable. They have a growth temperature range from 30 $^o$C to 45 $^o$C and pH range from 5 to 8; *C. jejuni, C. coli and C. lari* are thermophilic with optimum growth at 42 $^o$C and 43 $^o$C, but they do not grow at temperatures below 25 $^o$C. The species of medical and veterinary importance are *C. jejuni, C. coli* and *C. lari*. *C. jejuni* is divided into two subspecies: *C. jejuni jejuni*, referred to simply as *C. jejuni*, and *C. jejuni doylei*. The infectious disease caused by *Campylobacter* spp., is called Campylobacteriosis (Cabello, 2007; CDC, 2017; Cervantes García & Cravioto, 2007; Elika, 2013; Epps et al., 2013; Gutierrez Castillo, Paasch Martinez & Calderon Apodaca,

2008; Lapierre, 2013).
*Campylobacter* spp., is a ubiquitous microorganism and can be found in water, soil, and the intestinal tract of cats, dogs, birds, cattle, swine, rodents, monkeys, wild birds and humans. In animals, bacteria passing through the body are deposited in the feces and circulate through the environment, with birds being one of the main reservoirs and sources of infection (Cecilia et al., 2013; Cervantes García & Cravioto, 2007; Epps et al., 2013; USDA, 2011).
The factors related to the ability to cause disease to a host by these bacteria are as follows: motility through the presence of flagella, which is required in the colonization of the small intestine and subsequent transfer to the colon of a host; adhesion through external membrane proteins, flagellins and capsules; invasion and toxigenicity through the production of toxins; in addition, lipopolysaccharide (LPS) is endotoxic (Cabello, 2007; Cecilia et al., 2013; Cervantes García & Cravioto, 2007; Lapierre, 2013).
Campylobacteriosis is considered one of the main zoonoses with the highest prevalence of diarrhea and gastroenteritis in the world and is the second most frequent foodborne disease in countries such as the United States of America, with 1.3 million cases each year, where *C. jejuni* is the strain regularly associated with human infections (CDC, 2017; Gutierrez Castillo et al., 2008; Lapierre, 2013; USDA, 2011; WHO, 2017). In the European Union (EU), this foodborne disease is the most frequently reported with more than 190,000 cases in humans per year, estimating a cost for public health systems and loss of productivity of 2.4 billion euros per year (EFSA, 2018).
This disease is caused by the consumption of contaminated water and food of vegetable or animal origin, such as unpasteurized milk, seafood, fish, raw or undercooked meat, and fruits and vegetables irrigated with contaminated water or in contact with feces of infected animals (EFSA, 2018; Elika, 2013; Lapierre, 2013; Soares & Gonçalves, 2012; USDA, 2011; WHO, 2017).
It is estimated that an inoculum of $10^4$ cells is sufficient for infection by *Campylobacter* spp. to occur, and in some cases, *Campylobacter* spp., is highly infectious, giving rise to disease with only 500 cells depending on the strain, damage to the cells by a stressful environment and host susceptibility (Hunt Jan, Abeyta & Tran, 2001; Mardones P. & Lopez-Martin, 2017). Campylobacteriosis is characterized by symptoms such as diarrhea, which is generally bloody, abdominal pain, dehydration, weakness, malaise, fever, headache, nausea and/or vomiting that appears between 5 and 10 days after infection with a usual duration of 3 to 6 days; mortality is low but occurs in a greater proportion in groups considered high-risk populations, such as children, elderly, and people with chronic conditions and immunosuppressed systems. Campylobacteriosis can generate complications manifesting as bacteremia, urinary tract infection, pneumonia, peritonitis, hepatitis, pancreatitis, abortion, reactive arthritis and neurological disorders, such as Guillain-Barré syndrome (Cabello, 2007; CDC, 2017; Epps et al., 2013; WHO, 2017).
The therapeutic treatment of this disease is not usually required since the disease is short-lived and self-limiting. However, when the symptoms are prolonged or very serious, antimicrobial therapy is necessary. The antibiotics of first choice are macrolides, such as erythromycin, and fluoroquinolones, such as ciprofloxacin. However, some species of *Campylobacter* spp., have been shown to be resistant to glycopeptides, penicillin, ampicillin, cephalosporins, chloramphenicol, and fluoroquinolones, among others, making the clinical management of Campylobacteriosis complicated (Cecilia et al., 2013; Garcés Vega, Klotz Ceberio, Mantilla Pulido, Ramírez Rueda & Romero Prada, 2013; Weiler et al., 2017).

## 4 Fish and health

Fish is a food with a special dichotomy. On one hand, it is considered highly nutritious, has highly digestible proteins and a high biological value, and contains lipids (polyunsaturated), vitamins and minerals, which make it part of a healthy diet. On the other hand, these properties also make it a highly perishable food, susceptible to deterioration (microbial, autolytic and chemical) and contamination, making it high-risk to the health of consumers (Amanda Thaís Ferreira et al., 2017; FAO, 1998; Soares & Gonçalves,

2012). Capture fisheries and aquaculture are important sources of food, nutrition, income and livelihoods for millions of people globally (FAO, 2016). Fish acquire the microbiota of the natural environment where they live, and this microbial population may include different human pathogens. If fish are captured in areas near the coast or regions with a high human population density or produced through inappropriate practices in aquaculture and fisheries, including postharvest phases, handling, processing, storage, transport and marketing, the result may be foods that deteriorate faster, are of low quality and are vehicles for various pathogenic microorganisms such as *C. jejuni, E. coli, L. monocytogenes, Staphylococcus spp., Salmonella spp., V. cholerae*, as well as viruses (enteroviruses) and parasites (Protozoa, Trematodes, Cestodes and Nematodes). This places human health at high risk due to the consumption of raw meat or food subjected to inadequate preparation or cooking procedures (Ferre, 2016; Fos Claver, 2000; Frasao, Marin & Conte-Junior, 2017; Manuel Romero-Jarero & del Pilar Negrete-Redondo, 2011; REMA, 2017; Silva et al., 2016; Soares & Gonçalves, 2012). In some countries in South America, such as Chile, an epidemiological study conducted in the metropolitan regions found that 12,196 cases of foodborne diseases occurred from 2005 to 2010, of which 30.5% had fish and shellfish as the causal agents. Illness was of biological origin, mainly from bacteria such as *Salmonella* spp., *Shigella* spp., *Vibrio parahaemolyticus, Listeria* spp., *Staphylococcus* spp., and parasites (i.e. Giardia spp. and *Sarcocystis* spp.) (Alerte et al., 2012). In European countries, such as Spain, in the period from 2008 to 2011, 30,219 cases of food-related diseases were recorded, and fish was involved in 6% of all cases, with bacteria being identified as the main biological agents, including *Salmonella* spp., *Campylobacter* spp., *Staphylococcus* spp., and *Clostridium perfringens* (Espinosa, Varela, MartInez & Cano, 2014).

## 5 Control and prevention of foodborne diseases

Around the world, different standards, guidelines and certifications have been developed and implemented that assume an increasingly important role in the international food trade. Evidence of their use is commonly requested to guarantee food safety, quality and environmental sustainability in the growing food industry, including that of fish and fish products. Some of these prevention and control measures have been developed and are issued by international organizations such as the World Health Organization (WHO), which suggests good practices in sanitary control of operations in the production of safe food (good agricultural practices, good practices in manufacturing, and the Hazard Analysis and Critical Control Point (HACCP) system). The Food and Agriculture Organization of the United Nations (FAO) through the Codex Alimentarius has developed guidelines and codes of practice for food production of unprocessed, semi-processed or processed foods for distribution to the consumer or as raw material (PAHO, 2016; Racua, 2018). In addition, the International Organization for Standardization (ISO) developed standards such as ISO 22000, as well as other recommendations and certification programs accepted globally, such as Safe Quality Food (SQF), British Retail Consortium (BRC), International Food Standard (IFS), PrimusGFS, Global G.A.P, and Quality Certification Services (QCS), among others, with the purpose of producing, distributing and commercializing safe foods for the health of the consumer (PAHO, 2016; Racua, 2018).

It is considered that food production animals (poultry, cattle and swine) are the main source of infections by *Campylobacter* spp. in humans (Epps et al., 2013). Throughout the food processing chain (from the farm to the consumer´s table) from slaughter, through food processing, until its preservation and manipulation prior to consumption, there are numerous possibilities for transmission of infection by *Campylobacter* spp.; therefore, it is necessary to implement procedures for good hygiene practices during all stages of the food processing chain, including the implementation of control systems, such as HACCP, the establishment of microbiological criteria in raw materials and finished products and the provision of information and/or continuous training of food handlers and the general population on the handling, preparation and preservation of

food prior to consumption (Elika, 2013; USDA, 2013).

In food legislation, as a measure of prevention and control of foodborne diseases, microbiological specifications have been established for foods considered to be at risk of contamination by *Campylobacter* spp. to safeguard public health in Europe, for example, in 2003, the Recommendation of the Commission 2004/24 / CE (DOCE 19/12/03) in the program of official control of food products was established through the official journal of the European Union. It was announced that starting in 2004, the member states of the community would carry out inspections and controls and, if applicable, collect and analyze samples to assess the bacteriological safety of dairy products, such as cheeses made from raw milk or under thermal treatment, and assess the bacteriological safety of refrigerated poultry meat related to thermophilic *Campylobacter*. In addition, the latest version of the standard developed by the International Standardization Organization, ISO 10272, was recommended as a method for the detection of this pathogen. It also established food safety criteria in foods, with the microbiological limit of "absence" in 25 g samples of cheese based on raw milk, cheese based on milk subjected to heat treatment less than pasteurization and fresh poultry meat. Subsequently, the regulation (CE) 2073/2005 of the commission was presented through the official journal of the European Union on November 15, 2005, relating to the microbiological criteria and methods of analysis applicable to different food products and microorganisms. In addition, the regulation (CE) 853/2004, by which specific recommendations of hygiene of foods of animal origin are established, regulation (CE) 852/2004 on the hygiene of alimentary products, and regulation (CE) 1935/2004 on materials and objects intended to contact food were established. This legislation regarding food hygiene should be implemented by food producers and processors, including those of fish and shellfish, to provide safe food for the health of the population.

In Latin American countries, such as México, the consumption of meat products, including those from poultry, is considered a common source of various food pathogens, including *Campylobacter* spp. Sanitary control measures are usually carried out on different food products, including meat, for food pathogens such as Salmonella spp., but not for the control of *Campylobacter* species (Rodriguez Ceniceros, Gomez Hernandez & Vazquez Sandoval, 2016). For example, the official Mexican standard "NOM-213-SSA1-2002" focuses on sanitary specifications and testing methods for processed meat products, and the "NOM-243-SSA1-2010" standard states the sanitary specifications and test methods for foods of animal origin, such as milk and different products. The standard "NOM-242-SSA1-2009" focuses on fresh, chilled, frozen and processed fishery products regarding sanitary specifications and test methods, and finally, the standard "NOM-210-SSA1-2014" outlines microbiological methods for the determination of pathogenic indicators and pathogenic microorganisms. These standards should be considered in order to strengthen the measures of surveillance and control of this pathogen in food, contributing to the health protection of the population. Standard "NOM-251-SSA1-2009" establishes the minimum requirements of good hygiene practices that must be observed in the processing of food, beverages or food supplements and their raw materials in order to avoid contamination throughout the process, which includes the HACCP system and guidelines for its application. The detection of microorganisms in food is an essential part of any quality control and safety process and is an important element in epidemiological research in order to carry out surveillance, control microorganisms and prevent disease (Rodriguez Ceniceros et al., 2016).

## 6 Analysis of food in the laboratory

The measures for the control and prevention of Campylobacteriosis include analysis in a microbiological food laboratory, where the detection and isolation of *Campylobacter* is generally performed by isolation and confirmation is by biochemical, molecular and proteomic tests (Dudzic et al., 2016; Mandrell et al., 2005; Rodriguez, Guzmon Osorio & Verjan, 2015; Weiler et al., 2017). Different standardized methods have been developed that involve enrichment

Table 1: Different tests for phenotypic identification of species of the genus *Campylobacter* (Hunt Jan, Abeyta & Tran, 2001).

| Characteristics | Microorganism | | | | | |
|---|---|---|---|---|---|---|
| | *C. jejuni* | *C. jejuni* subsp. Doylei | *C. coli* | *C. lari* | *C. fetus* subsp. fetus | *C. hyointestinalis* |
| Growth at 25 $^{o}$C | - | ± | - | - | + | A |
| Growth at 35 $^{o}$C to 37 $^{o}$C | + | + | + | + | + | + |
| Growth at 42 $^{o}$C | + | ± | + | + | A | + |
| Nitrate reduction | + | - | + | + | + | + |
| NaCl 3.5% | - | - | - | - | - | - |
| $H_2S$, TSI | - | - | A | - | - | +H |
| Catalase | + | + | + | + | + | + |
| Oxidase | + | + | + | + | + | + |
| Motility | +(81%) | + | + | + | + | + |
| Hippurine hydrolysis | + | + | - | - | - | - |

Symbols: +, 90% or more of strains are positive; -, 90% or more of strains are negative; A, 11-89% of strains are positive; H: Small amount of $H_2S$ on fresh (<3 days) TSI slants; $H_2S$ hydrogen sulfide, Triple sugar iron agar (TSI).

and isolation stages in selective culture media as well as the management of specific culture conditions related to temperature, O2 requirements and subsequent biochemical confirmatory tests (Table 1). Methods developed by Hunt Jan et al. (2001), are reported in the Bacteriology Analytical Manual (BAM) of the Food and Drug Administration of the United States of America (US FDA) for the isolation, identification and confirmation of *Campylobacter* spp., the laboratory guide of the United States Department of Agriculture (USDA) (method MLG 41.04) and the Food Safety and Inspection Service (FSIS) (USDA, 2016). Methods for the detection (method 10272-1) (Figure 1) and colony counts of the food pathogen (method 10272-2) (Figure 2) are reported by the International Organization for Standardization (ISO); the latter method being the gold standard for detection and isolation. However, in order to avoid the inconvenience of the time required for analysis by traditional microbiological methods and to obtain information more quickly for decision-making regarding aspects of the health and safety of water and food, molecular methods have also been developed, which involve the polymerase chain reaction (PCR) and its different variants to identify genes, such as *hypO, glyA, sapB2, fla, rpoB,* 23S rRNA, and 16S rRNA, among others, and immunoassays (de Boer, Rahaoui, Leer, Montijn & Van der Vossen, 2015; Frasao et al., 2017; Park et al., 2011; Rodriguez et al., 2015; Rojas-Herrera & González-Flores, 2006). Molecular methods, such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), multilocus sequence typing (MLST), whole-genome sequence (WGS) and pulsed-field gel electrophoresis (PFGE) can be used in the typing of food pathogens; the latter being common for the typing of *Campylobacter* spp., useful and relevant in the epidemiological analysis of outbreaks of foodborne diseases (Behringer, Miller & Oyarzabal, 2011; Frasao et al., 2017; Lahti, Löfdahl, Ågren, Hansson & Olsson Engvall, 2017; Rodriguez et al., 2015; Taboada, Clark, Sproston & Carrillo, 2013).

## 7 Resistance to antimicrobials

Resistance to antimicrobials is the ability to resist and survive the action of antimicrobial molecules by microorganisms, this phenomenon is observed mainly in bacteria. Resistance to antimicrobials is considered a global threat to human

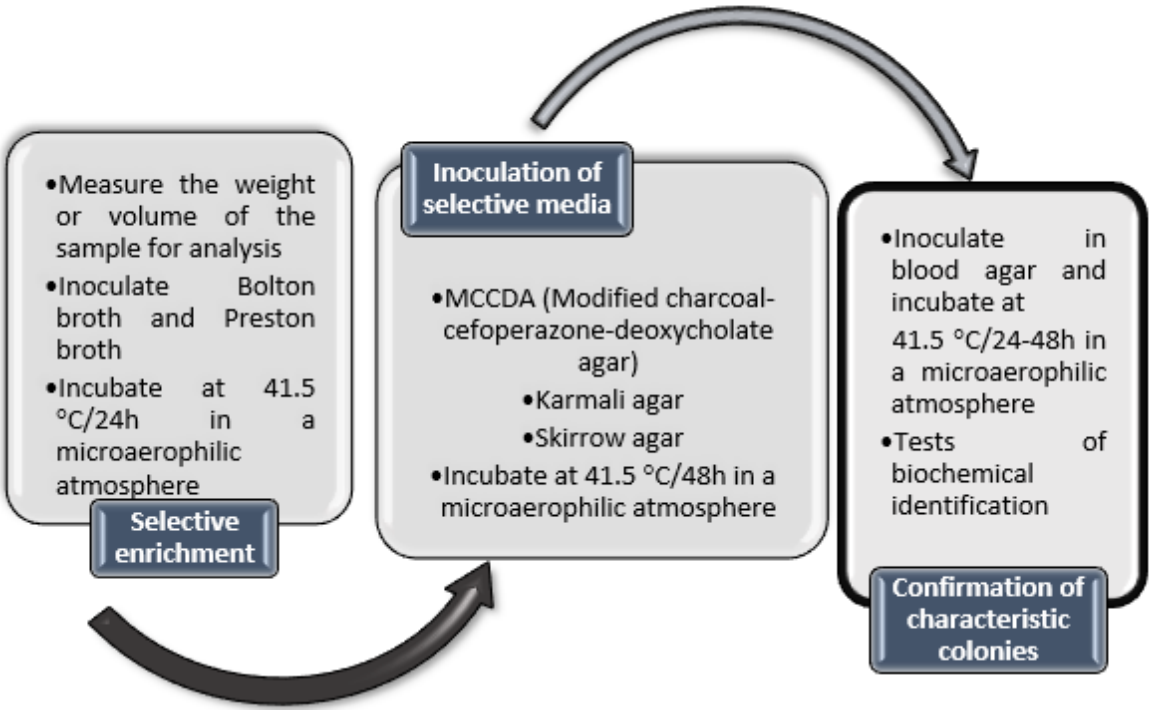


Figure 1: General diagram of detection of *Campylobacter* spp., in food (ISO 10272-1, 2017); (SAA, 2017a)

and animal health. It is estimated that 500,000 people die each year from causes related to antimicrobial resistance (Cires, 2002; FAO, 2018). In addition, this phenomenon influences clinical procedures, such as organ transplant, cancer chemotherapy, diabetes treatment and major surgery, which increases the cost of health care, hospital stays, the need for more intensive care and mortality (WHO, 2018b). This characteristic of microorganisms also impacts areas of food safety, food security, and economics, since food contributes to the development and spread of antimicrobial resistance as a potential route of exposure for the entire population (FAO, 2018). Reported mechanisms of antimicrobial resistance are genetic variability, modification of membrane permeability, excretion pumps, enzymatic modification of the compound, and modification of the ribosomal target or composition alteration of the site of action; several of these mechanisms have been developed and observed in strains of resistant *Campylobacter* spp., of clinical or food origin (Becerra, Plascencia Hernandez, Luevanos, DomInguez & HernAndez, 2009; Cires, 2002; Tafur, Torres & Villegas, 2008; Wieczorek & Osek, 2013). Antimicrobial resistance is transmitted between microorganisms through the acquisition of genetic material by conjugation, transformation or transduction processes of plasmids, transposons and integrons that contribute to the incorporation of resistance genes between microorganisms of the same genus (horizontal transmission) or different genera (vertical transmission) (Becerra et al., 2009; Cires, 2002; Pérez-Cano & Robles-Contreras, 2013).

In recent years, the resistance of different pathogens in foods, such as *Salmonella* spp., *E. coli*, and *L. monocytogenes*, has been reported (De Nes, Riboldi, Frazzon, d'Azevedo & Frazzon, 2010; Puig Peña, Espino Hernández

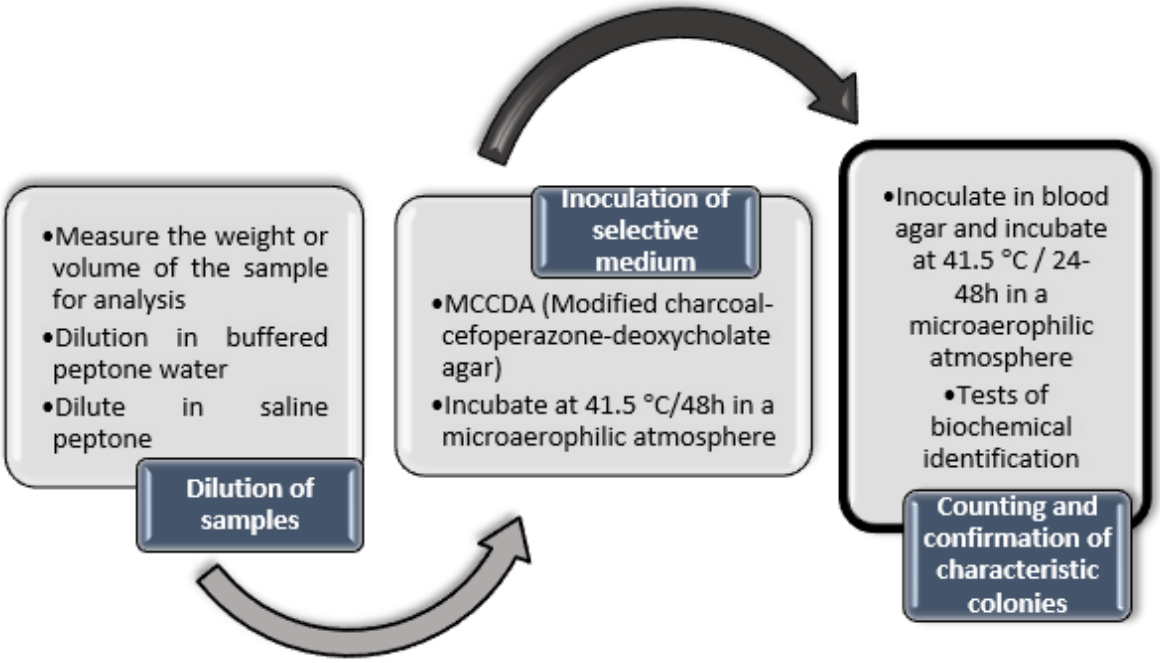


Figure 2: General diagram of quantification of *Campylobacter* spp., colonies in food (ISO 10272-2 (2017); SAA (2017b))

& Leyva Castillo, 2011). Studies around the world on *Campylobacter* spp. of human and animal origin have shown resistance to antimicrobials, such as erythromycin, tetracycline, ampicillin and quinolones (Dallal et al., 2010; Fernandez, 2011; Gonzalez-Hein, Cordero, Garcia & Figueroa, 2013; Wieczorek & Osek, 2013).
Microorganisms are reservoirs of antimicrobial-resistance genes which can potentially be exchanged between other pathogenic and commensal bacteria. The indiscriminate use of antibiotics in humans (clinical) and in animal production has been established as protective or preventive, therapeutic and growth promoting, resulting in the emergence and spread of antibiotic resistance among various pathogens, including *Campylobacter* spp., in areas involved in animal production for food (mainly in poultry) and the environment. This resistance can, therefore, be transmitted through food production and consumption, becoming a global risk for human health (Epps et al., 2013; Mardones P. & Lopez-Martin, 2017; Wieczorek & Osek, 2013). To control this phenomenon in microorganisms, action plans, strategies, guidelines, recommendations and codes of practice have been developed at the global level by the World Health Organization (WHO), the United Nations Food and Agriculture (FAO)-Codex Alimentarius and the World Organization for Animal Health (OIE) with the aim of promoting best practices that reduce or control the emergence and spread of antimicrobial resistance through the optimal use of these compounds in human and animal health, with regulations for the use of medicine and waste management, as well as in the production of food with good hygiene practices in the agricultural, livestock, and aquaculture sector, which are considered key to achieving food safety and combating resistance to antimicrobials (FAO, 2018; WHO, 2018b).

## 8 Final comments

Foodborne diseases caused by different agents of biological origin constitute a serious problem for the health sector at a global level due to their incidence, mortality and negative repercussions in the economic and productive sector, in addition to the appearance of causal agents (mainly bacteria) resistant to antimicrobials, which aggravates the problem. Governments and international organizations have developed and implemented control and surveillance strategies and actions that differ in the production of safe food for the health of consumers.

*Campylobacter* spp. is considered one of the zoonoses, called Campylobacteriosis, with greater incidence around the world. It is a disease transmitted by foods such as fruits, fish, vegetables, meat mainly of avian origin, dairy and derivatives for which bacteria have also shown resistance to different antibiotics.

Global measures for the control and prevention of Campylobacteriosis and resistance to antimicrobials by their causative agents are mainly focused on the application of hygiene practices along the food production chain, including good practices for agriculture and livestock production, fisheries, aquaculture, and manufacturing, as well as the implementation of operational control systems as HACCP. Also important is regular reporting and promotion of handling, preparation and correct conservation of foods at home directed to the food handler and final consumer. Finally, the joint action of governments, the food industry, and academia in the continual updating, development and optimization of legislation and analytical methods for detection of food pathogens, in regard to the control and prevention of foodborne diseases, should be applied to protect public health.

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