INAUGURAL ADDRESS

Dr. Wazir Hussain Shah

President, Pakistan Society of Food Scientists and Technologists

Honorable Chief Guest, Prof. Dr. Bashir Ahmed, Vice-Chanceller, University of Agriculture, Faisalabad, Prof. Dr. Muhammad Shafiq Chaudhry, Founder President, Pakistan Society of Food Scientists and Technologists, Colleague Food Scientists & Technologists, Delegates from the Food and Allied Industries, Distinguished Guests, Ladies and Gentleman.

Assalam-o-Alaikum!

The food industry in Pakistan has developing steadily over the past several decades. Status of this industry in terms of international competitiveness has, however, remained in infancy. The availability of raw materials in abundance is under exploited. The avenues of growth and potential are plentiful. These may be broadly categorized in the areas of product diversification, prevention of raw materials wastage, reduction in process line losses and product quality improvement. Other areas such as packaging materials and systems development corresponding with the diversity of products and tapping of export potentials remain underexplored. There is a shortage of trained manpower commensurate with the needed initiatives. This has inadvertently held back the entrepreneurs from venture capital investment. The net result of these two most essential inputs has not met the level of food technology applications and process facilities have by and large remained primitive and outdated. This scenario is likely to seriously jeopardize the very existence of food industry, let alone further development in this vital sector of our economy. Urgent measures are needed to overcome these barriers. I take this opportunity of the presence of a large body of national and international food scientists and technologists representing the academia, R&D organizations, the food industry, and commercial enterprises associated with this sector of economy, to share these concerns. I will, therefore, briefly indicate the areas that need our most urgent attention.

First and foremost is the utilization, processing and preservation of all that is produced in the agricultural fields and the orchards. This indirectly means that we must make every effort to prevent losses. Nothing can be more emphasized than an old saying that "a grain saved is a grain produced". An estimated 20-40% of raw production, depending upon the nature of the commodity, is wasted during various post-harvest stages.

This brings to focus the second aspect to our attention. This particularly relates to the produce management of perishable commodities during the glut period. The concept of village-head technology needs to be refined to meet this challenge. This concept envisages semi-processing of the produce close to the site of production in the rural areas, later to be fine processed into finished products at the urban industrial centers. However, this is heartening to note that Government has taken initiative to address this aspect. It has started training technicians through National Vocational and Technical Education Commission (NAVTEC) programme, which may lend helping hand in covering this aspect of perishable food commodities.

The third area inviting our attention is the development of storage facilities suited to our climate and cultural traditions and improvement in the transportation systems of perishable commodities. Product packaging is another very important input for the commerce. Packaging technology of food products has undergone tremendous evolution during recent decades in the developed countries. This, however, has remained a highly neglected area in Pakistan.

All the foregoing are essential for food industry in the country. Academic institutions and R&D organizations have a major role to play in their up-gradation. It is incumbent on these that they innovate to develop technologies suited to the processing of our raw materials into quality value-added products. Human resource development for handling of these technologies must be given top priority so that whenever entrepreneurs step in to establish food industries their efforts are not handicapped for want of adequate trained manpower.

Pakistan is signatory to the WTO regime, which, as per agreement has been implemented in the year 2005. With the implementation of WTO protocols our industries will no longer be protected by the national barriers and are likely to become extremely vulnerable. Only the most competitive will survive the international competition. The danger is that our food industry, still in infancy, may not prove strong enough to face the pressure and may wilt. This is not a dilemma for the food industry alone, but may have serious implications for the entire nation.

Through the forum of experts present today, I wish to speak out about the work that lies ahead of us to lend a supporting hand to the food industry for its march forward. Before concluding my address, I would like to forward my appreciations and congratulations to the present hierarchy and previous officials of the institution which started as a Section, then upgraded to a Department, further progressed to an Institute and finally attained a position to be known as the National Institute of Food Science and Technology. The efforts put in by various dignitaries towards this process of progress are much appreciated. I would especially congratulate Prof. Dr. Faqir Muhammad Anjum, Director General of the Institute and his team whose efforts resulted in to give it the present status.

In the end, on behalf of the Pakistan Society of Food Scientists and Technologists and on my own behalf, I wish to thank the Chief Guest, Prof. Dr. Bashir Ahmed, Vice-Chancellor University of Agriculture Faisalabad to have graced this occasion and honor the Society. My special thanks to the foreign delegates from France and USA who accepted our invitation and are here with us to share the innovations in Food Science and Technology.

Thank you all.

WELCOME ADDRESS

Prof. Dr. Bashir Ahmed (Izaz-e-Fazeelat)

Vice Chancellor, University of Agriculture, Faisalabad

The honourable Governor of the Punjab and Chancellor of the University, Lt Gen. Rtd. Khalid Maqbool, Eminent Scholars and Food Scientists from France and the United States of America, Learned Scholars, Speakers and Guests, Ladies and Gentlemen

Aslam-o-Alaikum

It is a privilege for me to welcome you all here on this auspicious occasion of the International Symposium on "Emerging Trends in Food Science and Technology" being organized by the National Institute of Food Science and Technology in collaboration with the Pakistan Society of Food Scientists and Technologists. The University of Agriculture Faisalabad, formerly Punjab Agricultural College and Research Institute, founded in 1906, is more than a century old premium agricultural research and academic Institution.

The national economy of Pakistan is growing at the GDP rate of 6.6 % with agriculture growth of 2.5 %. The growth of overall manufacturing sector is 8.6, while the value added sector contributes 32.3 billion Pak Rupees or 5% of the total value of food production. The food industry in Pakistan is the 2nd largest industry in terms of manpower employed which signifies the importance of this sector in Pakistan.

Honorable Guests: I would also like to draw your attention towards the development of food sector. Pakistan annually produces 16 million tones of fresh produce (fruits 46% and vegetables 54%). Its market value at current factor cost is estimated around US$ 2.1 billion. The annual export of fresh produce is estimated around half a million tons which earns foreign exchange worth US$ 180 million. It is a growing industry and is expected to grow with 23% annually onward from the year 2009.

Ladies and Gentlemen: The role of the academia in general and higher education in particular has been tremendous in the last decade which is abridging the territorial boundaries of the advanced institutions of the world with Pakistan. This country is gifted with natural abundant resources. It is imperative that we reform our agricultural practices to maximize the potential of the country. Food Science and Technology is the key area which will determine the national growth in terms of GDP in the near future. Today we can see positive shift in the growth of value added sector. The food scientists are also working for the utilization of agro-industrial wastes for the production of valued products that will increase foreign exchange earnings and will enable Pakistan enter into the global trade as a leading country. The food industry in Pakistan with the support from academia and research has also introduced new technologies for processing, preservation and storage to reduce post-harvest losses. The country is also witnessing emerging technologies which have been introduced in almost every food sector.

Ladies and Gentlemen: We are aware that the process of globalization has profound impacts on all regions, nations, societies and cultures. In a global village, as the world has become now, no nation can continue to live in isolation. Agreements on the application of Sanitary and Phytosanitary measures and Technical Barriers to Trade are heralding a new era of quality and safety management in food production, handling, and

trade operations. Some key issues which are challenges to our food scientists include those related to food additives, contaminants, pesticide residues and hygienic practices. In this global regime, it is imperative for the food industry in Pakistan to come up to the challenges posed by post WTO global scenario.

We are all aware of the situation that consumer's health is the driving force reshaping their choices. The current food industry is challenged to supply the consumers a wide range of products in almost all food sectors in Pakistan. The health diets, exotic fruits and vegetable products, ready to eat foods, nutarceuticals, organic produce and fresh chilled products are on high demand. This requires strengthening linkages between the international and national institutions, public and private sectors as well as academia and industry to meet the challenges of the food industry in Pakistan. The National Institute of Food Science and Technology has been striving hard to offer its services with active support and collaboration from the food industry to meet the research and education related needs to overcome the emerging challenges in food sector.

Honorable Guests and Scholars: I am sure that this "International Symposium on Emerging Trends in Food Science and Technology" would help create a new sense of wisdom and responsibility amongst us. This will enable us to multiply our efforts to introduce these new technologies to our food and food products to enter into the highly competitive global trade. In this regard I assure you that the National Institute of Food Science and Technology, University of Agriculture, Faisalabad will play its due role as a vehicle of higher education and research to bolster our food industry to achieve ever higher standards of performance.

I once again welcome the distinguished participants and guests and hope that their stay in the University of Agriculture will be a memorable one.

Thank You.

KEY NOTE ADDRESS

Prof. Dr. Faqir Muhammad Anjum

Director General, National Institute of Food Science and Technology

Lt. Gen. (R) Khalid Maqbool, Honorable Governor of the Punjab, Prof. Dr. Bashir Ahmed, Vice-Chancellor, University of Agriculture, Faisalabad, Mr. Bryan D. Hunt, Principal Officer, US Consulate Lahore, Dr. Muhammad Nawaz, Vice-Chancellor, University of Veterinary and Animal Sciences, Lahore, Mr. Ronald Decovert, Managing Director, Nestle Pakistan, Jean Francois Grongrett, Professor Agrocampus, Rennes, France, Dr. Mian Nadeem Riaz, Director, Food Protein Research & Development Center, Texas A&M University, USA, Deans, Directors, Distinguished Guests, Ladies and Gentlemen!

ASALAM-O-ALAIKUM

It is a matter of proud privilege for me to thank you all for sparing your precious time to be present in this concluding session of the International Symposium on "Emerging Trends in Food Science and Technology" and making this event more valuable.

As an agricultural country, Pakistan is bestowed with excellent climate and fertile land favorable for the production of good quality crops, fruits and vegetables. Despite the fact that we are not far behind the world in production of different foods, we are still facing problems of food shortage due to improper handling of our produce. The magnitude of post-harvest losses in vegetables and fruits is about 40% while 20% in case of grains. Moreover, we are not effectively exploring the potential of our produce through value addition.

Food Scientists and Technologists are busy for the optimum utilization of available food resources through post-harvest management, product development, process modifications and value addition. The National Institute of Food Science and Technology (NIFSAT) since its inception is actively involved in the development of trained and competent manpower to face the challenges in food sector at national and international levels.

This International Symposium has been organized to share the experiences of developed countries and introduce emerging trends in our food science sector. It is a good opportunity for our local scientists, researchers, academicians and industrialists to exchange their knowledge and experiences with renowned experts from France and the US. The papers presented in the symposium are in accordance with the current needs of the Pakistani food industry. The symposium highlighted the importance of dairy science, meat science and technology, functional foods, nutraceuticals, food safety, biotechnology and extrusion technology. I am thankful to all the speakers for making valuable presentations and active deliberations of participants in the five technical sessions of the symposium.

Honorable Chancellor!

It is a matter of great pleasure for me to mention few significant achievements of the NIFSAT. This Institute is the leading Institute at National level. It is actively involved in establishing linkages with public and private sectors at national and international levels.

Sir!

1. National Institute of Food Science and Technology has closely worked with Food Protein Research & Development Center, Texas, A&M University, USA to establish **Center of Extrusion Technology** at the University Campus under the umbrella of the Pak-US joint academic and research project funded by Higher Education Commission (HEC) and United States Agency for International Development (USAID). This technology is still not common in Pakistan. I hope this will play a major role in food quality improvement and value addition by utilizing our local resources. The Extrusion Center will also act as a hub for training at SAARC level to promote teaching, research and product development. I am thankful to Mr. Bryan D. Hunt, Principal Officer, US Consulate, Lahore for his support in the establishment of this center and his presence at the occasion of its inauguration.
2. The Institute is actively engaged in establishing linkages with private sector. We have signed two **MoU's** worth Rs. 40 million with Vita Pakistan Ltd. and Néstle Pakistan Ltd. in your kind presence at Governor House, Lahore. Two more industries have committed to sign MoU's with this Institute to promote Food Science & Technology in Pakistan.
3. Vita Pakistan Ltd. is establishing **Manji Food Technology Center** to train manpower for food industries. Néstle Pakistan Ltd. has also developed a world class air conditioned Néstle Hall with modern teaching facilities. Besides these, they will also provide scholarships, internships and jobs for our students. Néstle Pakistan will also contribute to strengthen the laboratories of the Institute and execute different research projects.
4. It is a matter of great pride that "Iron Fortification Technology" developed by the National Institute in collaboration with PINSTECH and NIFA has been launched nationwide from October 26, 2007 to fortify wheat flour under "**Wheat Flour Fortification Project**" by the Ministry of Health, Pakistan.
5. NIFSAT is also executing several projects funded by the Higher Education Commission and various other funding agencies on different aspects.

Sir!

REGARDING OUR FUTURE VISION

We intend to:

1. establish Center of Excellence in Dairy Technology at NIFSAT, University of Agriculture, Faisalabad.
2. establish NEW SECTIONS like
    - Food Packaging
    - Food Engineering
    - Post-harvest Technology

3. initiate two new Degree Programs, viz., B.Sc. (Hons.) Human Nutrition and Dietetics and M.Sc. (Hons.) Meat Technology as well as some DIPLOMA and SHORT COURSES.

4. establish out-reach facilities for our farmers and the industry. In this regard, Food Technology Transfer Center (FTTC) being set up in collaboration with France will play an important role to uplift the economic conditions of the farmers.

Lastly, I wish to express my gratitude to Honorable Governor of the Punjab, Lt. General (R) Khalid Maqbool for sparing his time to make this event special.

I am also very thankful to the participants of this International Symposium, especially our guests from France and US who traveled long distances to share new and novel ideas with our local researchers, scientists and industrialists. I wish a safe journey to all the participants back to their homes.

THANK YOU VERY MUCH

CONCLUDING ADDRESS

Lt. General (R) Khalid Maqbool

Governor of the Punjab

Chancellor, University of Agriculture, Faisalabad

Prof. Dr. Bsashir Ahmed, Vice-Chancellor, University of Agriculture, Faisalabad, Prof. Dr. Muhammad Nawaz, Vice-Chancellor, University of Veterinary and Animal Sciences, Lahore, Mr. Roland Decovert, Managing Director, Tetrapak Pakistan, Prof. Dr. Jean Francois Grongrett, Agrocampus, Rennes, France, Mr. Bryan D. Hunt, Principal Officer, US Consulate Lahore, Prof. Dr. Faqir Muhammad Anjum, Director General, National Institute of Food Science and Technology, Deans, Directors, Distinguished Guests, Ladies and Gentlemen

Asalam-o Alaikum

It gives me great pleasure to be among this galaxy of scientists and industrialists participating in the International Symposium on "**Emerging Trends in Food Science & Technology**" organized by the National Institute of Food Science and Technology, University of Agriculture, Faisalabad in collaboration with the Pakistan Society of Food Scientists and Technologists.

Pakistan is bestowed with abundant natural resources. In Pakistan the growth of manufacturing sector is 8.6 % with a total value of 693 billion Rupees. The value added sector contributes 32.3 billion or 5% of the total value. Pakistan is producing 16 million tones of fruits and vegetables annually but 30-40% produce is lost due to improper handling. This can only be saved if we shift towards introduction of new and emerging technologies for processing and value addition. The Government of Pakistan is emphasizing on value addition in agricultural produce. There is also need to adopt utilization of agro-industrial wastes to reduce environmental pollution and increase production of value added products that will enhance our earnings and enable Pakistan enter into the global trade as a leading country.

The dairy sector has been neglected since long. Annual milk production in Pakistan is more than 34 million tones but only 2-3% is being processed. There is a need for improvement in this sector. The Government of Pakistan has approved **Dairy Sector Development Plan** to bring **White Revolution** by establishing 11,000 model dairy farms by 2015. The establishment of "Livestock and Dairy Development Company" by the Ministry of Food Agriculture and Livestock and "Pakistan Dairy Development Company" by the Ministry of Industries are the initiatives recently taken by the Government of Pakistan for the development and growth in this important sector.

Pakistan has significantly improved its crop productivity after launching Green Revolution in the mid 60's. Food industry in Pakistan has continuously introduced new techniques, processes, preservation techniques, storage and distribution facilities of food. However, there is still great potential for adoption of the most emerging and modern technologies in this sector to make a product competitive in the international market.

Food industry can play a major role in the economic growth of Pakistan as huge gap exists between production and value addition. This can be achieved only by

establishing modern and technologically advanced food processing units with good quality assurance systems in place to fulfill local and international trade requirements in the WTO scenario.

Our food industry should focus more on export oriented products rather than imports. The relatively low cost agriculture in the country has a competitive advantage in the global market place, which can help to generate additional markets. Pakistan needs to invest heavily in upgrading its existing production facilities – from the farm to the processing units – to have large market benefits.

Ladies and Gentlemen!

I am impressed with the pivotal and dynamic role being played by the National Institute of Food Science & Technology in bringing local and foreign scientists, academicians and food industry people together on a common floor. This Institute has provided a platform to discuss emerging trends in food processing for improvements in food handling, processing, storage and distribution systems.

I am glad to learn that technical sessions were programmed according to various aspects of food science & technology. I appreciate such efforts and hope that participants have benefited from this symposium. I have been briefed that all the speakers delivered excellent and informative presentations. Our guests from France shared their knowledge on nutrition, dairy and meat quality which are major concern areas in Pakistan. In many parts of the world including Pakistan, problems of undernutrition and micro-nutrient deficiencies are prevalent, especially in rural areas. Food-based approaches are increasingly recognized as an effective and sustainable solutions to overcome such problems. The French scientists highlighted three examples of strategies on the improvement of food processes likely to allow nutritionally vulnerable groups of population to increase their essential nutrient intakes. They also proposed a global approach to identify and improve relevant food processing methods to reduce under nutrition and micro-nutrient deficiencies and to define and validate appropriate transfer to the potential beneficiaries.

The dairy industry must adapt to the increasing dairy production in different regions of the world and the increasing needs of the consumers. According to the level of industrial development of the country considered, the dairy industry is able to treat and transform few percent of the collected milk to totality. Pakistan is the 4th largest milk producing country in the world but only little is being processed. There is a need to introduce emerging trends for milk processing. They suggested innovating technologies in dairy sector to produce better quality products with controlled microbiological quality, desired rheological, textural properties and taste need to be adopted.

The French scientists also discussed recent developments in meat processing chains. They addressed the production system through the use of modern biotechnology, automation in slaughterhouses, rapid non-destructive online detection systems, presentation of new technologies incorporated by the meat industry, functional meat compounds, bacteriocins against meat-borne pathogens, latest developments in bacterial starters and recent product packaging systems.

Extrusion technology has become popular in many developed countries and is now emerging in Pakistan. This technology has numerous merits including energy saving, higher production, variety of products, wide range of ingredient usage and increased nutritive value. Currently extrusion technology is being used in processing such food products as breakfast cereals, direct expanded snacks, snack pellets, pasta, etc. The honorable speaker from the US has highlighted latest trend in food processing using extrusion technology. By adopting these guidelines we can get better quality extruded products. This symposium is an opportunity for all of you fellow scientists, academicians and industrialists to get maximum benefits from the experiences of honorable delegates from the United States and France to utilize the indigenous resources.

There is a need to motivate and create awareness among stakeholders through conferences, seminars, symposia and workshops. This enables the stakeholders to appreciate the challenges, which they are to face in the coming years and update their knowledge and facilities in accordance with modern trends. I would, therefore, emphasize upon all the stakeholders and government functionaries to join hands in meeting the requirements of rapidly growing population for the sake of humanity.

Ladies and Gentlemen!

It is also very heartening to know that laboratories of the National Institute of Food Science and Technology have been upgraded to meet the international standards. It is a matter of immense pleasure to mention here that a leading food processor of Pakistan- Néstle Pakistan has extended its cooperation by signing an **MOU**. As a part of this understanding, model Néstle Hall has been developed and furnished. This hall is delightfully decorated with excellent seating arrangements, dual modern multimedia and other facilities.

I also appreciate Vita Pakistan for establishing Manji Food Technology Training Center in this University which will be completed soon. It will provide facilities for technical training of the students and industry personnel.

Another remarkable achievement of this institute is the establishment of Extrusion Center costing $3, 89,000. It is a joint venture between Pakistan and United States of America. It will provide opportunities for research in extrusion which is becoming an emerging technology in Pakistan.

The National Institute of Food Science and Technology is no doubt a model institute in the country. I am happy to know that the institute has started new degree programmes including B.Sc (Hons.) Food Science and Technology and M.Sc (Hons.) Dairy Technology. The institute is planning to start other degree programmes. I appreciate the Vice-Chancellor, Dr. Bashir Ahmad, for his consistent commitment to improve educational standards. I also complement Prof. Dr. Faqir Muhammad Anjum, Director General of the Institute, and his entire team for their untiring efforts towards promoting the cause of Food Science and Technology in Pakistan. He has made tremendous progress for the uplift of this discipline during the past few years which is really commendable. The Government of Punjab will continue supporting the University and the Institute in the national interest for the promotion of Food Science and Technology.

I assure you all that recommendations made in this symposium will be implemented at all levels in the Government.

I finally congratulate the National Institute of Food Science and Technology and Pakistan Society of Food Scientists and Technologists for organizing this wonderful symposium on a very relevant and current topic for the professionals, food industry and the business community.

I wish all of you success and good luck.

Thank you very much

Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

DEVELOPMENTAL HISTORY OF FOOD SCIENCE DISCIPLINE IN PAKISTAN

Muhammad Shafiq Chaudhry
Director Ramna Products (Pvt) Ltd, Kot Lakhpat, Lahore

BIRTH OF THE DISCIPLINE

The discipline of Food Science & Technology came to teaching and research institutes in Pakistan a little late. Not that this subject was unknown in academic circles but its inclusion in the curriculum of teaching institutions did not commence until 1960. Preservation of fruits and vegetables was included in B.Sc. Agric. with Horticulture major curriculum and was limited to the preparation of high sugar products (like jams, jellies and preserves), beverages (like squashes and syrups) and pickles (fruits and vegetables). The author had studied Horticulture (including preservation of fruits and vegetables) while pursuing his career at the undergraduate level.

The importance of Food Technology as a discipline began to be realized in mid fifties and a professor of Food Technology namely Mr. C.W. Eddy was invited to come to Pakistan under the Inter-college Exchange Programme (Project of the USAID) between Punjab Agricultural College & Research Institute and Washington State University. Mr. Eddy was assigned with a mandate to establish Food Technology Section in the then Punjab Agricultural College and Research Institute. The author joined hands with Mr. Eddy in 1957 whereas Mr. Riaz-ur-Rehman was already helping him since the day Mr. Eddy arrived in Pakistan. Together the trio made plans and assessment of the requirements in terms of trained teachers, pilot plant food processing equipment, laboratory equipment, apparatus and books, etc. Having identified these needs the processing equipment, laboratory equipment, apparatus and books were obtained with the finances provided by USAID. The equipments were promptly installed in a processing hall and in the laboratories assigned for this purpose. Two scholarships for higher studies in Food Technology in Universities in the USA were provided in this program. The author availed one scholarship and Mr. Riaz-ur-Rehman the other. The author obtained an MS degree in Food Science from University of California Davis and returned to Pakistan in August 1960, whereas Mr. Riaz-ur-Rehman obtained an MS (Food Science) from Washington State University, Pullman and returned to Pakistan in 1961.

Mr. Riaz-ur-Rehman (left), Mr. C.W. Eddy (center) and the author (right) at work

FIRST ENROLMENT

By mid 1960 the Food Technology Section was ready to offer a teaching programme and, therefore, selected a group of 20 students for Bachelors Programme and 3 students for Master's degree in August 1960. The first combined class of B.Sc. and M.Sc. students was initiated in the academic year 1960-61. One fine morning in late August 1960, these students assembled in a make shift lecture room in Fruit-Section of the Punjab Agricultural College. The author was honored with the privilege of delivering the first lecture in Food Technology discipline in Pakistan. Thus a new science discipline was born in Pakistan. It had barely learnt to crawl when it received a severe shock in the sad demise of Mr. C.W. Eddy who succumbed to a massive cardiac arrest and died instantly in early summer of 1961 (may his soul rest in peace). He was playing golf in Lahore at that particular instant. The teaching programme, however, continued with the addition of Mr. M.B. Bhatti (who had received training at MIT, USA) and the return of Mr. Riaz-ur-Rehman from Washington State University.

DEPARTMENT OF FOOD TECHNOLOGY

In 1962, the status of Punjab Agricultural College was raised to that of a University with the name West Pakistan Agricultural University. The Research Institute was separated and established as Ayub Agricultural Research Institute, Lyallpur. The name of the University was subsequently changed to University of Agriculture Lyallpur and finally to University of Agriculture Faisalabad as it is known at present. With this change, all "Sections" of the College were elevated in their status to that of "Departments". By this time, the first batch of students had graduated with B.Sc. or M.Sc. degrees with Food Technology as their major subject. Some of these graduates joined the faculty to help the senior teachers and to be groomed to become future teachers. In the reorganization process with change of College to University, some new staff from other sections was also transferred to the Food Technology Department. These included Dr. S.A. Tremazi an Oil Chemist. He, being senior to other faculty members, was made Head of the Department. The department continued its progress both in teaching and research. The teaching program changed from annual system to semester system affording versatility and additional courses for broader array of choices for the students. At the same time new equipment, apparatus and better physical facilities became available with the construction of new expanded campus of the University. Research efforts were diversified with the appointment of new faculty members with different specializations.

The Department has played the key role of a flagship institution. It continued its growth and expansion with quality input by the growing faculty to lay a strong and solid foundation of this subject for it to become a torch bearer institution. This distinguished role of the Department has led other teaching institutions in the country to include Food Technology in their curricula. Today we have 8 other Universities which offer undergraduate and post-graduate courses in this subject in their scheme of studies. Continued and prolonged discussions in respect of teaching and research programmes in the faculty meetings led to the inclusion of new innovations and ideas in teaching courses. Course contents were periodically revised and updated to equip the graduating students with recent developments in food processing techniques, quality control, food safety, food security and WTO and ISO regulations.

UP-GRADATION OF THE DEPARTMENT OF FOOD TECHNOLOGY

From Food Technology Section to Institute and National Institute of Food Science & Technology the leadership of the Department changed many hands till the year 2000 when a highly qualified academician and researcher, Dr. Faqir Muhammad Anjum

became the Chairman. It was he who initiated the move to further elevate the status of this Department to that of an Institute. His sincere and dedicated struggle bore fruit in 2003 when the Government of Pakistan granted this change with broader scope for research and teaching. Dr. Anjum continued his efforts relentlessly to further improve the status and image of the Institute until in 2007, the small section of Food Technology of 1960 achieved the ultimate status of National Institute of Food Science and Technology. In keeping with national and international developments this Institute shall have a teaching program to impart state of the art training to its students to cope with the demands of research, teaching and industrial organization. The 21st century has augured well for Food Science & Technology and it is hoped that its growth will continue to achieve greater heights both in terms of producing more and better trained manpower and the performance of its graduates in the market.

Teaching has remained the primary activity of the Institute from 1960, the starting year to date; the number and variety of courses has increase/altered/modified in keeping with the requirements of growing food processing industries and emergence of new specializations of research. Since 2004, the Higher Education Commission in consultation with all the Food Technology teaching institutions, formulated a uniform curriculum comprising a minimum of 20 courses for a B.Sc. (Hons.) degree in Food Science & Technology and 26 courses for post-graduate studies. The number of students enrolled for this subject has increased gradually. It is one of the choicest field of study in the University. This Institute has so far produced 976 graduates with Bachelors and 733 with Masters and 22 with Ph.D. degrees. It is a matter of pride and satisfaction that the graduates from this Institute have given outstanding performance in National and International institutions and industries.

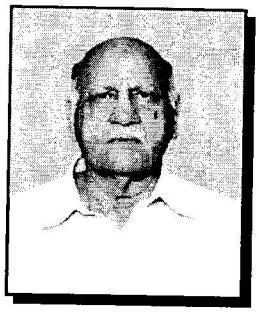
SIGNIFICANT RESEARCH DEVELOPMENTS

At the time of establishment of the Food Technology Section in 1960, it had the mandate to conduct both teaching and research. Formation of the University and up gradation to department, witnessed a shift in emphasis from teaching and research to only teaching. Research programmes continued as part of postgraduate teaching. Despite this change the faculty members continued with their research programmes through graduate students. These combined efforts of faculty members and graduate students have produced processes and products of commercial value. Some significant achievements include:

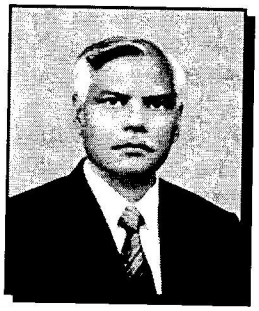
a. Development of mango drink which was launched in 1960 and continues to be produced and marketed continuously countrywide.
b. Development of drink from a blend of orange and mango pulp. This product was launched in 1970 and is considered to be as acceptable as mango drink.
c. Development of a formulation producing tandoori roti on an automatic roti plant. This formulation with few modifications has been adopted for the production of Arabic/Pita bread in Middle Eastern countries and elsewhere.
d. Local fabrication and introduction of reel type ovens for production of all types of bakery products. The availability of this oven has revolutionized the baking industry in Pakistan.
e. Selection of local wheat varieties for its flour to be used in pizza industry. This development has put a stop on the import of flour from other countries for pizza industry.

PAKISTAN SOCIETY OF FOOD SCIENTISTS AND TECHNOLOGISTS

This Society was formed to provide a forum for all stakeholders to get together and discuss issues of mutual interest and for the promotion of the subject of Food Technology. Being the parent institution, the Society was formed and registered at Faisalabad in 1979 with the author as its first President. Due to a host of reasons the Society did not function as envisaged for almost a decade until the arrival of Dr. Javaid Aziz Awan, as faculty member of the Food Technology Department. He had served several national and international institutions before returning to his almamater. It was he who acted as a catalyst to revive and rejuvenate the Society in 1989. We organized our first general meeting in 1990. The author and Dr. J. A. Awan served as President and Secretary of the Society for the initial several years. During this period the number of professional members rose to more than five hundred from an initial 83 in the first year. The members include Food Technologists, trading houses, engineering firms and food industries. The Society undertook the publication of its activities in the form of a newsletter with the name Food Science News. After four years, it was upgraded to Pakistan Journal of Food Sciences in 1995. Since then it is being regularly published. Dr. J. A. Awan was the first Editor in Chief who has been succeeded by Dr. F. M. Anjum. The Society maintains its secretariat in the National Institute of Food Science & Technology at Faisalabad. It is a matter of pride that the Society has had the moral and financial support of food industries, trading houses and other sources from the public and private sectors. These sources have been most magnanimous in promoting and supporting the activities of the Society.



Prof. Dr. Javaid Aziz Awan



Prof. Dr. Faqir M. Anjum

Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

IMPROVING TRADITIONAL AND SMALL-SCALE FOOD PROCESSING METHODS: AN EFFECTIVE FOOD BASED APPROACH TO PROMOTE HEALTHY NUTRITION

Serge Trèche, Claire Mouquet-Rivier, Sylvie Avallone
Research Unit "Nutrition, Food, Societies", Institute of Research for Development (IRD),
BP 64501, 911 Av Agropolis, 34394 cedex 5, Montpellier, France
E-mail: Serge.Treche@ird.fr

Abstract

In many parts of the world, problems of under nutrition and micro-nutrient deficiencies continue to persist, especially in rural areas, as a result of essential nutrient deficiencies in the daily diet. Food-based approaches are increasingly recognized as effective and sustainable solutions aimed at preventing such problems. They encompass a series of complementary strategies that involve the agriculture, food technology and education/communication sectors: increasing and diversifying food production and dietary intake; reducing post-harvest losses of perishable crops, increasing the content in bioavailable micronutrients of crops or diets by biofortification or fortification, modifying the processing of nutrient-rich foods. Amongst them, improving the food processing methods at household and community level or in small scale industry is a promising way which still merit to be developed. This paper gives three examples of strategies lying on the improvement of food processes likely to allow nutritionally vulnerable groups of population to increase their essential nutrient intakes. The first one concerns the development of a very low-cost extruder for the production of infant flours in Vietnam. The second one is relative to the improvement of traditional fermentation methods at small-scale production unit level to process cereal into appropriate gruels for infants and young children in West Africa. The last one deals with the transfer of improved recipes in different contexts at household level in order to upgrade traditional dishes to fortified and high energy dense dishes. From these examples, a global approach is proposed to identify and improve relevant food processing methods likely to contribute to reduce under nutrition and micro-nutrient deficiencies and to define and validate appropriate ways to transfer them to the potential beneficiaries.

Key-words: Food-based approach, healthy nutrition, traditional recipes, small-scale processing, transferability

INTRODUCTION

In many parts of the world, problems of under nutrition and micro-nutrient deficiencies continue to persist, especially in rural areas, as a result of essential nutrient deficiencies in the daily diet. In developing countries, respectively 32% and 11% of pre-school children suffer from moderate or severe stunting and wasting (Unicef, 2008). The situation is particularly alarming in South Asia and sub-Saharan Africa (figure 1).

In addition, more than half of pregnant women and school-age children suffer from iron deficiency anemia, as do more than 40 % of non-pregnant women and pre-school children. Some 100-250 millions of pre-school children are affected by severe vitamin A deficiency. 740 million people are affected by goiter, a symptom of iodine deficiency. Furthermore, evidence is increasing that other micronutrient deficiencies like zinc, folic acid, calcium and essential fatty acids may be as serious.

The consequences of malnutrition and micronutrient deficiencies are drastically detrimental. Malnutrition is responsible, directly or indirectly, for more than 50% of the 9.6 million deaths annually among children under five in developing country (Unicef 2008). It impairs immune function, increases rates and severity of enteric et al infections, delays motor development, stunts mental and physical growth, impairs cognitive function and school performance and retards national socioeconomic development (losses of GNP only due to iron deficiency anaemia was estimated to 1.3% in Pakistan in 1998-1999).

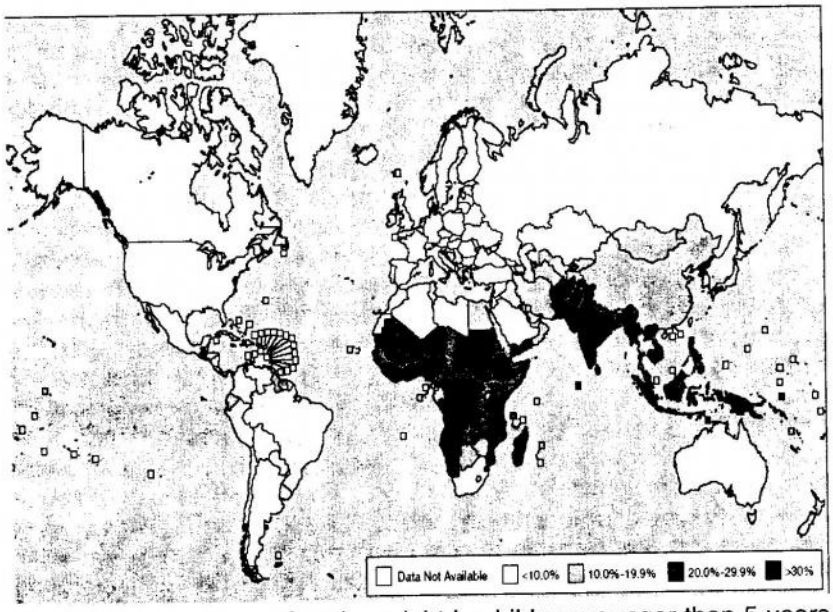


Figure 1. Geographical pattern of underweight in children younger than 5 years (de Onis *et al* 2005)

To prevent such problems, food-based approaches are increasingly recognized as effective and sustainable solutions (Brouwer *et al* 2005). Food-based strategies are defined as approaches that aim to increase the intake of nutrients (both macronutrients like energy and protein as well as micronutrients) through an increase of food intake and/or an improvement of nutrient content and bioavailability.

The objective of this paper is to examine the importance and role of food processing in the development of effective food-based approaches.

CRUCIAL ROLE OF FOOD PROCESSING IN THE DEVELOPMENT OF FOOD BASED APPROACHES

Food-based approaches encompass a series of complementary strategies and approaches that involve the agriculture, food technology and education/communication sectors. Food-based strategies include: increasing and diversifying food production and dietary intake; enhancing food processing, preservation and marketing of a variety of nutrient-rich foods; reducing post-harvest losses of perishable crops; increasing the content in bioavailable micronutrients of crops or diets by biofortification or fortification; promoting small-scale food fortification and nutrition education.

Amongst the possible food-based approaches, improving the food processing methods in small scale industry (Trèche *et al* 2002; Brouwer *et al* 2005) or at household and community level (Brouwer *et al* 2005) is a promising way which still merit to be developed.

Food processes affect not only effective supply but also sanitary and nutritional quality of foods. Taking into account that food processes strongly influence the retention and the bioavailability of nutrients, the use of appropriate food processing methods, not only in small scale industry but also at household or community level, is of crucial importance. At household and community level the main food processes which can have significant effects on vegetal foods are: decortication, dehulling, peeling, soaking, malting; fermentation and cooking (Lestienne *et al* 2005a, 2005b, 2007). At small scale industry levels, additional processes can be mentioned: formulation (food-to-food fortification, fortification); milling; roasting.

Their effects on bioavailable micronutrient contents of foods can be favourable or unfavourable and they can occur either:

- directly, by modifying their solubility, by leaching, or by thermal degradation or complexation (Besançon 1995)
- or indirectly, by changing the content of activators and/or inhibitors of bioavailability of the micronutrient (Lestienne *et al* 2005c, 2005d).

Some examples of processes likely to affect mineral bioavailability during cereal processing can be given:

- Formulation: Effects of molecules acting as activators (Ascorbic acid) or inhibitors (phytates, tannins, fibers) of mineral bioavailability
- Washing, soaking: losses by leaching (Lestienne *et al* 2005a, 2005b)
- Cooking: modification of in vitro digestibility of minerals resulting from the alteration of the food matrix (Besançon 1995)
- Germination (Traoré *et al* 2004, 2007), fermentation (Icard-Vernière *et al* 2005; Tou *et al* 2007a): degradation of antinutritional factors (phytates) and/or losses by leaching (figure 2).

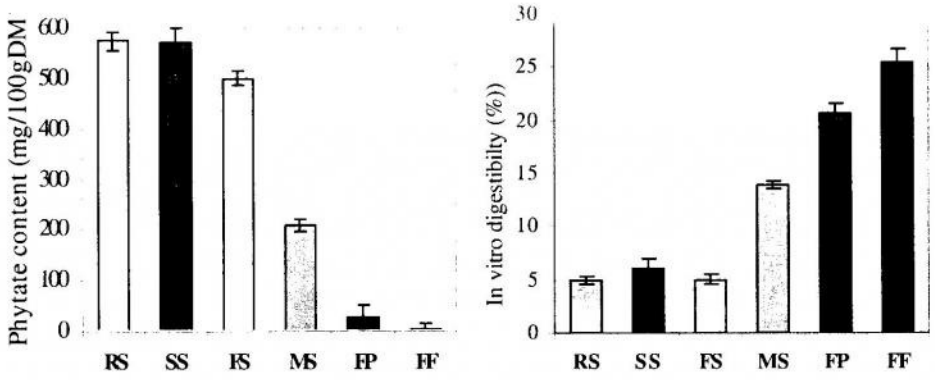


Figure 2: Comparison of the effects of various processes on phytate content and *in vitro* digestibility of iron in pearl millet *(sorghum glaucum)* seeds *(RS: Raw seeds; SS: Soaked seeds (15H, 30°C); FS: Flour from soaked/washed seeds; MS: Malted seeds (96°H, 30°C); FP: Flour treated by phytase; FF: Fermented flour (24H).* D'après Icard-Vernière et al 2005

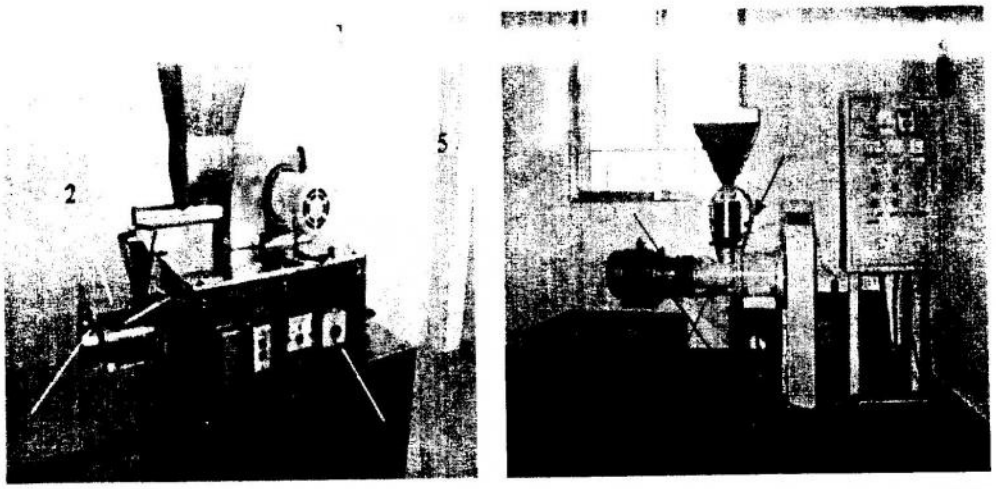
STRATEGIES AIMED AT IMPROVING NUTRITIONAL STATUS BASED ON FOOD PROCESSING ADAPTATION

Three examples of strategies likely to allow nutritionally vulnerable groups of population to increase their essential nutrient intakes can be given: development of a very low-cost extruder in Vietnam; improvement of traditional fermentation methods at small-scale production unit level in West Africa; transfer of improved recipes at household level in different contexts of the developing world.

DEVELOPMENT OF A VERY LOW-COST EXTRUDER IN VIETNAM

Extrusion cooking is well known to be an effective process to produce instant flour which can be prepared into gruels for infants after 6 months of age. But even if low-cost extrusion cookers have been proposed at the beginning of the eighties (Harper and Jansen 1985; Harper 1995), they are too expansive for small scale level production. In addition, in some contexts the hygienic practices during food preparation are too bad to promote the use of instant infant flour

In Vietnam, GRET (a French NG0), IRD and CTC (a Vietnamese Centre for Technology Transfer) designed simple single-screw autogenous "very low-cost extruders" (VLCE, figure 3) that can be manufactured locally and used for the production of rice-based instant flour (figure 4) or ready-to-cook flour (figure 5), which can be prepared into infant gruel with all the hygienic, nutritional and organoleptic characteristics required for young children after 6 months (Mouquet *et al* 2003; Bruyeron *et al* 2006).



First generation: 1 Feeding machine; 2 Screw / Barrel / Heating jacket, 3 Die; 4 Control box; 5 Speed variator of feeding machine

Second generation: 1 Feeding machine; 2 Screw / Barrel / Heating jacket, 3 Die; 4 Control box; 5 Speed variator of feeding machine)

Figure 3:The very-low cost extruders developed by Fasevie programme in Vietnam

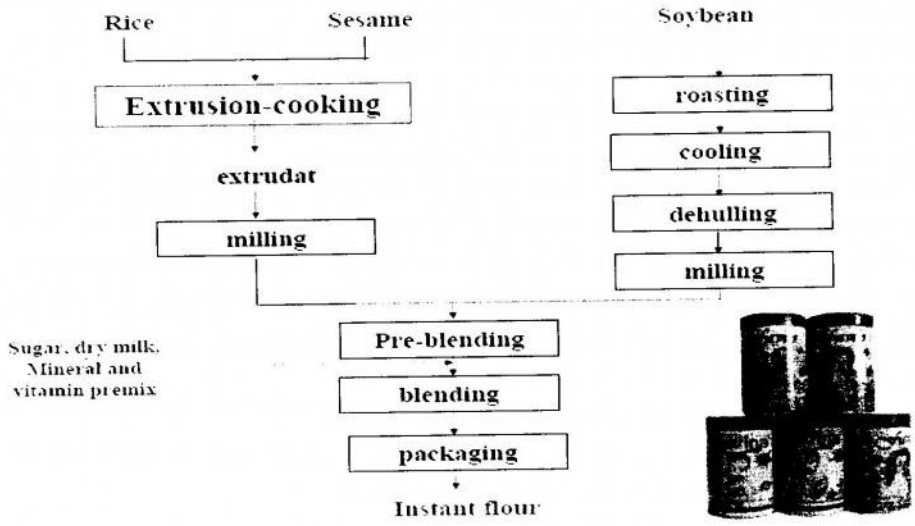


Figure 4: Flow-sheet for the production of instant infant flour using a VLCE.

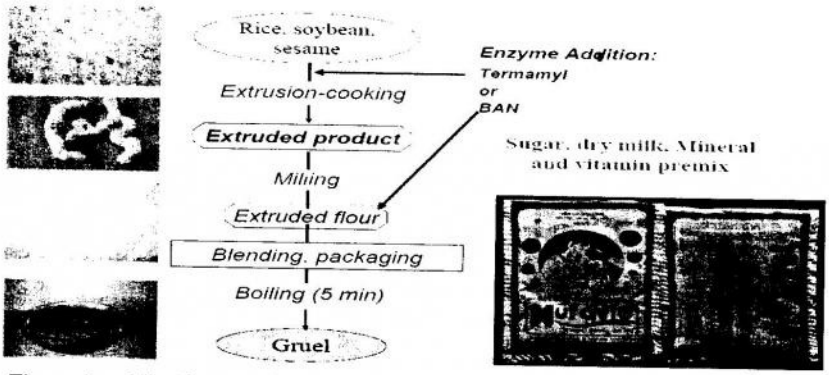


Figure 5: Flow-sheet for the production of ready-to-cook infant flour using a VLCE

These kinds of VLCE can work only if raw materials have a water content less than 15g/100g and a lipid content less than 5g/100g DM (Mouquet *et al* 2003). Consequently, to obtain instant infant flours, rice has to be extruded alone (figure 6) or in association with limited quantity of food having a high lipid content (e.g., sesame). When used with blends with high lipid content (e.g., rice/soybean/sesame, 65.0/27.6/7.4, w/w) very low-cost extrusion has to be used in combination with amylase treatments in order to sufficiently reduce viscosity of gruels (Hoan *et al* 2006) (figure 7).

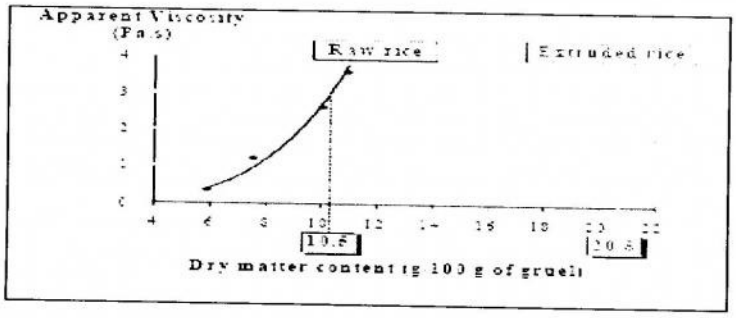


Figure 6: Effect of VLCE on the viscosity of gruel prepared from rice.

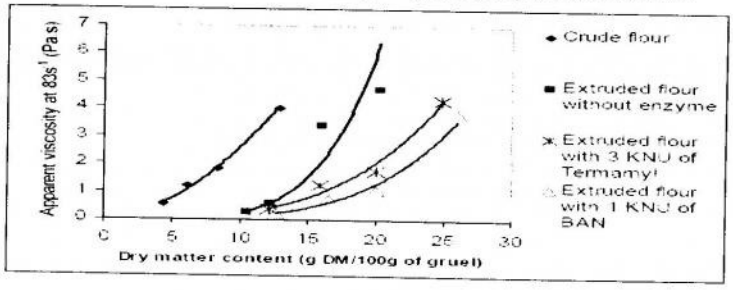


Figure 7: Apparent viscosity of gruels prepared at different concentrations from extruded flour including or not amylases

Interests of extrusion cooking are numerous (Björck and Asp 1983; Harper and Jansen 1985; Camire *et al* 1990). Concerning technological aspects, it allows simultaneous achievement of blending and pre-heating. Concerning nutritional quality, gelatinization and partial dextrinisation of starch during the process (Colonna et al 1984).increases starch digestibility and reduces gruel viscosity allowing the preparation of gruels having both an appropriate energy density and consistency. Heating treatment allows inactivation of certain antinutritional factors like trypsin inhibitor factors thus increasing protein digestibility. In addition, it induces a drastic reduction of the microbiological load and destroys insects. Furthermore, it gives products which only need short time of preparation with possibility to produce either instant flour or ready-to-cook flour and it improves organoleptic characteristics of the end products.

In conclusion, use of VLCE allows to produce infant flours at low production cost (1.2 € / kg in Vietnam), with all the required characteristics, particularly sufficient energy and nutrient densities and which can be consumed by children in sufficient quantities to complement breastmilk to meet their nutritional requirements (Bruyeron *et al* 2006).

IMPROVEMENT OF TRADITIONAL FERMENTATION METHODS AT SMALL-SCALE PRODUCTION UNIT LEVEL IN WEST AFRICA

Lactic acid fermented cereal gruels are currently used as complementary foods in West African countries (Tou *et al* 2006). Unfortunately, they do not meet the specific nutritional requirements of young children, mainly in terms of energy density and nutrient balance.

In Burkina Faso, IRD, Wageningen University and Ouagadougou University carried out studies in order to

- describe and characterise the processes and practices used during the traditional ways of preparation and consumption of ben-saalga, a gruel traditionally obtained by cooking a diluted fermented paste of pearl millet (Pennisetum glaucum) (figure 8).
- elaborate appropriate methods to improve and consolidate the nutritional and sanitary qualities of ben-saalga,
- Define the best ways to transfer to local producers the improved processes and to promote the consumption of the improved gruels by young children

At the end of the diagnostic phase, three ways of improvement have been identified and various solutions have been experimented in order to confer appropriate characteristics to ben-saalga (Tou *et al* 2007a; 2007b):

- For reducing the viscosity of the gruels in order to allow preparing gruel with higher energy density (> 85 instead of ≈ 40 kcal/100g), precooking the dough before fermentation to gelatinize starch and adding malt and/or an inoculum (back sloping) in the precooked dough have been proposed (figure 8).
- For increasing their protein (from ≈ 2.6 to >10 g/100gDM) and lipid (from ≈ 0.8 to >8 g/100g DM) contents, cofermentation of millet and groundnut (76/24; w/w) have been adopted (figure 8)
- For decreasing their antinutritional factor contents (phytate, tannins, α-galactosides) and improving content and bioavailability of micronutrients, optimization of the effect of enzymes (phytase, α-galactosidases) capable of degrading antinutritional factors and inoculation with bacterial strains selected for their functional properties have been done.

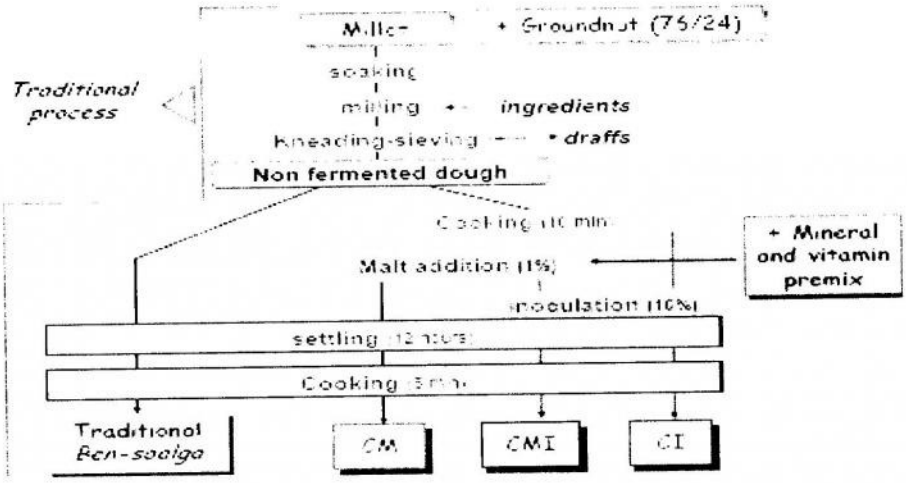


Figure 8: Modification of the flow-sheet of the production of Ben-saalga in order to obtain gruels with appropriate characteristics (CM= cooking + malt addition; CMI= cooking + malt addition + inoculation; CI= cooking and inoculation)

The comparison of the effects of various combinations of treatments on gruel consistency1 lead us to propose the use of CM (cooking+malt addition) or CMI (cooking+malt addition+inoculation) treatments in order to obtain gruels with both appropriate energy density and consistency (figure 9).

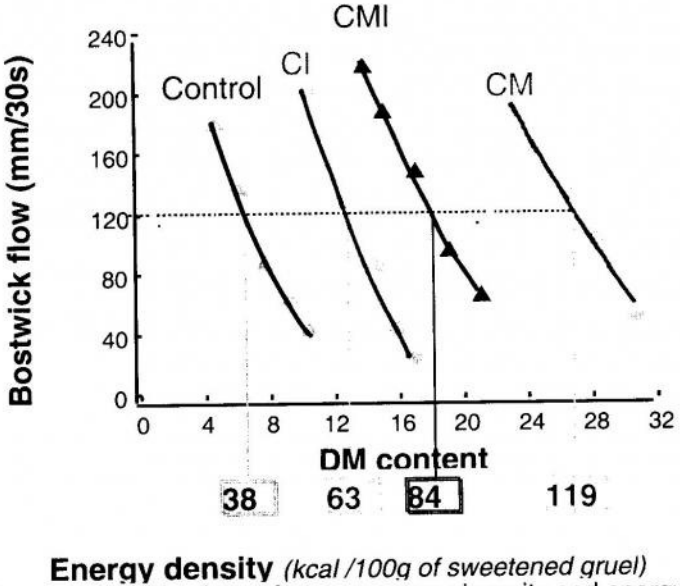


Figure 9: Effect of various combinations of processes on viscosity and energy density of gruels

Thus, by modifying traditional processes used in Africa to produce fermented cereal products, it is possible to obtain gruels with sufficient energy and nutrient densities and improved mineral bioavailability while keeping them the same advantages (well known, well appreciated organoleptic properties, low price, good hygienic quality...) than the traditional ones.

TRANSFER OF IMPROVED RECIPES AT HOUSEHOLD LEVEL IN DIFFERENT CONTEXTS OF THE DEVELOPING WORLD

In many contexts of the developing world, bioavailable micronutrient contents of usual foods prepared at household level and consumed by nutritionally vulnerable groups of population are often insufficient. Two approaches can be proposed in order to allow vulnerable groups to better meet their nutritional requirements:

The first one consists in modification of the traditional recipes in order to optimize micronutrient contents and to improve their bioavailability. For instance, Avallone *et al* (2007) have evaluated the effects of the formulation of the ingredients (okra, fish, soumbala, extract of wood ash) and of cooking time of dried okra (Hibiscus esculentus) sauce in Burkina Faso on total iron and zinc contents and on in vitro digestibility in order to propose an optimal recipe (mixture of 37.7% okra, 26.3% dried fish, 18.5% soumbala and 3.7% extract of wood ash cooked for 25 minutes) that enabled the quantity of digestible iron to be doubled and the quantity of digestible zinc to be increased by one third.

The second one consists in adding to traditional dishes food supplements (sprinkle) containing sufficient amounts of micronutrients to fill the gap between the nutritional requirements and intakes from usual foods. This approach is currently developed by our team in the south of Madagascar for 1-to-5-month-old children. It requires:

- characterization of the nutritional value and intake of usual dishes (i.e., in the south of Madagascar: Corn/cowpea, cassava/Dolochos Lablab, rice...) by children
- estimation of the % of recommended daily allowances (RDA) to bring in one bag considering the consumption of 1 bag per day
- choice of the more appropriate excipient taking intc account food preferences and nutritional value (Groundnut flour has been selected for the south of Madagascar)
- Verification of the acceptability of the product and of the way to use it

CONCLUSIONS

From these examples, it is possible to propose a global approach to identify and improve relevant food processing methods likely to contribute to reduce undernutrition and micro-nutrient deficiencies and to define and validate appropriate ways to transfer them to the potential beneficiaries.

- Step 1: Well identify the nutritional problems and well characterize the feeding practices of the concerned groups;
- Step2: Identify the available foods and food processing methods used in the context
- Step 3: Elaborate potential solutions to the nutritional problems taking into account available foods, culinary know-how and all the identified constraints
- Step 4: Test at pilot scale level the acceptability, the easiness of transfer and the biological efficacy of these potential solutions before to propose their extension at a larger scale.

Malnutrition and micronutrient deficiencies generally have various causes, but in most cases improvement of nutritional status in developing countries is not possible without a specific attention from food technologists.

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Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

EMERGING TRENDS IN PESTICIDE RESIDUES IN FOOD AND CANCER RISK

Altaf Hussain
National Institute of Food Science and Technology (NIFSAT)
University of Agriculture, Faisalabad

Abstract

Dietary pesticides are 99.99% all natural that plants produce to defend themselves. Only 52 natural pesticides have been tested in high-dose animal cancer tests and about half (27) are rodent carcinogen; these 27 are shown to be present in many common foods. Food has also been assayed for about 200 chemicals including the synthetic pesticides residues thought to be of greatest importance and the residues of some industrial chemicals such as polychlorinated biphenyls (PCBs), residues for 105 of these chemicals have been found. Intake of the sum of these 105 chemicals average about 0.09 mg per person per day, which we compare to 1.5 mg of natural pesticides (i.e., 99.99% natural). The intake of rodent carcinogens from synthetic residues is only 0.05 mg a day, averaging about 0.06 ppm in plant food. The finding that in high-dose tests, a high proportion of both natural and synthetic chemicals are carcinogens, mutagens, teratogenics and clostogenic undermines current regulatory efforts to protect public health from synthetic chemicals based on these tests

Key words: Cancer risk, pesticide residue, food

INTRODUCTION

Possible cancer hazards from pesticide residues in food have been much discussed and hotly debated in the scientific literature, the popular press, the political arena, and the courts. Consumer's opinion survey indicates that much of the public believes that pesticide residues in food are a serious cancer hazard. In contrast, epidemiologic studies indicate that the major preventable risk factors for cancer are smoking, dietary unbalances, endogenous hormones and inflammation. Other important factors include intense sun exposure, lack of physical activity, and excess alcohol consumption. There is, however, a paradox in the public concern about possible cancer hazards from pesticide residues in food and lack of public understanding of the substantial evidence indicating that high consumption of the foods that contain pesticide residue – fruits and vegetable – has a protective effect against many types of cancer. About 200 epidemiological studies reported a consistent association between low consumption of fruits and vegetables and cancer incidence at many target sites. The quarter of the population with the lowest dietary intake of fruits and vegetables has roughly twice the cancer rate for many types of cancer compared to the quarter with highest consumption of those foods. Keeping in view the protective effect of fruits and vegetables against

cancer and to create awareness in public about pesticide residues in food, this paper examines critically the pesticide residues content of different fruits, vegetables and grains consumed by Pakistani public.

Human exposures to natural and synthetic chemicals

Current regulatory policy to reduce human cancer risks is based on the idea that chemicals that induce tumors in rodent cancer bioassays are potential human carcinogens. The chemicals selected for testing in rodent, however, are primarily synthetic. The enormous background of human exposures to natural chemicals has not been systematically examined. This has led to an imbalance in both data and perception about possible carcinogenic hazards to humans from chemical exposures. The regulatory process does not take into account (i) that natural chemicals make up the vast bulk of chemicals to which humans are exposed: (ii) that the toxicology of synthetic and natural toxins is not fundamentally different: (iii) that about half of the chemicals tested, whether natural or synthetic, are carcinogens when tested using current experimental protocols; (iv) that testing for carcinogenicity at near-toxic doses in rodents does not provide enough information to predict that excess number of human cancers that might occur at low-dose exposures; and (v) that testing at the maximum tolerated dose (MTD) frequently can cause chronic cell killing and consequent cell replacement (a risk factor for cancer that can be limited to high doses) and that ignoring this effect in risk assessment can greatly exaggerate risks.

Of all dietary pesticides that human eat, 99.99% are natural (1). They are the chemicals produced by plants to defend themselves against fungi, insects, and other animal predators. Each plant produces a different array of such chemical. The amounts of synthetic pesticide residues in plant foods are low in comparison to the amount of natural pesticides produced by plant themselves. We estimate that the daily average exposure to natural pesticides in the diet is about 1500 mg and to burnt material from cooking is about 2000 mg. In comparison, the total daily exposure to all synthetic pesticide residues combined is 0.09 mg based on the sum of residues; humans ingest about roughly 5000 10,000 different natural pesticides and their breakdown products. Despite this enormously greater exposure to natural chemicals, among the chemicals tested in long-term bioassays in the cancer potency database (CPDB), 77% (1050/1372) are synthetic.

Concentration of natural pesticides in plants are usually found at parts per thousand or million rather than parts per billion, which is the usual concentration of synthetic pesticide residues. Therefore, because humans are exposed to so many more natural than synthetic chemicals (by weight and by number), human exposure to natural rodent carcinogens, as defined by high-dose rodent tests, is ubiquitous. It is probable that almost every fruit and vegetable in the super market contains natural pesticides that are rodent carcinogens. Even though only a tiny proportion of natural pesticides have been tested for carcinogenicity, 37 of 71 that have been tested are rodent carcinogens that are present in the common foods listed in Table 1 and 2.

Humans also ingest numerous natural chemicals that are produced as by-produced by cooking. For example, more than 1000 chemicals have been identified in roasted coffee, many of which are produced by roasting. Only 30 have been tested for carcinogenicity according to the most recent results in our CPDB, and 21 of these are positive in at least on test (Tables 2 & 3), totaling at least 10 mg of rodent carcinogens per cup of coffee. Among the rodent carcinogens in coffee are the plant pesticides caffeic acid (present at 1800 ppm) and catechol (present at 100 ppm). Two other plant pesticides in coffee, chlorogenic acid and neochlotoginic acid (present at 21.600 and 11,600 ppm, respectively) are metabolized to caffeic acid and catechol but have not been

tested for carcinogenicity. Chlorogenic acid and caffeic acid are mutagenic. Another plant pesticide in coffee, d-limonene, is carcinogenic. Some other rodent carcinogens in coffee are product of cooking, for example, furfural and benzo (α) pyrene. The point here is not to indicate that rodent data necessarily implicate coffee as a risk factor for human cancer, but rather to illustrate that there is an enormous background of chemicals in the diet that are natural and that have not been a focus of carcinogenicity testing. A diet free of naturally occurring chemicals that are carcinogens in high dose rodent tests is impossible.

It is often assumed that because natural chemicals are part of human evolutionary history, whereas synthetic chemicals are recent, the mechanisms that have evolved in animals to cope with toxicity of natural chemicals will fail to protect against synthetic chemicals, including synthetic pesticides. This assumption is flawed for several reasons.

- Various natural toxins, which have been present throughout vertebrate evolutionary history, nevertheless cause cancer in vertebrates. Mold toxins, such as aflatoxins, have been shown to cause cancer in rodents, monkeys, humans and other species. Many of the common elements, despite their presence throughout evolution, are carcinogenic to humans at high doses (e.g., the salts of cadmium, beryllium, nickel, chromium and arsenic). Furthermore, epidemiological studies from various parts of the world indicate that certain natural chemicals in food may be carcinogenic risk to humans: for example, the chewing of betel nut with tobacco is associated with oral cancer. Among the agents identified as human carcinogens by the International Agency for Research in Cancer (IARC) 62% (37/60) occur naturally: 16 are natural chemicals, 11 are mixtures of natural chemicals, and 10 are infectious agents. Thus, the idea that a chemical is "safe" because it is natural, is not correct.
- Humans have not had time to evolve a "toxic harmony" with all of their dietary plants. The human diet has changed markedly in the last few thousand years. Indeed, very few of the plants that humans eat today (e.g., coffee, cocoa, tea, potatoes, tomatoes, corn, avocados, mangoes, olives and kiwi fruit) would have been present in a hunter-gatherer's diet. Natural selection works far too slowly for humans to have evolved specific resistance to the food toxins in these newly introduced plants.
- Some early synthetic pesticides were lipophilic organochlorines that persist in nature and bioaccumulation in adipose tissue, for example, dichlorophenyltrichloroethane (DDT), aldrin, and dieldrin. The ability to accumulate is often seen as a hazardous property of synthetic pesticides; however, such bioconcentration and persistence are properties of relatively few synthetic pesticides. Moreover, many thousands of chlorinated chemicals are produced in nature. Natural pesticides also can bioconcentrate if they are fat soluble. Potatoes, for example, were introduced into the worldwide food supply a few hundred years ago; potatoes contain solanine and chaconine, which are fat soluble, neurotoxic, natural pesticides that can be detected in the blood of all potato-eaters. High levels of these potato glycoalkaloids have been shown to cause reproductive abnormalities in rodents.
- Because no plot of land is free from attack by insects, plants need chemical defenses either natural or synthetic to survive pest attack. Thus, there is trade-off between naturally-occurring pesticides and synthetic pesticides. One consequence of efforts to reduce pesticide use is that some plant breeders develop plants to be more insect resistant by making them higher in natural pesticides. A recent case illustrates the potential hazards of this approach to pest control: When a major grower introduced a new variety of highly insect-resistant celery into commerce, people who handled the celery developed rashes when they were subsequently exposed to sunlight. Some detective

work found that the pest-resistant celery contained 6200 parts per billion (ppb) of carcinogenic (and mutagenic) psoralens instead of 800 ppb present in common celery.

Toxic chemicals and human risk

Less important risk factors for cancer include occupation, sun exposure, medical interventions, pollution, hereditary and distractions. The major risk factors are endogenous damage, tobacco, chronic infection, inflammation, hormones and diet. Out of these diet is thought to account for about one-third of cancer. A brief overview of the dietary fruits and vegetables and cancer prevention is presented here.

Consumption of adequate fruits and vegetables is associated with a lowered risk of degenerative diseases such as cancer, cardiovascular disease, cataracts, and brain and immune dysfunction. Nearly 200 studies in the epidemiological literature have been reviewed and relate, with great consistency, the lack of adequate consumption of fruits and vegetables to cancer incidence. The quarter of the population with the lowest dietary intake of fruits and vegetables compared to the quarter with the highest intake has roughly twice the cancer rate for most types of cancer. Two servings of fruits plus three of vegetables per day are generally recommended. Tables 4, 5 and 6 give data on pesticide residues present in local fruits, vegetables and grains.

Antioxidants in fruits and vegetables may account for good part of their beneficial effect as suggested by mechanistic studies. However, the effects of dietary intakes of the antioxidants, ascorbate, tocopherol, and carotenoids are difficult to disentangle by epidemiological studies from other important vitamins and ingredients in fruits and vegetables. Also, it is unlikely that all compounds sharing antioxidant properties would have similar effects against all types of cancer, since each antioxidant has a unique function and distribution within the body. Further, even though a specific antioxidant may play a critical role in limiting cancer incidence, the levels already present in a particular population may be sufficient, so that greater consumption would not be of benefit.

Only a few randomized trials in humans have evaluated antioxidants as possible protective agents. In a trial conducted in rural China, a combination of antioxidant supplements appeared to reduce the incidence of gastric cancer, a disease which has been repeatedly associated with low intake of fruits and vegetables. However, supplements of β-carotene did not reduce recurrences of skin cancer, and vitamin C and E and β-carotene did not reduce recurrences of colon polyps. In a recent large study of 30-years, heavy smokers in Finland, β-carotene supplements appeared to slightly increase the risk of lung cancer, coronary heart disease, and total mortality, in contrast to the findings of protection by intakes of fruits and vegetables in many observational studies. A modest dose of vitamin E was unrelated to risk of lung cancer in this study, perhaps because vitamin C, which was not given, is necessary to regenerate vitamin E. The duration of the Finish study (six years may have been insufficient to observe a protective influence that might operate in the early stages of carcinogenesis. Present epidemiological evidence regarding the role of greater antioxidant consumption in human cancer prevention is thus inconsistent. Nevertheless, biochemical data indicating massive oxidative damage to DNA, proteins, and lipids, as well as indirect evidence, such as heightened oxidative damage to human sperm DNA with insufficient dietary ascorbate, indicate the need for further investigation of the wide variety of potentially effective antioxidants, both natural and synthetic.

Folic acid and other compounds in fruits and vegetables may contribute to the reduction of cancer. Low folic acid intake causes chromosome breaks in rodents and humans and increase tumor incidence in some rodent models. Folic acid is required for the synthesis of DNA nucleotides, and folate deficiency causes breaks in DNA through misincorporation of uracil. Low folate intake has been associated with several neoplasm,

including adenomas and cancers of colon. Dietary fiber obtained from foods of plant origin, may lower the risk of colon cancer. Vitamin A, which is derived from some carotenoids as well as from animal sources in the diet, regulates cell differentiation and reduces tumor incidence in many animal models and possibly humans. Fruits and vegetables may also reduce cancer risk because they contain antioxidants such as flavonoids, inducers of detoxifying enzymes such as indoles, and weak estrogens that act as antiestrogens (3-7).

Other aspects of diet

Cooking of food is plausible as a contributor to cancer. A wide variety of chemicals are formed during cooking. Four groups of chemicals that cause tumor in rodents have attracted attention because of mutagenicity, potency and concentration. (i) Nitrosamines are formed from nitrogen oxides present in gas flames or from other burning. Surprisingly little work has been done on the levels of nitrosamines in fish or meat cooked in gas ovens or barbecued, considering their mutagenic and carcinogenic potency. (ii) Heterocyclic amines are formed from heating amino acids or proteins. (iii) Polycyclic hydrocarbons are formed from charring meat. (iv) Furfural and similar furans are formed from heating of sugars. Heating fat generates mutagenic epoxides, hydroperoxides, and unsaturated aldehydes and may also be of importance. Epidemiological studies on cooking are difficult and so far are inadequate to resolve a carcinogenic effect in humans.

Alcoholic beverages cause inflammation and cirrhosis of the liver and liver cancer. Alcohol is an important cause of oral and esophageal cancer (and is also synergistic with smoking) and possibly contributes to colorectal cancer. Breast cancer is also associated with alcohol consumption.

Tobacco is the most important global cause of cancer and is preventable. Smoking contributes to about one third of cancer, and one quarter of heart disease. Tobacco is a known cause of cancer of the lung, bladder, mouth, pharynx, pancreas, kidney, stomach, larynx, esophagus and possibly colon. It causes even more deaths by diseases other than cancer. Tobacco is causing about three million deaths per year worldwide in the 1990s and will, if present rates of smoking continue, cause about 10 million deaths per year a few decades from now. The evidence of environmental tobacco smoke as a cause of cancer is much weaker. The carcinogenic mechanisms of tobacco smoking are not well understood. Smoking is a severe oxidative stress, and smoke contains a wide variety of mutagens and rodent carcinogens. The oxidants in cigarette smoke (mainly nitrogen oxides) deplete the body's antioxidants. Thus, smokers must ingest 2-3 times more ascorbate than nonsmokers to achieve the same level of ascorbate in blood, but they rarely do.

Natural defenses against normal exposure to toxins

The reason humans can eat the tremendous varieties of natural 'rodent carcinogens' in our food is that, like other animals, humans are well protected by general defense enzymes, most of which are inducible (i.e., when a defense enzyme is in use, more of it is made). Defense enzymes are effective against both natural and synthetic chemicals, such as potentially reactive mutagens. One does not expect, nor does one find, a general difference between synthetic and natural chemicals in ability to cause cancer in high-dose rodent tests.

Human has many natural defenses that buffer against normal exposure to toxins and these are general rather than tailored for each specific chemical. Thus they work against both natural and synthetic chemicals. Example of general defenses include the continuous shedding of cells exposed to toxins – the surface layer of the mouth, esophagus, stomach, intestine, colon, skin and lungs are discarded every few days, DNA repair enzymes, which repair DNA that was damage from many different sources and

detoxification enzyme of the liver and other organs, which generally target class of chemicals rather than individual chemicals. That human defenses are usually general rather than specific for each chemical, makes good revolutionary sense. The reason that predators of plants evolved general defenses is presumably to be prepared to counter a diverse and ever-changing array of plant toxins in an evolving world: if a herbivore had defenses against only a specific set of toxins, it would be at great disadvantages in obtaining new food when favoured foods became scarce or evolved new chemical defenses.

Defense system such as the glutathione transferases protects DNA against mutagens. These defenses are almost all inducible and thus, buffer cells from increments in reactive electrophile chemicals than can cause DNA lesions. DNA repair enzymes,, almost all of which are inducible, buffer the cell against increments in DNA lesions. Therefore, the effect of a particular chemical insult is dependent on the level of each defense, which in turn is dependent on the past history of exposure. Defense can be partially disabled by lack of particular micronutrients in the diet (e.g., antioxidants).

CONCLUSIONS

1. Hypothetical risk of one cancer in a million.
2. A diet free of naturally occurring chemicals that are carcinogen in high dose rodent tests is impossible.
3. The idea that a chemical is "safe" because it is natural is not correct.
4. It is probable that almost every fruit and vegetable in the market contains natural plant pesticides that are rodent carcinogens.
5. A diet rich in fruit and vegetables is associated with lower cancer rates. This may be because anticarcinogenic vitamins and antioxidants come from plants.
6. As an alternative to synthetic pesticides, it is legal for organic "farmers" to use natural pesticides from one plant species against pests that attack a different plant species, e.g., rotenone or the pyrethrins from Chrysanthenum plants. These naturally derived pesticides have not been tested as extensively for carcinogenicity (rotenone is negative, however), mutagenicity, or teratogenicity as have synthetic pesticides; therefore, their safety compared to synthetically derived pesticides should not be prematurely assumed.
7. Synthetic pesticides have markedly lowered the cost of plant food, thus increasing consumption. Eating more fruits and vegetables and less fat may be the best way to lower risks of cancer and heart disease, other than giving up smoking.
8. The tremendous variety of chemicals that occur naturally in food, some in high concentrations relative to their toxicity, may play some role in causing human cancer, and research is needed to identify potentially important human carcinogens.

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Table 1. Some natural pesticide carcinogens in food.

Rodent carcinogen	Conc., ppm	Plant food
5-/8-Methoxypsoralen	14 32 0.8 6.2 25	Parsley Parsnip, cooked Celery Celery, new cultivar Celery, stressed
p-hydrazinobenzoate	11	Mushrooms
Glutamyl p-hydrazinobenzoate	42	Mushrooms
Sinigrin (allyl isothiocyanate)	35-590 250-788 12-66 110-1,560 16,000-72,000 4500	Cabbage Collard greens Cauliflower Brussels sprouts Mustard (brown) Horseradish
D-Limonene	31 40 8,000	Orange juice Mango Pepper, black
Estragole	3,800 3,000	Basil Fennel
Safrole	3,000 10,000 100	Nutmeg Mace Pepper, black
Ethyl acetate	0.07	Pineapple
Sesamol	75	Sesame seeds (heated oil)
α-Methylbenzyl alcohol	1.3	Coca
Benzyl acetate	82 230 15	Basil Jasmine tea Honey
Catechol	100	Coffee (roasted beans)
Caffeic acid	50-200 >1,000 1,800	Apple, carrot, celery, cherry, eggplant, endive, grapes, lettuce, pear, plum, potato Absinthe, anise, basil, caraway, dill, marjoram, rosemary, sage, savory, tarragon, thyme Coffee (roasted beans)
Chlorogenic acid (caffeic acid)	50-500 21,600	Apricot, cherry, peach, plum Coffee (roasted beans)
Neochlorogenic acid (caffeic acid)	50-500 11,600	Apple, apricot, broccoli, Brussels, sprouts, cabbage, cherry, kale, pear, plum Coffee (roasted beans)

Table 2. Carcinogenicity status of natural pesticides tested on rodents

Carcinogens : N = 37	Acetaldehyde methylformylhydrazone, allyl isothiocyanate, arecoline, HCl, benzaldehyde, benzyl acetate, caffeic acid, capsaicin, catechol, clivorine, coumarin, crotonaldehyde, 3,4-dihydrocoumarin, estragole, ethyl acrylate, hexanal methylformylhydrazine, p-hydrazinobenzone acid HCl, hydroquinone, d-limonene, 3-methoxycatechol, 8-methoxypsoralen, N-methyl-N-formylhydrazine, α-methylbenzyl alcohol, 3-methylbutanal methylformylhydrazone, 4-methylcatechol, methylhydrazine, monocrotaline, pentanal methylformylhydrazone, quercetin, reserpine, safrole, senkirkine, sesamol, symphytine.
Noncarcinogens: N = 34	Atropine, benzyl alcohol, benzyl isothiocyanate, biphenyl, d-carvone, codeine, deserpidine, disodium glycyrrhizinate, ephedrine sulphate, eucalyptol, eugenol, gallic acid, geranyl acetate, glycyrrhetinic acid, isosafrol, nicotine, phenethyl isothiocyanate, pilocarpine, piperidine, protocatechuic acid, rotenone, rutin ulphate, sodium benzoate, tannic acid, turmeric oleoresin, vinblastine.

Table 3. Carcinogenicity status of natural chemicals in roasted coffee.

Positive N= 21	Aldehyde, benzaldehyde, benzene, benzofuran, benzo(α)pyrene, caffeic acid, catechol, ethanol, ethylbenzene, formaldehyde, furan, furfural, hydrogen peroxide, hydroquionone, isoprene, limonine, 4-methylcatechol, styrene, toluene, xylene
Not Positive: N = 8	Acroleine, biphenyl, choline, eugenol, nicotinamide, nicotinic acid, phenol, piperidine
Uncertain:	Caffeine
Yet to Test	~ 1000 chemicals

Table 4. Pesticide residues found in fruits

Pesticides	Banana	Grape	guava	Melon
Endosulfan	0.075-0.135 (2)	0.029-0.084	0.02-0.110	0.012-0.045
Methamidophos	0.052-0.089	0.059-0.078	0.02-0.086	0.016-0.065 (0.5)
Monocrotophos	-	0.052-0.087	0.069-0.147	0.085-0.164 (0.1)
Cypermethrin	0.026-0.089	0.016-0.088	0.110-0.145	0.025-0.140
Benomyl	0.025-0.056	0.060-0.118	0.051-0.105	-
Chlorpyriphos	0.025-0.048	-	0.020-0.086	0.016-0.052

Values in parenthesis are permissible maximum residue limits (FAO/WHO)

Table 5. Pesticide residues found in vegetables

	Spinach	Cauliflower	Tomato	Potato
Endosulfan	0.07-0.168 (2)	0.030-0.110	0.090-0.160	0.025-0.059 (0.2)
Methamidophos	0.055-0.570	0.052-0.078 (0.5)	0.062-0.178 (0.01)	0.056-0.262 (0.05)
Monocrotophos	0.058-0.076	0.056-0.069	0.050-0.068	0.057-0.110 (0.05)
Cypermethrin	-	0.131-0.196	0.098-0.248 (0.5)	0.058-0.186
Benomyl	-	0.02-0.058	0.022-0.270	0.068-0.140
Chlorpyriphos	0.076-0.314	0.019-0.270	0.034-0.155 (0.5)	0.018-0.124 (0.05)

Values in parenthesis are permissible maximum residue limits (FAO/WHO)

Table 6. Pesticide residues found in rice grains

Pesticide	**Paddy**	**Rice (Parboiled)**	**Brown rice**
Padan (cartap)	0.090	0.050	0.06 (0.10)
Furadan (carbofuran)	0.32	0.100	0.24 (0.50)
Gamma BHC (lindane)	0.030	traces	0.01 (0.20)

Values in parenthesis are permissible maximum residue limits (FAO/WHO)

Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

FOOD BROWNING CONTROL WITH COLLOIDAL TECHNIQUES

Alam Zeb
Department of Food Science & Technology, NWFP Agricultural University, Peshawar.

Abstract

Effect of various emulsifiers of SDS (anionic), Tween-20 (non-ionic) and CTABr (cationic) was studied on the formation of non-enzymatic browning occurring in food from ingredients such as ascorbic acid, sorbic acid, ascorbic acid-glycine (amino acid), sorbic-glycine and glucose-glycine. All these experiments were carried out in model systems at pH 5.0 (0.4 M acetate buffer), EDTA (0.2 mM, chelating agent) heated at 55°C. Absorbance of the solutions was measured at 470 nm. Ratio of the absorbance was obtained in fold of the reaction solutions (+emulsifier /-emulsifier). Ascorbic acid browning decreased with Tween-20 (-5%), SDS (-14%) and increased with CTABr (156%). Sorbic acid browning was found unaffected with Tween-20, increased with CTABr (+480%) and decreased with SDS (-12%). Browning of ascorbic acid-glycine decreased with Tween-20 (-7%), SDS (-11%) and increased with CTABr (+70%). Browning of sorbic acid-glycine decreased with Tween-20 (-10%), SDS (-30%) and slightly increased with CTABr (+6%). Browning of glucose-glycine well decreased with CTABr (-56%), SDS (-40%) and Tween-20 (-36%).

Key words: Maillard browning, emulsifiers [cetyltrimethylammonium bromide (CTABr), sodium dodecylsulfate (SDS), polyoxyethylene (20), sorbitan mono-olaurate (Tween-20)]

INTRODUCTION

In food browning occurs due to enzymatic and non-enzymatic reactions. However it is not easily determine whether the cause of the browning is enzymatic or other chemical reactions. In case the food has been earlier treated with heat on such a temperature which is enough to inactivate the enzymes then the cause of browning development will be a non enzymatic. Non enzymatic browning is generally occurs due to one of these reactions (1) Maillard browning, the reaction between a reducing sugar and amino compound (2) Ascorbic acid browning that is the spontaneous thermal decomposition of ascorbic acid under both aerobic and anaerobic conditions and either in the presence or absence of amino compounds (3) Caramelisation or the pyrolysis of food carbohydrate (4) lipid browning which is probably oxidative deterioration followed by polymerization. In many situations the term non-enzymatic browning is synonymous with Maillard browning (Mauron 1981) which is the most widely studied and the leading cause of the non-enzymatic browning. A common feature is the participation of carbonyl compounds as the reactive intermediates.

Maillard browning

This reaction was first described by Louis Maillard (1912) as the formation of colored products on heating a mixture of reducing sugars and amino acids (including peptides and proteins) and the reaction involving ammonia is widely used in the manufacture of

ammoniated caramels (Namiki and Hayashi, 1981; Kato and Tsuchida 1981). It takes readily at all pH values found in food systems. The reaction leading to the formation of color is independent of pH in the range expected for foods (pH 3-7).

1.2 Ascorbic acid (vitamin C) browning

Under anaerobic condition aqueous solution of ascorbic acid and ascorbate ion decompose spontaneously producing carbon dioxide, furfural (Herbert *et al* 1933) and 2,5-dihydro-2-furoic acid (Coggiola 1963). Ascorbic acid is relatively unstable in strongly acid media, the rate of decomposition decreasing with increasing pH to a minimum value at around pH 2.3. The decomposition of ascorbic acid is accompanied by the formation of color and the rate of color development is being increased in the presence of amino compounds (Mori *et al* 1967). On oxidation ascorbic acid is decomposed to dehydroascorbic acid. Mushran and Agrawal (1977) reviewed that autoxidation is strongly catalyzed by metal ions in trace amounts and the rate is pH dependent showing the maximum at pH 5. Dehydroascorbic acid is getting browning much more than ascorbic acid, the pathway involving opening of lactone ring to 2, 3-diketogulonic acid followed by degradation.

1.3 Sorbic acid (food preservative) browning

Sorbic acid (*trans, trans* -2, 4-hexadienoic acid) and its potassium salts are effective antimicrobial agents used to enhance stability of a wide variety of food products including intermediate moisture food (IMF) and beverages. Foods included different products of dairy, fruits & vegetables, and bakery. Being a diunsaturated acid sorbic acid can undergo autoxidation to yield malonaldehyde and other carbonyl compounds (Marx and Sabalitschka 1965; Arya 1980). The breakdown of sorbic acid can yield several volatile products (Saxby *et al* 1982). The degradation of sorbic acid in aqueous solution is accelerated by light and metal ions. Arya (1980) reported that in aqueous solutions sorbic acid develops browning and the rate of browning is accelerated in the presence of amino acids.

Non-enzymatic browning occurs in food in the presence of few major ingredients of carbohydrates, proteins and lipids and minor components of ascorbic acid, carotenoids and some food additives, which undergo chemical changes during processing and storage. Bura *et al* (1986) studied that the rate of Maillard browning of glucose-glycine amino acid reaction was enhanced with the presence of sorbate preservative. The similar effect of browining development of glucose reaction with sorbic acid was reported by Wedzicha *et al* (1991) and observed that some contribution in browning is due to the acid-base catalysis. Emulsifiers are amphiphilic molecules, which aggregate spontaneously in solution and form micelles. These association colloids play an important role in food, pharmaceutical and cosmetic products (solubilize non-polar materials in a polar medium). Emulsifiers increase solubility of food additives i.e. vitamins, colors, flavors and preservatives. Inhibition of non-enzymatic browning in food is desirable in view of the losses caused in nutritive value together with the possible generation of off-flavors, which diminished visual appeal of the product. The most effective method of controlling Maillard browning in food is the use of sulphur dioxide and its salts (Wedzicha, 1884). Non-enzymatic browning of the reaction of amino acids with glucose was reduced in 30% and above of glycerol solutions (Obanu and Ledward 1986). We previously studied the effect of emulsifiers on sorbic acid and thiols interaction (Wedzicha and Zeb 1990). In this research, influence of selected emulsifiers was studied on the formation of non-enzymatic browning in model systems containing reaction solutions of sorbic acid, ascorbic acid, glycine (amino acid) and glucose.

MATERIALS AND METHODS

Experimental work was carried out in the Laboratory of Food chemistry, Procter Department of Food Science, University of Leeds UK. All chemicals were of Analar grade, supplied by BDH, Sigma and Fluka. Measurement of pH was made by using a Jenway-3020 pH meter. The meter was calibrated with buffers (potassium hydrogen phthalate and anhydrous disodium hydrogen orthophosphate/sodium dihydrogen orthophosphate). Progress in browning of the reaction solutions was determined by measuring absorbance at 470 nm (1 cm cells) using spectrophotometer model No: CECIL CE-1020 1000-SERIES.This study was carried out in the following model systems.

Model system -I

In this system ascorbic acid solution and sorbic acid solution were separately mixed with individual emulsifier of CTAB (cationic), SDS (anionic) and Tween-20 (nonionic). Model system containing solutions of ascorbic acid (0.125 M) and CTABr (0.04 M), SDS (0.06 M) and Tween-20 (3% w/v), EDTA (0.2 mM), prepared in pH 5.0 acetate buffer (0.4 M), and heated at 55°C. Absorbance was measured at different time intervals (25-400 hr) at 470 nm.

Model system -II

This model system containing solutions of ascorbic acid (0.125 M), glycine (amino acid) (0.0625 M) with CTABr (0.04 M), SDS (0.06 M), Tween-20 (3% w/v), EDTA (0.2 mM), prepared in pH 5.0 acetate buffer (0.4 M), and heated at 55°C. Absorbance of the reaction solutions was measured at different time intervals (25-400 hr) at 470 nm.

Model system -III

This model system containing solutions of sorbic acid (0.02 M), glycine (amino acid) (0.0625 M) with CTABr (0.04 M), SDS (0.06 M), Tween-20 (3% w/v), EDTA (0.2 mM), prepared in pH 5.0 acetate buffer (0.4 M), and heated at 55°C. Absorbance of the reaction solutions was measured at different time intervals (25-400 hr) at 470 nm.

Model system -IV

This model system containing solutions of glucose (0.125 M), glycine amino acid (0.0625 M) with CTABr (0.04 M), SDS (0.06 M), Tween-20 (3% w/v), EDTA (0.2 mM), prepared in pH 5.0 acetate buffer (0.4 M), and heated at 55°C. Absorbance of the reaction solutions was measured at different time intervals (25-400 hr) at 470 nm

RESULTS AND DISCUSSION

Effect of CTABr, SDS and Tween-20 on ascorbic acid browning.

Browning of ascorbic acid was studied individually in the presence of emulsifier of CTABr (0.04 M), SDS (0.06 M) and Tween-20 (3% w/v). Absorbance (browning) was measured at 470 nm. Ratio of absorbance in fold of the reaction solutions (+emulsifiers / -emulsifiers) was obtained at a period of 400 hr heating Table-1 shows that CTABr increased the browning to 2.56 fold (156%), while decreased with SDS to 0.86 (14%) fold and Tween-20 to 0.95 fold (5%).

Effect of CTABr, SDS and Tween-20 on ascorbic acid-glycine browning

Effect of emulsifiers of CTABr (0.04 M), SDS (0.06 M) and Tween-20 (3% w/v), was studied on the development of browning of the solutions of ascorbic acid-glycine reaction. Absorbance (browning) of the reaction solutions was measured at 470 nm. Ratio of absorbance in fold of the reaction solutions (+emulsifiers/-emulsifiers) determined at a period of 400 hr heating. Table-1 shows that ascorbic acid-glycine browning increased with CTABr to fold 1.70 (70%), and decreased slightly with SDS to fold 0.89 (11%) and Tween-20 to fold 0.93 (7%).

Effect of CTABr, SDS and Tween-20 on sorbic acid browning

Browning of sorbic acid was studied individually in the presence of emulsifier of CTABr (0.04 M), SDS (0.06 M) and Tween-20 (3% w/v). Absorbance (browning) was measured at 470 nm. Ratio of absorbance in fold of the reaction solutions (+emulsifiers'/-emulsifiers) was obtained at a period of 400 hr heating (Figure 1). Table-1 shows that CTABr increased the browning to 5.80 fold (480%), while decreased with SDS to 0.88 (12%) fold and Tween-20 was found unaffected 1 fold (no increase).

Effect of CTABr, SDS and Tween-20 on sorbic acid-glycine browning

Effect of emulsifiers of CTABr (0.04 M), SDS (0.06 M) and Tween-20 (3% w/v), was studied on the development of browning of the solutions of sorbic acid-glycine reaction. Absorbance (browning) of the reaction solutions was measured at 470 nm. Ratio of the absorbance in fold (Figure 1) of the reaction solutions (+emulsifers/-emulsifers) determined at a period of 400 hr heating. Table-1 shows that sorbic acid -glycine browning remained unaffected with CTABr to fold 1.06 (6%), and decreased slightly with SDS to fold 0.70 (30%) and Tween-20 to fold 0.90 (10%).

Effect of CTABr, SDS and Tween-20 on glucose-glycine browning

Effect of emulsifers of CTABr (0.04 M), SDS (0.06 M) and Tween-20 (3% w/v), was studied on the development of browning of the solutions of glucose-glycine. Absorbance (browning) of the reaction solutions was measured at 470 nm. Ratio of the absorbance was determined in fold (Figure 1) of the reaction solutions (+emulsifers/-emulsifers) at a period of 400 hrs heating. Table-1 shows that the glucose-glycine browning was decreased with CTABr to fold 0.44 (56%), with SDS to fold 0.60 (40%) and Tween-20 to fold 0.64 (36%).

Table 1: Effect of CTABr, SDS and Tween-20 on the browning of the solutions of ascorbic acid (0.125 M), sorbic acid (0.02 M) and glucose (0.125 M) mixed with glycine (0.0625 M), EDTA (0.2 mM), pH 5.0 acetate buffer (0.4 M), heated at 55^0C. Browning of the reaction solutions was determined in fold and measured at 470 nm (+emulsifier /-emulsifier) and % difference in browning (increased or decreased) was obtained.

Reaction Solution	**CTABr (0.04 M)**	**SDS (0.06 M)**	**Tween-20 (3%w/v)**
	% Increase (+) or % Decrease (-) of browning at 470 nm		
Ascorbic acid	(+) 156	(-) 14	(-) 5
Sorbic acid	(+) 480	(-) 12	No effect
Ascorbic acid + glycine	(+) 70	(-) 11	(-) 7
Sorbic acid + glycine	(+) 6	(-) 30	(-) 10
Glucose + glycine	(-) 56	(-) 40	(-) 36

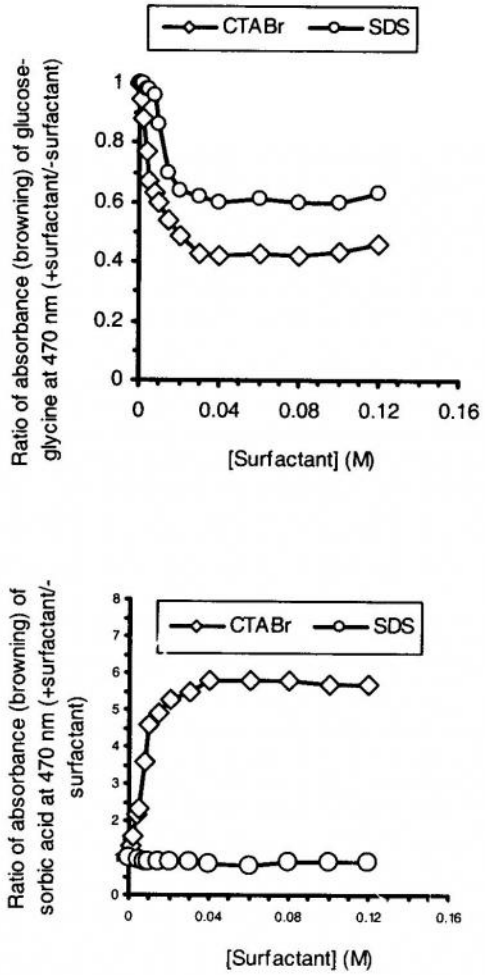

Figure 1: Effect of various concentrations of CTABr and SDS on the ratio of absorbance (browning) of the solutions of glucose (0.25 M) with glycine (0.125 M) and sorbic acid (0.02 M) [+emulsifier /-emulsifier] at 470 nm heated 400 hrs (55°C, EDTA (0.2M), pH 5 (acetate buffer 0.4 M). Absorbance without emulsifier was assumed =1

4. DISCUSSION

Non-enzymatic browning reactions are among the chemical alterations, which can affect the quality of shelf-stable intermediate and high moisture foods, pharmaceutical and cosmetic products. Such reactions decrease the protein bioavailability and exhibit antinutritive effects by mechanisms involving complexation with micronutrients (O'Brein and Morrissey 1989). Ingredients commonly used in food and pharmaceuticals are lubricants, antioxidants, preservatives, colouring agents and flavouring agents. Kumar and Banker (1991) reported that great majority of drugs has amine functionality and in the presence of sugars and other carbonyl compounds often produce extensive discoloration (browning). Inhibition of browning is desirable in view of the losses caused in nutritive value together with the possible generation of off-flavors, which diminished visual appeal

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of the product. Emulsifers are well known for acceleration and inhibition of chemical reactions. The solubility of organic compounds, which are sparingly soluble in water, can be increased by the addition of emulsifers (Wedzicha 1988; Zeb 2001). Previously we studied the influence of emulsifers on the interaction of sorbic acid with thiols, in which emulsifier DoTAB increased while SDS decreased the reaction rate (Wedzicha, and Zeb 1990). In this research work the influence of emulsifers of CTABr (cationic), SDS (anionic) and Tween-20 (non-ionic) was studied in model systems on the browning of the reactions solutions of ascorbic acid, sorbic acid, mixture of ascorbic acid with glycine, sorbic acid with glycine and glucose with glycine amino acid. CTABr increased while SDS and Tween-20 decreased the browning of ascorbic acid, sorbic acid and mixture solutions of ascorbic acid and sorbic acid with glycine amino acids. In case of glucose-glycine browning decreased with CTABr (56%), SDS (40%) and Tween-20 (36%). "*Aqueous ionic micelles affect the rates of bimolecular reactions; they can accelerate or inhibit the reaction by bringing reactants together at the micellar surface or keeping them apart (Martinek et al 1977; Fendler. 1982; Bunton, and Savelli. 1986).* Dunlap and Cordes (1968) studied that longer alkyl chain emulsifers are better catalysts. The micellar function is also depending on the nature of hydrophilic group and there is pronounced difference between the CMCs of ionic and non-ionic emulsifers with identical hydrophobic moieties. Non-ionic micelles have lower values because there is no interaction and repulsion of charges when the micelles are formed. Important factors controlling the micellar size are their counter ions and the mean distance of closest approach of a counterion to the charge centre of the emulsifier (Mukerjee, 1967; Geer *et al* 1971). Micellar catalysis is also depending on the substrate structure. For example the interaction between tri-p-anisylmethyl cation and aliphatic amines is catalyzed by sodium lauryl sulphate (NaLS). The effect of catalysis increases with alkyl amine chain length, but decreases when the chain is branched. It has been suggested that the larger n-alkyl groups bring the reaction centre of the amine group deeper into stern layer of the micelles because of favorable interactions between the n-alkyl groups of the reagent and the emulsifier. Bunton and Savelli (1982) reported a decrease in the reactivity with branched or cyclic groups and this can be explained using the same model because they may not fit easily between the n-alkyl groups of the emulsifier.

ACKNOWLEDGEMENT

The author is thankful to the Commonwealth Scholarship and Fellowship Commission, London UK, for awarding Post-Doctorate research-fellowship, Procter Department of Food Science University of Leeds UK for admission, and NWFP Agricultural University Peshawar Pakistan for granting study leave to complete this research.

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Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

EMERGING INTERNATIONAL COMPLIANCE ISSUES: CHALLENGE TO FRESH FRUITS AND VEGETABLE EXPORTS

Babar Ehsan Bajwa
Manager Technical, Pakistan Horticulture Development and Export, Board, 126/Y, Commercial Area, Phase III, DHA, Lahore, Pakistan.
E-mail:- behsan@phdeb.org.pk

Abstract

Pakistan annually produces 13 million tons of fresh produce (2004/05), divided into fruits 46% and vegetables 54%. Its market value at current factor cost is estimated around US$ 2 billion. The annual export of fresh produce is estimated around half a million tons which earns foreign exchange of worth US$ 140 million. Fresh fruits and vegetables export is a growing industry and pace of both production and exports can be much accelerated. In the Medium Term Development Framework 2005-10, production of fruits and vegetables has been projected at about 18 million tons by 2009/10 and exports at US$ 238 million. The major challenge being faced by the fresh fruit export industry is the increasing issues of international compliance mainly focused on food safety (SPS measures), traceability, residues of different agro-chemicals, lack of good agricultural practices, reduction of post harvest diseases and pests (quarantine treatments) and issues pertaining to safety of food packaging materials. Currently, the hazards analysis and critical control point (HACCP), Global GAP, British retailer's consortium (BRC) and monitoring of maximum residues limits (MRL's) are the major challenges. An integrated and comprehensive approach is required to address these issues from both the Government and Private sector to meet the export targets as addressed under the MTDF of the Government of Pakistan (US$ 238 million) by the year 2010.

Keywords: Fruits, vegetables, export, Pakistan

1. INTRODUCTION

The export value of fresh fruits and vegetables from Pakistan amounts to 216.5 million USD at the moment. These exports are growing at an incredible rate of 15% per annum. The Government of Pakistan has set the increase of export target to new markets like EU, East Europe, China, Canada, Africa and Australia. This goal has to be accomplished through foreign investment (branded companies) in value addition, implementing GAP at farm level, HACCP at industry level, establishment of processing plants/cold storages, reefers, and common facility centers (CFCs). A growing consumer and retailer concern about food safety is a global issue transcending national borders which is critical to be taken care off. Consumers throughout the world question food production conditions with respect to safety and hygiene. The commonly recognised reference standards of Good Agricultural Practices at farm level and HACCP at the processor level are emerging trends for Pakistan. For our growing horticulture economy,

there might be a very simple and obvious reason for adopting these practices as the international buyers have put these standards for further purchase as "inevitable requirements".

This problem is further compounded by food safety concerns leading to stringent quarantine measures, traceability and demand for international certifications of Global GAP, British Retailers Consortium (BRC) and HACCP. The Pakistan's horticulture industry is facing the multifaceted problems, which are briefly revealed below:

2. THE CHALLENGES

2.1 Diminishing profits in the wholes sales and retail focused trade.

2.2 EU, USA, Canada, Netherlands, France, Germany, Iran, China emerge as potential markets in which Pakistan's share is less than 1%.

2.3 The importing countries do not verify product rather systems through standards such as;

a. Food safety
b. Traceability
c. GMP
d. GAP
e. Production safety and onsite food security

3. BRIEF OVERVIEW OF THE EMERGING STANDARDS

3.1 Good Agricultural Practices

Global GAP is a single integrated standard with modular applications for different product groups, ranging from plant and livestock production to plant propagation materials and compound feed manufacturing. The standard serves as a global reference system for other existing standards and can also easily and directly be applied by all parties of the primary food sector. Global GAP operates like a satellite navigation system. It equips members with a reliable tool kit, which allows each partner in the supply chain to position themselves in a global market with respect to consumer requirements. Feedback from nearly ten years of working with the standard – with more than one hundred thousand completed audits – have been incorporated to make the new third version of 2007, more relevant to today's concerns and advanced production techniques. The comprehensive documentation of the system of the new Global GAP (former EurepGAP) is organized into five major blocks:

3.1.1 System rules referred to as General Regulations (GR).

3.1.2 Requirements referred to as Control Points and Compliance Criteria (CPCC).

3.1.3 Inspection documents referred to as Checklists (CL).

3.1.4 National GAP requirements referred to as Approved National Interpretation Guidelines.

3.1.5 Harmonization tools referred to as Bench marking Cross Reference Checklist (BMCL) and other guidelines.

3.2 The British Retailers Consortium (BRC) Global Standard

The British Retail Consortium is the trade association that represents the whole range of UK retailers including large multiples, department stores and independent shops, selling a wide selection of products through centre of town, out of town, rural and virtual stores. The BRC is directly involved in a number of important issues affecting retailing and the consumer, including product safety, all forms of legislation, e-commerce, the environment and retail crime. The BRC lobbies opinion formers in the UK and the EU, on these and many other issues. The BRC Standards have been utilized by a number of retailers outside the UK and other users, such as major food manufacturers and food

service organizations. BRC are working closely with other European and Global retailers to meet their requirements.

The key deliverables of BRC system are:

3.2.1 Establishment of an effective quality management system (QMS)

3.2.2 Operate a HACCP section as the cornerstone of a food safety management system in the processing

3.2.3 Eliminating issues of major industry importance, such as allergens and site security

3.2.4 Revision of product categories that focus on technology of food production

4. STRATEGIES AND RECOMMENDED ACTIONS

4.1. Developing Indigenous Gap Standards

The approach is to develop the GAP standards for Pakistan's produce against which, the different organizations in Pakistan shall apply to the Global GAP forum for acceptance and recognition of Pakistan's GAP standards. The process would be carried out through benchmarking which is one of Global GAP's core objectives. In order to ensure integrity and transparency of the system, Global GAP has adopted this benchmarking procedure and appointed external, recognized and competent organizations to undertake the technical review and witness audits. The purpose of this scheme is to define the procedure for establishing the equivalence of specified standards (and other normative documents) against the Global GAP set of standards, such that this procedure is completed in a consistent, reliable and transparent manner, thereby facilitating acceptance on a national and international basis and so furthering international trade. The expected benefits under this scheme launched by the PHDEB involve the harmonization of horticulture crops standards all over Pakistan to get them approved by Global GAP. This would entail the international acceptance of our good agricultural practices for the access of horticulture products from Pakistan to the best retail chains of the EU and other countries.

4.2. Increasing Global Gap And BRC Certifications

The Pakistan Horticulture Development and Export Board (PHDEB) at this moment has initiated a process to enhance the possibility of Global GAP certification as a means to demonstrate commitment to produce safe food in a sustainable manner. The Ministry of Commerce, Government of Pakistan has also announced incentives on the certification of GAP, BRC and HACCP in the trade policy 2007-08. The food industry (both fresh and processing) should take a lead role to up-grade the production and processing systems in line with these requirements.

Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

DETECTION OF GAMMA IRRADIATED FOODS THROUGH DIFFERENT TECHNIQUES

[1]Ijaz A. Bhatti, [2]J.H. Kwon, [3]Salim-ur-Rehman and [1]Shahid Adeel,
[1]Department of Chemistry, University of Agriculture, Faisalabad, Pakistan, [2]Department of Food Science & Technology, Kyungpook National University, Daegu 702-701 South Korea, [3]National Institute of Food Science & Technology, University of Agriculture, Faisalabad, Pakistan

Abstract

Treatment of food by ionizing radiation is a cold pasteurization technique which reduces the food losses and extends the shelf life during storage due to disinfestations of microorganisms. The development of new methods and improvement in already existing methods as well as their standardization are necessary for better understanding and confidence of consumers for their open choice in the market. In this study, physical and chemical methods have been employed on various types of foods of plants and animals origins in order to know about their radiation treatment. Pulsed photo stimulated (PPSL), Chemiluminescence (CL) and Gas Chromatographic technique (GC) were used to study the radiation treatment in various types of food-stuffs. The comparative study of the profile of fatty acids of control and irradiated oils extracted from the spices was performed in order to monitor the radiation induced changes which can serve as a marker for the detection of gamma-ray treatment. It was found that physical methods PPSL and CL were applied on different types of spices and dried vegetables in order to know their irradiation history. The results showed that in the irradiated samples due to the presence of paramagnetic species, produced upon irradiation and persists in the hard part of the food ,yield enhanced luminescence signals using the photosensitizers; luminol and lucigenin that discriminate between the irradiated from the unirradiated. The response of the PPSL signal for the un-irradiated and irradiated samples clearly discriminate them using their threshold values. The study of physicochemical values and the compositions of the oils extracted from un-irradiated and irradiated spices have also been carried out.

Keywords: Irradiation, Thermoluminescence, pulsed photstimulated luminescence, hydrocarbons, paramagnetic.

INTRODUCTION

After waging a constant battle against the spoilage of food caused by infestation, contamination and deterioration, food preservation technology by gamma radiation has made it possible to store the foods for longer period without the risks of pollution and induction of radioactivity (Wahid *et al* 1987). Now, there are 13 countries that are seriously engaged in commercial level food/feed irradiation not for manipulating the food/feed crisis inside but facilitating the international trade also and playing the vital role in the world wide economy. Now the wholesomeness of the irradiated food has been well established for the consumer's confidence. In spite of administrative control of

facilities licensed by food irradiation, compulsory labeling of treated food has been proposed by Codex Alimentarious commission (Anonymous 1984) for providing reliable control and confidence in irradiated food. In order to control and harmonize international trade, there is a dire need of identifying irradiated food materials. Some studies were concluded earlier to determine treated food qualitatively and quantitatively however, there is no single method available for the detection of categories of food items. Thermoluminescence and chemiluminescence for spices and dry herbs (Arun *et al* 1992; Ming-Shia *et al* 1992), viscometry for the starch based materials (Farkas et al 1990), ESR for the materials having seeds ,stones and bones (Farkas et al 1980), GC-MS (Darren *et al* 1983; Lee *et al* 1999; Kim and Seo 2003) for the fat based materials have been reported. The objective of this work is to standardize detection methods for the identification of dried vegetables and spices.

MATERIALS AND METHODS

Four spices such as cumin, coriander, black and red peppers were purchased from local market of Peshawar (Utility Store Corporation of Pakistan) and were irradiated to an absorbed dose of 10 kGy by Co-60 source present at Nuclear Institute of Food and Agriculture (NIFA) Peshawar, Pakistan. The oils of all irradiated and un-irradiated spices were extracted by Soxhlet apparatus in petroleum ether (40-60^{0}C B.P). The iodine values, peroxide value and acid value and total sterols contents were determined according to AOAC (David et al 1983). The refractive indices of oils of species were carried out at temperature of (35 ± C.5^{0}C) by Abbe's Refrectometer WS-1 (AOAC 1984).

Gas Chromatography Analysis

For gas chromatographic analysis the esterification of oils extracted from un-irradiated and irradiated spices to an absorbed dose of 10 kGy was carried out (Sehata *et al* 1970) and fatty acid composition of oil of cumin, coriander, black and red pepper were determined under following chromatographic conditions:

- Gas chrmatograph: Perkin –Elmer Model 3920
- Column: 2m×2mm (i.d.) glass packed with DEGS on chromosorb WAW 80-100 mesh
- Column temperature: 190 ^{0}C (isothermal)
- Detector: FOD (flam ionization detector)
- Flow rate of carrier gas (N_2): 25ml/min
- Air pressure: 50psi
- H_2: 20psi
- Injection value I:20µL
- Integrator: Shimadzu C-R4A Chromatopac.

Chemiluminescence Measurements:

Chemiluminescence of all the irradiated and un-irradiated samples of spices having mesh sizes, 20, 40 and 60, was measured by Bio-Orbit 1250 Luminometry system using luminol and Lucigenin sensitizers.The system was calibrated by C-14 source and the " Back ground" and " Gain " were adjusted to 0 and 10 mv respectively prior to routine measurements. For each measurement 20 mg of the sample was put in the Pyrex cuvette and transferred to the sample port of the system.

Pulsed Photostimulated Luminescence (PPSL) Measurements

Dried pumpkin slices, ginger, dried turnip slices and a mixture of Korean spices were irradiated to the absorbed doses of 0, 1, 3 and 6 kGy at Korea Atomic Energy Research Institute (KAERI), Daejeon, South Korea. The Pulsed photostimulated luminescence (PPSL) was measured for the irradiated and control samples at KAERI.

The PPSL signal was recorded for the control and irradiated samples at the rate of counts/60s, dark counts of 32.90±00, and light counts of 44.00±20 of the PMT noise

conditions. The PPSL Signal (photon counts, pc) emitting from the sample per second automatically accumulated in the PC and was presented as counts/60s. The accumulated PCs corresponding to the lower threshold of 700 counts /60s were classified as non-irradiated sample [Chung *et al* 1992). Signal levels between both thresholds were classified as an intermediate sample for which a further investigation was needed.

RESULTS AND DISCUSSION

The iodine value which is index of saturation and unsaturation; the peroxide value indicates the rancidity, the acid value means how much of the free fatty acids are present in the oil, the sterol contents reveal the level of the non-saponificable lipids in the fat and the refractive index is useful for the indication of isomerization and it increases with the increase in length of hydrocarbon chain and the number of double bonds. The data revealed to above mentioned values for irradiated and un-irradiated spices as given in Table-1. The results indicate that the iodine value decreased with the irradiation treatment. The radiation probably had broken some of the double bonds in the fatty acids which lead toward saturation. The peroxide value was found to increase with irradiation indicating that free radicals produced have oxidized the material. The literature on physiochemical constants of spices-oils is not available. The difference between peroxide values of irradiated and un-irradiated spice oils is quite interesting the refractive indices showed that there was very minute effect of gamma irradiation.

The gas chromatographic analysis of volatile fatty acids formed by esterification of oils of irradiated and control spices have been shown in Table-2. The peaks of fatty acids were identified by matching the relative retention times with respect to the standard ranging from C_6= 0 to C_{22}=0. The data indicate that in case of irradiated black pepper oil the C_6= 0, C_{20}=0 & C_{22}= 0 are eliminated while the present concentration of others were slightly affected as compared to the control. In case of red pepper only C_{20} = 0 was missing in case of irradiated sample. Although, fatty composition of fats and oils has been abundantly reported (Shehata *et al* 1970), however, the fatty acids composition of tropical spices has not been yet widely studied. The data given in Table-3 clearly shows qualitative and quantitative differences between the irradiated and unirradiated spices. As a result of these qualitative and quantitative tests, no consistent pattern was observed, although there was a definite indication of an increase in the total volatile contents as result of irradiation treatment in each spices .Further experiments is required to arrive at a definite conclusion.

The irradiated red pepper can also be clearly distinguished from un-irradiated on the basis of CL-intensity which is enhanced by Luminol and Lucigenin solution. The trend of increase of CL-signal recorded in red pepper. Here the decrease of CL-intensity with the increase of in radiation dose may be due to some impurities or moisture contents. The luminescence response of irradiated and un-irradiated rep pepper of different particle size has also been studied. It was revealed that surface area of the samples did play a role in causing CL- signals of different intensities. From the results, it is clear that the maximum CL-intensity by using luminol and Lucigenin photosensitizers was generally observed having particle size of 40 meshes. The effect of particle size on the response of luminescence intensity has been studied because the mesh size of red pepper may cause an ambiguity in irradiation identification (Sattar *et al* 1976).

The results of the PPSL photon count (PCs) for the control and irradiated samples are shown in Table 4. The PCs of all control samples were less than the lower threshold value (700 count/60s), indicating a clear negative result while the irradiated dried vegetables and spice powder samples show that the PCs are more than the upper threshold value (5000 count/60s) thereby easily screened out as irradiated.

ACKNOWLEDGEMENTS

We are very thankful to the authorities of Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan for providing the irradiation and CL measurement facilities and the Korea Science and Engineering Foundation (KOSEF) for the grant of Post-doctoral scholarship during this study.

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Table 1. Physiochemical constants of un-irradiated and irradiated spice oils.

Spices	Iodine value		Peroxide values		Acid value		Total sterol		Ref.index	
	Irrad.	Control	Irrad.	Control	Irrad.	Control	Irrad.	Control	Irrad.	Control
Cumin	98.7	114.03	41.8	32.35	4.34	1.90	3.51	3.68	1.4802	1.4662
Coriander	56.61	67.80	66.98	36.00	4.70	4.40	5.59	4.57	1.4697	1.4715
Black pepper	111.7	127.51	32.40	16.21	21.13	16.94	7.10	6.63	1.5369	1.5210
Red pepper	98.73	114.99	561	424	18.42	8.14	4.15	3.98	1.4705	1.4679
Values of average of 2-3 determinations Radiation dose given to the sample :10kGy										

Table 2. Fatty acids composition of un-irradiated and irradiated oils of spices

Fatty acid samples	C6=0	C 8=0	C10=0	C12=0	C14=0	C16=0	C18=0	C18=1	C18=2	C20=0	C18=3	C22=0
Standard	2.378	1.385	1.848	26.059	11.648	11.455	18.353	16.329	2.780	2.078	0.649	2.578
Un-irradiated Black Pepper	1.549	4.168	5.004	0.581	1.216	11.846	1.975	10.910	6.866	0.515	1.246	1.7674
Irradiated Black Pepper.(10kGy)		0.242	11.741	30.351	25.457	6.440	0.806	0.3974	1.467	-	3.485	-
Un-irradiated Red pepper	0.916	2.310	0.934	0.371	0.442	11.960	2.573	8.234	33.75	0.798	7.234	1.184
Irradiated Red pepper (10kGy)	0.77 4	2.285	0.127	0.5310	1.058	16.542	1.510	9.930	49.32	-	2.455	3.667
Un-irradiated Cumin	-	22.059	0.120	0.360	1.172	5.486	1.073	22.841	22.888	-	3.222	1.747
Irradiated Cumin.(10kGy)	-	0.316	0.462	0.547	7.334	3.912	1.579	29.072	16.500	2.913	1.39*	3.226
Un-irradiated coriander	-	0.691	0.122	0.399	0.443	5.096	1.096	68.676	19.889	-	0.453	0.112
Irradiated Coriander (10kGy)	0.972	0.84	0.230	0.661	3.997	10.981	1.520	53.230	23.422	-	0.572	0.152

Table 3. Effect of gamma irradiation and mesh size on the chemiluminescence intensity (cm) of red pepper using Luminol and Lucigenin sensitizer.

Irradiation	CL-Signal (cm) using Luminol sensitizer			CL-Signal (cm) using Lucigenin sensitizer		
Dose (kGy)	Mesh size20	Mesh size40	Mesh size60	Mesh size20	Mesh size40	Mesh size60
Un-irradiated	2.8±0.8	1.8±0.3	1.3±0.6	13.2±1.6	5.1±1.0	1.2±0.2
2.5	6.4±0.7	5.3±0.9	3.4±0.5	20.7±2.0	9.5±0.8	5.3±1.3
5	9.5±0.6	7.2±1.0	3.5±0.8	22.9±2.6	10.5±1.0	5.7±0.9
7.5	10.4±1.2	9.2±0.8	4.0±0.5	20.6±1.3	14.9±1.4	7.0±1.0
10	16.7±1.5	13.9±1.0	3.3±1.1	23.0±2.1	16.0±1.5	8.4±1.0

Tables 4. Pulsed photo stimulated luminescence (PPSL) signals of irradiated and control dried vegetables and spices mixture.

Sample	Radiation Dose				Regression and coefficient expression
	Control	1kGy	3kGy	6kGy	
Dry Pumpkins	180	5500	95325	12345	y = 4032x -3242.5 R2 = 0.9837
	210	4549	8399	7500	y = 2572x -1265.5 R2 = 0.8098
	350	6592	10065	93460	y = 28280x -43084 R2 = 0.686
Spice Powder	420	7221	63572	83411	y = 30532x - 37675 R2 = 0.9186
	661	5465	24523	68200	y = 22168x - 30707 R2 = 0.8651
	472	6600	32342	74300	y = 24723x - 33378 R2 = 0.9048
Ginger	333	992	4589	100031	y = 30269x - 49187 R2 = 0.6343
	543	7690	25921	200190	y = 61717x - 95707 R2 = 0.7033
	772	8187	18731	80335	y = 24923x - 35302 R2 = 0.7853
Dried Turnip Slices	412	43115	83592	105971	y = 7103.2x + 16671 R2 = 0.0598
	333	32840	92345	175212	y = 58414x - 70853 R2 = 0.9641
	842	23310	100210	95312	y = 36031x - 35159 R2 = 0.8534

Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

LATEST TRENDS IN FOOD PROCESSING USING EXTRUSION TECHNOLOGY

Mian N. Riaz[1], Faqir M. Anjum[2] and Muhammad Issa Khan[2]
[1]Food Protein Research & Development Center, Texas A&M University, College Station, Texas, 77843, USA. [2]National Institute of Food Science & Technology, University of Agriculture, Faisalabad-Pakistan

LATEST TRENDS AND EXTRUSION

Some of the latest trends in food processing and consumption mentioned by Sloan (2005) are healthy food and sense appeal. Right after fresh, crunch was the most frequent menu marketing claim on fine-dining menus last year (Archibald 2006). Third trend which she mentioned is snacking and sharing, whether at home or away from home, the snack business in on fire. A new late night eating occasion, light meals for seniors, women, teenage, after school get together, and a need to stay satiated until dinner have made snacks and mini meals the hottest opportunity in the restaurant world today (Sloan 2006).

During the recent years quite a number of technologies in food processing have been emerged and made an impact on the availability and variety of food products. Food extrusion is one of these latest multidimensional food processing techniques. Great possibilities are offered in food processing field by the use of extrusion technology to modify physicochemical properties of food components. The extruded food, besides its preserved and frequently even enhanced biological value, can be characterized by physicochemical properties superior to the original raw material. Extrusion cooking is defined as a unique tool to introduce the thermal and mechanical energy to food ingredients, forcing the basic components of the ingredients, such as starch and protein, to undergo chemical and physical changes. Extrusion combines several unit operations including mixing, cooking, kneading, shearing, shaping and forming so it is a highly versatile unit operation that can be applied to a variety of food processes. Extrusion has for years provided the means of producing new and creative foods. One major advantage of extrusion cooking is the capability to produce a wide range of finished products with minimum processing times and by using inexpensive raw material (Riaz 2000).

TYPES OF EXTRUDERS

There are several different types and styles of extruders available in the market. This may cause a difficulty for food or feed manufacturers to select a proper extruder for specific type production. In general, extruders are divided into two major categories: single-screw and twin-screw.

Single screw extruders

Single-screw cooking extruders have compressive screws with decreasing channel depth turning at high speeds to increase shear and mechanical energy input for heating. Heating of a product is induced by the resulting friction. The barrel is jacketed for steam to allow additional contact heating in the metering section. To increase capacity and efficiency, it is common to preheat ingredients in a pre-conditioner by adding steam

before they enter the extruder. Categories of single-screw extruders include (Harper, 1981):

- ***Cold forming (Pasta-type) extruder***: Deep flight, smooth barrel, low shear speed.
- ***Little or no cooking extruders***: Used for pasta, pastry dough, cookies, egg-rolls, ravioli, processed meat and certain candy.
- ***High-pressure forming extruder***: Grooved barrels to prevent a slip at the wall and greater compression in the screw design. Used for pre-gelatinize cereal and fried snack foods.
- ***Low-Shear cooking extruders***: Moderates shear machines with high compression machines and grooved barrels to enhance mixing. Soft-moist foods and meat like snacks such as simulated jerky.
- ***Collet extruders***: High shear machines with grooved-barrels and screw with multiple shallow flights. Used for puffed snacks and expanded curls or collets.

High shear cooking extruders: High shear machines, with screws of changing flight depth, HTST devices. Make pet food, aquatic feed, ready-to-eat cereal, candy, crisp breads, precooked food ingredients, pre-gelatinized corn flour, dried food mixes, instant beverage powder, croutons and breading, crackers and wafers, enzymes' deactivations of full fat soy flour, imitation nuts, famine relief feeding, texturized vegetable protein (TVP), and deactivation of enzymes in cereal and oilseeds. The first major commercial application of the single screw extruder in the food processing industry was the conversion of semolina into pasta. This low shear, low temperature forming process first found commercial production in the 1920's and 30's and remains a standard production process yet today. Conventional pasta products are processed with an extruder only to the level necessary to bind the moistened mass together and produce the desired shape.

Twin screw extruders

Twin screw consists of two parallel screws in a barrel with a figure-eight cross section. The use of twin-screw extruders for food processing started in the 1970s, with an expanding number of applications in the 1980s. Twin screw extruders are generally one and one-half times or more expensive than single a screw machine for the same capacity (Lusas and Riaz 1994). Yet the degree of quality control and processing flexibility they offer can make them attractive to food industries. Twin screws produce a more uniform flow of the product through the barrel due to the positive pumping action of the screw flights. Some other advantages of twin screw are:

- Handle viscous, oily, sticky or very wet material and some other products which will slip in single screw extruder, (it is possible to add up to 25% fat in a twin screw extruder)
- Less wear in smaller part of the machine than in single screw extruder.
- Wide range of particle size (from fine powder to grains) may be used, whereas single screw is limited to a specific range of particle size.
- Because of the self wiping characteristics clean up is very easy.

Four types of twin-screw extruders are possible:

1. Non-intermeshed, co-rotating
2. Non-intermeshed, counter rotating
3. Intermeshed, co-rotating
4. Intermeshed, counter rotating

From these four types of twin-screw extruders, co-rotating, intermeshed screw type has found the widest acceptance in food industry.

Function that an extruder can perform

Food extruders can perform one or several functions at the same time while processing food or feed (Riaz 2007).

Agglomerating ingredients: Human food, pet food, aquatic and livestock feed ingredients can be compacted and agglomerated into discrete pieces in an extruder process.

Degassing of ingredients: Snack food, feed and pet food ingredients that contain gas pockets can be degassed by extrusion processing.

Dehydration: During normal extrusion processing of feed or pet food moisture loss of 4-7% can occur depending upon the initial moisture contents.

Enzyme in-activation: By using extruders different enzymes present in the ingredients can be in-activated, like lipase enzyme in rice bran.

Expansion, puffing: Snack food, pet food or aquaculture feed density (i.e, floating and sinking) can be controlled by extruder operation conditions and configuration.

Grinding: Ingredients can be ground to some extent in the extruder barrel during processing of snack food, pet food and livestock feed.

Homogenization: An extruder can homogenize by restructuring unattractive ingredients into more acceptable forms during processing of human food, pet food, aquatic and livestock feed.

Mixing: A variety of screws are available for all kind of extruders which can cause the desired amount of mixing action in the extruder barrel during extrusion processing.

Pasteurization and sterilization: Ingredients can be pasteurized or sterilized using extrusion technology during processing of human and pet food.

Protein denaturation: Animal and plant protein can be denatured by extrusion cooking to make it more digestible for human and animals.

Shaping products: A special configuration within the extruder barrel can create the desired shearing action for a particular human and pet food, aquatic and livestock feed.

Shearing: A special configuration within the extruder barrel can create the desired shearing action for particular food and pet feet.

Starch cooking (gelatinization): Extrusion cooking improves starch gelatinization from all sources, i.e. tuber or cereal during the processing of food and feed.

Texture alteration: The physical and chemical texture can be altered in the extrusion system during processing of food, pet food, aquatic and livestock feed.

Thermal cooking: The desired cooking effect can be achieved in the extruder during processing of human food, pet food, aquatic and livestock feed.

Unitizing: Different ingredient lines can be combined into one product to give special characteristics by using an extruder for pet food, aquatic and livestock feed.

ADVANTAGES OF EXTRUDERS

The principal advantages of the extrusion technology as compared to the other traditional foods/feed processing methods include:

Adaptability: An ample variety of products are feasible by changing the minor ingredients and the operation conditions of the extruder. Extrusion process is remarkably adaptable in being able to accommodate the demand by consumers for new products.

Product characteristics: A variety of shapes, texture, color and appearances can be produced, which is not easily formed using other production method.

Energy efficient: Extruders operate at relatively low moisture while cooking food products, so less re-drying is required.

Low cost: Extrusion has lower processing cost than other cooking and forming processes. We can save 19% raw material, 14% labor, and 44% capital investment.

Less space: Extrusion processing need less space per unit of operation than other cooking system.

New foods: Extrusion can modify protein (vegetable and animal), starches (almost all sources), and other food material to produce a variety of new and unique snack food products.

High productivity and automated control: An extruder provides a continuous high-throughput processing and we can have a fully automated control for theses' extruders.

High product quality: Since extrusion is HTST heating process, it minimizes degradation of food nutrients, while improving the digestibility of proteins (by denaturing) and starches (by gelatinizing). Extrusion cooking at high temperature also destroys the anti-nutritional compound, i.e. trypsin inhibitors, and undesirable enzymes, such as lipases, lipoxidases and microorganisms.

No effluent: No or very few process effluents are produced.

EXTRUDER CAN MEET THE FOLLOWING LATEST FOOD TRENDS

Snack foods

Trends in the snack-food industry are numerous and ever-changing. The modern industrial snack was created in the early1940s with the manufacture of the first directly expanded snack from maize. In this process raw maize grits are fed into an extruder at low moisture to create a very hot melt within the barrel at temperatures of 140 to 180ºC. It was found that a snack product could be created by releasing a continuous stream of the hot melt fluid from a small hole. As the pressure is released the melt stream generates water vapor and expands in microseconds to form a foam, which can be cut into portions by a rotating knife. The ribbon of foam is cut into short lengths of highly expanded crispy snack known as corn curls or puffs.

Third-generation snack products or pellets are not new to the snack-food industry. In fact, they have been very popular in many regions of the world. Extrusion systems for the production of third-generation snacks are efficient, economical to run and result in a product with built-in marketing flexibility due to long shelf-life and high bulk density prior to frying or puffing.

Supercritical fluid injection, coupled with the continuous twin-screw extrusion cooking process, opens many opportunities for new engineered processing techniques for developing new products and product concepts. This supercritical fluid extrusion technology is a patented process that already has resulted in new developments in cereals, confectioneries, pastas, flavorings, pharmaceuticals, snacks and other products left only to the imagination.

Healthy snacks

An integral component in any snack line is an applicator/dryer. Originally designed for sugar-coating and frosting breakfast cereals, this system has gained significant value in the snack-food industry as a superior method of coating snack products with colors and flavors. Snack producers introducing low-fat snacks to the marketplace commonly include applicator/ dryer processing components. This equipment is used in conjunction with fat-free gums, which serve as the adhesion agent instead of fat, to greatly reduce the total caloric content from fat in the finished product. This technology makes it possible to add sweeteners and savory spices without adding fat.

Breakfast cereals

With increasing consciousness about healthy eating and changing lifestyles, cereals, especially extruded cereals, are becoming a standard feature in many households. "rice-crispies", "fruit flavored rings", "chocolate flavored cereal flakes" are some of the more commonly known extruded cereals.

Directly expanded extrusion-cooked breakfast cereals are prepared by extrusion technology as cereal flours and/or grits are cooked with other ingredients and with very low moisture content (usually below 20%). The process may use single- or twin-screw extruders, the configuration and operating characteristics of which generally lead to highly mechanical cooking. In pellet-to-flakes extrusion-cooked breakfast cereals preparation, cereal flours and/or grits are cooked with ingredients and at a moisture level in the range of 22–26%. They are usually processed in twin-screw extruders, the configuration and operating characteristics of which lead to a lower mechanical component of cooking, reinforcing the thermal component as opposed to the previous processing conditions.

Extrusion cooking has been sparked to a great extent by the perfection of twin screw extruders. This development redirected and brought under control the tremendous excesses in shear imparted to the grain formula during extrusion cooking in single screw extruder. The flexibility in set-up of the screw elements in a twin screw, along with greater flexibility in screw speed and heat input, have brought the extrusion cooking process under very exacting control.

Baby foods

Extrusion cooking is a new method for preparing baby foods. The type of extruders used, the particle size of the rice flour, the moisture content of the rice water mixture, and the extrusion conditions are some of the important factors influencing the properties of extruded rice baby foods.

Fortified ready to eat rice

Fortification of ready-to-eat rice with vitamins, minerals, and flavor compounds is now a very common practice. The usual approach is to add the minerals and more heat-stable vitamins such as niacin, riboflavin, and pyridoxine to the basic formula mix, extrude and then spray the more heat-labile vitamins such as vitamin A and thiamin on the product after extrusion before drying.

Extruded biscuits

Developments in extrusion cooking have led to some biscuit-like products or pieces. Where these are broad, flat and light in texture they offer interesting alternatives to plain crackers. Extrusion cooking is attractive to the manufacturer because of the relatively low capital cost of the plant and a great reduction in space required compared with conventional biscuit-making equipment. Where the appearance of the product is not critical the technique is particularly useful because difficulties of baking and drying are tackled in a more efficient manner.

Meat analog

An extrusion process utilizing one or two extruders in a serried can be employed to convert vegetable protein source directly into simplified verities of meat analogs. These meat analogs have remarkable similarity in appearance, texture and mouth feel to meat. Extrusion technology can form a fibrous matrix (analog) almost indistinguishable from meat and consumed as it is.

Meat extender

Meat extenders produced from the extrusion processing of defatted soy flour or flakes and soy concentrated, and they represent the largest portion of textured protein. Meat extenders are rehydrated to 60-65% moisture, blended with the meats or meat emulsions, to food product to a level of 20-30% protein.

Rice bran stabilization

Extruders are used to stabilize the rice bran just after milling and to reduce the FFA of rice bran oil. This stabilized rice bran can be used in human food and animal feed.

Ease in oil expelling

The extruder offers a convenient way of cooking the beans and breaking down the oil-bearing tissues in a fraction of the time required for conventional conditioning methods. The beans remain in the extruder system for less than 30 seconds at a temperature of approximately 135°C. The short cooking time at high temperature is adequate to satisfactorily destroy anti-nutritional agents such as the trypsin inhibitor and not so long as to damage important nutritional components such as protein.

Recycling food waste

Extrusion is used for the recycling of industrial and restaurant food wastes. The method of recycling waste food materials into useful byproducts comprising the steps of drying by dehydrating for a time interval necessary to reduce the moisture content to less than 25%, extruding food materials in an oxygen-free atmosphere at an elevated temperature level sufficient to sterilize food materials, cooling food materials, and thereafter tumbling and drying food materials to reduce said food materials to particle form.

Following are the areas of food industries where extrusion technology can be employed or is being used presently:-

Beverages powders
Boiled sweets
Breads (miscellaneous, expanded, dense)
Breading substitute
Candy sticks, caramel, chewing gum
Chocolates, cocoa and crump
Crisp bread
Confectionery
Cooked grains (barley, corn/sorghum, mixed)
Dairy products
Dried food mixes
Egg rolls
Fabricated potato chips
Flavoring, food additives
Frozen confectionery
Fudges
Full fat and partially de-fatted soy flour
Imitation nuts
Pasta products (noodles, spaghetti, macaroni)
Pastry dough
Precooked and modified starches
Pressed tablets, pretzels
Protein (textured and gluten)
Soup and gravy mixes
Sugar crust liqueurs, three dimensional confectionery and toffees

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Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

NUTRITIONAL TRENDS IN PAKISTAN WITH REFERENCE TO YOUNG CHILDREN

Riffat Aysha Anis[1], Faqir M. Anjum[2], Aurang Zeb[3], Pervaiz I Paracha[4]
[1]Nutrition Division, National Institute of Health Islamabad, [2]National Institute of Food Science and Technology, University of Agriculture, Faisalabad, [3]NIFA, Peshawar, [4] Faculty of Nutrition, NWFP Agriculture University, Peshawar

Abstract

Nutrition surveys have been conducted in Pakistan in the years 1970, 1977, 1988 and 2003 and young children (0-59 months) have been studied as a vulnerable group of population for malnutrition. The prevalence of chronic and acute malnutrition is indicated by stunting (low height-for-age) and wasting (low weight-for-height) respectively. The nutritional trends indicate that over the years, there has been a gradual decline in stunting 43.3 percent (1977) to 31 percent (2003) and a slight increase in wasting 8.6 percent (1977) to 11.6 percent (2003) in these children. Under nutrition is also indicated by low weight-for age and the percentage of underweight children has been decreased from 51.5 (1988) to 41.5 (2003). The decline in stunting and underweight and an almost negligible increase in wasting show an improvement in the overall nutritional status of the children in the past years. The percentage of anemia has been decreased from 65.2 (1988) to 50.9 (2003). Prevalence of goiter, as an indicator of iodine deficiency, was not estimated in children under 5 in any of the surveys. Vitamin A deficiency exhibits an increasing trend as apparent from the results of clinical and biochemical estimations. In 2003 survey, Zinc deficiency was assessed for the first time, which ranged from 26-39 percent. The trend of breast feeding has been decreasing continuously. The percentage of children given breast milk at the age of two years shows a sharp decline i.e. 60, 21 and 2.8 percent in 1970, 1977 and 1988 respectively. Pregnancy, which was one of the major reason of discontinuation of breast feeding in 1988 (34.3%) has been reduced considerably (13.4%) in 2003, thus indicating the effectiveness of family planning programs in the country. Infant feeding practices reveal that formula milk is preferred over buffalo milk in the urban areas, as compared to rural areas, where buffalo milk is given. Weaning is started after the age of three months and dalia, banana, khichri, roti, kheer, biscuits, halwa, rusk, choori, egg and sagoodana still continue to be the traditional weaning foods. Among childhood illnesses, incidence of diarrhea and acute respiratory tract infections (ARI) has decreased significantly; 79 (1970) to 28.3 percent (2003) and 79.5 (1970) to 3.05 percent (2003) respectively, over the past three decades. Comparison of all the malnutrition indicators was not possible as the same parameters were not measured alike in all the surveys. The nutritional trends in the surveys conducted so far show improvement in the nutritional status of young children, but still the situation is not satisfactory and needs improvement.

Key words: Nutrition, Children, Pakistan

INTRODUCTION

Four nutrition surveys have been conducted in Pakistan so far. These include West Pakistan Nutrition Survey (WPNS) 1970, Micro Nutrient Survey (MNS) 1977, National Nutrition Survey (NNS) 1988 and National Nutrition Survey 2003. The major nutritioinal problems in the country include low birth weight due to poor maternal nutrition and protein energy malnutritioin (PEM), and anemia across various groups of population and geographic areas. The entire population is at risk of one or other form of malnutritioin; young children, pregnant and lactating women are the most vulnerable. Despite an increase in per capita income, during the last two decades an increase in poverty has been observed and there has been increase in the prevalence of malnutrition as well.

To assess growth retardation among children under five years of age, anthropometric measurements were obtained which indicate stunting (low height-for-age), wasting (low weight-for-height) and underweight (low weight-for-age). Prevalence of stunting and wasting indicate chronic and acute malnutrition respectively. Clinical examination and biochemical estimations were made to assess the micronutrient deficiencies i.e. iron, iodine and vitamin A. Deficiencies of these micronutrients lead to anemia, goiter and night blindness respectively.

Breastfeeding and weaning practices information was collected, as discontinuation of breast feeding and improper weaning practices contribute to malnutrition. Data on diarrhea and acute respiratory tract infection (ARI) was obtained, these diseases also reciprocate malnutrition. This review has been done to know the changes, if any, in the nutritional status of young children over the years.

MATERIALS AND METHODS

The nutrition surveys conducted so far in the country have been reviewed and compared for malnutrition among young children. Trends have been established, where ever possible, to elucidate continuously changing parameters.

RESULTS AND DISCUSSION

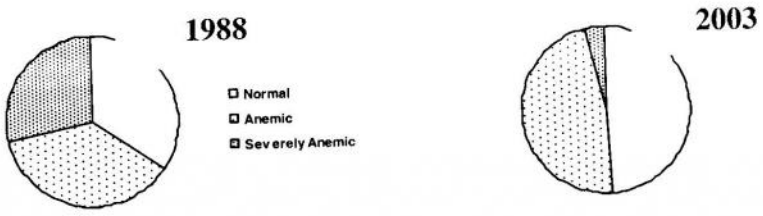
The anthropometric data is the first indicator of malnutrition in children. The statistics show that stunting has decreased from 43.3 percent in 1977 to 41.8 percent in 1988 and 31 percent in 2003. There is a slight increase in wasting which has raised from 8.6 percent (1977) to 11.6 percent (2003). The figures of stunting along with wasting were 7.2 percent in 1977 and 4.5 percent in 1988 which show a decline as well. The percentage of underweight children has also dropped from 51.5 in 1988 to 41.5 in 2003.

Table 1: Malnutrition in Young Children (Percentage) in 1977, 1988 and 2003

Parameter	1977	1988	2003
Stunting	43.3	41.8	31
Wasting	8.6	10.8	11.6
Stunting + Wasting	7.2	4.5	-
Underweight	-	51.5	41.5

Among the micronutrient deficiencies, iron deficiency is depicted by anemia. In 1988, 37 percent of the children were anemic and 28.2 percent were severely anemic while in 2003, 47.3 percent showed signs of anemia and only 3.6 percent had severe anemia. The percentage of normal children has been raised from 34.8 in 1988 to 49.1 in 2003.

Figure 1: Prevalence of Anemia in Children under 5 (Percentage) in 1988 and 2003



Vitamin A deficiency in young children as depicted by Bitot's spots has increased from 0.2 percent in 1988 to 1.2 percent in 2003. In the NNS 2003, various clinical symptoms of Xerophthalmia were spotted in the male and female children less than five years of age. Prevalence of Bitot's spots was 1.4 percent in male and 1.1 percent in female children. Corneal xerosis was 0.2 and 0.1 in male and female children respectively. Incidence of Keratomalcia was 0.1 percent in both sexes. 0.3 percent males and 0.2 percent females had corneal scars and the prevalence of Conjunctival xerosis was 1.0 and 0.8 percent in male and female children respectively.

The percentage of children being breastfed at the age of 21 months was 80 in 1970, 56 in 1977 and 34.8 in 1988. And the children being breastfed for two years or more were 60 percent in 1970, 21 percent in 1977 and 2.8 percent in 1988. Both these trends show a significant decline through the years. In the NNS-2003, the breastfeeding figures were taken only up to 13+ months and were split into "current" and "exclusive" breastfeeding. At the age of 13+ months, 60.7 percent children were breastfed out of which only 14.4 percent were exclusively breast fed.

Among the reasons of discontinuing breast milk, 34.5 percent cases in 1988 and 71.7 percent cases in 2003 were those where mothers thought that child was big enough to take breast feed. A highlight of the recent figures is that the occurrence of pregnancy, as a reason of discontinuing breastfeeding, has decreased from 34.3 percent in 1988 to 13.4 percent in 2003. The comparison of other causes is given in the table 2.

Table 2:Reasons for Discontinuing Breast Milk

Reason for Discontinuing Breast Milk	1988	2003
Child Big	34.5	71.7
Pregnancy	34.3	13.4
Insufficient Milk	19.3	7.6
Child Stopped Itself	4.6	2.1
Mother Working	0.8	0.3
Mother Sick	4.2	1.7
Child Sick	2.3	0.5
Other	0.0	0.8

Supplementary foods, which include dalia, banana, khichri, roti, kheer, biscuits, halwa, rusk, choori, egg and sagoodana, were mainly introduced at the age of 7-12 months according to the 1988 survey. In the nutrition survey of 2003, 54.7 percent children were given some solid supplementary food along with milk. Buffalo milk was given to 23.2 percent children, while liquids other than milk were given to 19.8 percent children. Formula milk and powdered milk were given as a supplementary food to only 1.6 and 0.7 percent children respectively. It is worth mentioning that the frequency of giving formula milk is more in urban areas while that of buffalo milk is more in rural areas.

The occurrence of diarrhea and ARI depict the overall health status of the young children. The less is the frequency of diarrhea and ARI, the better would be the nutritional status and vice versa. The figures illustrate a considerable decrease in the incidence of diarrhea and ARI over the years. In the surveys of 1970 and 2003, the percentage of children suffering from diarrhea and ARI has decreased from 79 to 28.3 and 79.5 to 3.05 respectively.

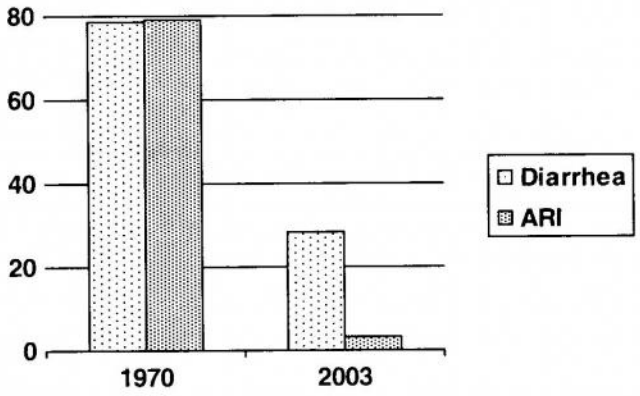


Figure 2: Percentage of children sufferir g from diarrhea and ARI in WPNS-1970 and NNS-2003

The dietary survey by 24-hours recall in children under five years of age was done in the NNS-2003. The consumption of wheat and rice was found to be 165 grams and 61 grams per child per day, respectively. The intake of eggs was 1.9 grams and that of meat was 31 grams per day. 231 grams of milk and milk products were taken per day by this group. At national level, the children <5 took 936 calories and 36 grams of protein per day.

The parameters used for the assessment of malnutrition were not measured alike in all the surveys. In the WPNS 1970, anthropometric measurements were not taken in the children under 5 years of age. Iron deficiency was also not assessed in the WPNS 1970 and MNS 1977. Vitamin A deficiency was measured in terms of only Bitot's spots in 1988 and in terms of various other clinical symptoms of Xerophthalmia in addition to Bitot's spots in NNS 2003. VAD was evaluated only in general population in 1970 and 1977. Iodine deficiency among children under five years of age has not been assessed in any of the surveys.

The prevalence and duration of breast feeding have declined in many parts of the world for a variety of social, economic and cultural reasons. Same is true for the situation in our country. From 1970 to 1988 the percentage of children being breastfed at the age of two years has decreased from 60 percent to 2.8 percent. However, this trend could not be followed further as in the NNS 2003, breastfeeding figures were taken only up to the age of 13 months.

Number of pregnancies, as a reason for discontinuing breastfeeding, has been significantly decreased during the last fifteen years. This highlights the effectiveness of the government's family planning programs in the country. Dietary intake (24 hours recall) of children <5 years of age was estimated only in the NNS-2003.

CONCLUSIONS

The over all nutritional status of the young children has been improved over the years, however, malnutrition still continues to be a major public health problem. The

interventions of vitamin A supplementation and distribution of ORS through national EPI program have reduced the incidence of childhood illnesses.

RECOMMENDATIONS

- Improvement in the socio-economic status of the population is necessary for achieving a better nutritional status.
- Priority should be given to food fortification programs so as to eradicate the micronutrient deficiencies.
- The health authorities should implement the health and social measures required to protect, promote and support breast feeding.

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Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

DIETARY HABITS OF ADOLESCENTS IN RELATION TO THEIR NUTRITIONAL STATUS

Asma Arshad , Riffat Ayesha Anis, Perveen Liaqat,
Syed Shahzad Shah and Hajra Ahmed
Department of Home & Health Sciences, Allama Iqbal Open University, Islamabad.

Abstract

Adolescence is an age group, which remains largely neglected, difficult to measure and hard to reach population. There are pressing research needs, notably to develop adolescent-specific anthropometric reference data, to better document adolescents' nutritional and micronutrient status. The objectives of this study were to assess the quality of adolescent's daily diet and their nutritional habits in relation to their nutritional status.413 students from 10 randomly selected (computer generated list) rural and urban public schools of Islamabad were recruited.. The tool was a study questionnaire that had anthropometric information, relevant clinical examination, and 24-hour recall method and food frequency questionnaire. The percentage of under-weight subjects was 33.7%. Under weights were found more in male subjects as compared to females (p-value<0.01). The percentage of pallor was significantly higher in females (p-value=<0.05). 6.7% had palpable goiter and 11.1% said that they had difficulty in seeing in the dark with more rural predisposition. The average caloric intake of girls was 2307kcal/day and protein intake was 51.3g/day. The average caloric intake of boys was 2434kcal/day and protein intake was 57.54g/day. Consumption of soft drinks, junk food deep fried foods was high among urban subjects and sweets and confectionaries was high among rural subjects. A majority of the population in Pakistan suffers from deficiency in nutritional intake but not enough research has been done in this area. Trends were similar in the present study.

Key words: Aldolescence, diet, nutritional status, Pakistan

INTRODUCTION

Assessment of the nutritional status comprises the state without assessing the process that lead to it. For example clinical examination and anthropometry may indicate that an individual is thin and under weight but may not give any indication about the reasons for his or her being in this state. It is widely accepted that for practical purposes anthropometry is the most useful tool to assess the nutritional status of children and adults alike. Nutrition for adolescents (teenagers) means giving them enough nutrients from age 12 to18 years of age. Teenagers will go through several growth spurts during this time. They will become taller and gain weight quickly. The amount of calories and protein that teenagers needs each day depends on their age and weight in kilograms. Divide teenagers' weight in pounds by 2.2 to figure out what they weigh in kilograms (kg). The calories and protein needed for growth are higher if the teenager is active in sports or fitness programs. (Nicholeand Kent 2004). Children and adolescent populations are very sensitive to inadequate nutrition.

Teenagers make many choices for themselves than they did as children. At the same time, social pressure thrusts choice at them. The consequences of these choices will influence their nutrition health both today and throughout life. Young girls are not fed according to their demand in the future (child bearing etc) due to social injustice prevailing in our society in particular (Irena *et al* 2000). The nutritional status of adolescents in often-measured in terms of weight-for height expressed as body mass index (BMI). The limited data available indicates that the average BMI among 11-18 years olds is considerably lower in developing world than in the industrialized countries. Often, adolescents' health and nutritional status is a direct reflection of the cumulative effects of childhood health nutrition. Adolescence is a time of rapid growth and change leading to an increased need for micronutrients such as iron. Physical changes including growth, the onset of menarche for girls and increases in the fat and muscle mass places extra nutritional requirements on adolescents. 25% of a person's height is achieved during adolescence, which typically marks the end of height growth. The growth depends on adequate nutrition, which is determined by availability of food of sufficient quality and quantity and the ability to digest, absorb and utilize food. Food availability is influenced by food practices, cultural traditions, family structure, birth intervals, and meal patterns, political environments and food allocation (WHO 2004). The main focus is developing countries, although this dichotomy of developed versus developing countries is becoming irrelevant with urbanisation and globalisation, particularly among adolescents. Those living in cities anywhere tend to have a common liking for fast food, and they increasingly have access to the same commercial outlets worldwide. Obesity among young people is a growing problem in most countries owing to eating patterns and sedentary lifestyles. Teen pregnancy is a problem anywhere. Furthermore, micronutrient intake inadequacies are not only to be found in developing country adolescent girls. Deficiencies or poor diets may be associated with poverty; they may also result from unhealthy eating behaviours, which are observed in well off and not so well off groups. Broadly speaking, adolescents' problems are malnutrition, micronutrient deficiencies, and nutrition-related chronic diseases.

The objective of the study was to assess the quality of adolescent's daily diet and their nutritional habits (number of meals, frequency of consuming foods) for which food frequency questionnaire is designed with some additional questions on habits. This research study will help find out the adolescent-specific nutritional problems, and highlight priority issues for the health sector, particularly in Pakistan.

MATERIALS AND METHODS

The research aimed at assessing the nutritional status of adolescents living in Islamabad (urban and rural). School children of 8^{th} to 10^{th} class in public schools were recruited. A structured questionnaire was developed, for assessing the nutrient intakes and dietary patterns (24-hour dietary recall and food frequency questionnaire). 413 students were recruited from public schools in Islamabad. Anthropometrics measurements and clinical signs of anemia, goiter, and vitamin A deficiency were observed in the interview.

During the planning stage, variables to be measured were selected and clarified on the basis of their relevance to the objectives of the study. In addition to variables with obvious relevance to the study objectives, other variables were also included; these included universal variables, such as age, sex. It included questionnaire, which had both open and close-ended questions. Questionnaire was adopted from the tool used in National Nutritional Survey 2001-02. Self-administered questionnaires were not used. A very extensive questionnaire was therefore developed which looked at a magnitude of demographic, socio-economic, therapeutic, lifestyle, clinical and other variables.

Form was developed to record the food consumed by the student during the previous day. Starting with 24-hour recall data on food consumption was collected by asking for food and drinks consumed by them during the previous day with details about ingredients and portion size. Standardized measures (utensils i.e. spoons, cups, plates and bowls) were used to assess the portion size of the food consumed by them. The energy and nutrient intakes of each subject were then calculated by using the "Food Composition Table for Pakistan", prepared by Department of Agricultural Chemistry and Human Nutrition, N.W.F.P Agricultural University, Peshawar (revised 1998). The average caloric intakes and protein intakes of the subjects were also compared with the Recommended Daily Allowance (RDA) of Pakistan (Liaquat 2000).

A Food Frequency Questionnaire (FFQ) carried out assessment of dietary pattern in this study. Though this subjective method is fraught with methodological problems including recall bias and under-reporting of certain types of food, it nevertheless, gives useful insight into the dietary pattern and was found to be useful for the Pakistani population for which, no such data existed.

Anthropometric measurements (human body measurements) are an objective technique for assessment. Weight (kg) and height (cm) of the respondents were measured by weighing machine and measuring tape respectively. The standing height (stature) was the distance from sole of feet to top of head and was measured with a vertical rod or measuring tape while the respondent was standing erect. Body Mass was calculated. BMI-for-age for ages 11 to 17 CDC growth chart was used for interpreting. For 18 years and above BMI international standards were used. For this purpose Grey scale was used.In general physical examination physical signs of nutrient deficiencies of iron iodine and vitamin A, were examined and noted in the questionnaire.

The habit of physical activity undertaken by the subject was assessed by questions that integrated daily physical activity, exercise, sports, worksite activity and personal chores. in questions relating to exercise, the respondent's type, level, frequency and duration of exercise was ascertained. Question regarding the number of hours spent in front of TV and computer was inquired in order to assess their sedentary habits.

Data collection and technique

Data were collected in the field visiting the specified schools. Approval of the school principals was obtained before the start of the study, and written; informed consent was taken from all the students through the tool of data collection itself. Principal investigator visited all the randomely-selected schools along with field officer who had previous experience of field surveys and was a public school teacher. Survey was done between April and mid June 2005.

Data entry and analysis

Data were entered and analyzed on SPSS 7.5 software package. Questionnaires were checked and coded, and analysis of data was carried out using *SPSS*. Missing values were allowed for in the analysis. Descriptive statistics was carried out where frequencies were calculated for all questions. Urban and rural comparison and gender comparison were done for different variables.

RESULTS AND DISCUSSION

Out of 413 subjects 48.2% were male and 51.8% were female students of public schools of Islamabad. The average income of the fathers of the subjects was 10970.06 RS. 54% of the rural and 27.5% of the urban mothers never went to school. The percentage of under-weight subjects was 33.7%, out of which 46.8% of the under weight subjects were children of illiterate mothers. 4.1% were over weight and 1.2% were obese. Under weights were found more in male subjects as compared to females (p-value <0.01).

Table 1 Comparison of rural and urban population

Category	Normal weight	Under weight	Over weight	Obese
Rural(%)	61(125)	31.7(65)	5.4(11)	2.0(4)
Urban(%)	61.1(127)	35.6(74)	2.9(6)	.5(10

p-value = 0.27

Gender comparison of weight on the basis of BMI was also done which is shown in table 2

Table 2 Comparison of gender population

Category	Normal weight	Under weight	Over weight	Obese
Male(%)	55.3(110)	40.7(81)	1.5(3)	2.5(5)
Female(%)	66.4(142)	27.1(58)	6.5(14)	None

P-value<0.01

Comparison of Girls of urban and rural areas was also done as shown in table 3

Table 3 Comparison of girls of urban and rural areas

Category	Normal weight	Under weight	Over weight	Obese
Urban (%)	61.3(68)	35.1(39)	3.6(4)	None
Rural (%)	71.8(74)	18.4(19)	9.7)	None

P-value<0.05

The percentage of pallor was found to be 40.2% in over all subjects. It was significantly higher in females (p-value=<0.05). Out of 413 subjects, 6.7% had palpable goiter while 0.6% had visible goiter. Among 413 subjects, 11.1% said that they had difficulty in seeing in the dark with more rural predisposition. The average caloric intake of girls was 2307kcal/day and protein intake was 51.3g/day. The average caloric intake of boys was 2434kcal/day and protein intake was 57.54g/day. Food frequency questionnaire revealed that daily consumption of eggs, milk, dairy products and yellow fruit consumption was high in urban subjects. Vegetables like carrot and sweet pumpkin was low in both groups. The daily consumption of vegetables was 75.5% in urban subjects and 75.1% in rural subjects. Consumption of soft drinks, junk food deep fried foods was high among urban subjects and consumption of sweets and confectionaries was high among rural subjects. Food preferences were more for chapatti (85%(, yellow fruit(84%), eggs(72.9%) soft drinks(69.2%), and poultry(68%) in all subjects . In homes of 33.2% of subjects, banaspati ghee was used as a cooking medium. 9% of the respondents were not involved in physical activities like walking etc. on a daily basis. An average screen hour for the subjects was two hours per day.

The age group of adolescents is neglected from research point of view in Pakistan. Not much work has been done for this particular age group especially from nutrition point of view. The nutrition surveys, which are conducted nation-wide like National nutrition surveys do not include this age group in particular for the surveys. Similarly a fundamental dilemma exists in trying to measure under nutrition in adolescents. This particular study has been carried out to observe the relation of dietary habits and their relation to nutritional status among adolescent.

In this particular study among 413 respondents, 48.2% were male and 51.8% were female students of public schools of Islamabad. The ages ranged from 12 to 19 years studying in class 8^{th} to 10^{th} of the recruited schools. 32.2% students were 15 years of age, 15.3% were 16 years of age and 13.1% were 13 years of age. It was made sure to take their exact ages as it was important for interpreting the BMI for every year on the basis of CDC international standards.

The household income, living standards and family compositions (larger or smaller families) does play a role in the nutritional well-being of the individuals of the family. Consumption of adequate quantity of food and affordability of quality food goods is affected by economic situation of the family. The average income of the fathers of the subjects was RS. 10970.06. 38% of fathers had income range of RS. 6000-10000 while 31.2% had income up to RS. 5000. 16.2% had income between RS. 11000 and 15000 RS. Only 3.1% had income RS. 26,000 and more.

Education levels of parents of the subjects as expected was better in urban areas as compared to rural areas of Islamabad. Females were less educated then males. Another striking finding was that 54% of the rural and 27.5% of the urban mothers never went to school. Education level and awareness level of mothers is important for the health of the children and family. The nutritional surveys done in the past revealed those children of mothers with better education and awareness level have better nutritional status. (National Nutrition Survey, 2001-2002). In this particular survey the percentage of un-weight subjects was 33.7%, out of which 46.8% of the under weight subjects were children of illiterate mothers.

Anthropometric measurements are extremely important to assess the nutritional status of the individuals. For this particular age group(adolescents) it may be more difficult because the anthropometric indices in normally nourished adolescents change in age and sexual development. Moreover, survey and reference population may differ in the age at which certain pubertal land marks are attained, requiring adjustment for difference between survey and reference population. Adolescent population may also differ by ethnicity in various body proportion that effect anthropometric indices. (Woodruff 2002)

For this particular study heights in cm and weights in kg were taken. The BMIs were calculated for each individual and the interpretation of weight (normal, under-weight, over weight and obese) was done by consulting the CDC standards for BMI for adolescents. 33.7% of the subjects were under weight. 4.1% were over weight and 1.2% was obese. The urban and rural difference on the basis of weight categories was not significant (p-value0.27). But under weights were found more in male subjects as compared to females (p-value<0.01). This can be explained by the fact that adolescents tend to eat selective food items and they may not be providing enough calories and quality nutrients.e.g, high intake of beverages among boys, which plays tend to make less consumption of other foods and drinks. Girls of the urban areas were more under weight then rural areas (p-value=<0.01). Meals skipping and unnecessary weight consciousness may play a part in this connection. Secondly the findings may be high because the appropriateness of using international reference data like CDC standards for assessing the growth of children and adolescents has been debated. Findings from studies in younger children indicate appropriateness of these standards but data from older children are lacking. (Hakeem *et al* 2004)

Clinical examination was done in order to assess the clinical sighs of anaemia(pallor), vitamin A deficiency(difficulty in seeing in dark and also iodine deficiency(goiter). Since clinical examination was not not complemented by biochemical analysis like haemoglobin levels, urinary iodine levels and serum retinal levels so the subjects with positive clinical findings for these deficiency disorders cannot be labeled as such. The percentage of pallor was found to be 40.2% in over all subjects. It was significantly higher in females (p-value=<0.05). These results are reflecting the over all scenario of anaemic women in countries. In order to halt the deadly consequences of anaemia in pregnant women in the form of high mortality rates this age group must be intervened for correction of anaemia.

The worm infestation among adolescents is also high so only nutritional deficiencies cannot be blamed for anaemia in adolescents.

Out of 413 subjects, 6.7% had palpable goiter while 0.6% had visible goiter. Prevalence of goiter is significantly high in rural subjects (p-value=<0.05) and found more in girls. Among 413 subjects, 11.1% said that they have difficulty in seeing in the dark with more rural predisposition. Prevalence was found high among male subjects of the study (p-value=<0.05). Accurate assessment of dietary intake in free-living human beings remains one of the most basic and fundamental unsolved problems in the study of nutrition. The choice of an appropriate method is critical in designing a nutritional study.

Reliability and validity of the intake measures need some attention. Free-living human beings almost never eat exactly the same meal twice, thee fore to measure the reliability of a measurement method may confound measurement error with variety in diet. This problem is acute in measure of single meals or 24-hrs intakes in which large amounts of within-subject variability may be seen. As for validity, it is often assumed that high correlations with other instruments designed to measure same the same variable indicate a valid instrument. Burke (1947) pioneered the development of methodology for assessing dietary intakes including 24-hour recall method.

A major challenge facing the nutritional epidemiologist is the correct measurement of dietary exposure. An apparently straightforward task is fraught with difficulties, and plagued by seemingly endless list of factors, which will introduce error in to the simplest measurement. If the aim is to measure current diet, the Heisenberg uncertainty principle rears its head: as you stop something to measure it, you change its behaviour. If the aim is to measure past dietary exposure, then one is reliant on the memory, conceptual abilities, and ruthless honesty on part of respondent. There are particular problems associated with the measurement of diet with in single populations where there is little dietary variation between individuals but large measurement error associated with each individual assessment. It would seem that no direct measure of what people eat would provide a true picture of their dietary habits.

Average caloric intake for both the subjects was 2369 kcal/day and protein intake was 45.27 g/day. For comparing the average caloric intakes and protein intakes with the Recommended Daily Intakes of Pakistani reference the average intakes of girls and boys were calculated separately. The average caloric intake of girls was 2307kcal/day and protein take was 51.3g/day. The average caloric intake of boys was 2434kcal/day and protein intake was 57.54g/day. The caloric intakes were almost equal to RDAs and protein intakes were somewhat high in both groups. This can be explained by the fact that major bulk of calories and protein comes from wheat flour in our setting. These figures reflect the trend seen in National Nutrition Survey 2001-2002.

Food frequency questionnaire revealed daily consumption of eggs, milk, dairy products and yellow fruit consumption is high in urban subjects. Vegetables like carrot and sweet pumpkin is low in both groups. The daily consumption of vegetables is 75.5% in urban subjects and 75.1% in rural subjects. It's a healthy trend but problems lying with cooking methods (over cooking and fat rich curries) cannot be over looked. Roti is the main staple food item. It is consumed on daily basis in almost all the subjects. The high consumption of soft drinks, junk food deep fried foods among urban subjects and sweets and confectionaries among rural subjects is a matter of concern.

Food preferences are more for chapatti (85%), yellow fruit (84%), eggs (72.9%) soft drinks (69.2%), and poultry (68%) in all subjects. Breakfast skipping is one of the bad habits of adolescents, which need attention. Among 413 subjects, 22% do not take break fast regularly and 17.2% take only two meals per day. Trends of going out for food are not very common in these subjects. 44% of the subjects go out for food only once in a

month. The trend of eating out with negative impact on health is more marked in adolescents of high affluent class. The use of banaspati ghee as a cooking medium in homes of 33.2% respondents is important from future point of view for the study subjects in terms of increase prevalence of cardiovascular diseases in our setting. 9% of the respondents are not involved in physical activities like walking etc. on a daily basis, while average screen hours for the subjects was 2 hours per day.

CONCLUSIONS

The available information from previous suggests that a majority of the population in Pakistan suffers from deficiency in nutritional intake. But not enough research has been done in this area. Iron deficiency and anemia are the main problem of adolescents; other micronutrient deficiencies may also affect adolescent girls. Trends were similar in the present study but for assessing the nutritional status especially in terms of micronutrient deficiencies, biochemical analysis was important which was not done due to cost constraints. The study did not represent the adolescents of whole Pakistan. There are pressing needs for research, for this age group. Research is required to develop adolescent-specific anthropometric reference data, to better document adolescents' nutritional and micronutrient status.

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Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

ESTIMATION OF GOITROGENOUS SUBSTANCES IN FOODS AND THEIR RELATIONSHIP WITH THE PREVALENCE OF GOITER IN PAKISTANI POPULATION

Ihsanullah, A. Zeb, *Riffat A. Anis and *Amjad Ali
Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar,
*National Institute for Health (NIH), Islamabad

Abstract

Tumorous overgrowth and inflammatory lesions are the most common types of thyroid gland disorders. It occurs endemically in certain geographic regions of Pakistan. Goitrogens are foods which suppress thyroid function. The present study was planned to identify and estimate the glucosinolate contents in edible portion of certain selected plant foods of Pakistani origin. Different teams visited Islamabad, Rawalpindi and different districts of NWFP. Samples of edible portions of selected plants were collected from different parts of NWFP and Islamabad/Rawalpindi, In addition to glucosinolate levels, these samples were also analyzed for other nutritional parameters e.g. moisture, ash, protein, fat, fibre and carbohydrates to see their concentration effects on glucosinolate levels. Data on prevalence of goiter prevalence from IRNUM, Peshawar and Institute of Nuclear Oncology and Radiotherapy (INOR), Abbottabad was collected. At INORE, various patients having goiter related problems were interviewed and asked questions about their food habits. The findings of the research would help the doctors to advise limiting the plant-oriented food items to the patients suffering from thyroid diseases.

Key words: Goiter, prevelance, Pakistani population

INTRODUCTION

Colloid goiter refers to an enlargement of part or all of the thyroid gland. It may be accompanied by altered production or release of the gland's principal hormones thyroxin and triiodothyrnine; or the lesion may be present without endocrine change (euthyroid). It occurs sporadically in certain individuals. The etiology of the disease is only partially known. Goitrogenous foods can further depress thyroidal function and stimulate the growth of the thyroid. The literature contains many reports on endemic goiter and suggestions that the condition may be caused by eating cruciferous plants. However, goiter in human beings caused by eating these plants has to be proved in Pakistan. Sulfur-containing phytochemicals of two different kinds are present in all *Brassica oleracea* (Cruciferae) vegetables (cabbage, broccoli, etc.). They are glucosinolates (previously called thioglucosides) and S-methyl cysteine sulfoxide. These compounds, which are derived in plant tissue by amino acid biosynthesis, show quite different toxicological effects and appear to possess anticarcinogenic properties. The majority of cultivated plants containing glucosinolates belong to the Cruciferae, although several other plant families produce glucosinolates (Ettlinger and Kjaer 1968; Kjaer 1973; Stoewsand 1999). Many of the crucifers that serve as sources of food and condiment belong to the genus Brassica. Examples of such foods are cabbage and related

vegetables, turnip, rutabaga and mustard greens. Condiment mustard seed is also a member of Brassica. Other genera of economic value include *Armoracia* (horseradish). *Eruca* and *Lepidium* (salad greens), and *Raphanus* (radish) (Kjaer 1966).

Glucosinolates were called thioglucosides in much of the literature before about 1970; many of the individual substances in this class are still known by such trivial name as sinigrin, sinalbin, and progoitrin (Waser and Watson 1963).Glucosinolates, commonly referred to as goitrogens, are uniform class of naturally occurring compounds found exclusively in the plant kingdom, and only in limited number of dicotyledonous families (Underhill 1980). All members of the cruciferae family, including rapeseed, contain glucosinolates (Fenwick and Curtis 1980). About 100 glucosinolates have been so far identified (Sörensen 1990, Fenwick and Heany 1983). Of the horticultural plants from Brassica, cabbage is the main crop. The genus Brassica includes plants that are used in many parts of the world as pasture, forage, or silage for livestock (Morrison 1959).

Although glucosinolates are present throughout the plant (root, stem, leaf, and seed), their highest concentration is usually in the seed. (Tookey and Wolff 1970). The basic structure for a glucosinolate was revised by Ettlinger and Lundeen (1956) and confirmed by the synthesis of benzyl-GS (glucotropaeolin). The glucosinolates are anions and occur in plants as salts. They are usually regarded as potassium salts, although the complex organic cation sinapine occurs widely among crucifers (Schultz and Gmelin 1953) and is the cation accompanying p-hdroxybenzyl-GS (sinalbin) as it is usually isolated (Kjaer 1960).

The present study was conducted to estimate the glucosinolate contents in edible portion of certain selected plant foods of Pakistani origin and data was collected on the prevalence of goiter in NWFP

MATERIALS AND METHODS

Samples

Samples of edible portions of cruciferous plants (generally vegetables) were collected from Islamabad, Rawalpindi, Abbottabad, Nathiagali, Mansehra, Murree, Swat, Karak and Bannu. The samples included cabbages, cauliflower, carrot, turnip, radish, pinenuts and peanuts. Seeds of rapeseed, carrot, radish, turnip, cabbage, cauliflower, pinenuts and peanuts were also collected. These were taken to the institute, cleaned, dried and stored in plastic jars for subsequent analysis.

Analysis

Proximate composition of the samples was determined by the standard methods (AOAC 1990; Ranganna 1978; James 1985) for the following parameters:

Moisture

Moisture content was determined by taking 5.0 g of well-mixed sample in a clean, dried and pre-weighed Petri dish. The dish was kept in an oven at 105°C till constant weight. After cooling in desicator and re-weighing, the moisture % was calculated as follow:

$$\text{Moisture content (\%)} = \frac{\text{Weight fresh sample} - \text{Weight dry sample}}{\text{Weight fresh sample}} \times 100$$

Ash

Dried sample 1g was taken in the pre-weighed crucible for the determination of ash. It was charred over a slow burning flame and then kept in muffle furnace at 550-600°C for two hours or till the appearance of a gray-white ash. The crucible was then transferred to a desiccator to cool. It was then weighed. The %-age of ash was calculated as follows:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Crude protein

Digestion: The sample was weighed (0.3 to 0.5g) on a filter paper and then put it in the digestion tube. Then 2-3 g of digestion mixture and 7 ml of concentration H_2SO_4 was added to each digestion tube. The samples were put in the Tecator digestion system (Kjeldtech digestion Block) at an initial temp. of 100^0C for about 1.5 hour. The temperature was raised to 150, 200, 250, 300 and 350 °C at regular intervals of 30-45 minutes until the digested samples became clear.

Distillation: For distillation 4% boric acid and 40% NaOH are used. The sample is automatically distilled after the addition of concentrated alkali solution (NaOH 40 %) to make the medium alkaline. The distillate was received in a conical flask containing 75 ml of 4% boric acid solution. The contents of this flask were titrated against standard H_2SO_4 solution i.e. (0.01 N), until the original color of boric acid reappeared.

$$\text{Nitrogen content of sample (\%)} = \frac{\text{Vol of acid used} \times \text{Normality of standard}}{\text{Wt of sample}} \times 0.014 \times 100$$

$$\text{Crude protein content (\%)} = \text{Nitrogen content} \times 6.25$$

Crude fat

Dry extraction method for fat determination was implied. It consisted of extracting dry sample with some organic solvent, since all the fat materials e.g. fats, phospholipids, sterols, fatty acids, carotenoids, pigments, chlorophyll etc. are extracted together therefore, the results are frequently referred to as crude fat. Fats were determined by intermittent Soxhlet extraction apparatus. Approximately 1g of moisture free sample was taken in fat-free thimble and fat was extracted repeatedly with anhydrous petroleum ether (40-60°C). The ether was distilled by rotary evaporator and the fat dried in an oven at 70°C. Percentage of fat in the sample was calculated as under:

$$\text{\% Crude fat} = \frac{\text{Wt of fat}}{\text{Wt of sample}} \times 100$$

Crude fiber

A moisture free and ether extracted sample of crude fiber made of cellulose was first digested with dilute H_2SO_4 and then with dilute NaOH solution. The undigested residue collected after digestion was ignited and loss in weight after ignition was registered as crude fiber.

After crude fat determination the sample was transferred to a dry 1000 ml conical flask, 200 ml of diluted hot sulphuric acid was added and brought to boiling with in one minute. Boiling was carried out under reflux condenser for exactly 30 minutes, rotating the flask every few minutes in order to mix the contents and remove particles from the sides. It was then allowed to stand for one minute.

The content was immediately transferred to a benched funnel attached to the suction assembly with a filter paper. The suction was adjusted so as to complete 200 ml filtration with in 10 minutes. The insoluble solids were washed with hot water until they were free from acid.

The sample was transferred back to 1000 ml conical flask, using 200 ml of 0.313 N NaOH from a wash bottle. It was boiled for 30 minutes with the save precautions, then filtered and wash with distilled water and then with 1% HCl. The insoluble material was dried

in an oven and weighed. It was ashed in a muffle furnace at a dull red heat. The loss in weight after ashing was the amount of crude fiber in the sample.

$$\text{Crude fiber \%} = \frac{\text{Loss in weight on ignition - f}}{\text{Sample weight}}$$

Nitrogen free extract

Nitrogen free extract (N.F.E), which represents the digestible carbohydrates, was calculated by difference. This value was obtained by subtracting the sum of the percentages of moisture, crude protein, crude fat, crude fiber and ash from 100.

% NFE = 100- (%moisture + % crude protein + % crude fat + % crude fiber + % ash).

Glucosinolates

There are over 90 different glucosinolares but they share a common backbone structure:

$R\text{-}C{=}SO_4\text{-}K^*$ Where R = variable side chain

This means that they all have a glucose moiety. This method takes advantage of this fact to determine glucosinolate content by measuring the glucose released by endogenous myosinase (thioglucosidase) activity. Lmyosinase is ubiquitous in the plant family cruciferae and it has a broad specificity for glucosinolates. It is normally compartmintalized away from the glucosinolates in the seed but when the seed is crushed the membranes are broken and it attacks them. Sodium ascorbate is used in the assay to enhance myosinase activity. After release from the glucosinolates by myosinase activity, the glucose is measured colormetrically after oxidation with glucose oxidase. Glucose oxidase is specific for glucose, eliminating possible interference by other sugars and it releases hydrogen peroxide which is reduced by horseradish peroxidase affecting the oxidation of the chromogen, 4-aminoantipyrine and phenol to a red colored compound which is measured by the spectrophtometer at 510 nanometers. Charcoal is used in the assay to remove interferences before the colour reaction, including sodium ascorbate which can prevent colour development by acting as a non – chromogenic oxygen acceptor. This procedure determines the total glucosinolate contents as sinigrine equvilants in µmol/g (Olsen and Sörensen 1981).

Procedure

1. Place 200 mg sample into 7 mL plastic scintillation vials containing the small size steel rods. Use scoop provided for this. It is the cap of a 2 mL glass vial. Use foam racks to hold vials. In every rack of 50 include a low glucosinolate check and a high glucosinolate check. For best accuracy it is necessary to the sample to ± 5 mg. Record
2. Add 3 mL the of myosinase activation buffer to the vials. Cap vials and shake for 20 minutes in Eberback shaker.
3. Uncap vials and add 1 mL charcoal suspension. Use Eppendorf repeater with 12.5 ml tip. Keep charcoal suspension continuously stirred on magnetic stirrer while pipetting.
4. Centrifuge vials at 4000 rpm for 10 minutes.
5. Sample 40 uL from each vial and put into semi – micro cuvettes. Also prepare a calibration set of cuvettes with the glucose standards, sampling 40 µL from each standard solution. Make a separate calibration set for each 100 unknowns. Also make water blank, sampling 40 µl of distilled water.
6. Add 1.0 ml colour reagent to cuvettes. Use a 30-minute delay between each 100 unknowns and they respective calibration set. Let reactants incubate at room temperature for > 45 minutes.

7. Measure absorbance in at 510 nm against the water bland. Measure the unknowns along with their respective calibration sets and keep track of which calibration set is for which unknowns.

Calculations

Construct a calibration curve from the standards. The 8 mM glucose solution is equivalent to 160 µmole glucosinolates per gm sample for 200 mg samples. Estimate the glucosinolate content of the unknowns from the standard curve. Check the glucosinolate content values observed from the high and low check samples and correct the unknown values as necessary.

Statistical analysis

Data were analyzed statistically by measuring average and standard deviation, minimum, maximum, range and coefficient of variability for the analyzed parameters (SPSS 1999).

Collection of data on prevalence Goiter

Data on the prevalence of goiter in NWFP was collected from Institute of Radiotherapy and Nuclear Medicine IRNM, Peshawar and Institute of Nuclear Oncology and Radiotherapy (INOR), Abbottabad.

RESULTS AND DISCUSSION

A. Biochemical Analysis

Dietary iodine deficiency is of great importance but is probably not the sole cause of the endemic form and probably less important in the sporadic form. Certain families in highly inbred populations have been found to have defective synthesis of thyroid hormone (Schone et al 1990). The present study was planned to identify and estimate the glucosinolate contents in edible portion of certain selected plant foods of Pakistani origin. Other nutritional parameters e.g. moisture, ash, protein, fat, fibre and carbohydrates (proximate composition) were also studied. Botanical names and edible portion of common cultivated vegetables and fruit plants are listed Table 1.

Table 1. Botanical names and edible portion of common cultivated cruciferous plants

Common Name	Botanical Name	Edible Portion
Turnip	Brassica rapa.	Root, leaf
Cabbage	Brassica oleracea L.	Leaf (Head)
Cauliflower	-do-	Leaf, flower bud or stem
Radish	Raphanus sativus L.	Root
Mustard (Brown or Indian)	Brassica juncea L.	Seed
Mustard (Black)	Brassica nigra L.	Seed
Feed (rape, turnip rape, polish rape, navette, rubsen)	Brassica campestris	Seed meal

Data on the proximate composition and glucosinolate contents of the food samples collected from various locations are presented in Tables 2-4. Table 5 shows a summery of the glucosinolate contents of all the analyzed plant food samples. The largest and the most commonly consumed group of edible plants within the family Cruciferae are the vegetables of Brassica genus. On the basis of glucosinolate levels, we have categorized various studied cruciferous plants into high, medium and low cyanogenic foods as shown below:

Category 1 (< 10 µmole/g): Groundnut, pinenut, radish, turnip, carrot, cabbage seeds.

Category 2 (10-50 µmole/g): Cabbage, cauliflower

Category 3 (> 50 µmole/g): Rapeseed and mustard seeds and plants

The variability in the contents of glucosinolates of various cruciferous plant food samples was fairly high and ranged from 77 to 205% among various locations indicating highly varied distribution of these anti-nutritional compounds in nature. Broadly speaking groundnut, pine nut, radish, turnip carrot and cabbage seed fall in the glucosinolate range of below 10 µmol/g, cabbage and cauliflower contain the intermediate amounts (10-50µmol/g) while rapeseed and mustard seeds and plant parts are high in glucosinolate contents (>50umol/g). Inter location variability was minimum in case of radish (CV%=3.0%), followed by turnip (CV%=4.05%) while the highest variability was noted in the case of cauliflower (CV%= 63.73%). These varied degrees of variability in the glucosinolate contents in a plant are indicative of environmental as well as genotypic influences.

The values reported in Tables 2-4 for the glucosinolate contents of various plant food samples fall well in the reported values for these samples. The variation in the glucosinolate contents of a single food from various locations and also the variability among different food samples collected from a single location is understandable. These variations represent true variation due to the measurement of different cultivars of particular vegetables and different growing conditions such as soil, climate and cultivation practices (McNaughton and Marks 2003).

In view of an almost completely lacking food composition tables for glucosinolate contents epidemiological studies on the intake of these compounds become difficult. This was the first attempt to analyze and report the glucosinolate contents of different vegetables in order to provide basis for any such large level study and for conducting epidemiological studies on Pakistani population for the consumption of goitrogenic substances in their foods. Samples collected from market in one geographical location may represent wide regions (McNaughton and Marks 2003). However a much large scale study covering the entire country divided into zones as well as the cooking and consumption pattern of these vegetables may yield more applicable data.

B. Goiter Prevalance

Data on the prevalence of goiter and Cancerous (Ca) thyroid patients in NWFP was collected from IRNM, Peshawar and given in Tables 6-7. Data collected from INOR Hospital Abbottabad are presented in Table 8. At IRNUM Peshawar a total 5081 goiter patients were registered from various divisions of NWFP, FATA and Afghanistan during 2004. The total number of Ca thyroid patients was 43, with female dominating in number (28) than males (15). The distribution of Ca thyroid cases was almost evenly distributed across the various geographical locations of the province (Table 6). As far the distribution of Ca thyroid cases across age groups, the cases tended to be more in the upper age groups than in the young age (Table 7). During the year 2005 a total of 5039 goiter patients were registered at IRNUM Peshawar. The Ca thyroid cases were 38. Again the number of females dominated (24) than the males (14). The geographical distribution again was almost even, although the plain areas of Peshawar, Charsadda and Swat had relatively high number of cases. Age wise distribution did now show a trend, although the highest number of cases (n = 5) was recorded in the age group of 65.

Data available from the Institute of Nuclear Oncology and Radiotherapy (INOR), Abbottabad, on the prevalence of goiter cases was related to 2005 (January to December) and 2006 (January- August). During 2005 a total of 1140 thyroid patients were registered with majority coming from the Districts of Abbottabad, (n =510), Mansehra (n =303) and Haripur (n =123). During the year 2006, a total of 981 thyroid patients got registered. Abbottabad had the highest number of patients (n =606) followed by Balakot (n =180).

Table 2 . Proximate composition and glucosinolate (GS) contents in samples of vegetables/fruits collected from Abbottabad, Nathiagally and Mansehra

Sample Name	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	NFE (%)	GS µmole/g
Abbottabad							
Groundnut	5.1	2.5	37.2	38.1	1.7	15.4	2.04
Peanut	4.5	2.3	21.5	46.5	0.9	24.3	1.94
Radish	86.2	0.9	1.02	0.12	0.8	10.9	5.45
Carrot	78.1	1.2	1.13	0.19	1.02	18.4	4.28
Turnip	90.2	0.93	1.03	0.18	0.95	6.7	5.60
Rapeseed (Seed)	5.9	0.9	22.7	31.6	6.9	32.0	74.00
Radish (Seed)	5.4	1.0	2.1	3.2	1.2	87.1	3.90
Turnip (Seed)	4.6	4.0	3.5	2.1	1.1	84.7	4.95
Cabbage (Seed)	3.9	1.8	2.0	1.5	1.2	89.6	3.18
Cauliflower (Seed)	3.4	1.7	1.7	1.3	1.1	90.8	2.90
Mean	**28.73**	**1.72**	**9.39**	**12.5**	**1.69**	**46.0**	**10.82**
St. Dev	**38.83**	**0.99**	**12.94**	**18.5**	**1.85**	**36.9**	**22.24**
CV %	**135.16**	**57.74**	**137.83**	**148.1**	**109.54**	**80.2**	**205.43**
Nathiagali							
Carrot	78.5	0.9	1.02	0.2	1.02	18.4	4.36
Cabbage	81.9	0.79	1.03	0.22	1.15	8.9	16.95
Cabbage seeds	3.9	1.1	1.7	1.2	1.00	91.1	4.0
Mean	**54.77**	**0.93**	**1.25**	**0.54**	**1.06**	**39.5**	**8.44**
St. Dev	**44.08**	**0.16**	**0.39**	**0.57**	**0.08**	**45.0**	**7.37**
CV %	**80.50**	**16.90**	**31.18**	**105.86**	**7.71**	**114**	**87.42**
Mansehra							
Groundnut	5.2	2.6	37.2	39.2	1.5	11.1	2.50
Peanut	4.7	2.1	20.8	46.7	1.2	14.2	2.85
Cauliflower	83.2	1.1	3.2	0.19	1.2	24.5	18.80
Radish	86.7	0.8	1.02	0.13	0.9	10.5	5.20
Cabbage	88.2	0.75	1.04	0.2	1.1	8.7	19.80
Mean	**53.60**	**1.47**	**12.65**	**17.28**	**1.18**	**13.8**	**9.83**
St. Dev	**44.45**	**0.83**	**16.03**	**23.58**	**0.22**	**6.30**	**8.71**
CV %	**82.93**	**56.69**	**126.74**	**136.42**	**18.37**	**45.7**	**88.65**

Table 3. Proximate composition and glucosinolate (GS) contents in samples of vegetables/fruits collected from Murree, Karak and Kohat

Sample Name	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	NFE (%)	GS µmole/g
Murree							
Groundnut	4.97	2.1	38.2	39.4	1.2	14.1	2.46
Peanut	4.5	2.2	21.2	47.2	0.9	24.0	2.45
Cabbage	85.1	0.9	2.7	0.2	1.4	9.7	21.60
Radish	86.2	0.9	0.9	0.12	1.2	10.7	5.50
Turnip	92.1	0.87	1.2	0.17	1.1	4.6	5.46
Carrot	78.3	1.25	1.14	0.196	1.07	18.6	4.48
Mean	**58.53**	**1.37**	**10.89**	**14.55**	**1.15**	**13.6**	**6.99**
St. Dev	**41.90**	**0.62**	**15.54**	**22.41**	**0.17**	**6.90**	**7.29**
CV %	**71.59**	**45.33**	**142.73**	**154.03**	**14.55**	**50. 7**	**104.21**
Karak							
Cabbage	81.2	1.03	3.2	0.2	1.31	13.1	19.80
Carrot	75.2	1.2	1.32	0.2	1.2	20.8	4.95
Turnip	85.1	0.98	1.02	0.19	1.1	11.6	5.36
Rapeseed local	5.9	1.0	23.8	31.8	7.1	30.4	72.8
Rapeseed Desi	6.0	1.2	24.6	30.9	7.2	30.1	7.2
Rapeseed oil	1.3	0.1	10.5	86.9	0.1	1.1	33.60
Mean	**42.45**	**0.92**	**10.74**	**25.03**	**3.00**	**17.8**	**23.95**
St. Dev	**41.84**	**0.41**	**10.98**	**33.94**	**3.24**	**11.5**	**26.40**
CV %	**98.55**	**44.93**	**102.23**	**135.57**	**108.02**	**64.3**	**110.21**
Kohat							
Cabbage	80.1	1.1	2.9	0.21	1.2	14.5	18.00
Carrot	75.1	1.23	1.23	0.2	0.95	21.3	5.10
Turnip	84.2	1.2	0.9	0.2	1.1	12.4	4.95
Mean	**79.80**	**1.18**	**1.68**	**0.20**	**1.08**	**16.1**	**9.35**
St. Dev	**4.56**	**0.07**	**1.07**	**0.01**	**0.13**	**4.65**	**7.49**
CV %	**5.71**	**5.78**	**63.95**	**2.84**	**11.62**	**29.0**	**80.12**

Table 4. Proximate composition and glucosinolate (GS) contents in samples of vegetables/fruits collected from Islamabad, Rawalpindi and Swat

Sample Name	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	NFE (%)	GS µmole/g
Islamabad							
Groundnut	4.7	2.7	38.0	38.4	1.6	14.6	2.00
Peanut	4.9	2.1	22.2	45.9	1.1	23.8	-
Cabbage	84.2	0.9	2.7	0.17	1.4	10.6	19.94
Carrot	75.1	1.7	1.1	0.2	1.1	20.8	5.26
Rapeseed-i	6.1	1.1	24.3	33.1	7.4	28.0	72.5
Rapeseed-ii	6.4	1.2	24.7	32.9	7.2	27.6	75.0
Turnip	89.1	1.3	1.2	0.19	1.2	7.01	5.20
Cauliflower	82.5	1.2	2.8	0.20	1.3	13.5	17.90
Mean	**44.13**	**1.53**	**14.63**	**18.88**	**2.79**	**18.2**	**28.26**
St. Dev	**41.44**	**0.61**	**14.36**	**20.38**	**2.79**	**7.93**	**31.80**
CV%	**93.92**	**39.77**	**98.21**	**107.9**	**100.1**	**43.5**	**112.55**
Rawalpindi							
Peanut-i	4.6	2.8	37.3	35.9	1.3	18.1	2.46
Peanut	4.3	2.1	21.9	47.1	0.9	23.7	2.70
Cabbage	85.2	1.2	1.9	0.2	1.2	10.3	19.88
Carrot	78.2	1.6	1.3	0.19	1.2	17.5	5.60
Turnip	87.8	1.1	0.9	0.2	0.9	9.1	5.50
Peanut-ii	5.1	3.5	28.5	56.1	1.2	-	2.48
Rapeseed-i	5.9	1.1	24.6	32.80	7.3	28.3	74.6
Rapeseed-ii	5.8	1.2	24.2	33.00	7.2	28.6	74.8
Mean	**34.61**	**1.83**	**17.58**	**25.69**	**2.65**	**19.4**	**23.50**
St. Dev	**40.77**	**0.90**	**14.19**	**22.49**	**2.84**	**7.92**	**32.11**
CV %	**117.78**	**49.51**	**80.73**	**87.56**	**107.28**	**40. 9**	**136.63**
Swat							
Ground nuts	5.1	3.4	29.2	53.0	0.9	8.4	3.1
Cauliflower	82.0	0.82	2.5	0.2	1.0	13.5	18.2
Radish	92.8	0.8	0.92	0.1	0.7	5.4	5.2
Soybean	9.8	4.6	32.0	16.3	3.90	33. 7	9.6
Cabbage	90.0	0.7	1.28	0.2	0.8	7.02	18.96
Pine nut	5.2	3.5	28.6	56.0	1.0	5.7	2.45
Mean	**47.48**	**2.30**	**15.75**	**20.97**	**1.38**	**12.3**	**9.59**
St. Dev	**44.85**	**1.73**	**15.59**	**26.73**	**1.24**	**10.9**	**7.41**
CV %	**94.45**	**75.05**	**98.97**	**127.50**	**89.52**	**88.6**	**77.27**

Table 5. Gluconinolate contents (µmol/g) of cruciferous food samples from various locations

Location	Ground nuts	Pine nut	Radish	Carrot	Turnip	Cabbage	Cauli-flower	Rape-seed	Seeds
Abbottabad	2.04	1.94	5.45	4.28	5.6	16.95	18.2	72.50	3.90
Kohat	2.50	2.45	5.20	4.36	5.46	18.00	18.80	75.00	4.95
Karak	2.46		5.50	4.48	4.95	19.80	17.90	72.80	2.90
Nathiagally	2.00	2.70	5.20	5.10	5.50	19.80	-	7.20	3.18
Mansehra	2.46	2.85	-	4.95	5.40	19.94	-	74.60	4.00
Murree	2.48	2.45	-	5.26	5.36	19.88	-	74.80	-
Islamabad	3.10	-	-	5.60	5.20	18.96	-	74.00	-
Rawalpindi	2.56	-	-	4.50		19.88	-	46.90	-
Swat	-	-	-	-	-	21.60	-	72.50	-
Mean	**2.45**	**2.48**	**5.34**	**4.82**	**5.35**	**19.42**	**12.61**	**63.37**	**3.79**
Minimum	**2.00**	**1.94**	**5.20**	**4.28**	**4.95**	**16.95**	**1.33**	**7.20**	**2.90**
Maximum	**3.10**	**2.85**	**5.50**	**5.60**	**5.60**	**21.60**	**18.80**	**75.00**	**4.95**
Range	**1.10**	**0.91**	**0.30**	**1.32**	**0.65**	**4.65**	**17.47**	**67.80**	**2.05**
St. Dev	**0.34**	**0.35**	**0.16**	**0.48**	**0.22**	**1.33**	**8.04**	**22.88**	**0.80**
CV %	**13.87**	**13.96**	**3.00**	**9.98**	**4.05**	**6.83**	**63.73**	**36.10**	**21.15**

- = Not analyzed

Table 6. District-wise distribution of cancerous thyroid cases registered at IRNUM Peshawar

District	Female	Male	District	Female	Male
2004			**2005**		
Bunir	0	1	Bunir	0	1
Bannu	2	0	Bannu	1	0
Bajor	2	1	Bajor Agency	2	1
Charsadda	1	1	Charsadda	1	2
Dir	1	0	Chitral	0	1
Karak	1	0	Karak	1	1
Kohat	1	1	Khyber Agency	1	0
Kurrum Agency	1	1	Kohat	2	2
Mardan	1	1	Kurrum Agency	1	1
Nowshera	2	1	Mardan	0	1
Peshawar	3	2	Mohmand Agency	1	0
Swat	3	1	Nowshera	1	0
Swabi	3	0	North Wiziristan Agency	0	1
S. Waziristan Agency	0	1	Peshawar	3	2
Tank	1	0	Swat	4	0
Afghanistan	6	4	Aurakzai Agency	1	0
			Afghanistan	5	1
Total	**28**	**15**	**Total**	**24**	**14**

Table 7. Age-wise distribution of thyroid patients registered at IRNUM Peshawar

Age	Female	Male	Age	Female	Male
2004			**2005**		
20	0	1	11	1	0
24	0	1	20	1	0
25	0	1	21	1	0
28	0	1	22	1	0
30	1	0	25	2	0
33	1	0	26	0	1
40	0	1	30	0	1
45	4	0	35	2	0
48	0	1	36	0	1
50	5	2	40	2	1
52	2	0	45	0	1
54	1	0	50	2	2
55	3	0	55	1	0
56	1	0	58	1	0
60	4	2	59	0	1
62	1	0	60	2	0
63	1	0	65	3	2
65	3	3	70	2	2
70	1	1	75	1	0
75	0	1	80	1	0
			85	0	2
			100	1	0
Total:	**28**	**15**	**Total:**	**24**	**14**

Table 8. Number of thyroid patients registered at INOR, Abbottabad

S. No.	District	No. of person	*No. of person*
		January – December 2005	January – August 2006
1	Abbottabad	510	606
2	Mansehra	303	065
3	Muzafarabad/Ghari Habibullah / Gilgit	040	030
4	Ellai / Battagram	044	025
5	Kohistan	025	058
6	Balakot/Kaghan	010	180
7	Haripur	123	002
8	Charsadda	002	004
9	Peshawar	005	003
10	Rawalpindi/Wah Cantt/Taxila	007	003
11	Malakand	002	003
12	Dera Ismail Khan	004	002
	Total Thyroid Patients	**1140**	**981**

The majority of cases belonged to the lower districts of Hazara Division, partly because of the proximity with the hospital. However other reasons related to the geography, lifestyle and nutrition can not be totally excluded and need to be studied in detail. Dependence on vegetable diets (cabbage, cauliflower, rapeseed/mustard, turnip and radish) mainly because of non affordability of meat by majority of citizens in Northern areas, leads to the high prevalence of goiter problem in the population. In short, consumption of cyanogenic foods has been considered as one of the etiological factors in certain instances for the persistence of endemic goiter.

CONCLUSIONS

- Goitrogens are foods that suppress thyroid function with the likelihood of causing goiter disease. Very limited data on this subject is available in worldwide reports. Therefore, PAEC's Nuclear Institute for Food and Agriculture (NIFA) initiated R&D on a new project in collaboration with National Institute of Health (NIH) which aims to estimate goitrogenous substances in foods and the prevalence of goiter in Pakistani population.
- Groundnut, peanut, radish, turnip carrot and cabbage seed were classified as low glucosinolate category ranging below 10 µmol/g,
- cabbage and cauliflower contain the intermediate amounts (10-50µmol/g)
- Rapeseed and mustard seeds and plant parts are high in glucosinolate contents (>50umol/g).
- There was high variability in the contents of glucosinolates of various cruciferous plant food samples, with CV% ranging from 77 to 205% among various locations
- Inter location variability was minimum in case of radish (CV=3.0%), followed by turnip (CV=4.05%) while the highest variability was noted in the case of cauliflower (CV= 63.73%).
- Prevalence of goiter was gauged from the goiter patients registered at two cancer hospitals.
- The number of patients registered at these hospitals was very high, but give only the clinical situations. Such cases are normally only the tip of the iceberg, with a presumably vary large prevalence of the sub-clinical cases. These sub-clinical

cases never reach the hospitals but are responsible for much of the un-reported physiological damage within the population.

- Excessive use of the cruciferous vegetables and fruits like cabbage, cauliflower, turnip, rapeseed, radish, pinenuts, peanuts, is undesirable in individuals having tendency to goiter.
- The data will help in establishing a database of goitrogenous substances in Pakistani foods. It may subsequently help in epidemiological surveys on the intake levels of these substances by the various population groups in the country. The relative contribution of these substances towards the etiology of goiter can then be easily worked out.
- The findings of the research and the methodology established for the analysis will also help the breeders working on cruciferous plants to develop varieties with lower levels of these toxic substances.
- In view of the variation of glucosinolate content from plant to plant, in different parts of the plant, and due to genotypic and locational variation, new vistas of R & D in the field were opened in the fields of biochemical analysis, nutrition, epidemiology and plant breeding.

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Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

NUTRITION AND FOOD SAFETY – ITS IMPORTANCE AND APPLICATION IN PAKISTAN FOOD INDUSTRY

Aijaz Sheikh
Head of Development & Quality, Unilever Pakistan Limited-Avari Plaza,
Fatima Jinnah Road, Karachi – 75530 Pakistan

Abstract

Food safety is a scientific discipline describing the handling, preparation, and storage of food in ways that prevent food borne illnesses which encompasses absence or acceptable and safe levels of contaminants, adulterants, naturally occurring toxins or any other substance that may make food injurious to health on an acute or chronic basis. "We are what we eat" is an old proverb. Our nutritional status, health, physical and mental faculties depend on the food we eat. Access to good quality food has been man's main endeavour from the earliest days of human existence. Food quality can be considered as a complex characteristic of food that determines its value or acceptability to consumers. Besides safety, quality attributes which include nutritional value; organoleptic properties such as appearance, color, texture, taste; and functional properties cannot be neglected. Food systems in developing countries are not always as well organized and developed as in the industrialized world. Moreover, problems of growing population, urbanization, lack of resources to deal with pre- and post- harvest losses in food, and problems of environmental and food hygiene mean that food systems in developing countries continue to be stressed, adversely affecting quality and safety of food supplies. People in developing countries are therefore exposed to a wide range of potential food quality and safety risks. This paper discusses the special problems of food quality and safety in developing countries as well as their impact on food security and presents ways and means of dealing with these problems. The paper also includes the existing scenario in Pakistan regarding food safety, implementation of standards in industry and contribution of Pakistan's legislative bodies. How strictly the food industries here are following good manufacturing practices and incorporating food safety in their manufacturing and distribution chains to provide the quality that consumers deserve. Food safety challenges and their impact on industry and consumers will also be touched upon. The paper also includes the, conclusions and recommendations including utilization of appropriate equipment and processes are employed to ensure delivery of 'safe' food to our consumers. Unilever Pakistan Limited's role so far and the future expectations from it shall also be covered. What will be the implications of the paper and how it is going to change the world and to have a significant 'win'. How taking it all further by making sure that the food laws, new technologies and changing needs of the consumer make all the difference in regards to nutrition and food safety.

Key words: Nutrition, safety, food industry, Pakistan

INTRODUCTION

Pakistan is the most populated country in the WHO Eastern Mediterranean Region, accounting for 30% of the regional population. It has an estimated population of over 169

million inhabitants in 2007 (Source: www.wikipedia.com Demography of Pakistan). During 1951-98, Pakistan's urban population expanded sevenfold and by the next decade the population is expected to exceed 176 million. Dramatic social changes have led to rapid urbanization and the emergence of mega cities. During 1990-2003, Pakistan sustained its historical lead as the most urbanized nation in South Asia, with city dwellers making up 34% of its population. Pakistan has a multi-cultural and multi-ethnical society and hosts one of the largest refugee population in the world as well as a young population.

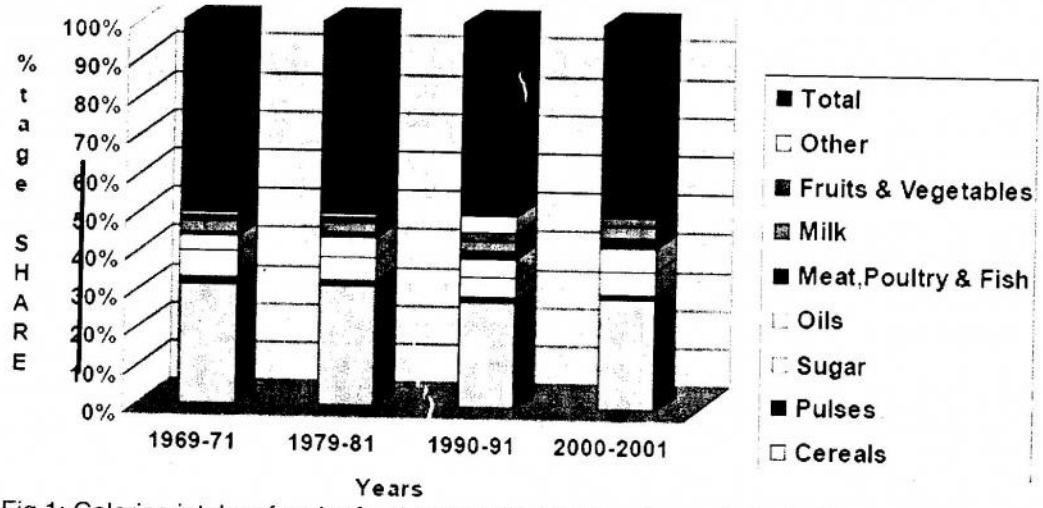


Fig 1: Calories intake of major food groups (Asian Development Bank 2000 – 2001)

Malnutrition

It is a general term for a medical condition caused by an improper or insufficient diet. It most often refers to under nutrition resulting from inadequate consumption, poor absorption, or excessive loss of nutrients. It is typically associated with extreme poverty in economically developing countries. Poverty levels in Pakistan are comparable to those in Sub-Sahara Africa, Malnutrition levels in women and children are sizably worse.

Fig. 2: Percentage population undernourished world map (United Nations World Food Programme 'hunger map' 2006)

FORMS OF MALNUTRITION

Common forms of malnutrition include:-

Protein-energy malnutrition (PEM): it is inadequate availability or absorption of energy and proteins in the body.

Micronutrient malnutrition: this refers to inadequate availability of some essential nutrients such as vitamins and trace elements that are required by the body in small quantities.

GOVERNMENT INITIATIVES

Based on nutrition data the following table shows Pakistan's standing in a study done by UNICEF.

Nutrition (Pakistan)	%
% of infants with low birth weight, 1998-2005*	19
% of children (1996-2005*) who are: exclusively breastfed (<6 months)	16
% of children (1996-2005*) who are: breastfed with complementary food (6-9 months)	31
% of children (1996-2005*) who are: still breastfeeding (20-23 months)	56
% of under-fives (1996-2005*) suffering from: underweight, moderate & severe	38
% of under-fives (1996-2005*) suffering from: underweight, severe	13
% of under-fives (1996-2005*) suffering from: wasting, moderate & severe	13
% of under-fives (1996-2005*) suffering from: stunting, moderate & severe	37
Vitamin A supplementation coverage rate (6-59 months), 2004	95
% of households consuming iodized salt, 1998-2005*	17

(Source: Nutrition data of Pakistan (UNICEF))

The government is aware of the national situation concerning malnutrition and thus has taken a number of initiatives in this regard. To name a few, wherever possible it incorporates nutritional objectives into development policies, it promotes nutrition awareness and healthy nutritional practices in rural households via local lady health workers programme. It encourages public and private sector partnerships to promote good eating habits / good nutrition amongst the general public and plays its part by encouraging food fortification and supplementation, eg. vegetable oil is fortified with vitamin A, edible salt with iodine and wheat flour with iron. It strives to prevent and mange infectious diseases and protects consumers through improved food quality and consumer safety.

With respect to above the Government of Pakistan has set the following targets:-

- Decrease vitamin A deficiency in children below five years;
- Reduce protein energy malnutrition amongst children to 25% from 39%;
- Bring down iron deficiency anaemia in women from 45% to 30% and in children from 65% to 55%

(Source:Asian Development Bank 'National Nutrition Plan – Targets 2001)

Pakistani government has taken heaps of steps to make sure that the quality produced and consumed by the Pakistani people, if not great, is good. The steps have been taken through National Food Security Policies, systems and agenda to ensure that food quality and safety considerations form an integral part of their food security system. Nutrition goes hand in hand with food safety because if the food is unsafe for consumption then it is of no use to the consumer hence emphasis should also be laid on food safety.

FOOD SAFETY

It is a scientific discipline describing the handling, preparation and storage of food in ways that prevent food borne illness. At home, prevention of food borne illness mainly consists of:

- Washing and drying hands before handling foods
- Thoroughly washing fresh fruits and vegetables with clean water, especially when not cooked (e.g. fruits, salads)
- Separating foods while preparing and storing seperately to prevent cross contamination.
- Keeping the kitchen and cooking utensils clean and dry
- Washing dishes after use, rinsing them well in hot water and storing them clean and dry
- Keeping work surfaces and chopping boards clean and dry
- Observing food storage methods (hot foods hot and cold foods cold) and food preservation methods (especially refrigeration), and checking the expiration date
- Avoiding over-long storage of left-overs.
- Not preparing food when sick or recovering from recent illness
- Not relying on disinfectants or disinfectant-impregnated cloths and surfaces as a substitute for good hygiene
- Preventing pets from walking on food-preparation surfaces.

REPUTABLE FOOD SAFETY SYSTEMS

FDA (Food and Drug Administration) is an agency of the United States Department of Health and Human Services and is responsible for the safety regulation of most types of foods, dietary supplements, drugs, vaccines, etc.

The Codex Alimentarius is a collection of internationally recognized standards, codes of practice, guidelines and other recommendations relating to foods, food production and food safety under the aegis of consumer protection. The Codex Alimentarius is recognized by the World Trade Organization as an international reference point for the resolution of disputes concerning food safety and consumer protection. The Codex Alimentarius officially covers all foods, whether processed, semi-processed or raw, but far more attention has been given to foods that are marketed directly to consumers. In addition to standards for specific foods, the Codex Alimentarius contains general standards covering matters such as food labeling, food hygiene, food additives and pesticide residues, and procedures for assessing the safety of foods derived from modern biotechnology. It also contains guidelines for the management of official (i.e., governmental) import and export inspection and certification systems for foods.

HACCP (Hazard Analysis and Critical Control Points) is a systematic preventive approach to food and pharmaceutical safety that addresses physical, chemical and biological hazards as a means of prevention rather than finished product inspection. HACCP is used in the food industry to identify potential food safety hazards, so that key actions, known as Critical Control Points (CCP's) can be taken to reduce or eliminate the risk of the hazards being realized. The system is used at all stages of food production and preparation processes. HACCP s 7 principles are:

Principle 1: Conduct a hazard analysis.
Principle 2: Identify critical control points.
Principle 3: Establish critical limits for each critical control point.
Principle 4: Establish critical control point monitoring requirements.
Principle 5: Establish corrective actions.
Principle 6: Establish record keeping procedures.

Principle 7: Establish procedures for ensuring the HACCP system is working as intended.

Verification ensures the HACCP plan is adequate, that is, working as intended. Verification procedures may include such activities as review of HACCP plans, CCP records, critical limits and microbial sampling and analysis.

ROLE OF INDUSTRY

Interaction and cooperation between industry and government on food control matters is often lacking in Pakistan. The basic responsibility of industry is to produce and market good quality and safe food. It is the duty of the government to ensure that industries comply with national food quality and safety requirements. At present the Pakistan Standards and Quality Control Authority Act (PSQCA) is a standards framing body and adopts international standards. Its prime responsibility includes the enforcement of standards in Pakistan and has the mandate to inspect and test products and services, including food items, for their quality, specification and characteristics during use, and for import and export products.

National food control systems suffer from serious inadequacies, including:

They are not based on modern scientific and management concepts using compliance policies, risk assessment, HACCP, transparency, and broad-based involvement of industry, trade and consumers.

Insufficient involvement of scientific expertise from the academia.

Lack of suitable facilities such as laboratories.

Lack of resources such as trained inspectorate and laboratory staff.

Inflexibility of the system making it difficult to cope with developments in food science and technology, changing consumer demands, and newer requirements of trade and industry. Institutional obstacles to reforms can be formidable and can create disincentives for development of industry causing serious damage to national economy.

Lack of Research and Development driven industries.

Lack of coherence and co-ordination amongst different governmental activities concerning agriculture, food, trade, industry and health to achieve optimal results.

ENFORCING FOOD CONTROL SYSTEM IN PAKISTAN

Food systems are not only complex but are also highly fragmented and predominated by small producers. This has its own socio-economic advantages. However, as large quantities of food pass through a multitude of food handlers and middlemen extending the food production, processing, storage and distribution chain, control is more difficult and there is a greater risk of exposing food to contamination or adulteration. Lack of resources and infrastructure for post-harvest handling, processing and storage leads to severe diminishing of quality and avoidable contamination and food losses.

There are several causes due to which the general public is the recipient of unsafe and unhygienic food; some of these are listed below:

Expertise
Education
Systems
Tools
Developments

Food preservation, processing and packaging systems can be minimal or highly sophisticated, but assuring food quality and safety in all situations should be a constant. Pakistani industry must play its role in assuring food quality and safety through the application of quality assurance and risk-based food safety systems utilizing current scientific knowledge. Implementation of controls throughout production, handling,

processing, distribution and marketing leads to improved food quality and safety, increased competitiveness and reduction in cost of production and wastage.

Regulation and standards alignment with international requirements

The world has become a global village resulting in increased trade between countries. In order to compete with the rest of the world, it is imperative that Pakistan aligns its food and consumer safety standards to global requirements. By doing this it will be in a position to raise the quality of products manufactured in Pakistan and be in a position to compete with other countries for trade.

First and a vital step in this regard would be formulation and implementation of standards which are in line with Codex standards as this harmonization of standards will enable Pakistan to compete in international trade. Very recently on 30th Oct 2007 European Commission has banned sale of food items exported from Pakistan to European Union due to non compliance of food standards notified by European Commission (EU). EU has informed that as a result of market control in the UK peanut snacks exported from Pakistan were found contaminated with Aflatoxins and therefore detained / rejected for distribution in the UK (Source: Business Recorder 'EC bans sale of Pakistani food items', 30th Oct 2007 Tuesday). Aflatoxins are naturally occurring mycotoxins that are produced by many species of Aspergillus, a fungus, most notably Aspergillus flavus and Aspergillus parasiticus. Aflatoxins are toxic and carcinogenic. After entering the body, aflatoxins are metabolized by the liver to a reactive intermediate, aflatoxin M1, an epoxide. (Source: www.wikipedia.com, 'Aflatoxins')

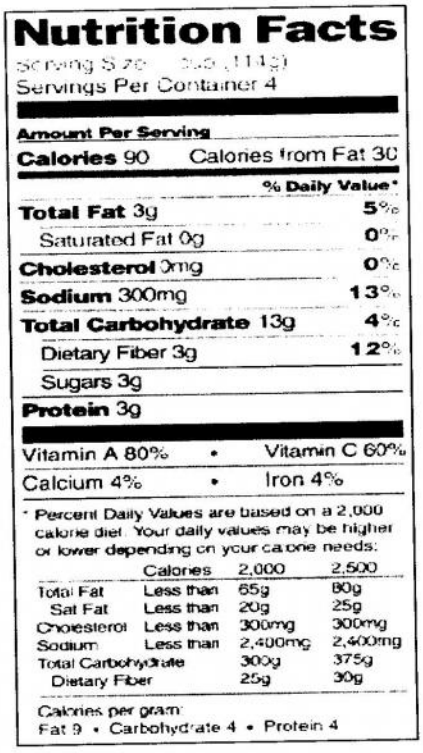
Probably just due to one peanut snack supplier, now the rest of the Pakistani traders will suffers. As PSQCA deals with the development of standards, the necessary action should be taken against the one company exporting substandard products to Europe. A proper checking would reduce their share of exports in markets where standards do not matter greatly. Food security has always been an issue for nations plagued by anarchy, droughts or war. However, in the backdrop of recent food shortages and soaring prices, it has become a global concern, affecting both rich and poor nations. Since the importance of food in overall consumption is negatively correlated with income levels, food shortage and price hike affect poor countries more severely. According to the UN estimates released on the annual World Food Day on Oct 16, there are 654 million malnourished women, men and children around the globe and 10 million people die every year of reasons associated with hunger.

Complex factors are causing global demand for food to rise faster than the supply. Without prompt and well considered action, the problem will compound with passing time. For one, government must ensure increased investment in agriculture to augment supplies. Secondly, more funding for crop research, combating climate change and improving marketing of agriculture products is required. Thirdly, water conservation and management are critical for sustainable agriculture.

NUTRITION FACTS PANEL

Many people obtain most of their nutrition information from a food label called the Nutrition Facts Panel. This label is mandatory for most foods in all first world countries which contain more than one ingredient and these foods are mostly processed foods.

Placement of the nutrition panel on the pack enables the consumer to choose what he desires as the panel highlights a product's content of fat, saturated fat, cholesterol, sodium, dietary fiber, vitamins A and C, and the minerals calcium and iron. The stated content of these nutrients is based on a standard serving size.



Nutrition Facts

Serving Size [illegible] (114g)
Servings Per Container 4

Amount Per Serving

Calories 90	Calories from Fat 30
	% Daily Value*
Total Fat 3g	5%
Saturated Fat 0g	0%
Cholesterol 0mg	0%
Sodium 300mg	13%
Total Carbohydrate 13g	4%
Dietary Fiber 3g	12%
Sugars 3g	
Protein 3g	

Vitamin A 80% • Vitamin C 60%
Calcium 4% • Iron 4%

* Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

CONSUMER RIGHTS INPAKISTAN

Pakistan, the third fastest growing economy in Asia, has recently received Foreign Direct Investment (FDI) of around US$ 6.0 billion in the year 2007. With rising per capita income at USD 925 the demand for consumer products is growing by 25% on an annual basis. In Pakistan, with an enormous population along with high levels of poverty, unemployment and poor literacy levels, consumer awareness continues to remain low. The ordinary citizens still continue to be victims of bad governance and are being denied the basic rights and facilities. (Source: www.plastipacpakistan.com, Power of Integral Sectors - 2007)

Consumer protection laws in Pakistan need to be more effective. Laws such as the Pakistan Penal Code, the Contract Act, the Sale of Goods Act, the Drug Act, the Standard of Weight and Measurement Act are not sufficient to protect consumers. The laws deal with adulterated foods and substandard drugs but their enforcement is poor. As a result, the markets are flooded with substandard and poor quality goods and products. There are defective, over-priced and expired products in the market, but there is no place where complaints can be redressed. A citizen in Pakistan rarely sues a company if it supplies substandard products although Consumer Protection Act 2004 has been enforced and Consumer Courts established.

FOOD SAFETY AND UNILEVER

Our basic aim is assuring the safety of our products for all our consumers, wherever they are in the world and our vision is to seek to continuously improve the health, safety and well being of everyone working for, or on behalf of Unilever to a standard which is at least comparable with the very best companies in our industrial sector. Nutrition is a key growth driver for our business. As a leader in the global food market, Unilever is

committed to market great tasting foods that will make the healthy choice an easy choice. We will help our consumers every where to achieve nutritional balance leading to a vital and healthy life.

Our brand teams and operating divisions, supported by the Unilever Health Institute and our Global Nutrition and Health Network, are responsible for implementing this policy and for strengthening our continuing efforts to:

- Develop a deep understanding of consumers' nutrition and health needs and wants
- Know the nutritional composition and dietary role of our products and label our products in a consumer-friendly and meaningful way
- Optimize the nutritional composition of our products to meet consumer needs and wants
- Undertake and support scientific research to provide evidence for benefit claims for our product
- Ensure responsible communication about product benefits to health care professionals and consumers
- Seek external partnerships to develop mutual understanding and agree common approaches in nutrition and health programmes.
- As part of the Unilever Vitality Mission, we want to meet the everyday needs for nutrition; hygiene and personal care with brands that help people feel good, look good and get more out of life.
- The WHO guidelines per nutrient are the bases for Unilever's vitality passport.

WHO guidelines per nutrient

Ranges of population nutrient intake goals

Dietary factor	Goal (% of total energy unless otherwise stated)
Total fat	15-30%
Saturated fatty acids	<10%
Polyunsaturated fatty acids (PUFAs)	6-10%
n-6 Polyunsaturated fatty acids (PUFAs)	5-8%
n-3 Polyunsaturated fatty acids (PUFAs)	1-2%
Trans fatty acids	<1%
Monounsaturated fatty acids (MUFAs)	By difference[a]
Total carbohydrate	55-75%[b]
Free sugars[c]	<10%
Protein	10-15%[d]
Cholesterol	<300 mg per day
Sodium chloride (sodium)[e]	<5 g per day (<2 g per day)
Fruits and vegetables	≥400 g per day
Total dietary fibre	From foods[f]
Non-starch polysaccharides (NSP)	From foods[f]

All our innovations in the area of food and health are increasingly addressing one or more of these Vitality Life Goals. This work is led by the Unilever Food and Health Research Institute. Our work at the Institute is part of our wider commitment to research and development across both our Foods and Home and Personal Care categories. Our knowledge of health and nutrition grows daily and as new research about the health properties of individual ingredients comes to light, we change our product formulations. We do not just try to create and market foods. We also help raise awareness about the benefits of a healthy lifestyle – not just diet – through our partnerships and collaborations with healthcare professionals and leading global health bodies like UNICEF and the

World Health Organization (WHO). We also use these benchmarks in our claims validation procedure that takes into account if a food promising a health benefit is contributing to a healthy diet.

We would like to work with the Pakistani government to bring those innovations to Pakistan which we use as a base nutrition benchmarks on established international dietary guidelines. To be fully effective, nutrition information needs to be supported by continuous education on nutrition, health and lifestyle. Unilever will contribute to these programs and co-operate with NGOs and public authorities in promoting a healthy lifestyle. Unilever stresses the need for harmonized guidelines on tolerances for the provided nutritional data, resulting from variations in raw materials, prices and product composition and during shelf life.

Unilever supports efforts to come to a simple, consumer friendly system that indicates the contribution of a specific food product to the diet. Such a system should be on a voluntary basis though as well as flexible and open for improvements/adaptations. We stress the need for further consumer education on health, nutrition and lifestyle and the role a balanced diet can play in maintaining health. Unilever is prepared to contribute to educational programs. Unilever advocates the need for more extensive consumer research to find out what consumers expect from nutritional labeling and how current, by and large voluntary, systems are being perceived. This would allow improvements to better accommodate consumer needs.

A passion for vitality, health and nutrition are at the heart of Unilever and we will continue to work hard to develop new products that make healthy eating enjoyable, convenient and affordable for people everywhere. Innovation is right at the heart of Unilever, and our dynamic R&D environment attracts top-class scientists who enjoy the best of both worlds being at the cutting edge of technology and seeing their work deliver real benefits daily. Unilever is committed to meeting the needs of customers in an environmentally sound and sustainable manner, through continuous improvement in environmental performance in all our activities and ensuring the safety of its products and operations for the environment. We are committed to supplying products that will not harm customers and consumers, and the environment.

RECOMMENDATIONS

The Pure Food Rules in Pakistan are enforced through health service delivery channels of the provincial governments. The District Health Officer and Deputy Health Officer function as food inspector for sampling and inspection. On the other hand, the Municipality Corporation may also appoint food inspectors and sanitary inspectors for sampling purposes. Any other public servant can also be appointed as inspector and can execute the power of food inspector. The existing food regulations and food safety procedures in Pakistan do not completely cope with the emerging requirements.

Pakistan does experiencing difficulties in meeting the requirements of developed countries and concerns are expressed about the way in which the agreement has been implemented to-date. What Pakistan needs to do is to harmonies the quality of its products to internationally accepted standards. Information dissemination to farmers on higher standards should be promoted, financial assistance extended and training imparted to them on methods of attaining these standards. There is dire need to arrange conferences, seminars and talks on electronic and print media to educate the scientists, policy makers, farmers and other stakeholders about various aspects of WTO. Pakistan, amongst South Asian countries, has a very narrow base of agricultural exports, which are directed largely to South East Asia and the Middle East.

Given the current situation we do believe that a voluntary system is out of the question and defending that would automatically and immediately take us out of the loop. A better

approach might be to ask Commission and Council to allow for flexibility in the way the Regulation will be implemented. This would give Industry room to experiment with various forms of labeling, i.e. a mix of voluntary/mandatory and on pack/via other carriers. A review of different types of measures that can be applied to address particular problems and their relative impact on agricultural and food exports from Pakistan should be undertaken. This needs to be performed in collaboration with agencies responsible for the promulgation and enforcement measures at both the national and international levels.

A study of different options for facilitating participation of Pakistan in the Codex Alimentations, OIE and IPPC should be undertaken. This needs to be performed in collaboration with the WTO and international standards organizations and should feed into the on-going review of participation in organizations such as Codex Alimentarius. Constraints are present that limit level of co-operation on matters amongst Pakistan and other developing countries and identification of the mechanisms through which these constraints can be alleviated should be undertaken. This should be performed in collaboration with other countries and/or inter-governmental agencies.

Further research should be conducted about impact on export of agricultural products from Pakistan, should be undertaken to generate a more rigorous and, preferably, quantified assessment.

These will help us build our food industry locally and internationally, which will consist of our basic needs, safety, information, choices, representations and consumer education.

Basic needs: our rights to basic good and services, which guarantee survival. It includes adequate food, clothing, shelter, health care, education and sanitation.

Safety: The right to be protected against products, production processes and services, which are hazardous to health and life.

Information: The right to be given the facts needed to make an informed choice or decision. Awareness raising amongst consumers needs to be ensured and media could be employed for this purpose.

Choice: The right to choose products and services at competitive prices and in the case of monopolies to have an assurance of satisfactory quality and service at a fair price.

Representation: The right of representation in government and other policy making bodies as well as in the development of products and services before they are produced or set up.

Consumer Education: The right to acquire the knowledge and skills to be an informed consumer throughout life.

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Proceedings:
International Symposium on
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5th to 8th November, 2007
National Institute of Food Science and Technology

WHEAT FLOUR FORTIFICATION WITH IRON AND FOLIC ACID

Abdul Wahid Khan and Khalid Iqbal,
The Micronutrient Initiative (MI) Pakistan, Islamabad

Abstract

Iron Deficiency Anemia (IDA) and folic acid deficiency are major public health problems across the globe. In Pakistan, deficiencies of these two micronutrients are severely affecting large sections of the population, particularly young children, and women of child bearing age, in particular pregnant and nursing mothers, and female adolescents. National Nutrition Survey conducted during 2001-2002 to ascertain the benchmark of the nutritional status of women and children indicated that about 38% of the children between the age of six months to five years are under weight while 36.8% are stunted. 12.5 % of women are malnourished while it is 16.1% in the case of lactating mothers. Similarly, nearly half of the population is suffering from the micronutrient deficiencies i.e., iron, iodine, vitamin A and zinc. All this ultimately leads to impaired immunity, diminished strength, vitality and lower mental and physical activity. Similarly a large number of births take place with problem of neural tube defects (NTDs). Food fortification is considered as cost effective nutritional intervention for control micronutrient malnutrition. Wheat flour has been successfully fortified with iron and folic acid in more than 60 countries for prevention of iron deficiency anaemia and NTDs. In Pakistan, wheat flour is a staple food and is a good vehicle for iron and folic acid fortification. With the financial and technical assistance of Micronutrient Initiative (MI), feasibility studies have been conducted to in Pakistan. This project provided a strong foundation for starting a large scale flour fortification projects in Pakistan. MI has successfully launched the 1st ever flour fortification project in AJ&K. Under this project about 10500 MT of fortified flour is being produced and distributed monthly. MI has also launched the Commercial Wheat Flour Fortification Project in Earthquake Affected Districts of NWFP with financial assistance from WFP through CIDA funding. Additionally MI has providing Task Based Technical Assistance (TBTA) to the Ministry of Health, Government of Pakistan in implementation of GAIN funded National Wheat Flour Fortification Project in large roller mills of Pakistan. Pakistan is one of the countries where legislation for mandatory fortification is lacking. There is an urgent need of this important law to ensure sustainable and effective intervention.

Key words: Anemia, iron fortification, folic acid

INTRODUCTION

Consumption of adequate amount of iron ameliorates iron deficiency, the most prevalent nutritional deficiency in the world, affecting approximately 2 billion persons (WHO 2006). Adequate intake of folic acid by women before pregnancy and during early pregnancy decreases their risk for having a pregnancy affected by neural tube defects (NTDs) (Wald *et al* 2001), the most common preventable type of birth defects worldwide. There is an urgent need to address these micronutrient deficiencies across the globe.

Pakistan being one of the most populous countries has a high prevalence of these micronutrient deficiencies that need to be addressed on priority.

Causes, consequences, magnitude of the problems and the cost-effective strategy to eliminate deficiency of these two important micronutrients are discussed below.

IRON AND FOLIC ACID DEFICIENCIES

IRON DEFICIENCY AND IRON DEFICIENCY ANAEMIA (IDA)

Iron deficiency is defined as decreased total iron body content. Iron deficiency anemia occurs when iron deficiency is sufficiently severe to diminish erythropoiesis and cause the development of anemia. Iron deficiency is the most common and widespread nutritional disorder in the world and is a public health problem in both industrialized and non-industrialized countries.

CAUSES OF IRON DEFICIENCY AND IRON DEFICIENCY ANAEMIA

Iron deficiency occurs when an insufficient amount of iron is absorbed to meet the body's requirements. This in-sufficiency might be attributed to a low intake of haem iron (which is present in meat, poultry and fish), an inadequate intake of vitamin C (ascorbic acid) from fruit and vegetables (the presence of vitamin C enhances the absorption of iron from the diet), poor absorption of iron from diets high in phytate (including legumes and cereals) or phenolic compounds (present in coffee, tea, sorghum and millet), periods of life when iron requirements are especially high (i.e. growth and pregnancy), heavy blood losses as a result of menstruation, or parasite infections such as hookworm, ascaris and schistosomiasis. The presence of other micronutrient deficiencies, especially of vitamins A and B12, folate and riboflavin, also increases the risk of anaemia (Allen *et al* 2000). If prolonged, iron deficiency leads to IDA.

CONSEQUENCES OF IDA

IDA has profound effects on psychological and physical development, behavior, and work performance and eventually on productivity and socioeconomic development (WHO, 1998). During pregnancy it increases maternal morbidity, and mortality as well as prenatal mortality, and increases the risk of low birth weight (WHO 1989). Iron deficiency has been shown to reduce physical endurance, even in the absence of anaemia (Brownlie *et al* 2002), and severe anaemia has been associated with an increased risk of both maternal and child mortality (Brabin *et al* 2001; Brabin *et al* 2001)

RISK GROUPS

Risk group include, children, adolescents, women of child bearing age and pregnant and lactating women. Women of childbearing age are a highly risk group of ID because of the effects of menstruation and pregnancy. Women of childbearing age usually require additional iron to compensate for menstrual blood loss (an average of 0.3 – 0.5 mg daily during their productivity years), and for tissue growth during pregnancy and blood loss at delivery and postpartum (an average 3 mg daily over 280 day's gestation) (CDC 1998).

PREVALENCE OF IRON DEFICIENCY ANEMIA

Approximately two billion people are iron deficient (WHO 1998). IDA, one of the most important implications of ID, is widely prevalent in young women all over the world especially in pregnant women in Pakistan (Isselbacher *et al.* 1987). Approximately 40 % adult women and 41 % pregnant women in Pakistan are suffering from iron deficiency anemia (MI 2004). According to the National Nutrition Survey 2001-02, it is estimated that more than 50% of pregnant women are anemic in the country. Evidence also suggests that a high percentage of adolescents, children and men suffer from anemia.

FOLIC ACID DEFICIENCY

Folic acid deficiency, an abnormally low level of one of the B vitamins, results in anemia characterized by red blood cells that are large in size but few in number. Low intake before and during pregnancy results in birth of the new born with neural tube defects.

CAUSES OF FOLIC ACID DEFICIENCY

Besides ID, folic acid (FA) deficiency is also an important public health concern. Nutritional deficiency of folate is common in people consuming a limited diet (Chanarin, 1979). This can be exacerbated by malabsorption conditions, including celiac disease and tropical sprue. Besides increased folate requirement such as during rapid fetal growth and lactation put women at risk of folate deficiency. Pregnant women are at risk for folate deficiency because pregnancy significantly increases the folate requirement, especially during periods of rapid fetal growth (i.e. in the second and third trimester) (McPartlin *et al* 1993). During lactation, losses of folate in milk also increase the folate requirement.

CONSEQUENCES OF FOLIC ACID DEFICIENCY

Inadequate intake of folic acid before and during the first 8 wk of pregnancy has been associated with an increased incidence of giving birth to an infant with Neural Tube Defect (NTD) such as spina bifida or anencephaly (Jacques *et al* 1999). The benefits of folate in reducing the incidence of neural-tube defects (NTDs) have been well documented (Czeizel *et al* 1992; MRC 1991).

PREVALENCE OF FOLATE DEFICIENCY

Folic acid deficiency results in megaloblastic anemia with alterations in bone marrow and peripheral blood (Machlin & Hüni, 1994). Inadequate intake of folic acid before and during the first 8 wk of pregnancy has been associated with an increased incidence of giving birth to an infant with Neural Tube Defect (NTD) such as spina bifida or anencephaly (Jacques *et al,* 1999). The benefits of folate in reducing the incidence of neural-tube defects (NTDs) have been well documented (Czeizel *et al* 1992; MRC 1991; Food and Nutrition Board 1998).

STRATEGIES FOR ELIMINATION OF MICRONUTRIENT MALNUTRITION

There are three basic food system approaches to eliminate micronutrient malnutrition. These include Supplementation, Food Fortification and Dietary Diversification (Individual change in eating habits and crop diversity). Additionally, many public health measures are successful in combating MM (e.g., preventing & treating helminthes infections to prevent Fe deficiency).

Micronutrient malnutrition is not effectively dealt with through the existing health care system, nor can it be dealt with exclusively by the health care system as large parts of the population, or in some cases the entire population, must be reached. The sectors that must be involved in successful elimination of MM include:

1. Health care sector -- Supplements
2. Private industry -- Food fortification
3. Education system -- dietary change
4. Agriculture -- crop diversification

CHOICE OF AN INTERVENTION

Several approaches exist to prevent and treat micronutrient malnutrition, each with its own strengths and limitations, but which are highly effective if applied in complementary ways. Food fortification is a cost effective strategy to combat micronutrient malnutrition.

FORTIFICATION OF STAPLE FOODS

Food fortification is one of the strategies to address these micronutrient deficiencies. It is the addition of vitamins and/or minerals to a vehicle with the goal of increasing the nutritional content (FAO/WHO 1994). Clear evidence exists that fortification is the most cost effective and sustainable strategy in the battle against micronutrient deficiencies, especially iron (MMWR 1998) and folic acid fortification are the optimal approach for reducing iron and folate deficiencies in developing countries.

FORTIFICATION OF WHEAT FLOUR WITH IRON AND FOLIC ACID

The fortification of wheat flour with iron and folic acid is an important strategy that has been proved to help improve the nutritional status of people globally. Studies in United States have shown that wheat flour fortification is a cost-effective strategy (Grosse 2005). Currently, wheat flour fortification strategies are being implemented in more than 60 countries across the world. At present more than 60 countries are adding iron and 37 countries are adding both iron and folic acid (FA) to wheat flour at national level (MI 2004). Folic Acid fortification of wheat flour has been attributed to reduction in the incidence of neural-tube defects (MRC 199; Czeizel *et al* 1992; Food and Nutrition Board 1998). Young children, Women of childbearing age and pregnant and lactating women are the major risk groups of these micronutrients deficiencies. In order to deal with the growing burden of iron and FA deficiency it is critical to fortify wheat flour with iron and folic acid in the large public interest.

EFFECTIVENESS OF WHEAT FLOUR FORTIFICATION WITH IRON AND FOLIC ACID

NTDs affect approximately 200,000 births each year, resulting in the death of fetuses or newborns or in lifelong disabilities that result in tens to hundreds of thousands of dollars per year in direct costs per person. In the United States, folic acid fortification has an estimated economic benefit of $312--$425 million annually. The estimated benefit-cost ratio of U.S. folic acid fortification is 40:1 (Grosse 2005). Worldwide, iron deficiency is associated with approximately 861,000 deaths, approximately 35 million disability-adjusted life years lost, and billions of dollars in indirect costs annually (Stoltzfus *et al* 2006). The benefit-cost ratio for iron fortification is approximately 36:1 (Horton 2006).

Ecological studies from the United States (Williams *etal,* 2002), Canada (De Wals *et al,* 2007), and Chile (Hertrampf *et al,* 2004) have documented decreases of 26%, 42%, and 40%, respectively, in the rate of NTD-affected births after implementation of national regulations mandating wheat-flour fortification with folic acid. Investigators in Ireland documented that small increases in red blood cell folate levels reduce the risk for NTDs, indicating that small increases in folic acid consumption might result in substantial reductions in NTD incidence in the population (Daly *et al* 1997). No adequate ecological studies have examined the health impact of fortifying wheat flour with iron; however, research trials have demonstrated an association between the consumption of wheat flour fortified with iron and increased haemoglobin levels and decreased prevalence of anaemia (Sun *et al* 2007).

REQUIREMENTS OF AN EFFECTIVE FORTIFICATION INTERVENTION

Successful wheat-flour fortification worldwide requires adoption and enforcement of legislation for mandatory fortification at the national level, and industry and public-sector commitment for such legislation. Mandatory fortification places the same requirements on all flour producers and is more likely to succeed if the milling industry is well organized and supports fortification (Allen 2006). Concomitant consumer education and social-marketing programs are important to ensure consumer acceptance of fortified flour products. The development and implementation of consumer education and communication strategies that include evidence of the health benefits of fortification

require commitment from the public sector and is strengthened by the support of civic organizations. Through public, private, and civic collaboration, advocates and public health agencies are promoting wheat-flour fortification and the fortification of other food items (e.g., other cereal grains, sugar and cooking oil) to increase worldwide consumption of vitamins and minerals.

In 2007, the number of countries with documented national regulations for mandatory wheat-flour fortification increased was 54. Fifty of the 54 countries with mandatory fortification in 2007 required fortification with both iron and folic acid, two with folic acid but not iron, and two with iron but not folic acid. Twenty-four of those countries also mandated wheat-flour fortification with thiamin, riboflavin, and niacin; two with thiamin and riboflavin; and two with thiamin.

GLOBAL REACH OF IRON AND FOLIC ACID FORTIFIED WHEAT FLOUR

The percentage of wheat flour processed in roller mills that was fortified increased from was 27% in 2007. It has been reported that nearly 540 million additional persons, including 167 million additional women aged 15--60 years, had access to fortified wheat flour in 2007 compared with 2004, and the annual number of newborns whose mothers had access to fortified wheat flour during pregnancy increased by approximately 14 million (CDC 2008). By region, the greatest increase in the percentage of wheat flour being fortified in the last few years was in the Eastern Mediterranean Region where the figures increased from 5% in 2004 to 44% in 2007. In the same year, the portion of wheat flour being fortified was 97% in the Americas Region (the region with the highest percentage of wheat flour being fortified), 31% in the African Region, 21% in the South-East Asia Region, 6% in the European Region, and 4% in the Western Pacific Region (CDC 2008)

STATUS OF WHEAT FLOUR FORTIFICATION

To reduce the burden of deficiencies of these two important micronutrients, fortification of an appropriate food vehicle is critical. As Pakistan is a high wheat consuming country, with an average wheat flour consumption of 125 kg/capita/year (FAO 2002); therefore, wheat flour is a good vehicle for iron and folic acid fortification. Wheat flour is recommended to be fortified with 10 ppm Sodium Iron EDTA and 1.5 ppm folic acid (GAIN 2004). This level of fortification is much lower than the upper safe levels allowed (MI 2004) thus averting any danger of toxicity.

There is no legislation in Pakistan for mandatory fortification of wheat flour with iron and folic acid. Only regulations that exist in Pakistan are Pure Food Rules of 1965, but it is silent on wheat flour fortification.

WHEAT FLOUR FORTIFICATION EFFORTS IN PAKISTAN

MI has conducted stability, acceptability and bioavailability studies of the Iron Fortification of Wheat Flour in Pakistan through the renowned research institutes of the country. In January 2007 MI Pakistan Program has successfully launched 1st ever flour fortification project in Azad Jammu & Kashmir (AJ&K). Under this project about 10500 MT of fortified flour is being produced and distributed monthly. MI is the lead implementing partner in this project. MI has also launched the Commercial Wheat Flour Fortification Project in Earthquake Affected Districts of North West Frontier Province (NWFP) of Pakistan. Under this project about 500 tons of fortified flour is being produced and distributed in the earthquake affected areas of NWFP. MI has also assisted the Ministry of Health (MoH), Government of Pakistan in submitting a successful bid of US$ 3.3 million for a GAIN funded National Wheat Flour Fortification Project in large roller mills of Pakistan. MI is providing continuous advisory services and task based technical assistance to MoH for the successful implementation of this project. GAIN assisted

NWFFP would cater needs of 22% of Pakistan population through fortification of 3,081,767 MT wheat flour over three years.

CONCLUSIONS

Iron and folic acid are two important micronutrients essential for a healthy life in adequate amounts. One third of global population is suffering from iron deficiency and many a more are at risk. Folic Acid deficiency is taking heavy tolls on human lives in the form of NTDs. Large number of people in Pakistan are at risk of Iron and Folic Acid Deficiencies. Pakistan has Fortification is a cost-effective strategy to prevent deficiencies of these two important micronutrients. While legislation for mandatory fortification is needed for iron and folic acid fortification, Pakistan has yet to work on it. Wheat flour fortification program in Pakistan is in its infancy and needs a lot of support.

RECOMMENDATIONS

1. Data on wheat flour consumption should be collected. The available data are based on estimates.
2. Iron and folic acid intake from other sources should be quantified.
3. Mandatory legislation for wheat flour fortification for iron and wheat flour should be enacted.
4. Consumer education and promotion of wheat flour consumption should be done through continuous education program.

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Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

FUNCTIONAL FOODS: EMERGING TREND IN NUTRITIONAL SUPPORT PROGRAMS

Masood S. Butt*, Tauseef Sultan, Faqir M. Anjum, Mian K. Sharif and Saima H. Khan
National Institute of Food Science and Technology, University of Agriculture, Faisalabad.
E-mail: drmsbutt@yahoo.com

Abstract

Nutritional support is a recent advancement in the domain of diet based therapies, prior to which food was just recognized for its basic nutritive value. Modern era has seen the coinage of terms like functional foods, nutraceuticals and pharma foods; all aiming to provide health benefits along with basic nutrients they contains and indeed are important in combating various health risks. Scientific investigations in the field of nutrition led to discovery of various phenomena playing key role in induction of various maladies. Oxidative stress and improper immune responses has been credited to the prevalence and pathogenesis of many diseases in humans. These are influenced by numbers of genetic and environmental factors however ingestion of appropriate diet is helpful in combating their associated health risks. Presently, use of allopathic medicines is at climax nevertheless interest in medicinal plants has flourished due to effectiveness of new plant derived drugs. Functional foods particularly of plant origin may open up several therapeutic avenues. Plants produce complex compounds to meet their own requirements; some of these metabolites have health promoting properties often termed as phytochemicals. Recent research findings elucidate the importance of phytochemicals like omega-3-fatty acids, dietary fibers, vitamins, antioxidants, plant sterols and flavonoids all have certain health benefits. There are increasing evidences that dietary phytochemicals may play important roles as chemopreventive or chemotherapeutic agents. Amelioration of oxidative stress, antimutagenic and immunomodulatory perspectives of various functional foods have been explored recently but still mechanism of their action needs thorough scientific investigations. Current paper focus on the use of human friendly phytochemicals and their possible inclusion as diet based therapies not only to improve nutritional status but also to cure various maladies.

Key words: Functional food, health benefits

INTRODUCTION

Food is one of the most important necessities of life. Variety of foods consumed by humans has changed greatly over the centuries focusing on balanced diet. During the 20th century, science of nutrition discovered essential nutrients, established nutrient standards, dietary guidelines and food guides, with the aim of preventing deficiencies and of supporting body growth, maintenance, and development (Rayner *et al* 2003). Recommendations aiming to avoid excessive consumption of some of these nutrients after recognizing their potential role in the prevalence and progression of diseases were also highlighted. During that era concept of balanced diet flourished to meet the minimum

requirements of nutrients and a few other food components needed to support growth and maintain body weight, to prevent the development of deficiency diseases and to reduce the risk of diseases associated with deleterious excesses (Mueller 1999).

Rise of 21st century faced new challenges in the form of exponentially growing costs of health care, increase in life expectancy, improved scientific knowledge and development of new technologies to major changes in lifestyles. Nutrition has to adapt to these new challenges by developing new concepts. Optimum (optimized) nutrition is one of these new concepts that aims at maximizing physiologic functions of each individual to ensure both maximum well-being and health, and, at the same time, confers minimum risk of disease throughout lifespan.

On the way to optimum (optimized) nutrition, the term *functional food* refers to a new and stimulating concept. "Functional Foods" are foods or dietary components that may provide a health benefit beyond basic nutrition. Functional foods, according to Zeisel (1999), are not dietary supplements but rather consumed as part of normal diet and deliver one or more active ingredients within the food matrix.

Functional Foods are predicted to become one of the biggest dietary trends of the coming decades. Examples include fruits and vegetables, whole grains, fortified or enhanced foods and beverages, and some dietary supplements. Botanicals with medicinal properties have been used from time immemorial in all cultures and, with the introduction of ingredient assays and standards in the early 20th century, form the foundation for modern Western pharmacology (Goldman 2001).

Concept of functional foods some times intermingles with others such as nutraceuticals, chemopreventive agents and phytochemicals, thus. The American Dietetic Association has put forward the following definitions,

- Chemopreventive agent: nutritive or non-nutritive food component that has been scientifically investigated as a potential inhibitor of carcinogenesis
- Functional food: any altered food or ingredient that could give a beneficial effect beyond the nutrients that traditionally it contains
- Phytochemical: substances found in edible fruit and vegetables that can be ingested daily (in quantities of grams) by man and that exhibit a potential to modulate human metabolism to prevent cancer and other diseases
- Nutraceutical: any substance considered a food or part of this and offers health or medical benefits, including prevention and treatment of diseases
- Novel foods; any food which originated after 1997 and possesses health benefits
- Designer foods; these are foods in which specific gene or functional component has been added or those concentrations has been raised with artificial means and hold some specific purpose

For thousands of years, humankind has relied on plant derivatives for prevention and treatment of a wide variety of ailments. For example, in China, various teas have been used as a crude medicine for over 4,000 years. And more recently, there has been considerable interest in taking advantage of various plant extracts as a source of health promoting substances such as natural antioxidants, phenolic compounds, flavonoids, tocochromanols and beneficial fatty acids. In part, this trend is due to a growing body of evidence demonstrating that some of these compounds have beneficial properties that may be advantageous in preventing or delaying the onset of disease.

Indeed, several epidemiological studies considering the effect of diet on disease such as cancer and cardiovascular disease have provided leads in the search for naturally-occurring anti-cancer or anti-cholesterol agents. Some studies suggest that plant-based diets, rich in whole grains, legumes, fruits and vegetables, may reduce the risk of various types of cancer (Steinmetz *et al* 1991; World Heath Report 2000; World Health

Organization 2000). Various phenomenons occurring in nature result in excessive production of metabolites that target human health. Release of free radicals, improper immune functions, high cholesterol level and ingestion of toxicants are important (Barta *et al* 2006).

Free radical-induced damages and subsequent lipid, protein and DNA peroxidations are implicated in many human pathologies (Ferrari 1998; Halliwell 2000). Dietary ingestion of fruits and vegetables or administration of synthetic antioxidants, which neutralize free radicals, could be used to decrease certain chronic diseases of aging. Healthy dietary practices from ancient Oriental populations originated these concepts and the influence of Oriental and Mediterranean diets (rich in fruits, vegetables and grains) on cancer and cardiovascular disease morbidity and mortality is remarkable, since those nations present lower total and cardiovascular mortality rates in comparison to the United States. Based on ancient ethnopharmacological knowledge, German physicians usually prescribe herbal medicines to treat common diseases and Japan, China, South Korea and India are leaders in functional food and herbal use and research. In Mediterranean nations, such as Greece, Italy, France, Spain and Portugal, nutritional traditions have been associated with healthy living and aging.

Similarly, other studies report that populations consuming large amounts of fruit and vegetables have a lower incidence of cardiovascular disease and reduced risk of several types of cancer. Such studies have attributed the beneficial properties of diets rich in fruits and vegetables to the presence of naturally occurring compounds, including various vitamins and minerals, and these compounds have been found in a wide variety of plant sources.

Moreover, additional studies suggest that fruit products are a source of a number of health promoting phytochemicals. Cancer and cardiovascular disease (e.g., cholesterol-related diseases) are two of the major causes of death in the United States, additional research on identification of fruit-derived therapeutic compounds which, for example, are useful in treating or preventing such diseases, would be of great benefit.

Nutritional support is a recent development, prior to which the value of food was recognized for its medicinal benefits as nutraceuticals. The value of such "alternative" therapy is now being rediscovered by many patients who enhance their dietary intake with these traditional remedies. In Western culture, conventional medicine until recently has largely rejected the use of such "alternative" therapeutic intervention. Based on an increasing database, though, insight has been gained concerning the scientific validity of many previously termed established nutraceuticals. Honey has proven antimicrobial activity. Green tea enhances humoral and cell-mediated immunity while decreasing the risk of certain cancers and the risk of cardiovascular disease. Ginseng enhances production of macrophages, B and T cells, natural killer cells, and colony-forming activity of bone marrow. Vitamin supplementation is associated with increased antibody titer response to both hepatitis B and tetanus vaccines as a result of macrophage and T cell stimulation. Because of these findings, nutraceuticals are becoming more widely accepted as an adjunct to conventional therapies for enhancing general well-being (Klein *et al* 2000).

Nutritional support programs are meant to provide appropriate nutrition based on concepts of optimum nutrition. These programs can be run in hospitals and at homes too. Hospitalized patients seek somewhat more attention as complication during disease conditions that could also results in complexities. Enteral nutrition and parenteral nutrition are terms used to feeding diet to those ill patients which are not able to engulf or digest food. Malnutrition is another complication in these conditions and appropriate diet rich in several food components required by the body required that could facilitate proper health

of humans. Recent technological advances in parenteral and enteral nutrition coupled with increasing clinical experience have greatly improved the care of the malnourished patient in the hospital, and have fostered the development of programs for outpatient nutritional management as well. Along with more sophisticated methods of providing nutritional care, various strategies are evolving for coordination and administration of nutritional assessment and support in the long-term care facilities based on experience with hospitalized patients and home nutritional support programs. Clearly, there is a need for research and further attention to providing such services to these individuals. Pharmacists are now finding great professional opportunity in responding to the demand for specialized nutritional support services in this rapidly growing elderly segment of our population (Barber 1988).

It has allowed successful management of patients with diseases that would otherwise have resulted in repeated or prolonged hospitalization. Nutritional therapy in the home can save health care dollars and improve clinical outcome. However, these services require careful assessment, planning, monitoring, and follow-up in order to be successful.

NUTRITION SUPPORT CARE PLAN

Once it is decided that a patient is a candidate for home nutrition support, the specifics of care should be established through a nutrition plan of care. Factors that should be included in this plan are:

1. Highlighting the individual's nutritional goals
2. Creating a patient-specific nutrient prescription
3. Selection of appropriate route for providing nutrients
4. Selection of appropriate access device
5. Establishing schedule for infusion of enteral or parenteral nutrition therapy
6. Defining appropriate preparation and administration techniques for the patient caregivers.
7. Laying out plan for safe storage and preparation of formulas, for management of equipment, and for site care.
8. Establish and document a plan for monitoring nutrition therapy

Different organizations are working on establishing recommendations for the use of dietary supplements with special consideration to nutritional support Programme. Food and Drug Administration (1997) classified health claims into four types. Statements or claims are sometimes referred to as structure or function claims.

1. They claim a benefit related to classic nutrient deficiency diseases but must disclose the prevalence of such disease in the United States.
2. They describe the role of a nutrient or dietary ingredient intended to affect structure or function in humans.
3. They characterize the documented mechanism by which a nutrient or dietary ingredient acts to maintain such structure or function.
4. They describe the general well-being from consumption of a nutrient or dietary ingredient.

The frequent nut consumption decreased coronary heart disease risk by 34%. This could be explained by higher nut content of tocopherols, Omega-3 fatty acids and selenium. Walnut polyphenols inhibit plasma and LDL oxidation. This is the same protective mechanism executed by lycopene-rich foods (tomato juice, spaghetti sauce and tomato oleoresin), fruits and vegetables (β-carotene, A, C and E) and soy isoflavonoids (genistein, daidzein). Intake of an isoflavone extract, without soy protein,

diminished atherosclerotic lesions of the aorta by 26.3–36.9% (lower and higher supplemented groups, respectively, comparing to control) and decreased the number of positive foam cells. Tea ingestion decreased by 23% aortic atherosclerotic injury and decreased aortic cholesterol and triglyceride levels.).

ROLE OF NUTRITIONAL SUPPORT PROGRAMS IN AMELIORATION OF OXIDATIVE STRESS

Oxidative stress results from the excessive production of reactive oxygen species overrides the antioxidant capability of the target cells (Vina *et al* 2006), resulting in DNA damage, production of mutated tumor-suppressor genes and inducing cell death. These pathological events are involved in pathogenesis of various diseases. Improved antioxidant defence may therefore protect against these diseases rendering intake of antioxidant vitamin and trace elements important (Berger 2005).

Various functional foods or their components possesses the ability to scavenge free radicals that indeed result in amelioration of oxidative stress (Barta *et al* 2006). Alpha-Lipoic acid is used both in the prevention and treatment of various oxidative stress related diseases and now days important dietary supplement (Durrani *et al* 2007). Karlsen *et al* (2007) suggested anthocyanin supplementation that have a role in the prevention or treatment of chronic inflammatory diseases by inhibition of NF-kappaB transactivation and deceased plasma concentrations of pro-inflammatory chemokines, cytokines, and inflammatory mediators.

Ginkgo biloba leaves extract is among the most widely sold herbal dietary supplements in the United States. Its purported biological effects include: scavenging free radical; lowering oxidative stress; reducing neural damages, reducing platelets aggregation; anti-inflammation; anti-tumor activities; and anti-aging. Boveris et al (2007) also enumerated the importance of Ginkgo biloba (Gb) extracts and showed results that Gb limit lipid peroxidation and scavenge lipid radicals by 39% and reduces the production of thiobarbituric acid reactive substances (TBARS) generation by 30% after Gb supplementation (100 mg/kg/day) for 10 days that reveals that it actively protect membranes from oxidative damage.

Cocoa powder is rich in polyphenols such as catechins and procyanidins and has been shown in various models to inhibit LDL oxidation and atherogenesis (Baba *et al* 2007). Supplementation of tomato products, containing lycopene, has been shown to lower biomarkers of oxidative stress and carcinogenesis in healthy and type II diabetic patients, and prostate cancer patients, respectively. Processed tomato products like tomato juice, tomato paste, tomato puree, tomato ketchup and tomato oleoresin have been shown to provide bioavailable sources of lycopene, with consequent increases in plasma lycopene levels (Basu and Imrhan 2007).

Green tea catechins (GTCs) are polyphenolic flavonoids formerly called vitamin P. GTCs, especially (-)-epigallocatechin-3-gallate (EGCG), lower the incidence of cancers, collagen-induced arthritis, oxidative stress-induced neurodegenerative diseases (Moon *et al* 2007). So many other reports also suggest the consumption of fruits and vegetables rich in antioxidants results in amelioration of oxidative stress and restore the balance between free radical production and their scavenging activities. As several metabolic diseases and age-related degenerative disorders are closely associated with oxidative processes in the body, the use of herbs and spices as a source of antioxidants to combat oxidation asks further attention. Immediate studies should focus on validating the antioxidant capacity of herbs and spices after harvest, as well as testing their effects on markers of oxidation (Tapsell *et al* 2006).

ROLE OF NUTRITIONAL SUPPORT PROGRAMS IN CARDIOVASCULAR PROTECTION

Functional foods can promote relevant vasodilatory effects by stimulation of nitric oxide production. Several important has the credential to fall into this category. Few of them and their effects are presented here like ginsenosides from ginseng trigger vascular relaxation by nitric oxide releasing beyond inducible effects of ginseng on SOD-1, and stimulation of calcium–potassium channels and subsequent cascade events of guanylate-cyclase/GMP system actions that make ginseng and its components promising candidate in hypertension therapies. Black tea intake also improved circulation by potent endothelial-dependent dilation of brachial artery in coronary artery disease patients. Psidium guajava (guava) leaves could control hypertension, decreasing myocardial force and inducing atrial relaxation by inhibition of cell inward calcium current (Conde-Garcia *et al* 2003), confirming a previous report. Quercetin administration (10 mg/kg; during 5 weeks), an onion and garlic flavonoid, to spontaneously hypertensive rats increased antioxidant status and decreased arterial blood pressure and heart rate, without vasodilatory effects. Allicin/ajoene from garlic inhibit macrophage nitric oxide synthase activity decreasing NO• and NOO), resulting in lesser atherosclerotic effects. Atherosclerosis could also be reduced by antioxidants that inhibit vascular endothelial adhesion molecules. Curcumin administration (200 mg/kg) inhibited (30 mg/100g) rat myocardial necrosis, decreasing collagen degradation and re-synthesis, effects mediated by scavenging of free radicals and blocking of lysosomal enzymes releasing. Propolis and grape extracts with high 280 antioxidant activities blocked myocardial ischemic-reperfusion injuries.

Homocysteine, a metabolite from methionine, is an independent cardiovascular disease risk factor, which causes thrombosis and oxidative-stress damage. It had induced increase blood coagulation and endothelial adhesion molecules, and impaired endothelial responses to arginine, both blocked by vitamin E and C supplementation. Higher ingestion of fruit and vegetables (500 g/day) by human subjects was associated with 11% decrease in homocysteine and 15% increase in folate plasmatic levels when compared to the low consumption subject group (100 g/day). Whole grain intake increases bioavailability of folate and is inversely associated with homocysteine plasma levels, contributing to decrease diastolic blood pressure by their high content of fiber, potassium and magnesium.

High cholesterol risk factor in cardiovascular diseases that further gives raise to atherosclerosis coupled with inflammation. Consumption of soy protein due to low methionine content reduces serum homocysteine concentration and its results in decreased risk of acquiring a cardiovascular disease. Furthermore, soy protein isoflavones stimulate the mechanism that is required for serum cholesterol clearance. Thus, soy protein consumption may reduce the clinical and biochemical abnormalities in diseases mediated by lipid disorders (Torres *et al* 2006). Functional foods rich in PUFA also results in reduction in LDL cholesterol and thus contributing towards protection against atherosclerosis and cardiovascular disease prevalence and progression (Gidding *et al* 2005). American heart Association (AHA) devised dietary recommendations for achieving desirable blood lipid profile as follows;

1. Replace saturated fats with lower-fat foods
2. Introduce variety of foods with unsaturated fat
3. Carefully monitor intake of food high in cholesterol
4. Severely limit foods containing trans fatty acids
5. Increase foods rich in viscous fiber

6. Increase foods containing stanol/sterol esters (special margarines, fortified orange juice, special cocoa/chocolate bars)
(Van Horn 1997; Erdman 2000; Lichtenstein and Deckelbaum 2001).

Cho et al (2006) studied the anti-atherosclerotic effect of a new synthetic functional oil containing mono and diacylglycerol from corn oil and their finding suggested that the functional oil possesses blood cholesterol-lowering effect in mouse model and inhibition effects against the atherogenic enzymes such as liver acyl-CoA:cholesterol acyltransferase and serum lipoprotein-associated phospholipase A2.

Overall, it can be concluded that consumption of diet containing functional components can reduce the risks associated with prevalence and progression of cardiovascular disease. Intentions are clearly needed for proper incorporation of these functional components in diet or in nutritional support programs meant to take care communities at high risk of CHD.

ROLE OF NUTRITIONAL SUPPORT PROGRAMS IN CANCER PREVENTION AND CURE

Malnutrition is a common problem among patients with cancer, affecting up to 85% of patients with certain cancers (e.g. pancreas). In severe cases, malnutrition can progress to cachexia, a specific form of malnutrition characterised by loss of lean body mass, muscle wasting, and impaired immune, physical and mental function (Strasser and Bruera 2002). The pathophysiology of cancer cachexia is not fully understood; however, studies have shown that cytokines are important in the alteration of carbohydrate, lipid, and protein metabolism (Tijerina 200; Skipworth *et al* 2007). Early intervention with nutritional supplementation has been shown to halt malnutrition, and may improve outcome in some patients. However, increasing nutritional intake is insufficient to prevent the development of cachexia, reflecting the complex pathogenesis of this condition (Argilés 2005; Skipworth and Fearon 2007).

Diet enriched with fruits and vegetables or their active constituents can reduced the prevalence of cancer. Diet containing active functional components can even reduce the progression of various cancer lines however; there are certain limitation in the use of several components. Staying nutritionally fortified is one positive way to take control of your life and your well-being during these conditions. Optimal nutrition allows body to function at its best. Maintaining optimal nutrition can provide several benefits for people living with cancer, including:

- Support immune function
- Improve strength and increase energy
- Preserve lean body cell mass
- Rebuild body tissue
- Decrease your risk of infection
- Improve your tolerance to treatment
- Help you recuperate faster after treatment
- Improve quality of life

Nutritional supplements containing anti-inflammatory agents, for example the polyunsaturated fatty acid (PUFA) eicosapentanoic acid (EPA), have been shown to be more beneficial to malnourished patients than nutritional supplementation alone. EPA has been shown to interfere with multiple mechanisms implicated in the pathogenesis of cancer cachexia, and in clinical studies, has been associated with reversal of cachexia and improved survival (Argilés 2005; Skipworth and Fearon 2007).

Functional food biomolecules can exert anticarcinogenic effects through diverse pathways. Modulation of cytochrome P450 enzymes, antioxidant protection of DNA, and

induction of apoptosis of cancer cells constitute the most important anticancer mechanisms of functional foods. Increasing DNA repair (folic acid); changing immunological response (carotenoids, vitamins C and E, selenium and zinc); inhibition of cyclooxygenase (resveratrol); restriction of caloric intake and absorption; decreasing time for transit of intestinal bulk, avoiding carcinogen formation and absorption (fibers); inhibition of angiogenesis; and abrogation of tumor cells proliferation (by suppressing telomerase or induction of apoptosis) also constitute important anticancer properties of functional foods.

During life span there is an enhancement of body free radical production, which is associated with oxidative DNA damage and increased risk of cancer. Tocopherols, carotenoids (from fruits such as apples) and lycopene can decrease cancers' risk. Many compounds from functional foods could suppress DNA oxidation. Apoptosis, a genetic cell death program, is important to kill undesirable cells (old, degenerated and oncogenic) avoiding inflammatory reactions. Catechin, epicatechin, quercetin, and resveratrol, which account for more than 70% of polyphenolic compounds in red wine, were shown to inhibit growth of human breast cancer cells at picomolar concentrations. The same compounds were also shown to potently inhibit human prostate cancer cells (Kampa *et al* 2000). Retinoids and carotenoids also have inhibitory activity on breast cancer cell proliferation in vitro (Prakash *et al* 2000).

Dietary bioactive food components that interact with the immune response have considerable potential to reduce the risk of cancer. Reduction of chronic inflammation or its downstream consequences may represent a key mechanism that can be reduced through targeting signal transduction or through antioxidant effects. Major classes of macronutrients provide numerous examples, including amino acids such as glutamine or arginine, lipids such as the omega-3 polyunsaturated fatty acids, DHA or EPA, or novel carbohydrates such as various sources of beta-glucans. Vitamins such as C and E are commonly used as antioxidants, while zinc and selenium are minerals with a wide spectrum of impacts on the immune system. Some of the most potent immunomodulators are phytochemicals such as the polyphenols, EGCG or curcumin, or isothiocyanates such as PEITC. There is accumulating evidence for cancer prevention by probiotics and prebiotics, and these may also affect the immune response. Genomic approaches are becoming increasingly important in characterising potential mechanisms of cancer prevention, optimising the rational selection of dietary bioactive food components, or identifying humans with differing nutrient requirements for cancer protection. Dietary flavonoids and other polyphenols have the potential to be developed as effective food supplements as well as drugs for the prevention, as well as treatment of, cancer and other disease conditions.

IMMUNOPOTENTIATING PROPERTIES OF FUNCTIONAL FOODS

Immunity is considered as ability of organism to fight against any abnormal event occurring in the body. Several scientific studies depicted the link between diet and proper health. Proper functionality of immune system is essential in maintenance of body defence against microbes and malignant cells. Recent research findings illustrated that omega-3-fatty acids, dietary fibers, vitamins, antioxidants, plant sterols and flavonoids have beneficial effects on the health aspects of humans (Manach *et al* 2004). Dietary phytochemicals play important roles in prevention of many diseases. Human diet containing these phytochemicals possesses antimutagenic and immunomodulatory properties and have shown anti-inflammatory, anti-stress and anti-cancer effects by altering immune functions (Barta *et al* 2006; Ramaa *et al* 2006).

Newborn babies possess a functional but immature immune system as a defense against a world teeming with microorganisms. Breast milk contains a number of

biological, active compounds that support the infant's immune system. A number of these ingredients can be used as supplements for infant formulas based on cow's milk (Niers *et al* 2007). Some additional components such as probiotics and prebiotics can also added in the diet to enhance the immunopotentiating properties. Lara-Villoslada *et al* (2007) reported that consumption of a probiotic product containing L. coryniformis CECT5711 and L. gasseri CECT5714 improves intestinal flora of healthy children, enhancing the defence against gastrointestinal aggressions and infections both by inhibiting pathogen adhesion to intestinal mucins and enhancing the immune function.

Fructo-oligosaccharides (scFOS) are prebiotic ingredients that improve protection against pathogens probably through promoting the growth of gastrointestinal bacteria-like Bifidobacteria and Lactobacilli: this stimulation may lead to a better development of immune repertoire and/or stimulation of the local immune response (Adogony *et al* 2007). Supplementation of minerals also imparts differential impact upon immune system (Son *et al* 2007).

Probiotics (PRO) modulate immunity in humans, while the effect of prebiotics (PRE) and synbiotics (SYN) were reported as minor but still has stimulatory and should be considered (Roller et al 2007). Kim et al (2006) suggested the supplementation of B. polyfermenticus as it has potentially positive effect on immune function. It enhances enhancing IgG production along with modulating the number of immune cell population such as CD4+ and CD8+ T cells and NK cells.

These results suggest that some probiotic bacteria have the potential to augment or modify the host immune function through the regulation of host immune cells (Matsuzaki *et al* 2007). Orally applied nondigestible carbohydrates (NDC) have been associated with immune-modulating effects and other health benefits. The effects of prebiotic carbohydrates have recently received much attention, but other NDC have been reported to induce immune modulation as well. Many different effects have been shown on parameters of innate and specific immunity, mostly in animal experiments or in vitro (Vos *et al* 2007). Immune and epithelial cells can discriminate between different microbial and bioactive plant species and knowledge generated by such studies extended the known mechanism(s) of action of nutraceuticals and probiotics beyond simple nutrition and/or antimicrobial effects. The progressive unravelling of these plant and bacterial effects on systemic immune and intestinal epithelial cell function has led to new credence for the use of probiotics and nutraceuticals in clinical medicine (Penner *et al* 2005).

CONCLUSIONS

The aim of this paper is to review important evidence-based mechanisms of functional foods with special interest on prevention of chronic diseases. However the major emphasis was given to amelioration of oxidative stress, anticarcinogenic properties and immunopotentiating behavior of functional foods and their active ingredients along with other allied health benefits. Ultimately Food scientists are responsible to convince manufacturers in the direction of new functional technologies to develop healthier foods with life-saving, life-extending potential.

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Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

IMMUNONUTRITION: THE BASES FOR DEVELOPING NUTRACEUTICAL FOODS

S. M. Ghufran Saeed*, S. Abudiat Abidi*, M. Ahmed Mesaik°,
S. Umer Abdullah* and Rashida Ali,
*H.E.J. Research Institute of Chemistry, °Dr. Panjwani Centre for Molecular Medicine and Drug Research, International Centre for Chemical and Biological Sciences, (ICCBS)
University of Karachi, Karachi-75270, Pakistan.

Abstract:

Food components have been advocated as the remedy for treatment and prevention of not only infectious but also of chronic diseases such as cancer, atherosclerosis, rhemotoid arthritis etc. The therapeutic mode of action of various food components, although still in its infancy, has been attributed to many bioactive sites of the molecules and the combinations of such molecules. The presentation reveals a mini-review of the selected foods, their components and brief insight mechanism of action at the molecular level. Recently the diet therapy has crossed many dimensions in applications in view of the advanced soft instrumentations available and the novel isolation, purification and identification technologies adopted for procuring extracts or pure components. The use of biopolymers such as β-D-glucans from mushrooms and cereals are now well known for their multi immunoceutical effects. The Lentinans being immunopotentiators, are widely sold at health shops. The LC-PUFAs (long chain polyunsaturated fatty acids) produce eicosanoids which regulate variety of reactions in immune system. Membrane phospholipids and their role in functions of prostaglandins, prostacyclins and thromboxanes is being gradually understood. The biopolymers, such as lectins from legumes help understanding the glycodes in immune modulations. The glycobiology of foods play critical role in understanding biomarkers in the immune system. Lectins are known to be active at cell surface because of their specific binding or understanding the language encoded in sugar molecules. Lectins are the key components of primary importance in complementary pathway which is a key function always occurring in human body as natural immunity. Macromolecules as citrus pectins bind with glyconjugates of cellular matrix that promotes metastasis and acts as anti cancer agent. Cereal lectins regulate body weight. Lectilns interact with insulin and the functionality of the fat cell is affected. The types of lectins and their functions will be elaborated during discussions. Carotenoids are another components of food governing the immune system, β-Carotene, Lycopene, Leutin, circulate in blood with variety of lipoproteins as carriers and are health indicators. Carotenoids participate in apoptosis, angiogenesia and in cell differentiation. The role of lycopene in prevention and cure of prostrate cancer is well registered in clinical nutrition. Vitamins specially vitamin C is extensively involved in many immunoactivation reactions. The structure function relationship of food components, their immunomodulations and health benefits are briefly discussed in the present paper.

Key words: Food components, action mechanism

NUTRACEUTICAL FOOD COMMODITIES

The father of medicine, Hippocrates has rightly said "Disease does not occur unexpectedly, it is the result of constant violation of nature's law". However, God has designed human body as an efficient and perfect self healing system that promotes auto-regeneration of metabolites of strong defensive abilities. Our immune system has the excellent capacity to fight against the diseases through a network of cells which produce the antibodies and other bioactive components for controlling the invasion. The cells of the immune system need appropriate nutrients from our diet for their nourishment, survival and proliferation. We have to understand the close association, inter-relationship and dependency of the immunity and the bioactivefood components for achieving and maintaining healh. The immunonutrition is the emerging field in recent research which finds applications in clinical nutrition and in preventive measures against variety of diseases (Trichopolou et al 2003 and Sorensen et al 1999). Strong immune system of our body is the safest defence against all sorts of illness and the production of nutraceutical foods is therefore based on relying the search for immune modulating foods. Food industries face heavy responsibilities on their shoulders to produce foods of high nutritive value and effective healing power to reduce the prevailing diseases.

Nutraceuticals, specially phytonutraceuticals (plant foods of medicinal importance) have a stable historic bond with human health that began its story in 1897 when aspirin (acetyl salicylic acid) was introduced to the world as a remedy for aches, fatigue, flue and fever without knowing the fact that salicylic acid and its derivatives are widely distributed in plants including the edible plants (Pierpoint 1994). Although healing properties of plant foods and wild plants are known to mankind since the beginning of life, this branch of nutraceuticals is reviewed in modern literature as botanical therapeutics. Functional foods, another name for the nutraceuticals also include genetically modified (GM) foods such as golden rice, deep coloured corn, soy beans, canola, rapeseeds and sunflower oils. They are rich in β-carotenes with elevated level of monounsaturated fatty acids, reduced level of trans fatty acids and appropriate ratio of omega 6 and 3 fatty acids (Raskin et al 2002). Nutraceutical foods demonstrating clear, target oriented and direct health benefits with increasing public acceptance are making their place in food markets. New chemical entities (NCEs), the synthetic drugs described in western paradigm are more expensive with many side effects will gradually and partially be replaced by Asian herbal medicines or nutraceuticals; being natural, inexpensive, safe and widely available in view of the biodiversity of plant kingdom (Cordell 2000).

BIOACTIVE COMPONENTS OF FUNCTIONAL FOODS

Foods with multidimentional health benefits mostly have more than one bioactive components present, and often are recommended for identified biomarker present in a particular food. The foods therefore reveal their healing properties against numerous diseases simultaneously. Here we will discuss some of the well known foods for the presence of specific nutrients.

Cereals health benefits

Whole grain was the entirety of peasant diet in the primitive age, offering health benefits through antioxidant rich compounds as phytates, tannins, lignans, flavonoids, phenolic acid and phytoestrogens with wide biodiversity related to functional properties. Cereals show the fantastic mechanism in controlling the chronic diseases. The epidemiological studies have revealed strong relationship between whole grain intake and reduced risks of coronary heart diseases (CHD). It is noted that an inverse association persists with the risk and the CHD among those who consumed whole grain for three or more times daily. Cereals, consisting bran and germ reduce risk of CHD by 30%. (Jensen *et al* 2004 and Erkkila *et al* 2005). The inverse relationship also exists

between whole grain consumption and the risk of developing type-2 diabetes that is dose dependent (McKeown et al 2004).

Body weight may also be reduced by constant consumption of whole grains. Studies have unveiled the evidences that suggest the role of soluble dietary fibers such as the β-glucans from oat and barley to affect the management of body weight, blood pressure, blood cholesterol and blood sugar. The diet rich in whole grain reduces BMI (body mass index) as it lowers dietary calories (Koh-Banerjee et al 2004).

The cereals contribute a bioactive compound of high nutritional status that is universally accepted as the immune modulator i.e. β-glucan. The major biological function of β-glucan is related to enhancing the immune system and reducing the serum cholesterol. The WBC are activated by β-glucan, specially macrophages and neutrophils which then are able to recognize and kill tumor cells, remove cellular debris as a result of oxidative damage, heal the damaged tissue and further activate the cells of immune system (Ross et al 1999; Wakshull et al 1999). The cereals phenolic compound including ferulic acid with purported antioxidant activity have been identified to serve as stimulator of the mammalian immune system.

Spices

Turmeric (*Curcuma longa)*, a plant of tropical Asia belonging to the ginger family is one of the spices which are known as anti-inflammatory antibacterial, antiviral, anticancer, antidiabetic etc. The active components in turmeric are the curcumins, comprising 2-5% of the solids and it also consists many other chemically related structures that are reported for health benefits (Huang et al 1992). Curcuma suppress carcinogenesis of skin, stomach, colon, liver and mammary glands by blocking tumor cells from proliferation. It acts on many molecular targets such as protein kinases and acts as medicine for bilary disorders, anorexia, cough, diabetes, wounds, hepatic disorders, rheumatism and sinusitis etc. (Aggarwal et al 2005).

Fruits and Vegetables

The fruits specially coloured fruits and leafy vegetables mentioned in "**Nutrition Five Color theory**", are the potential source of vitamins and minerals. Vitamins are arguably beneficial to our complex immune system, however, molecular details for their mode of action need new approaches to explain the immune pathology. The Vitamin C being an outstanding hydrophilic antioxidant with the ability to scavenge many reactive oxyen species (ROS) plays distinct protective role to control endothelial dysfunctions. The interaction of ROS with nitric oxide (NO), the excretory constituent of endothelium fluid is a principal vasodilator and its deficiency causes variety of disorders related to coronary arterial diseases. Vitamin C, by suppressing ROS provide fair chances to NO for playing its protective role to regulate the smooth muscle vascular tone (Korantzopoulos and Galaris 2003). Vitamins act synergetically, for example, Vitamin C and K_3 kills cancer cells through autoschizis which is a novel form of cell-death. These vitamins are used as coadjuvants in anticancer therapy (Verrax et al 2003; Sanchez-Moreno et al 2004). The role of vitamin D as an environmental factor involving immune system to reduce the prevalence rate of autoimmune diseases such as multiple sclerosis, arthritis and juvenine diabetes is gradually understood (Cantorma 2000).

Nearly all parts of the vegetables are rich in sugar beet roots, cucumber, spinach and tumeric were effectively used to prevent chemically induced skin tumor (Irene et al 2002).

Fats and Oils

Fatty acids are known to modify immune responses including organization of cellular lipids and interaction with nuclear lipids. The n-3 PUFA (polyunsaturated fatty acids through their anti-inflammatory properties are able to protect arterial wall by controlling deterioration of plaque lipids and fatty acids are well recognized as gatekeeper of

immune cell regulation (Yaqoob 2003, 2004). Several studies have supported that high fat intake suppresses natural killer (NK) cell activity, specially dietary supplementation with eicosapentaenoic acid. However the other long carbon-chain n-3 or n-6 polysaturated fatty acids (PUFAs) reduces the NK activity and do not support such reactions (Thies et al 2001).

Dietary Lectins

Cereal lectins are reported to lower plasma glucose, insulin, cholesterol and triglycerides (Slavin et al., 1999). Whole grains responsible for bulking the feaces, promoting laxation and reducing transit time of feaces affect the functions of colonic wall and process of fermentation which are significant in immune system. Soluble dietary fiber fermentation in the colon affects the highly integrated immune system by increasing CD4 + T-cells in lymph (Lim et al 1997).

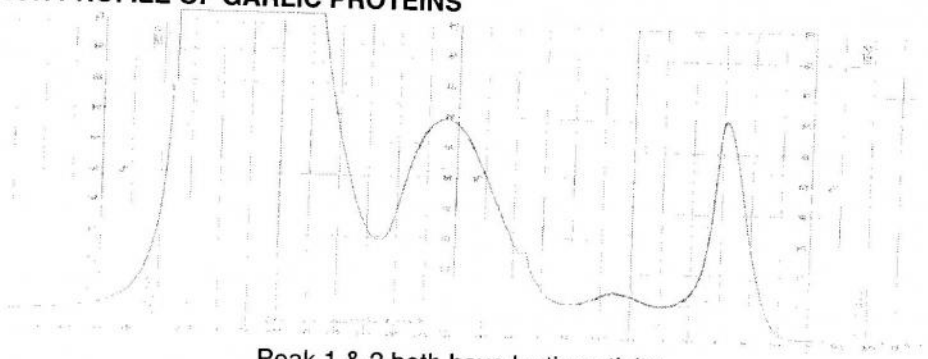
β-glucan from mushrooms have been used as immunomodulating foods for years and extracts of many mushrooms are available as nutraceutical food on health shops. Lentinan is sold as immune potantiator. The biological superiority of garlic proteins with multiple health benefits are widely referred in literature.

Garlic Lectins

Lectins, the extremely active biopolymers are closely related to immune system through a number of factor including cytokines, the proteins controlling biological responses to transduction molecules (Jean-Pierre et al 2002). The advances in glycobiology and in view of the carbohydrate recognition capacity of cytokines has generated a new concept of lectin-like activity (LLA) in glycoimmunology. Plant lectins are widely reported including the lectins from the family Alliaceae which are mannose specific. Garlic lectins, the dimeric proteins of 12 kDa from leaves and of 15 kDa from bulb are reported earlier and some of the *Alliaceae* lectins possess HIV inhibiting activity. Although garlic bulb lectins are not involved in controlling retroviruses, however the garlic extract is known for its therapeutic activities for years (Xia and Ng 2005). We therefore isolated some proteins and identified one of them showing lectin like activity.

The garlic bulb extract was obtained by crushing 100 gm of bulb in 200 ml of 20 mM Na-phosphate buffer of pH 7.6 after shaking constantly in an automatic incubating shaker for 24 h at 4ºC. After cloth filtration it was contrifagat at 100 g and the proteins were separated by sorting out method with the addition of $(NH_4)SO_4$ at 70% saturation, the precipitated proteins were redesolved in the same buffer and lypholysed for storage at 7-8°C. The proteins were dissolved in the same buffer consistilng 5 mg of protein/ml. A solution of 500/µg ml was loaded on the Sephadex G-100 column (70 x 2.5 cm) equaliberated with the same phosphate buffer and proteins were separated with a flow rate of 0.1 mL/min. as shown in Fig. 1.

ELUTION PROFILE OF GARLIC PROTEINS



Peak 1 & 2 both have lectin activity.

Haemagglutination Assay

The heamagglutination assay was carried out according to the method of Shi et al, (2007) and Burger (1974). The phosphate buffer saline (PBS) solution was prepared by dissolving 8 g of sodium chloride, 0.2 g of potassium chloride, 1.15 g of disodium hydrogen phosphate and 0.2 g potassoium dihydrogen phosphoate in 1 liter of DDD water. The standard lectin (soya) was prepared by taking 1 mg in 1 mL PBS, RBPs (0.9 mg) were dissolve in 1 mL PBS and trypsin solution was obtained by dissolving 1 mg enzyme in 1 mL of PBS. The RBC suspension was prepared by drawing 2 mL blood from the healthy volunteers in the anticoagulant fluid (sodium citrate solution). The suspension was washed twice with 15 mL PBS and 100 µL blood was diluted to 5 mL by adding PBS to finally produce 2% suspension of RBC.

The above figure showing Agglutination activity of garlic lectin.The peak one shows lectin activity. Presently the peak is subjected for further immunomodulating actilvities using chemoilluminiscence assay for exploiring the oxidative burst response. However as lectins are involved in immune modulation it seems that part of the nutraceutical properties of the garlic is due to the presence of lectin like proteins.

CONCLUSIONS

While evaluatilng the chemopreventive properties of various phytonutrients, it may be summarized here that both the biopolymers and low molecular weight bioactive substances equally are involved in variety of immunoenhancing properties. Such phytochemicals having positive impact on defensive mechanism of immune system should be the bases of formulating nutraceutical foods. Garlic lectins, as one of the many components present in garlic extract make it the most potent nutrient-dense food that claims the multiple therapeutic claims. Foods consisting immune active components as biomarkers seem to be the appropriate candidates to play their role in diet therapy and production of nutraceutical foods.

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Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

MICRONUTRIENT FORTIFIED MULTI CEREAL BISCUIT: AN EMERGING AND COST EFFECTIVE FOOD MATRIX FOR SCHOOL NUTRITION PROGRAME

Mahboob Aly Manji
Vita Pakistan Ltd, Lahore.

INTRODUCTION

Over 1.5 billion school aged children around the world suffer from hunger and deficiencies of vitamins and minerals (micronutrients) which are needed in small quantities but are very essential for good health. Due to micronutrient deficiencies antibodies are not developed to the required level in children therefore these children are susceptible to disease. The micronutrient deficiency is a hidden hunger which in turn also acts as a barrier to learning. The retaining faculty of learnt lessons is hampered. School Feeding programs throughout the world have successfully attracted children to school and retained them by offering them what they would probably not get elsewhere: hot food or nourishing snacks. The primary objective of a School Feeding program is to provide nutritious snacks to alleviate short-term hunger, enabling children to learn. A hungry child cannot concentrate. A hungry child cannot perform. Hungry children are unlikely to stay in school. School-based feeding programs have proven effective in encouraging enrollment, increasing attentiveness spans and improving attendance in school.

In the developing world it is expedient to develop a cost effective school feeding and nutrition program through which millions of malnourished children between five years to twelve years of age can benefit. As most of the children in the developing countries live in miserable poverty, have not had enough to eat since birth and will never complete primary school. Sadly, without education, their future will undoubtedly be as bleak as their past and their children's future will be a distressing echo of their own. The intergenerational transmission of poverty is insidious and persistent. All the governments in developing countries strive to improve their national economies, but where majority of people are poor, underfed, insecure and uneducated the economic development is difficult and slow.

Only in Pakistan due to non implementation of school feeding and nutrition program, around 1,150,000 student years are wasted, the losses impact on the GDP is around 4.5% – the resultant losses are approx. in USD 3200 million or annual losses per capita is USD 20.00.

Malnutrition is one of the major public health challenges in Pakistan. Malnutrition occurs throughout the lifecycle resulting in low birth weight, wasting and stunting. National Nutrition Survey 2001-2002 provides information on various nutritional aspects of the vulnerable group of population. Micronutrient deficiency in Pakistan is common and reflects a combination of dietary deficiency, poor maternal health and nutrition, high burden of morbidity and low micronutrient content of the soil especially for iodine and zinc.

To fulfill the nutritional requirement of a common citizen of Pakistan is the prime objective of the government as Pakistan's international commitment under Millennium Development Goals (MDGs). The Government of Pakistan plans to bring down the prevalence of underweight children of less than 5 years of age by 20% and reduce up to 13 percent the proportion of population below minimum level of dietary energy consumption. At present around 20% people of Pakistan are suffering from depleted dietary energy.

The 5-12 years children are most vulnerable. On average 38% children are underweight by age and if split between boys and girls; over 44% girls are underweight. To address these challenges; one of the major interventions is school feeding / nutrition program. It has been found that a total of 38.6% children are underdeveloped and 45.3% were underweight; 25.2% were below standard weight for height. Among the urban children, 30% were below the standard for height (undersized), and 32.3% were below the standard for weight (underweight); 32.7% were thin. Of the rural children, 40.9% were underdeveloped, 64.7% were underweight and 33.3% were thin. The rural female group was the most affected and malnourished with 61.8% underdeveloped and 84% underweight; 67.1% were thin(WHO).

NUTRITIONAL BENEFITS

There is little evidence to suggest that school feeding programs have a positive impact on nutrition for participating children. In some instances, parents may provide less food at home, and the school meal simply replaces a home meal rather than adding food to the child's diet. (There is some evidence, however, that providing breakfast rather than lunch could diminish this substitution effect.) In other instances, the food provided by the feeding program may not adequately address the complex nutritional deficiencies in the children's diets (protein-energy malnutrition and lack of micronutrients). Also, the programs may be too irregular to have a meaningful effect on the long-term nutritional status of the children. On the other hand, there is some merit in the argument that in the most impoverished settings, even seemingly minimal, short-term efforts are worthwhile (food as a human right)—even though it is not clear that school feeding programs reach the neediest children.

IMPACT ON EDUCATION AND THE LINK BETWEEN HUNGER AND LEARNING

Much evidence suggests that children who are hungry or chronically malnourished are less able to learn, regardless of the setting. But the converse—that children in school feeding and food for education programs are better able to learn—only holds true when the food is accompanied by other inputs related to school quality.

IMPACT ON ATTENDANCE

The evidence strongly suggests that school feeding programs can increase attendance rates, especially for girls. School feeding or take-home ration serve as incentive for putting children in school and is promoting and encouraging daily attendance. This is like a short-term solution, however, because if there is no change in the quality of schooling (or increase in intrinsic demand for education) attendance is likely to drop once the food incentive is removed. The promise of at least one nutritious meal each day attracts children to school, boosts enrolment, promotes regular attendance and enhances student performance. In the poorest district of Pakistan this simple strategy can double primary school enrolment in one year.

The School Feeding program with above stated three major objectives will help in improving nutritional status and educational performance of primary school children, reducing gender disparity in enrolment and dropout rates and creating awareness to adopt healthy life style. The Government of Pakistan through ministry of social welfare and special education started a program namely "TAWANA PAKISTAN", Under this

program a special Nutrition package has been designed for the school girls which comprises nutritious biscuits (100 gm.) and nutrient milk drink (250 ml) enough to meet 1/3rd requirements of the school girls for calories, vitamins and trace elements. These nutrition packs were specially designed by Institute of Food Science and Technology, UAF, Faisalabad. The nutrient snack pack contains 650 Kcal with 24g of Fat and 20 gram protein with 60% requirement of micronutrients. The cost of program is worked out to be USD 52.00 per child per school year which includes the cost of snack pack and 20% is the logistic and program supervision cost – thus 100 Kcal was costing PKR: 2.42 (1 USD = PKR 60.50).

Now the concerned Ministry has changed the Tawana Pakistan snack pack which is now to contain one bottle of fruit juice drink and a pack of 50g biscuit. The nutrition dense is 250 Kcal with 5 gram Fat and 5 gram protein. This new program is costing government around USD 85.00 per child per year as reported in the Daily Time newspaper in its 21.03.2007 edition and the Ministry of Social Welfare News letter that Government of Pakistan is launching Tawana Pakistan program by spending Rs. 7.63 billion in three years to provide free packs to 550,000 girls in 50 districts of Pakistan. – Thus 100 kcal will cost PKR: 5.86 (1 USD = PKR 60.50).

At present around the world there are 110 known school nutrition and feeding program implemented by national government, international donor agencies and international non-governmental organizations. Except for the few programs, most of the on site school feeding and nutrition program comprise of free distribution of biscuits. The biscuit is easy to produce, has long shelf life, is liked by the children of all ages, is easy to transport without fear of leakage or breakage and thus it is easy to monitor and supervise the program. Children Health Environment and Nutrition (CHEN) Foundation applied Triple- Helix approach by involving industry (Vita Pakistan) and academia (National Institute of Food Science and Technology – University of Agriculture Faisalabad) to develop Micronutrient Fortified Multi-cereal Biscuits for school feeding and nutrition program across Pakistan by utilizing / using indigenous raw-material. The philosophy behind this development is to prepare a guide line for those professionals who are involved in writing and planning a school feeding and nutrition program for Pakistan and for other nations / areas similar to Pakistan. The following were the prime objectives in development strategy:

- To ascertain the actual nutritional gap
- To utilized available resources and raw-material to its best to get the best nutritional results of the developed product
- To use most cost effective production technology, which is at present under utilized by the industries
- The product so designed should have long shelf life, easy to transport, palatable, enjoyable, pleasant in taste, easy to eat in one go, easy to digest, have an all day lasting micronutrient effect and have enough quantity to address short term hunger
- The product individual pack should be from locally available raw-material and packaging possible on locally fabricated equipment / machine.
- The product outer packaging should be robust so to sustain long distance transport without damage or breakage.
- The production nutritionally so dense that it could cover the current macronutrient gap in effected regions
- The last but not the least the newly designed and developed product should be cost effective.

In various meetings it was decided to develop a micronutrient fortified multi-cereal biscuit to overcome the daring threat of malnutrition as at present 25% of the installed

capacity of around 300,000 metric tons per year of Pakistan biscuit industries is going unutilized and wasted. The micronutrient fortified multicereal biscuits major ingredient is wheat flour- almost 50% of the weight of biscuit is available around the year. While developing the product following nutritional gap in Pakistani children (from low income families) aged 5-12 years after daily food intake at home will have to be taken into account:

In terms of nutritional intake average caloric availability per day and current gap is approximately 20% of the combination of dietary deficiency of Fat, Protein and Carbohydrates. The micronutrient deficiency could not be worked out without complete nutrition survey and eating habits of various regions of Pakistan and official recommended daily dietary intake (RDI). But moderately the group feels that our Pakistani child is taking almost 50% of the required micronutrient in a day and leaving behind 50%. Thus the micronutrient deficiencies in all categories be it vitamins, minerals or trace elements are 50% with exception of Vitamin A, Iron, Zinc and Iodine.

NURIENT	UNIT	DAILY		
		NEED	INTAKE	GAP
Energy	**Kcal**	2000	1600	400
Fat	**gram**	65	50	15
Proteins	**gram**	50	40	10
Carbohydrates	**gram**	300	250	50
Sodium	**Mg**	2400	2000	400
Potassium	**Mg**	3500	3000	500
Dietary Fiber	**gram**	25	20	5

WHY MICRONUTRIENT FORTIFIED MULTICEREAL BISCUITS

Hunger is the major barrier to learning. School Feeding and nutrition programs throughout the world have successfully attracted children to school and retained them by offering them what they would probably not get at home - a nourishing pack of biscuit. The primary objective of a School Feeding program is to provide these biscuits pack to alleviate short-term hunger, enabling children to learn.

A hungry child cannot concentrate. A hungry child cannot perform. Hungry children are unlikely to stay in school. School-based feeding programs have proven effective in encouraging enrollment, increasing attention spans, and improving attendance in school. The symptoms of the micronutrient deficiency related diseases are often not felt or visible. This is known as hidden hunger and is rampant in all segments of society – rich and poor alike. The basic reason is imbalanced dietary intake. This hidden hunger leads to mental impairment, poor health and productivity and in some cases deficiency of certain micronutrients may be fatal. The children and teenagers in developing countries suffer most due to malnutrition and hidden hunger.

Well balanced, wholesome and nutritious diet during childhood and teen years is essential for normal growth and mental development. Nutrient intake, both macro and micro, during childhood and teen years is of critical importance because this is a period of life during which the velocity of growth accelerates in both a linear and body cell mass fashion. While the role of macro nutrients in food like protein, fat, carbohydrates and water is well known and accepted, the importance of micro nutrients such as Vitamins, Minerals and trace elements is often neglected.

- Growing children are at high risk for malnutrition, undernourished children often have deficient or delayed cognitive and mental development.

- Malnutrition dulls motivation, curiosity, reduces playing and physical activities.
- Iron deficiency impairs brain development and working stamina.
- Iodine deficiency causes cretinism (deficiency of thyroid secretion resulting deformity and idiocy) as well as other forms of learning disability.
- Deficiencies of essential fatty acids (DHA, EPA) affect intelligence and vision.
- Low intakes of vitamin B1 (Thiamine) show an inability to concentrate, confusion of thought, uncertainty of memory, anorexia, irritability and depression.
- Diets lacking in vitamin B2 (Riboflavin) are associated with changes in personality.
- Low vitamin C intake may result in poor reaction time and increased fatigue.
- Poor cognitive development is linked to deficiencies of zinc and selenium.
- Calcium is vital for the growth and strength of the children. Milk based products are excellent source of calcium since they also contain Vitamin D which is necessary for the body to be able to absorb the calcium.
- Each form of micronutrient deficiencies may have independent as well as cumulative, interactive effects on learning and behavior.
- Lysine is an essential amino acid needed for growth and to help maintain nitrogen balance in the children. It helps the body absorb and conserve calcium. Lysine supplementation in biscuits helps to increase memory and improve cognitive and learning skill and also improves retention of learnt lesson for longer duration.

An 80 gram pack of micronutrient fortified multi-cereal biscuits is a well balanced, completely wholesome, nutritious and delicious dense food matrix for school feeding and nutrition program. An 80 gram biscuit pack contents 400 Kcal. The ingredient composition of micronutrient fortified multi-cereal biscuits is high quality wheat flour 40%, blend of cereal flour (rice, barley, soya, peas, 15%, hydrogenated vegetable fat 22.5% (blend of cotton seed, canola, sunflower, soya-bean), white cane sugar 15%, biscuit premix 4% micronutrient premix in soya protein and lysine 3.5%.

Table 2.The micronutrient fortification in biscuits

MICRONUTRIENT VALUES		
Energy per 80 gram serving size **Calories: 400 Kcal** **Calories from Fat:162 Kcal**		
Nutritional Contents	**Per 80 g**	**Per 100 g**
Energy	400 Kcal	500 Kcal
Fat	18.0 g	22.5%
Proteins	10.0 g	12.5%
Carbohydrates	45.0 g	
Sodium	600 mg	24% of DRV
Potassium	800 mg	23% of DRV
Dietary Fiber	3.0 g	3.8%
Moisture (when packed)	2.6 g	3.5 % max

The micronutrient premix is out-sourced in two different matrix, vitamins and few mineral are blend in soya protein isolate whereas second micronutrient premix is locally available comprising of fine minerals such as calcium, choline, chloride, iodine, magnesium and phosphorus blended with lysine and mixed in double refined iodized salt.

Table 3: Premix formulation of biscuits

PREMIX FORMULATION

Vitamin A	300 mcg
Vitamin B1	0.7 mg
Vitamin B2	0.6 mg
Vitamin B3	8 mg
Vitamin B5	3 mg
Vitamin B6	0.3 mg
Vitamin B7	12 mcg

Vitamin B9	180 mcg
Vitamin B12	1 mcg
Vitamin C	30 mg
Vitamin D	2 mcg
Vitamin E	5 mcg
Vitamin K	25 mcg
Chromium	5 mcg

Copper	150 mcg
Fluoride	0.6 mg
Iron	5 mg
Manganese	0.8 mg
Molybdenum	20 mcg
Selenium	12 mcg
Zinc	8 mg

MINERAL PREMIX FORMULATION

Calcium	500 mg
Choline	100 mg
Chloride	300 mg

Iodine	75 mcg
Iron	3 mg
Magnesium	150 mg

Phosphorus	250 mg
Potassium	800 mg
Sodium	600 mg

Micronutrient fortified multi-cereal biscuits weigh 10 g per biscuit packed in multi layer BOPP /VMCPP with one year shelf life, manufactured in conformity with applicable food safety standards and HACCP / codex standards. Micronutrient fortified multi-cereal biscuit is most cost effective nutrient dense food matrix for school feeding program as 98% ingredients (raw-material, packaging material and certain micronutrient) are locally produced /manufactured.

Micronutrient fortified multi-cereal biscuit for school feeding and nutrition program is divided into six segments, to enhanced transparency level during the process of procurement and for future price inflation:

- Cost of Raw-material and packaging material
- Production overhead
- Logistics and transportation
- Delivery and distribution
- Monitoring and supervision
- Manufacturer profit

The cost break- down of Micronutrient fortified multi-cereal biscuits 80 gram pack for school feeding and nutrition program is as below:

Table 4. The cost breakdown of Micronutrient fortified multi-cereal biscuits

			Cost per unit		Cost per child per year	
#	Particular	Unit	PKR	USD	PKR	USD
1	Cost of raw material including packaging material cost and cost of micronutrient	Kg	Rs 59.250	$0.979	Rs 948.00	$15.670
2	Production overhead, fuel, labor and maintenance	12%	Rs 7.110	$0.118	Rs 102.00	$1.686
3	Local transport from production unit to district HQ. Subject production unit is within range of 700 kilo meters	Unit	Rs 0.150	$0.0025	Rs 30.00	$0.495
4	Distribution cost from district HQ to primary schools. Subject schools are within 50 km from warehouse	Unit	Rs 0.090	$0.0015	Rs 18.00	$0.298
5	Manufacture profit, taxes and other misc expenses.	Kg	Rs 7.000	$0.1157	Rs 112.00	$1.851
6	Government monitoring and supervision cost (Cost 5% of program cost as per PC1)	Unit	Rs 0.303	$0.0050	Rs 60.50	$1.000
Total cost per child per school year					Rs 1,270.50	$21.000

If the Ministry adopts this new proposed food matrix for school nutrition program, this would reduce the cost of the government to around Rs.698.5 million per year to serve same number of girls; thus saving almost 75% of the present proposed cost (Rs.7.63 billion for three years). In other words 100 Kcal would cost Rs: 1.27 instead of presently proposed cost of 100 kcal which is Rs: 5.86. Or From the same allocated Rs.7.630 billion funds over 2,000,000 students can feed for three years instead of presently proposed 550,000 students.

Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

PRODUCTION OF XYLOOLIGOSACCHARIDES FROM AGRICULTURAL WASTES

Semee Mumtaz*, Salim-ur-Rehman*, Nuzhat Huma*.
*National Institute of Food Science and Technology, University of Agriculture, Faisalabad.
E-mail: semee.mumtaz@gmail.com

Abstract

Xylooligosaccharides are now long been acknowledged as therapeutic and food ingredients with several direct and indirect health effects. In present study, four agricultural wastes i.e corncob, rice husk, almond shells and sheesham wood were used to produce xylooligosaccharides through chemical and enzymatic action. The methodology comprised of chemical pretreatment, enzymatic hydrolysis, refining of crude xylooligomers solutions which were then spray dried. Pretreatment with 1g/L H_2SO_4 at 65°C for 10 hours and 2g/L H_2SO_4 at 70°C for 15 h (for sheesham wood) followed by filtering and washing to pH 5.0 was done before heat treatment. Slurry was subjected to steaming at 145°C for 30 min. Steamed substrates were pounded to facilitate enzymatic hydrolysis at 55°C at pH 5.0. After refining, the xylooligosaccharides solution was spray dried. Both crude and refined xylooligosaccharides were measured through HPLC. Maximum yield of xylooligosaccharides was obtained from corncobs, followed by rice husk, almond shells and sheesham wood. Sheesham wood, a new substrate tested revealed potential results.

Keywords: Xylooligosaccharides, almond shell, sheesham wood dust, corncob, rice husk, agricultural wastes

INTRODUCTION

Xylooligosaccharides (XOs) are newly invented functional food ingredients which have immense impending to improve the quality of numerous food products. Moreover, these sugars acquire beneficial health properties, such as low cariogenicity, non-digestibility, improving anti-freezing property, enhancing the effects of intestinal flora, dietary fiber like action and water retention activities (Koga and Fujikawa 1985). XOs are usually produced by enzymatic hydrolysis from xylan, which is a major component of plant hemicellulose. Among the several potential biomass resources, almond shells may be the finest substrate for XOs production, because it allows processing at a high concentration of xylan, offers an elevated yield, and have relatively low lignin content (Nabarlatz *et al* 2007).

Pakistan is an agricultural country and there are number of wastes/byproducts from this sector which can be explored for further processing. Biomass whether as sugar crop, starch crop, or lignocellulosic waste material, provide a range of valuable ingredients that can be separated by chemical, and or enzymatic treatments. These wastes if untreated add to environmental pollution, and with this many useful components of this material

fritter away. Some conventional uses of such materials are in practice e.g corncobs and bagasse are used as fuel, similarly wheat straw and rice husk are used as animal feed; but still many go untreated like almond and peanut shells, wood dust etc. Lignocellulosic biomass is a renewable resource that can be treated via fractionation process to yield precursors for the chemical, food, and pharmaceutical industries. Fractionation of lignocellulosics is the core of "biomass refinery" concept, the aim of which is to separate the main polymers that form biomass (i.e cellulose, hemicelluloses, and lignin) and further process them into marketable final products (Overend and Chornet 1987).

Xylan is a kind of heteropolysaccharide. The main chain is composed of D-xylose, and the branches consist of L-arabinofuranose linked to the *O*-3 position of D-xylose residues and D-glucuronic or *O*-2-methyl-D-glucuronic acid linked to the *O*-2-position (Eriksson *et al* 1980, Das *et al* 1984). Some of the D-xylose residues are acetylated. Xylan appears to be a major interface between lignin and other carbohydrate components in plant cell walls (Selvendran 1985). Physical access to xylosic linkages in xylan is restricted by the surrounding lignocellulosic components as well as substituents on its backbone. Consequently, the raw materials are conventionally treated before enzymatic degradation (Dekker 1985). There are several pretreatment methods that can expose the polysaccharide components to enzymatic hydrolysis, including high-concentration alkaline extraction (Kusakabe *et al* 1976a), low-concentration alkaline treatment (Kusakabe *et al* 1976b), acidic pretreatment (Olsson and Hahn-Hagerdal 1996) and cooking (Sasaka and Ozer 1995). Some of these methods are successfully used in the xylose, xylitol or butanediol production. However, alkaline extraction and acidic pretreatment are not suitable for the production of XOs because the former causes serious corrosion and alkali pollution and the latter produces a lot of xylose in the hydrolysate.

The market for functional ingredients is increasing (Pizzoferrato 2003) since awareness regarding health, nutrition and diet has increased immensely among consumers. Thus there is great potential for XO production based on growing demand of new functional ingredients. Due to numerous health and technological benefits associated with xylooligosaccharides, there is a potential to incorporate XOs in novel health foods. In Pakistan, there are number of agroindustrial wastes with great potential to be used as feedstock for production of xylooligosaccharides. The mandate of this research was to explore this potential by extraction of XOs from different agricultural wastes using xylanase enzyme.

MATERIALS AND METHODS

All four substrates were purchased from the local market of Faisalabad. Corncob, rice husk, almond shells and were ground with grinder. The ground material was sifted through 1mm screen. Chemicals were purchased from Sigma- Aldrich exportation, Box 14508, St. Louis, Missouri, 63178, USA.

Proximate analysis of the all substrates was conducted using AACC Methods (2000). The ground substrates were soaked in dilute acid (1.0 g/l H_2SO_4) at 65°C for 10 hours and 2g/L H_2SO_4 at 70°C for 15 h (for sheesham wood) at 60°C for 12 h, filtered, washed with water and adjusted to pH 6.0. The pretreated samples were autoclaved at 145°C for 30 min. When the desired processing time (30 min) was attained, electricity was turned off. After cooling, the autoclave was opened and the steamed almond shells were collected. Water was added in steam treated almond shells in the almond to water ratio of 1:12 and mashed with blender at 3500 rpm for 15 min. Part of the resulted slurry was used for chemical analysis, and the rest was for enzymatic hydrolysis (Yang *et al* 2005).

For chemical analysis, the slurry was filtered. The filtrate, named as extract hereinafter, was analysed for xylose and XOs. About 1000 ml of almond shells slurry was adjusted to pH 4.5. Hydrolysis of xylan in the slurry was initiated by the addition of xylanase at 5 U/g. The sample was placed in the shaker at 150 rpm and 55°C for 5 h, followed by centrifugation at 3500 rpm for 15 min to get supernatant. The XOs were adsorbed by the activated charcoal powder that was packed in the column (400 mm) (Yang *et al* 2005).

Reducing sugars were analysed by the DNS method (Miller, 1959). The monosaccharides and XOs in the raw and hydro1ysed extracts were determined by HPLC (Izumi *et al* 2005). The analysis was done in HPLC model LC 200 (Perkin Elmer, Life and Analytical Sciences, 710. Bridgeport Avenue Shelton, CT 06484-4794. USA) using an Aminex HPX-87H column at 30°C. The solvent was 0.001N H_2SO_4 and flow rate was 0.6 ml/min. Refractive index (RI) detector was used for quantification (Suwa *et al* 1999).

XOs were recovered from the hydrolyzed solution by drying in a (Niro Atomizer, model no 1293, Copenhagen, Denmark) spray drier operated at feed and air flow rates of 8 mL/min and 670 L/h, respectively. The inlet and outlet temperatures of the air were maintained at 150 and 85°C, respectively. The dried XOs were stored at 5°C (Nabarlatz *et al* 2005).

Data obtained from these analyses was analyzed statistically using analysis of variance technique as described by Steel *et al* (1997).

RESULTS AND DISCUSSION

The main purpose of this research was to produce xylooligosaccharides from different substrates which are locally available and to analyze their potential as raw material for processing. According to the findings of the present study, the xylan and cellulosic contents of substrates are highest which was the criterion for their selection. Xylan rich feedstocks are potential raw material for XOs production. Mean values for proximate analysis as given in table 1, shows the moisture, ash, xylan, cellulose and lignin contents of all substrates to be used for XOs production. These values confirm the findings of Jenkis *et al* (1998) who studied the combustion properties of different biomass. The corncob is of highest xylan contens with 36.5%, this led to the highest extraction of XOs as the % yield of spray dried XO shows at temperature of 85°C. The sheesham wood dust is second most rich in xylan composition (30.5%). The lignocellulosic bond is strong enough in hard woods, that is why the resultant production of XOs after chemical and enzymatic hydrolysis is far less than that of almond shells and rice husk. The residual chains with xylan polysaccharide in almond shells result in the production of undesirable compounds like lignin (25.3%), though the initial xylan contents are 27.5% given in table 1. These results are in accordance with work previously reported by researchers like (Nabarlatz *et al* 2005; Babu 2007).

The amounts of XOs in the all four substrates before and after enzyme hydrolysis are exhibited in figure 1 and 2. HPLC analysis of monosaccharides and oligosaccharides analysis showed that before enzymatic hydrolysis, the content of XOs was very low (Figure 1, Table 2); the highest concentration was found in corncobs (15.9%) followed by rice husk (14.8) and lowest in sheesham wood dust which was 0.9%. When the hydrolysis was initiated with the addition of xylanase, the relative content of xylose and glucose decreased rapidly, but the relative amount of xylose containing oligosaccharides increased rapidly which indicated that the main products of the reaction were xylose-containing oligosaccharides. The content of XOs after enzymatic hydrolysis was 49% (Figure 2, Table 3) in case of corncob which was lower than the previous studies by Yang

et al (2005) and same trend was observed in other three feedstocks e.g extraction from almond shell was higher through autohydrolysis reported by Nabarlatz *et al* (2005). Before enzymatic hydrolysis, the amounts of glucose, arabinose and xylose in the extract were not in considerable quantities which were further reduced during hydrolysis. The ratio of arabinose: glucose: xylose of hydrolyzate was 2.4: 2.06: 3.6. Nabarlatz *et al* (2005) reported that the yield of oligosaccharides in the almond shell at 150°C was 42% and concentration of arabinose was 2.4% in hydrolysed almond shell. Yang *et al* (2005) carried out the enzymatic hydrolysis of corncobs and found the similar trend. The difference in the concentration of XOs may be due to varietals differences. Local hard shell almond variety was used in present study and the enzyme may not cause the complete degradation of xylan due to hardness. To overcome this problem, higher doses of enzyme are considered necessary for efficient xylan degradation.

The recovery of XOs at different temperatures is given in table 3. To optimize the temperature conditions, three different temperatures i.e 75°C, 85°C and 95°C were selected for drying the xylooligomer solutions. The most suitable temperature observed was 85°C for all four substrates and this trend of maximum yield is shown in figure 3. These observations are in line with the previous work of Nabarlatz *et al* (2005) who spray dried the refined XOs solutions using almond shells through autohydrolysis technique.

CONCLUSIONS

Objective of study was utilization of indigenous agricultural byproducts to produce functional ingredient to be used in processed food by exploring new substrate for XOs production. The present study concludes that agricultural substrates used are useful source of XOs. Improvement in xylan hydrolysis is helpful to increase the yield of XOs. The cumulative yield of XOs after enzymatic hydrolysis was 49.0%, 38.0%, 36.0%, 25.5% for corncob, rice husk, almond shells and sheesham wood respectively for and the purity of XOs was over 70 g/100 g. The xylan-lignin complex present in almond shells was affected during steaming at 145°C for 30 min. Almost, all the xylan was extracted from the lignin after steam application and subsequent enzymatic hydrolysis.

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Table1: Mean values for proximate composition of substrates (%)

Substrate	Moisture	Ash	Xylan	Cellulose	Lignin
Corncob	7.9	1.3	36.5	33.0	15
Rice husk	6.5	10.4	17.7	39.4	22
Almond shells	6.8	2.6	27.5	35.9	25.3
Sheesham wood dust	7.2	7	30.5	38.5	22

Table 2: Concentration of different components before enzymatic hydrolysis*

Substrate	Xos (%)	Glucose (%)	Arabinose (%)	Xylose (%)
Corncob	15.9	9.7	3.7	8.7
Rice husk	14.8	8.5	4.6	7.8
Almond shells	9.8	2.4	2.2	3.6
Sheesham wood dust	0.9	4 9	6.4	2.9

*Values expressed are mean values.

Table 3: Concentration of different components after enzymatic hydrolysis*

Substrate	Xos (%)	Glucose (%)	Arabinose (%)	Xylose (%)
Corncob	49	2.7	-	1.3
Rice husk	38	3.8	-	1.2
Almond shells	36	1	-	1.8
Sheesham wood dust	25.5	1.6	0.9	5.7

*Values expressed are mean values.

Table 4: % yield of spray dried XO recovery at different temperatures*

Temperature	Corn cob	Rice husk	Almond shells	Sheesham wood dust
75 °C	61	47	40	25
85 °C	65	45	42	26
95 °C	57	40	36	21

*Values expressed are mean values.

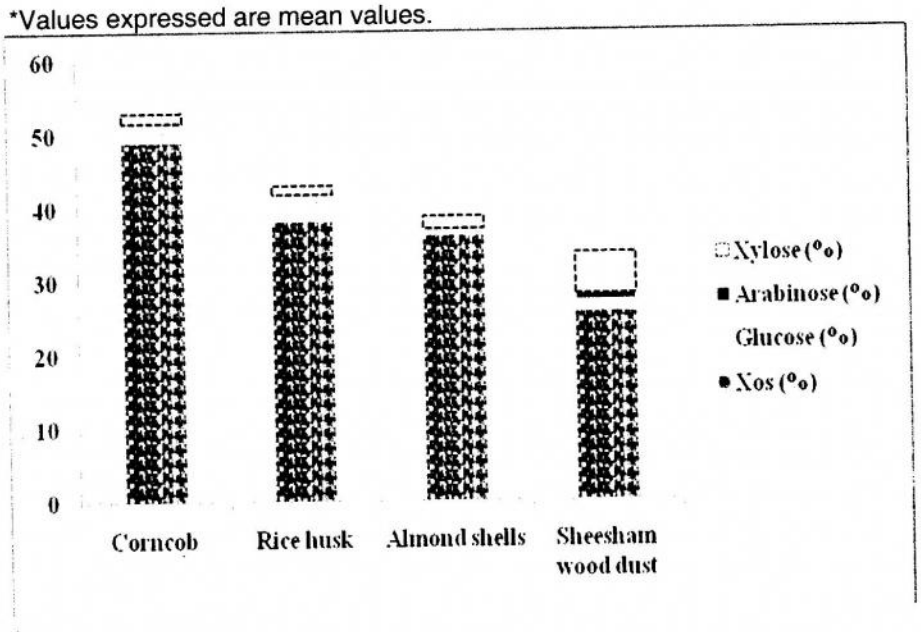

Figure 1: Concentration of different components before enzymatic hydrolysis

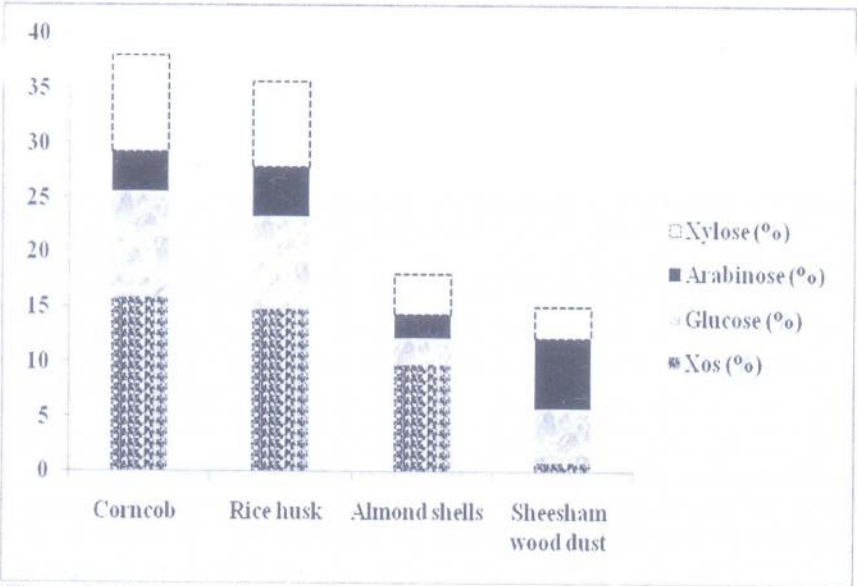

Figure 2: Concentration of different components after enzymatic hydrolysis

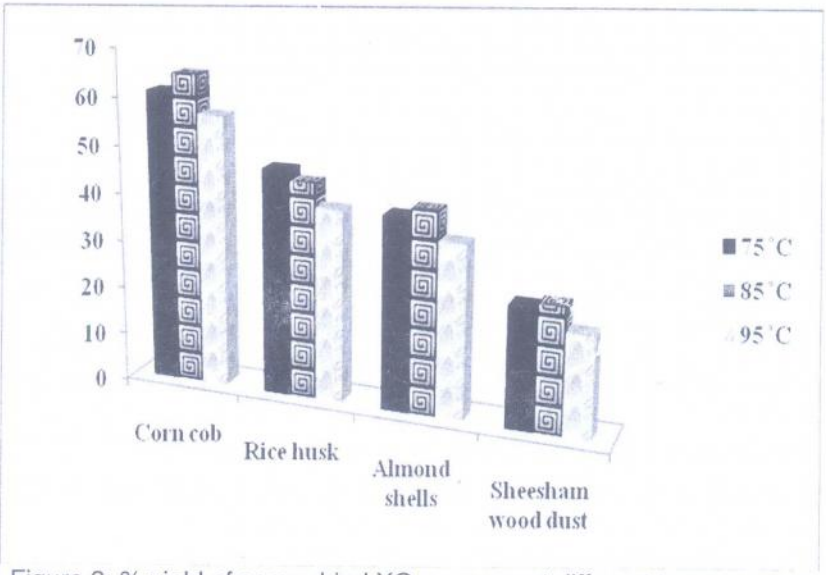

Figure 3: % yield of spray dried XO recovery at different temperatures

Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

PAKISTAN DAIRY DEVELOPMENT COMPANY & DAIRY DEVELOPMENT PROGRAMME

Geoff Walker
Chief Executive Pakistan Dairy Development Company, Lahore

INTRODUCTION

PDDC is a non-profit company established by the Government of Pakistan in order to lead dairy development in the country. It was established after sector-wide consultation. PDDC is a good example of public-private partnership, in that while funding is primarily from the Federal Government of Pakistan, PDDC co-operates closely with the private sector. Reflecting this, the Board of Directors is comprised of four representatives from Government, four from the processing industry, two farmers, one from the University of Agriculture, Faisalabad, plus the Chief Executive Officer.

PDDC has been operational for around 18 months, with the Chief Executive taking up his position in May 2006.

For many of PDDC's programmes, the role of the company is to provide significant assistance in order that private sector investment in dairying is encouraged. The Articles of Association of the company do not allow it to process and market milk on its own account.

PDDC operates with three key philosophies:

- Dairying and dairy development need to be integrated across a broad range of activities:

1. Successful dairy farming requires integration of a number of inputs, including water, feed, animal health, breed, and shelter among others.
2. The challenges facing the dairy sector are considerable and broad, hence dairy development needs to be integrated across a broad front; otherwise, success in one area of development will be negatively impacted by limitations elsewhere.
3. Desirably, the activities of the many organisations with roles to play in dairy development, it is desirable to achieve integration among them in order to avoid duplication of effort and ensure gaps are identified and closed.

- Dairy farming in Pakistan must be developed in a low cost manner. Pakistan has relatively low tariffs by the standards of the international dairy market; hence farming systems must focus on low cost methods, which tend to be found in the Southern Hemisphere. The alternative is to leave the Pakistani dairy sector vulnerable to ever increasing competition from imported dairy products.
- A farm management approach to the development of dairying must be introduced to Pakistan. To date, most development efforts have focused on vaccination and breed development only.

STRATEGIC DEVELOPMENT PROGRAMMES AND TIMELINES

PDDC has classified its development programme into three Strategic Timeframes:

- Horizon one covers the programmes for which PDDC has funding today.

- Horizon Two includes programmes which PDDC consider to be important to achieve development across the necessary broad front.
- Horizon Three includes programmes which, in the judgement of PDDC, can be postponed for a few years.

HORIZON ONE

PDDC has funding for two programmes at present:

- Cooling Tank Programme
- Model Farm Programme

Cooling tank programme

This programme seeks to assist the provision of cooling tanks to the sector, in order to improve the supply chain and thus improve the quality of milk available in Pakistan today. Under this programme, a successful applicant is able to receive a soft loan on which PDDC pays mark-up. The capital cost of the tank is paid off over five years by the applicant.

At the time of the symposium, PDDC had assisted the installation of 678 Cooling Tanks, while another 70 have been shipped prior to installation. This is after around one year of operation of the programme.

To put this in context, prior to the PDDC programme, over 20 years the dairy industry had installed about 1,500 tanks.

Model farm programme

The Model Farm Programme is primarily a technology transfer programme. It is targeted exclusively at existing dairy farmers. The programme is not intended to create "show farms" which may be excellent for presentation purposes, but rather to illustrate how with more modern farm management practices, enhanced productivity and profitability can be achieved.

The programme is open to existing farmers nationwide, and PDDC is active in all four provinces plus Azad Jammu Kashmir. The company is presently considering how to become more active in the Northern Areas.

While the programme is about the introduction of new, more modern farm management practices, on most farms, some assets will be required in order to allow the implementation of those new practices. Hence, there is a financial component to the programme. The structure of this financial component is that once applicants, vetted by PDDC, are recommended to one of our partner banks, the bank must ensure the applicant meets its credit criteria. The assets are funded by a commercial loan; neither PDDC nor the Government of Pakistan provides any guarantee to the financial institution. PDDC, however, does meet the mark-up on the loan. In addition, after three years and subject to good performance (which includes implementing the advice of PDDC and meeting loan repayment schedules), a grant of 50% of the original value of the loan is paid by PDDC. It is further noted that no cash is paid to the applicant; rather the assets approved by PDDC for that individual farm are purchased on account of the farmer, and installed under PDDC supervision.

The more modern farm management practices recommended by PDDC focus firstly on quite basic matters, but with a view towards improved productivity and profitability. The recommended techniques include:

- Free access to water
- Improved feeding
- Basic animal health care
- Temperature mitigation
- Separation of animals by class, including the improved feeding of calves

The programme is proving its worth: within a week or two of PDDC intervention and implementation of the farm upgrades, milk production is increasing by 25-35%, and in some cases by 100%. At the same time, production costs per litre of milk are reducing. The net result is a more profitable farm.

At the same time as PDDC is assisting individual farmers through this programme, the company is also learning how to adapt the knowledge of its international experts to local conditions. In this manner, models for profitable dairy farming under different conditions across the country are being developed.

HORIZON TWO

The suite of dairy development programmes included in "Horizon Two" is as follows:

- Community farms
- Milk pocket development
- Extension
- PDDC management of breeding and training farms
- Upgrade of military farms
- Bulk vending
- Rural service providers
- Link to finance
- Biogas

At the time of the seminar, this suite of programmes has been approved by the Government of Pakistan, but no funding has been received.

Community farms

Reaching the smallest dairy farmers, in a sector where there may be 8 million households with some dairy farming activity, is difficult and expensive. In addition, it is likely that there will be difficulty in providing the assets necessary to implement PDDC recommended farm management practices on the smallest farm operations, some of which are conducted by farmers who are effectively landless.

This programme is designed to group the smallest and poorest dairy farmers, in a manner which facilitates the provision of technical and financial assistance, while allowing the beneficiaries to build stakes in society.

PDDC is flexible on the structure of each individual community farm, depending on the needs of the particular community. Further, it is planned that some community farms are operated by women in the community.

The programme design includes the provision of seed finance, the payment of mark-up on a soft loan, and most importantly management assistance in terms of both business and farm operations.

Milk pocket development

One of the lessons from other developing dairy industries is that it is essential to provide a stable market to farmers, in order to create the environment in which the farmer is prepared to invest time and money to achieve growth. This programme is intended to provide linkages to markets in areas where such links are not sufficiently effective today. In practice, this probably means areas where the formal processors are not active today.

The programme will assist the establishment of processing facilities to provide the market function. The facilities are likely to be pasteurising plants, and the programme allows either larger scale plants to be established, or smaller, probably community based plants.

PDDC will provide management assistance as well as financial assistance to encourage the necessary private sector investment.

Extension

Over time, especially once PDDC has developed the models for profitable dairy farming through the Model Farm Programme, there will be a requirement to move towards more traditional forms of extension. As implied, our extension activity will be geared towards rolling out the lessons of the Model Farm Programme. It is noted that virtually all extension activity in Pakistan today is focused on vaccination, which does not touch many of the ingredients for successful and profitable dairy farming.

PDDC management of breeding and training farms

It is intended that PDDC manages a selection of large dairy farms across Pakistan, probably provincial government owned farms. The primary intent will be two-fold:

- Use of these farms for training purposes, to provide master trainers who are equipped to become the extension workforce for the nation.
- Building herds which will be able to undertake breed development, with the medium term goal of being able to offer to the farmers of Pakistan around 2,000 good quality dairy animals per year.

Upgrade of military farms

The Army is the largest dairy farmer in the country. Upgrading Military Farms and implementing more modern farm management practices on these farms will have a positive impact on the situation of the dairy sector. In addition, in combination with the PDDC Management of Breeding and Training Farms, this programme will allow the development of a model for corporate dairy farming in Pakistan.

Bulk vending

The bulk vending concept is practiced in parts of India, most successfully in Delhi. Pasteurised milk is not packed into individual packages, but transported in bulk to specially equipped outlets. Consumers bring their own containers to these outlets, and receive good quality pasteurised milk after the purchase of a token. In this manner, quality is enhanced at reasonable cost.

The programme will provide technical and financial support to introduce this concept to Pakistan.

Rural service providers

In developed dairying countries, farmers do not in general own all equipment necessary to operate the farm. Rather, specialised and expensive equipment is owned by a "contractor" who supplies his expert labour and equipment to a group of regional farmers. In this manner, costs for all farmers are lowered. The programme is consistent with the objective of low cost dairying, therefore.

It is easy to perceive that labour is available cheaply in Pakistan, but in reality many of our farmer clients report difficulty in recruiting and retaining staff. Harvesting of crops is especially challenging, in terms of labour needs.

This programme will seek to establish businesses which operate under the rural contractor model as seen in other countries, with a focus on harvesting equipment.

Link to finance

Formal finance is not readily available to the millions of smallholder farmers in Pakistan. Much seasonal finance is supplied by middlemen, who in return take the milk from dairy farmers. This system results in very high effective interest rates, perhaps 60% or even higher. Milk production, owing to the daily nature of its supply and sale, is an excellent cash flow generator. The intent of this programme is to use this cash flow generation to facilitate the provision of formal credit. Most likely the programme will need to be matched with supply of milk to processors, in order that loan repayments can be made in an effective manner. If successful, this programme will provide significant poverty alleviation.

Biogas

Pakistan is short of energy, especially in rural areas. Dung is often used an energy source, resulting in poor sanitary conditions and impaired air quality in the home. This programme utilises PDDC's rural penetration to provide additional benefits to our farmer clients. At the same time, the by-product of biogas production is fertiliser of superior quality.The PDDC Biogas Programme will have significant environmental benefits.

CONCLUSIONS

PDDC is a new company, only one-and-a-half years old, but it has developed a vision for effective dairy development. Through its two programmes for which it has funding at present, the company has demonstrated that it is able to drive development effectively. In particular, where PDDC is interacting with farmers, it is able to point towards significant productivity gains, which over time will lead to further investment and growth in the sector.

Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

A COMPARATIVE STUDY OF CASEIN MICELLES FROM BUFFALO AND COW MILKS IN THREE DISPERSING PHASES-COMPOSITION AND PHYSICO-CHEMICAL CHARACTERISTICS

Sarfraz Ahmad[1]*, Michel Piot[1], Daniel Mollé[1], Jean-François Grongnet[2], Frédéric Gaucheron[1]
[1]UMR 1253 Science et Technologie du Lait et de l'Œuf, Inra-Agrocampus Rennes, 65 rue de St. Brieuc, 35042 Rennes Cedex, France. [2]Agrocampus Rennes, 65 rue de St-Brieuc, 35042 Rennes Cedex, France. e-mail: sarfraz.ahmad@rennes.inra.fr

Abstract

The group of caseins forms largest protein components in most milks of industrial significance. This protein plays an essential role in the technologies of several milk protein based dairy products such as cheese, yoghurt etc. Caseins are environment dependent proteins, so we can modify the product technologies by changing the environment as done in this work. Composition and physico-chemical characteristics of casein micelles from buffalo and cow milks were compared in three dispersing phases (water, NaCl and their respective ultrafiltrates) at similar concentration of caseins (23 ± 1 g.kg-1). The composition of the suspensions was determined by analyzing the contents of different nitrogen fractions (total nitrogen (TN), non casein nitrogen (NCN), non protein nitrogen (NPN), amino acids, protein fractions by ESI-MS) and minerals (calcium, potassium, sodium, chloride and inorganic and organic phosphate). Physico-chemical characteristics such as size and charge of the casein micelles by Zetasizer apparatus and water content of ultracentrifuged pellets were also determined. The composition and sequencing of amino acids of caseins coming from both species were very similar, but the concentrations of micellar inorganic phosphate and calcium were more important for the buffalo milk. The influence of the nature of the dispersing phases on the mineral equilibrium and on the micellar properties were very similar for both species. In the presence of NaCl in suspension of casein micelles, an increase in the diffusible calcium concentration was determined due to an increase in the ionic strength. The consequences resulted were reduction in the zeta potential and increase in the water contents of ultracentrifuged casein micelles. Compared to water, the dispersion of casein micelles in ultrafiltrates induced a binding of calcium to the micellar phase with as consequences, an increase of the micellar size and a decrease of zeta potential. It seems appropriate to scrutinize such kind of studies which provide a substantial platform for discussion as an emerging trend in the dairy sector to know the physical and technological behaviour of the casein micelle system.

Keywords: Casein micelles, milk, physico- chemical characteristics.

INTRODUCTION

In cow milk, casein micelle is a supramolecular structure containing different casein molecules (αs1-, αs2-, β- and κ-caseins) and calcium phosphate which plays a significant role in the maintenance of the integrity of casein micelles (Holt 1992; Horne 2006). Due to the associations of casein molecules between themselves by hydrophobic interactions and with calcium phosphate by electrostatic interactions, the casein micelles have an average size around 200 nm and are highly hydrated. Their zeta potential, essentially due to the presence of κ-casein at the periphery of micelle is about – 20 mV. The colloidal properties of the casein micelles are very dependent on the physico-chemical

conditions and especially the mineral environment. It is admitted that decrease in pH, additions of NaCl, calcium, phosphate or citrate and removal of minerals affect the mineral distribution between diffusible and micellar phases. As consequences of such modifications, the physico-chemical characteristics of casein micelle are affected (Walstra and Jenness 1984 ; Holt 1992; Gaucheron 2004).

This knowledge concerning the structure and stability of casein micelles has been essentially described on casein micelles from cow milk. Casein micelles coming from other mammalian species can be considered as different and a comparative approach is interesting to understand the factors that govern the structure and the stability of casein micelles. Knowing that data about composition, structure and physico-chemical properties of casein micelles from buffalo milk exist but are very limited (Ganguli 1973), we compared in this work the properties of casein micelles suspensions from buffalo and cow milks in the same conditions. The composition of the suspensions was determined by analyzing the contents of different nitrogen fractions (total nitrogen (TN), non casein nitrogen (NCN), non protein nitrogen (NPN), amino acids, molecules of caseins) and minerals (calcium, potassium, sodium, chloride and inorganic and organic phosphate). Size, charge of the casein micelles and water content of ultracentrifuged pellets were also determined as their physico-chemical characteristics. These comparisons were carried out in different environments (water, NaCl and ultrafiltrates) at a similar casein concentration.

MATERIALS AND METHODS

Milk samples

Fresh raw bulk whole buffalo milk (Murrah breed of Bubalus bubalis) and cow milk (Holstein breed of Bos taurus) were obtained from the Cantal region (Coopérative de Bufflonnes, Zone Artisanale, 15600 Maurs, France) and from Société Laitière (35590, l'Hermitage, France), respectively. 0.3 g.L-1 thimerosal (Sigma-Aldrich, St Louis, USA) was added as a preservative against micro-organisms.

Preparation of casein micelles suspensions

Buffalo and cow milks were ultracentrifuged at 20°C during 1 h at 100 000 g (Sorvall, Discovery 90SE, Hitachi, USA) with a T-865 rotor. Their supernatants were ultrafiltered at 20°C with a membrane Vivaspin 20 (Vivascience - Sartorius group, Germany), (molecular mass cut-off: 10 000 Da) during 2 h at 1 800 g in a centrifuge SV 11 TH (Firlabo, Lyon, France). Micellar pellets were drained, washed with distilled water and dispersed in three different phases (water, NaCl and ultrafiltrates of respective milks). The volume of the dispersing phases was defined to have similar final concentration of casein. Microbial growth was inhibited by addition of thimerosal (0.3 g.L-1). All these suspensions were left overnight under stirring at ambient temperature.

Compositional analysis of casein micelles suspensions

Nitrogen fractions

TN, NCN and NPN fractions were prepared according to IDF (1964). For NCN, 40 mL of each suspension was acidified to pH 4.6 with a mixture of 10 % (v/v) acetic acid and 1 M sodium acetate. For NPN, about 40 mL of 15 % (v/v) trichloroacetic acid was added into 10 mL of suspensions. NCN and NPN samples were filtered through Whatman papers (Whatman Int. Ltd., Maidstone, UK) No. 42 and 40, respectively. TN, NCN and NPN were determined by Kjeldhal method (IDF 1993). Nitrogen content was converted into equivalent protein content using 6.38, 6.25 and 3.60 as converting factors for TN, NCN and NPN contents, respectively (Karman and Van Boekel 1986). Casein nitrogen concentration [CN] was calculated as [CN] = [TN] – [NCN].

Amino Acids Determination

Contents of different amino acids were determined after hydrolysis of suspensions by 6 N HCl at 110°C for 24, 48 and 72 h in vacuumed sealed glass tubes according to Davies and Thomas (1973). Sulphur amino acids were obtained in the same conditions but after 16 to 20 h hydrolysis time and the samples having been previously oxidized by performic acid according to De Belsunce and Pion (1963). The amino acids analysis was then carried out by cation exchange chromatography on a Biochrom 30 automatic amino acid analyzer (Biochrome Ltd, Cambridge, UK) according to Spackman, Stein and Moose (1958) using lithium citrate buffers as eluants and ninhydrin post-column reaction system. Tryptophan determination was done on pronase hydrolyzed samples by colorimetric measure at 590 nm of the compounds resulting from tryptophan and p-dimethylaminobenzadehyde (PAB) reaction (Spies 1967).

Chromatography and mass spectrometry of proteins

This analysis was carried out as reported by Léonil, Mollé, Gaucheron, Arpino, Guénot and Maubois (1995). Proteins in the sample were reduced with 10 mM dithiothreitol in the presence of 6 M urea; after standing at 37°C for 1 h, each sample was diluted twice with 0.1 % trifluoroacetic acid before chromatographic separation. Reduced proteins (200 µg) were separated on chromatographic system (Agilent 1100 series, USA) using a reversed-phase column (4.6 × 250 mm, filled with 5 µm C4 particles; Vydac 214 TP 54, Hesperia, USA). The conditions of separation were as temperature 40°C, flow rate 250 µl/min and linear gradient elution with acetonitrile. Solution A was 0.1 % TFA in de-ionized water (v/v) and solution B was 0.1 % TFA in 80/20 acetonitrile/de-ionized water (v/v). The chromatographic column was conditioned by flowing 37 % of solution B and the elution was carried out by increasing solution B concentration, using the following sequence: 0-5 min, 37 %; 5-42.5 min, 37-55 %; 42.5-44 min, 57-90 %; 44-47 min, 90 %; 47-48 min, 90-37 % and 48-58 min, 37%. The effluent from the RP-HPLC column was splitted using a low dead volume T-connector, with 15 % entering the electrospray ion source, and the rest was routed towards an UV detector (214 nm). This arrangement permitted parallel recording of the UV and MS signals.

The mass spectrometer was a Perkin Elmer-Sciex (Thornhill, Ontario, Canada), Model API III Plus Biochemistry Mass Analyzer, single-quadrupole mass spectrometer equipped with a pneumatically assisted electrospray ion source (or ion spray source). Positive multicharged protein ions were generated by spraying the sample solution through a 75 µm ID fused-silica capillary located into stainless steel capillary held at a high potential of 4800 V. A coaxial air-flow along the sprayer delivered at an inlet pressure 0.3-0.4 MPa, assisted liquid nebulisation.

The interface between the sprayer and mass analyzer consists of a small conical orifice with a 100 µm diameter, held at 75 V. A gas curtain, formed by a continuous flow (0.8-1.2 l/min) of N2 in the interface region, prevents the penetration of neutral molecules into the mass spectrometer, and breaks sample ion clusters formed at atmospheric pressure. Protein mass spectra were obtained by averaging the signals from multiple scans. Each scan was acquired by the mass-to-charge (m/z) range of 800-1800, with a step size of m/z = 0.33 and a dwell-time of 0.5 ms. The charge number of the multi-charged ions, the deconvulated mass spectra, and the protein molecular mass determination were automatically obtained using an Apple Macintosh computer and a Tune version 2.5 for acquisition of data and Biomultiview 1.3.1 software for treatment of data. The proteins were identified by comparison of molecular masses determined by ESI-MS to molecular masses described in protein data bank (www.expasy.org).

Total and diffusible concentrations of minerals

Cations concentrations (calcium, potassium and sodium) were measured by atomic absorption spectrometry (Varian 220FS Spectr AA, Les Ulis, France) (Le Graët and Brulé 1993). Anions concentrations (chloride and inorganic phosphate) were determined by ion chromatography coupled with suppressed conductivity detection (Dionex DX 500, Dionex, Voisin-le-Bretonneux, France) (Gaucheron Le Graët, Piot and Boyaval 1996). Phosphorus contents were analyzed by colorimetric method using ascorbic acid and sodium molybdate (IDF 1987). This method was carried out after wet mineralization of samples in the presence of sulphuric acid and hydrogen peroxide. Ion concentrations determined in the ultrafiltrates were converted into diffusible concentrations by multiplying a correcting factor 0.96, as described by Pierre and Brulé (1981). This correction took into account the excluded volume effect.

Analyses of physico-chemical characteristics of the different casein micelles suspensions

Determination of size and zeta-potential

Hydrodynamic diameter of casein micelles was measured by dynamic light scattering on a Zetasizer 3000HS (Malvern Instruments, Worcestershire, UK). Before measurement, each suspension was diluted in its respective diffusible phase (water, NaCl and ultrafiltrate) and filtered on a membrane with a pore size of 0.8 µm. Measurement was carried out at a scattering angle of 90°, wavelength of 633 nm and temperature of 25°C. The refractive indexes of the dispersing phases were 1.333, 1.333 and 1.342 for water, NaCl and ultrafiltrate, respectively. The viscosities of dispersing solutions were 0.89 x 10-3, 0.89 x 10-3 and 0.99 x 10-3 Pa.s for water, NaCl and ultrafiltrate, respectively.

Zeta-potential of casein micelles was determined with the same apparatus with an applied voltage of 125 V at 25°C. Measurement of the negative charge of micelles was based on electrophoretic mobility using equation reported by Smoluchowski (1917). Samples were prepared with the same method as for micellar size determination. The viscosities of the dispersion solution were also kept same.

Water content of ultracentrifuged pellets

Suspensions were ultracentrifuged at 100 000 g during 1 hour (Sorvall, Discovery 90SE, Hitachi, USA) with a T-865 rotor. The pellets were weighed and then dried at 103°C for 7 h. The difference of weight before and after drying, expressed as g of H2O by g of dry pellet was taken as the water content of ultracentrifuged pellets.

RESULTS

Composition of casein micelles suspensions

Nitrogen fractions

Results concerning the contents of the different nitrogen fractions are presented in Table 1. For both species, the TN, NCN and NPN contents were similar for casein micelles suspended in water and NaCl, but slightly higher in ultrafiltrates. Consequently, the casein concentrations, calculated by the difference between [TN] – [NCN], were also similar for the various types of suspensions and independent of the origin of the milk.

Amino acids

The determination of total amino acids contents in the casein micelle suspensions from buffalo and cow milks (Fig. 1A and B, respectively) showed that there was no qualitative and quantitative difference between both species and the dispersing phases have logically no influence on the amino-acids composition. For both species, glutamic acid had the highest concentration and cystein had the lowest. The amino acid

compositions of buffalo milk were comparable with the findings of Ganguli, Prabhakaran and Iya (1964).

Table 1. Composition of casein micelles suspensions as a function of dispersing phase (water, NaCl and respective ultrafiltrates). TN, NCN and NPN correspond to total nitrogen, non casein nitrogen and non protein nitrogen contents, respectively

	Buffalo			Cow		
	Water	**NaCl**	**Ultra-filtrate**	**Water**	**NaCl**	**Ultra-filtrate**
TN (g.kg-1)	25.07 ± 0.05	25.51 ± 0.03	26.39 ± 0.03	23.66 ± 0.06	24.27 ± 0.04	25.00± 0.04
NCN (g.kg-1)	1.06 ± 0.05	1.06 ± 0.02	2.78 ± 0.03	1.10 ± 0.01	1.24 ± 0.01	2.51 ± 0.01
NPN (g.kg-1)	0.14 ± 0.02	0.13 ± 0.01	1.37 ± 0.12	0.15 ± 0.02	0.16 ± 0.01	1.00 ± 0.04
CN (g.kg-1)	24.01 ± 0.01	24.45 ± 0.05	23.61 ± 0.01	22.56 ± 0.07	23.03 ± 0.04	22.49 ± 0.04

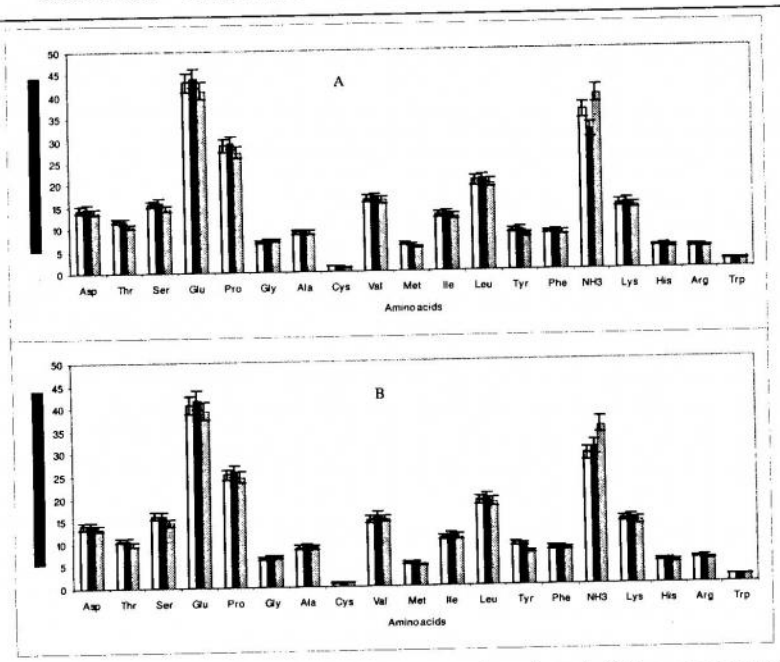

Figure 1. Amino acids composition of casein micelles suspensions from buffalo and cow milks as a function of dispersing phase. A: Casein micelles suspensions from buffalo milk and B: Casein micelles suspensions from cow milk (□: casein micelles suspensions in water, ■: casein micelles suspensions in NaCl and casein micelles suspensions in respective ultrafiltrates).

Chromatography and mass spectrometry of proteins

For the suspensions of both species in water, the chromatographic profiles contained 6 major chromatographic peaks (noted 1 to 6). Qualitative and quantitative differences between both species (Fig. 2) existed because the retention times and areas of the different chromatographic peaks were different. The proteins were identified by comparison of molecular masses determined by ESI-MS (Table 2) to those calculated

from amino acid composition deduced from primary structures reported in the protein data bank (www.expasy.org). For casein micelles from buffalo milk (Fig 2A), the majority of casein molecules was identified. For this specie, the molecular mass determined for the peak 1 was 19 169 Da. This molecular mass has not been described in the literature or in the data bank. However, by analogy to the chromatographic profile of casein micelles from cow milk (Fig 2B); it would correspond to κ-casein. Four molecular masses with a difference of about 80 Da between themselves were found for the peak 2. These proteins were not identified but the differences of about 80 Da correspond probably to different levels of phosphorylation suggesting the presence of αs2-casein which is highly phosphorylated casein. Peak 3 correspond to κ-casein with one phosphorylation (www.expasy.org). The peaks 4 and 5 contained two proteins with a difference of 80 Da suggesting also a difference of one phosphorylation. In the protein data bank, these molecular masses correspond to αs1-casein with 6 and 7 phosphoseryl residues, respectively. Different molecular masses were determined for the peak 6. One of these masses, reported in the protein data bank (23 986 Da), corresponded to β-casein with 5 phosphoseryl residues. The other proteins in the chromatographic peak 6 having molecular masses of 23 041 and 23 575 Da were not identified. Concerning casein molecules from cow milk (Fig. 2B and Table 2), all the chromatographic peaks were identified except peak 3. Chromatographic peaks 1, 2, 4, 5 and 6 correspond to κ-CN A-1P, κ-CN B-1P, αs1-CN B-8P, β-CN A1-5P and β-CN A2-5P, respectively.

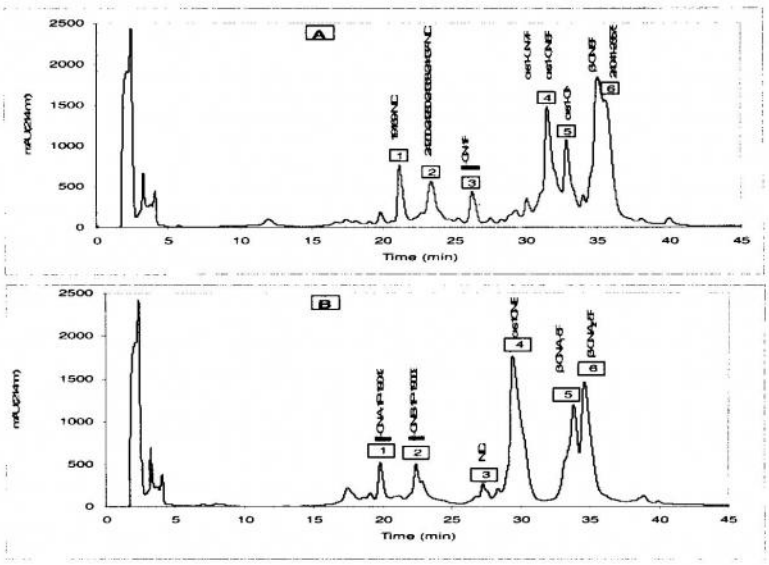

Figure 2. Reverse-phase chromatographic profiles of casein molecules from buffalo (A) and cow (B) milks. These profiles corresponds to the analysis of casein micelles suspensions in water. The attribution of each chromatographic peak was determined by mass spectrometry and results are reported in table 2. Molecular masses (Da) and types of casein molecules are indicated for each chromatographic peaks.

Major mineral contents

The total and diffusible concentrations of the major minerals are reported in Table 3. Whatever the nature of the dispersing phases, the diffusible and total calcium concentrations were always higher in buffalo casein micelles suspensions compared to

cow. On the other hand, the total concentrations of calcium were higher in the corresponding ultrafiltrates compared to water and NaCl. The total and diffusible phosphorus and inorganic phosphate contents were also always higher in buffalo casein micelles suspensions compared with those of cow. Moreover, these concentrations were superior in the ultrafiltrates compared to water and NaCl. Concerning potassium (total and diffusible concentrations), casein micelles suspensions from cow milk were more concentrated than the suspensions from buffalo milk. It is noteworthy that the potassium concentration was always higher when the casein micelles were suspended in their respective ultrafiltrates compared to water and NaCl. The total and diffusible sodium were similar for the both species but were very different as a function of the dispersing phases (NaCl > ultrafiltrates > water). For chloride, the total and diffusible concentrations were similar for both species except in the ultrafiltrates where a higher concentration was observed in casein micelles from cow milk than buffalo milk. Moreover, and as observed with sodium, there were important differences of chloride contents as a function of the dispersing phases (NaCl > ultrafiltrates > water).

pH

The pH values, reported in Table 3, were different as a function of the nature of the dispersing phases (water > NaCl > ultrafiltrates) and the trend was similar for both species.

Table 2. Retention times (determined on the chromatographic profiles presented in Fig. 2), observed masses (with a precision of ± 2 Da) and corresponding casein molecules in water suspension of buffalo and cow milks presented in Figure 2. (N.D Not Determined). *The proteins were identified by comparison of molecular masses determined by ESI-MS to molecular masses reported in the protein data bank www.expasy.org. In some cases, addition of 80 Da (or multiple) was necessary to deduce the level of phosphorylation

Peak No.	Retention time (min)	Observed mass (Da)	Attribution*
Buffalo			
1	21.1	19169	N.D.
2	23.4	24200, 24280, 24358, 24437	N.D.
3	26.2	19182	κ-CN-1P
4	31.5	23337 23257	α_{s1}-CN-7P α_{s1}-CN-6P
5	32.8	23364, 23285 (-1P), 23443 (+1P)	α_{s1}-CN
6	35.0	23986	B-CN-5P
	35.6	23041, 23575	N.D.
Cow			
1	19.8	19042	κ-CN A-1P
2	22.4	19009	κ-CN B-1P
3	27.2	N.D.	N.D.
4	29.4	23620 23700	α_{s1}-CN B 8P N.D.
5	33.8	24029 23653	B-CN A^1-5P N.D.
6	34.6	23989 23122	B-CN A^2-5P N.D.

Table 3. Total and diffusible mineral concentrations (mM) in casein micelles suspensions from buffalo and cow milks as a function of dispersing phase (water, NaCl and respective ultrafiltrates). *Micellar concentrations were calculated as the difference between the total and diffusible concentrations. The values between brackets are expressed in mM of ions per gram of casein **Micellar organic phosphate concentrations were calculated as the difference between micellar phosphorus concentration and micellar inorganic phosphate concentration

		Buffalo			Cow		
		Water	**NaCl**	**Ultra- filtrate**	**Water**	**NaCl**	**Ultra-filtrate**
pH		7.28 ± 0.02	7.12 ± 0.02	6.92 ± 0.02	7.32 ± 0.02	7.15 ± 0.02	6.89 ± 0.02
Calcium	**Total**	28.8 ± 0.2	28.9 ± 0.5	36.6 ± 0.1	21.5 ± 0.1	21.8 ± 0.1	29.9 ± 0.1
	Diffusible	1.7 ± 0.1	3.7 ± 0.1	6.9 ± 0.1	1.1 ± 0.1	3.2 ± 0.1	6.8 ± 0.1
	Micellar*	27.1 (1.13)	25.2 (1.03)	29.7 (1.26)	20.4 (0.90)	18.6 (0.81)	23.1(1.03)
Inorganic Phosphate	**Total**	14.2 ± 0.3	14.3 ± 0.1	23.9 ± 0.1	9.5 ± 0.2	9.8 ± 0.1	20.0 ± 0.1
	Diffusible	2.4 ± 0.1	2.7 ± 0.1	9.0 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	9.4 ± 0.2
	Micellar*	11.8 (0.49)	11.6 (0.47)	14.9 (0.63)	7.3 (0.32)	7.4 (0.32)	10.6 (0.47)
Phosphorus	**Total**	25.7 ± 0.2	26.5 ± 0.3	38.1 ± 0.2	21.4 ± 0.2	21.1 ± 0.3	32.9 ± 0.4
	Diffusible	6.0 ± 0.1	6.7 ± 1.4	13.6 ± 0.3	5.3 ± 0.6	6.0 ± 0.6	12.8 ± 0.2
	Micellar*	19.7	19.8	24.5	16.1	15.1	20.1
Organic phosphate	**Micellar****	7.9	8.2	9.6	8.8	7.7	9.5
Potassium	**Total**	3.3 ± 0.1	3.4 ± 0.1	31.4 ± 0.1	5.1 ± 0.1	5.2 ± 0.1	42.8 ± 0.1
	Diffusible	2.8 ± 0.1	3.2 ± 0.1	27.3 ± 0.1	4.1 ± 0.1	4.7 ± 0.1	37.9 ± 0.1
Sodium	**Total**	3.4 ± 0.1	102.1 ± 1.9	20.4 ± 0.1	3.2 ± 0.1	102.4 ± 1.2	19.6 ± 0.1
	Diffusible	2.7 ± 0.1	95.7 ± 1.1	18.0 ± 0.1	2.6 ± 0.2	94.6 ± 1.8	17.7 ± 0.1
Chloride	**Total**	1.0 ± 0.1	88.5 ± 0.6	19.3 ± 0.1	1.8 ± 0.2	89.6 ± 0.2	28.7 ± 0.2
	Diffusible	1.2 ± 0.2	89.7 ± 1.8	20.6 ± 1.2	1.8 ± 0.1	93.7 ± 2.2	29.7 ± 0.6

Analysis of physico-chemical characteristics of casein micelles

Determination of size and zeta-potential

The size of the casein micelles was similar for both species (about 200 nm) when they were suspended in water whereas in the ultrafiltrates, the size of micelles from buffalo milk was higher than that of the cow's milk (Table 4). In the presence of NaCl, no stable values were obtained. Indeed, the preparation of the sample for measurement required important dilution with NaCl solution which probably had a dissociating action on casein micelles.

The nature of the dispersing phase had a significant influence on the zeta potential of casein micelles (Table 4). Indeed, the casein micelles were more negative in water than in the presence of NaCl and ultrafiltrates. On the other hand, these tendencies observed on micelles coming from the buffalo milk were similar as those observed with casein micelles from cow milk.

Water content of ultracentrifuged pellets

The water content of the ultracentrifuged pellet depended on the specie and composition of the dispersing phase (Table 4). For the same dispersing phase, the water content of ultracentrifuged pellet coming from buffalo milk was always smaller than those from cow milk. In the presence of NaCl, the water content was higher for both species compared to water and ultrafiltrates. In the presence of ultrafiltrates, the water content was similar to those obtained in water.

Table 4. Physico-chemical characteristics of casein micelles from buffalo and cow milks as a function of the dispersing phase (water, NaCl and respective ultrafiltrates)

	Buffalo			Cow		
	Water	**NaCl**	**UF**	**Water**	**NaCl**	**UF**
Size (nm)	203 ± 5	N.D.	253 ± 5	206 ± 5	N.D.	231 ± 5
Zeta potential (mV)	-26 ± 1	-23 ± 1	-21 ± 1	-26 ± 1	-21 ± 1	-20 ± 1
H2O content of ultracentrifuged pellet (g of H2O/g of dry pellet)	1.9 ± 0.1	2.4 ± 0.1	1.9 ± 0.1	2.4 ± 0.1	2.9 ± 0.1	2.2 ± 0.1

DISCUSSION

The composition and physico-chemical properties of casein micelles from cow milk are described in the literature with a lot of precision. On the other hand, casein micelles from buffalo milk are less studied and with the purpose to have new data on these last, we have suspended caseins micelles from buffalo milk in different environments (water, NaCl and its ultrafiltrate) and compare their compositions and physico-chemical properties with those determined in the same conditions with cow milk.

Proteins and minerals compositions of the casein micelles

The determination of the different nitrogen fractions (Table 1) showed that the casein concentrations were similar (23.5 ± 1 g.L-1) in the different studied suspensions. The slight differences for TN, NCN and NPN contents between water and NaCl against ultrafiltrates can be explained by the presence of nitrogenous compounds such as urea, ammonia, free amino acids present only in the ultrafiltrates (Walstra and Jenness 1984; Taha and Kielwein 1989). Compared to milk, the NCN values of casein micelles suspended in ultrafiltrates were very low (2.8 and 2.5 g.kg-1 for suspensions in ultrafiltrates of buffalo and cow milk, respectively against 7 to 8 g.kg-1 for milk of both species). These low contents revealed that the suspensions were essentially composed

of caseins as the majority of whey proteins were removed during sample preparation by ultracentrifugation and ultrafiltration. The analyses of amino acids of different suspensions with similar casein concentrations (Fig. 1) revealed that casein molecules were qualitatively and quantitatively close for both species. This was confirmed by the identification of the main caseins by mass spectrometry and their attribution (Table 2). Casein molecules from buffalo milk were very similar to their counterparts cow milk. By comparing the sequence of each casein molecule between both species in the protein data bank (www.expasy.org), the differences were less than 5 % whatever the type of caseins.

The minerals present in the casein micelles are essentially composed of calcium and phosphorus (which can be divided into organic and inorganic) commonly named micellar calcium phosphate. In cow milk, this micellar calcium phosphate exists predominantly in the form of thermodynamically stable amorphous calcium phosphate nanoclusters (Holt 2004). From the results reported in Table 3, especially those obtained with casein micelles suspended in water, it appears that the micellar calcium phosphate is quantitatively different for the both species because the amounts of calcium and inorganic phosphate associated to casein micelles were significantly higher in casein micelles from buffalo milk than cow milk. Assuming that the casein molecules existed mainly in the micellar form (Ganguli 1973), the amounts of calcium and inorganic phosphate associated to casein were 1.13 and 0.90 mM calcium per gram of casein and 0.49 and 0.32 mM inorganic phosphate per gram of casein for suspensions of casein micelles from buffalo and cow milk, respectively. Moreover, the results also indicated that the chemical nature of this micellar calcium phosphate was not exactly the same because the molar ratio of micellar calcium / micellar inorganic phosphate was 2.3 and 2.8 for suspensions of casein micelles from buffalo and cow milk, respectively. As this micellar inorganic phosphate calcium is associated to organic phosphate (phosphoseryl residues) in the casein micelles, so it was possible to evaluate the amount of organic phosphate in casein micelles from both species. This quantity was calculated as the difference between concentration of micellar phosphorus and concentration of micellar inorganic phosphate (Table 3). The calculated concentrations of micellar organic phosphate were very close suggesting that the level of casein phosphorylation was very similar between both species.

Influence of the nature of the dispersing phase on the mineral composition of casein micelles

It is known that casein micelles are in equilibrium with the dispersing phase (Gaucheron 2004) and some mineral exchanges between serum and casein micelles can occur depending on the composition of this phase.

When casein micelles were suspended in water, the transfers of calcium and phosphate from the casein micelles to the diffusible phase were relatively limited and consequently the concentrations of these minerals in its diffusible phase were low (Table 3). The presence of NaCl in the suspensions caused a reduction of micellar calcium and consequently diffusible calcium content increased. These changes can be explained by an increase of the ionic strength which induces a decrease in the activity coefficient of the different ions present in the diffusible phase and an increase in the solubility of calcium phosphate in the milk serum. On the other hand, it is known that addition of NaCl to milk induces only a solubilisation of calcium (Le Graët and Brulé 1993) which can be due to exchange of sodium by calcium directly attached to caseins rather than the calcium present in the calcium phosphate nanoclusters. In suspensions made with ultrafiltrates, the exchanges were different as those observed in the presence of NaCl. The ultrafiltrate contained minerals in important quantities and it is admitted that this phase is

supersaturated in calcium phosphate. One small part of the calcium ions can interact with negative charges of the casein micelles and consequently the concentration of calcium associated to casein micelle increased (Table 3).

As resulting of these different exchanges depending on the nature of the dispersing phases, the pH of the suspensions were also different (Table 3). The lower pH in the presence of NaCl compared to the suspension in water can be related to an exchange of Na+ for H+ attached to charged group of casein, thereby increasing the concentration of H+ in the serum phase (Grufferty and Fox 1985; Van Hooydonk, Hagedoorn and Boerrigter 1986; Famelart et al 1996; Le Ray et al 1998). The lowest pH values observed for suspensions in ultrafiltrates was due to the presence of diffusible salts especially inorganic phosphate in ultrafiltrates. For Lucey et al (1993), these diffusible salts are responsible for about 50 % of the buffering of the milk and consequently it was not surprising that buffering of micelle suspension was greater in ultrafiltrates.

The objective of this work was also to compare the casein micelles from buffalo and cow milks. The casein micelles from these two species are slightly different in term of protein and mineral compositions but the influence of the dispersing phase on the mineral exchange was very similar.

Modification of the physico-chemical properties of casein micelles

In milk, the majority of casein molecules are combined to form casein micelles. A variety of molecular forces are involved in this structure and the stability of casein micelles depends on the balance between electrostatic repulsions and hydrophobic interactions. Moreover, micellar calcium phosphate crosslinks the casein molecules and neutralizes negatively-charged phosphoseryl groups, allowing the formation of hydrophobic interactions between casein molecules (Walstra and Jenness 1984; Holt 1992 and 2004; Horne, 2006). Size and charge of casein micelles and water content of ultracentrifuged pellets are some physico-chemical characteristics of casein micelles. As reported in Table 4, the nature of the dispersing phase had an influence on these physico-chemical properties of casein micelles and the trends observed with casein micelles from buffalo milk were very similar to those observed with casein micelles from cow milk.

In water, the size of casein micelles from buffalo and cow milks were very similar with a value close to 200 nm. In the presence of NaCl, the size determination was not possible because the dilution with this phase had a dissociating effect on the casein micelle. In the presence of their respective ultrafiltrates, the size of casein micelles was more important as compared in water (253 and 231 nm for casein micelles from buffalo and cow milks, respectively). These differences between water and ultrafiltrates can be due to an increase of the mineral contents associated to micellar phase (Table 3) with as consequences a reorganization of the casein micelle and a size increase. On the other hand, no clear and adequate argument can be given to explain the size variation between both species but differences in the mineral composition (especially monovalent ions) of the diffusible phase can be suggested as an explicatory factor. Indeed, the diffusible phase of buffalo milk contains less potassium and chloride ions than the diffusible phase of cow milk (Table 3). Owing to these differences, molecular modifications of interactions inside and outside of casein micelles can be suggested.

Zeta potential corresponds to the electrical charge at the surface of the casein micelle and is related to the presence of glycosylated residues present on the κ-casein. In water, the zeta potential of casein micelles for both species was similar and very negative (Table 4). The absence of difference between the two species suggested a similar composition in glycosylated residues for their κ-caseins. On the other hand, this low value (-26 mV) was due to a highest pH (Table 3) in water compared to other

dispersing phases and also to a low shielding of the negative charges present at the surface of casein micelles by cations which are at low concentrations when the diffusible phase was water (Table 3). The dispersion of casein micelles in NaCl induced a significant reduction of this parameter as compared in water (Table 4). Similar decreases in the zeta potentials of casein micelles from cow milk after NaCl addition were also observed by Dalgleish (1984) and Huppertz and Fox, (2006). This reduction can be due to the changes in the casein micelles but also in the diffusible phase. As reported in Table 3, sodium ions being mainly in the diffusible phase, they can interact with negative charges of casein micelles and contribute to the decrease of the zeta potential. When the ultrafiltrates were used as dispersing phases, the effect on the zeta potential was similar to those observed with NaCl. However, the presence of important calcium concentration in the suspensions (36.6 and 29.9 mM for buffalo and cow, respectively) must be considered as the interpretation of the decrease in zeta potential. One part of the calcium ions can interact with negative charges located on the surface of casein micelles and contribute to a decrease in zeta potential. Similar results and interpretation were done by Philippe et al (2003) to explain the decrease of negative charge on casein micelles with increasing calcium content of the suspending medium. Alternatively, the reduction of this value may arise from a new distribution of charged chains at the micellar surface as a consequence of calcium binding to other parts of the surface, with changes in the overall thickness of the steric layer.

Whatever the composition of the dispersing phase, the water content of ultracentrifuged pellets from buffalo milk was lower than that of cow milk (Table 4). Kuchroo and Malik (1976) have found similar differences between casein micelles coming from buffalo and cow milks. The most influencing factors which could contribute to these significant differences between species are structure of caseins, glycosylation of κ-casein and mineralisation of casein micelles. In this work, we showed by analyzing the molecules of caseins in term of their amino-acid composition (Fig. 1), molecular masses and sequences (Fig. 2 and Table 2) that they were very similar with more than 95% of homology of sequence. These results suggested that the structure of caseins would not be taken into account to explain these differences in water content of ultracentrifuged pellets. Differences of glycosylation of κ-casein seems exist because Sabarwal and Ganguli (1977) indicated that this protein from cow milk exhibited higher sialic acid value than κ-casein from buffalo milk. Moreover, the same authors indicated that the glycopeptide released from cow κ-casein had a higher molecular weight than those released from buffalo κ-casein. On the other hand, the higher mineralisation of casein micelles from buffalo milk (Table 3) could also be an important explanatory factor. Compared to water and ultrafiltrates, the presence of NaCl in the micellar suspension caused an increase in water content of ultracentrifuged pellets about 0.50 g of H2O/g of dry pellet. These results, which were in agreement with the results obtained only with cow milk (Grufferty and Fox 1985; Creamer 1985; Van Hooydonk et al. 1986) suggested that the nature of the dispersing phase could induce modifications of structural organisation of casein micelles with changes in the interactions between water, minerals and casein molecules.

CONCLUSIONS

These results improve our understanding on the composition and physico-chemical properties of casein micelles from buffalo milk as a function of ionic environment. The comparison of the casein micelles from these both species showed that their qualitative and quantitative compositions in amino-acids and caseins were very similar. In the opposite, their contents in calcium and inorganic phosphate were higher for casein

micelles from buffalo than from cow milk. In spite of these differences of mineralisation, the modifications of their physico-chemical characteristics as size, zeta potential and water content of ultracentrifuged casein followed the same trends.

ACKNOWLEDGEMENTS

We thank Mr. Joël Guillemin from Cantal region (Coopérative de Buflonnes, Zone Artisanale, 15600 Maurs) for facilitating us with buffalo milk and Higher Education Commission of Pakistan and INRA of France for providing funds to do this research.

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Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

TRENDS IN DAIRY SCIENCE AND TECHNOLOGY

Frédéric Gaucheron, Sarfraz Ahmad and Gaëlle Tanguy
INRA, UMR Science and Technology of Milk and Egg 65 rue de Saint-Brieuc, 35042 Rennes cedex, France. E-mail: Frederic.Gaucheron@rennes.inra.fr

INTRODUCTION

At present, there are tendencies to manufacture dairy products :

- with a controlled microbiological quality
- desired rheological, textural properties and taste adapted to the needs of the consumers as well as
- having good effects on the human health (*e.g.* of supplemented dairy products with specific proteins, peptides, minerals or beneficial bacteria).

The objectives of this communication are to provide an up-date on scientific knowledge in dairy science and technology.

1. TRENDS IN DAIRY SCIENCE

Milk is a unique liquid composed of proteins (caseins and whey proteins), lipids, lactose, minerals and vitamins. The composition of cow milk is presented in Table 1.

Table 1. Milk composition

Compounds	Concentration (% w/w)
Water	87.1
Lipids	4.0
Lactose	4.6
Proteins	3.3
Caseins	2.6
Whey proteins	0.6
Non Protein Nitrogen	0.06
Minerals	0.7

Each of these compounds is relatively well known. However, a lot of research is still focused on a better understanding of milk. These works concern mainly casein micelles, whey protein and lipids. Casein micelle is a supramolecular edifice composed of different casein molecules (α_{S1}-, α_{S2}-, β and κ-caseins) associated with calcium phosphate named nanocluster. This structure is highly hydrated and negatively charged. The negative charge is especially located at the surface of casein micelles on κ-casein (Fig. 1).

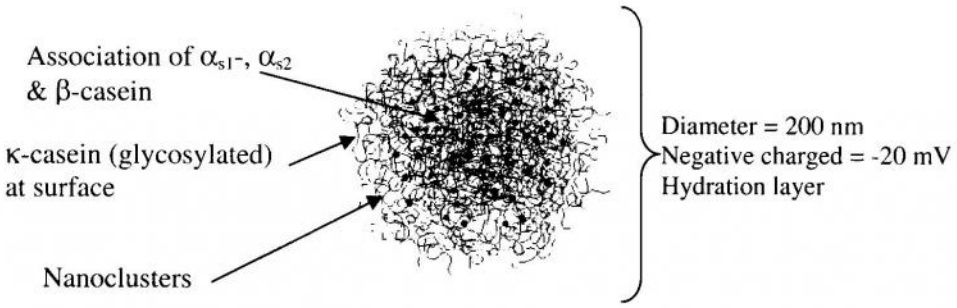

Figure 1. Model of casein micelle (Holt's model)

This superstructure is not completely elucidated and different questions still exist such as: What types of interactions between casein-casein and casein-minerals exist in the native casein micelles ?

- What are the physico-chemical modifications of casein micelles induced by different conditions (ionic environment, acidification, heat treatments, concentration, spray-drying, etc) ?

The major whey proteins (α-lactalbumin, β-lactoglobulin, serum albumin, lactoferrine, etc) are globular well structured proteins containing α-helix and β-sheet in important proportions. Their structures are relatively well known. However, the influences of processes on the structural modifications and functionalities of these proteins are not precisely known. Moreover, the identification and quantification of minor proteins and the knowledge on their biological activities constitute new trends for the dairy research.

Lipids of milk are essentially composed of triglycerides (98% of the total lipids). They are associated to form fat globules having a size of 4-5 μm and a negative charge (- 15 mV) (**Fig. 2**). Each fat globule is covered by a native membrane constituting 2% of lipids as shown in Figure 2.

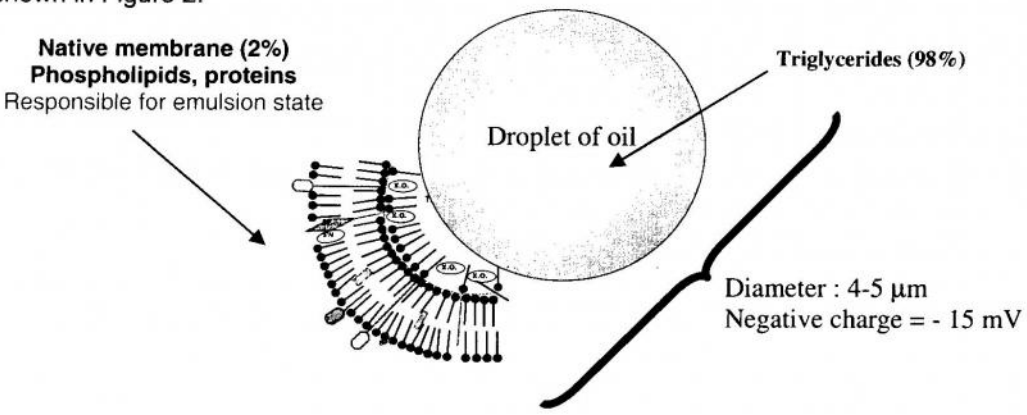

Figure 2: Fat globule

Like proteins of milk, the composition, structure and functional properties of the fat globules and the native membrane in different dairy products are not completely elucidated.

2. TRENDS IN DAIRY TECHNOLOGIES

The dairy industry must adapt to the increasing number of dairy products in the different regions of the world. The level of industrial development of a particular country determines the extent to which milk is collected from dairy farms and processed. The scales of transformation of milk in to different products by application of technological treatments varies. Small dairies may process a few hundred litres per day compared to large plants that can handle up to a million litres per day. Their contributions are essential for achieving the following goals :

1- Better conservation of the components of milk (microbial destruction by heat treatments, water removal by concentration and drying, cheese-making technology etc.)
2- Modification of properties of some milk compounds (decrease of the fat globule size by homogenisation, protein denaturation by heat treatments and high-pressure) ;
3- Valorisation of co-products coming from the dairy industry and a fractionation-purification-extraction of some interesting compounds of milk (membrane-separation technologies).

In this presentation heat treatments, membrane filtration, spray drying and cheese manufacture are described because these have significance for the dairy sector in Pakistan.

2.1 Heat treatments

The aims of heat treatments are to destroy pathogenic micro-organisms in raw milk. Consequently, the storage time of milk is increased and better control of industrial process is possible. Different intensities of heat treatments are given. The most common are

- Thermisation, pasteurisation (70°C, few seconds) (for cheese)
- High pasteurisation (90-95 °C, 5-10 minutes (for yoghurts))
- Sterilisation (> 100°C, few minutes)
- Ultra High Temperature (140°C, 2-15 secondes)

These different heat treatments induce several biochemical modifications depending on the intensity (Fig. 3). For intense heat treatments (>100°C, several seconds), changes in taste and nutritional value occur.

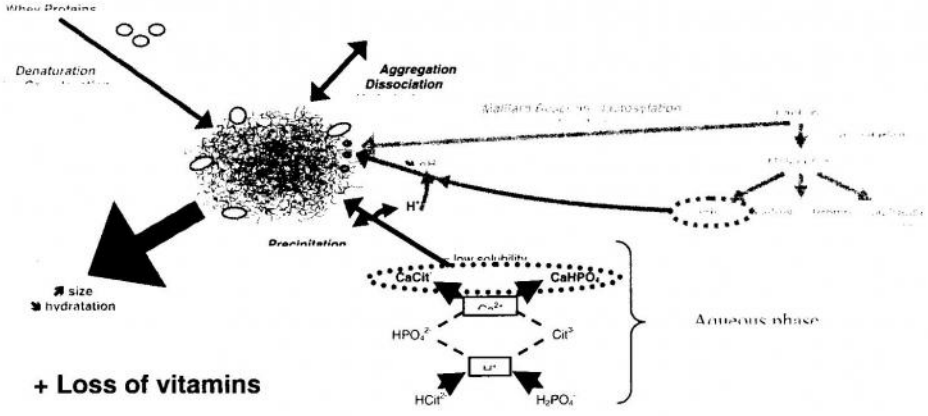

Figure 3: Recent results concerning the modifications induced by heat treatments

In some cases, instability of UHT milk during storage occurs. There are multiple causes for this problem but the reasons are not precisely known and different hypotheses concerning zootechnical, technological biochemical aspects have been made (Fig. 4).

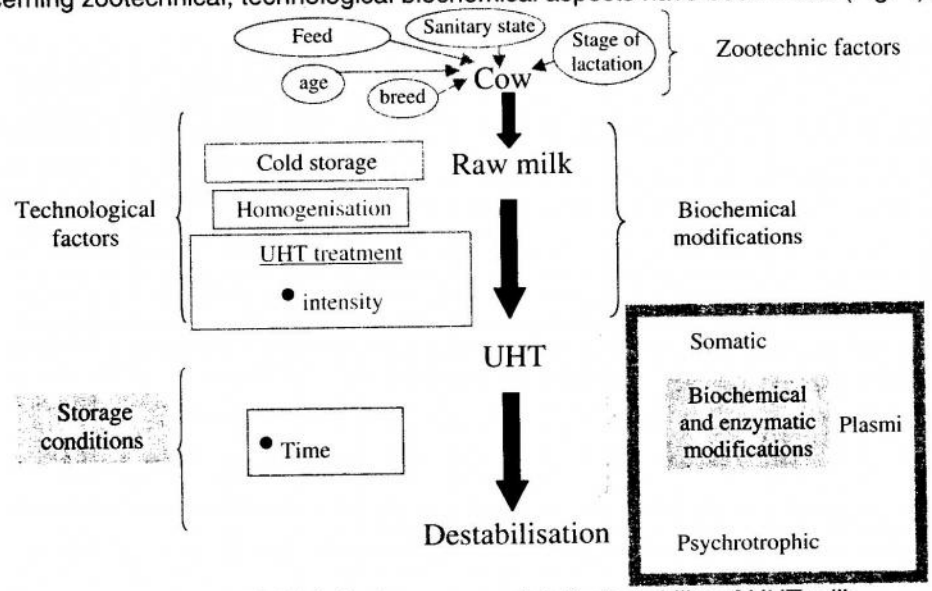

Figure 4: Multiple factors responsible for instability of UHT milk

2.2 Membrane filtration

Based on the size differences of milk constituents (Table 2), it is possible to separate them by membrane filtration.

Table 2. Size of different milk constituents

Somatic cells	6 - 15 µm
Fat globules	4 - 5 µm
Bacteria	0.5 - 2 µm
Casein micelles	150 - 300 nm
Whey proteins	10 nm

This separation is a physical process using a semi permeable membrane (with different sizes of pores). To avoid fouling, the filtration is tangential. Microfiltration, ultrafiltration and nanofiltration are commonly applied. Some applications are as under :

- Milk concentration
- Standardisation
- Manufacture of new cheeses
- Demineralisation of whey
- Separation of bioactive peptides or proteins
- Delipidation of whey
- Fractionation of fat globules
- Bacteria epuration of milk (Fig. 5)
- Purification of casein micelles and whey proteins (Fig. 6).

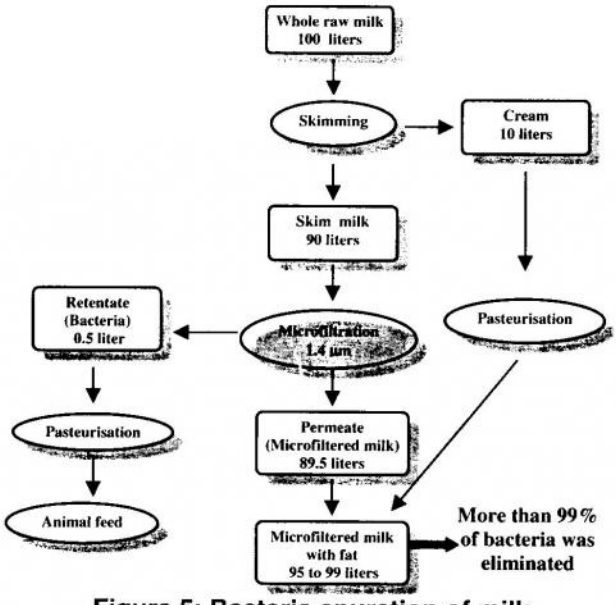

Figure 5: Bacteria epuration of milk

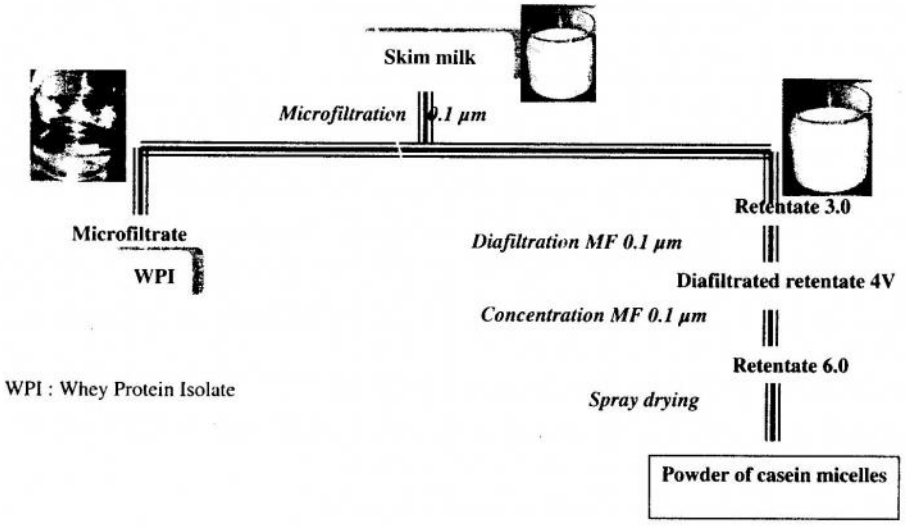

Figure 6: Purification of casein micelles and whey proteins

2.3. Spray Drying

Spray drying starts with the atomisation of liquid feedstock as small droplets into the hot air. The quality of the powder will depend on:

- Milk and concentrate quality (composition and viscosity)
- Drying conditions (temperature, droplet size, humidity)
- Storage conditions (humidity, oxygen, temperature)
- Rehydration conditions

Different types of the milk powders are available in the market(Fig. 7).

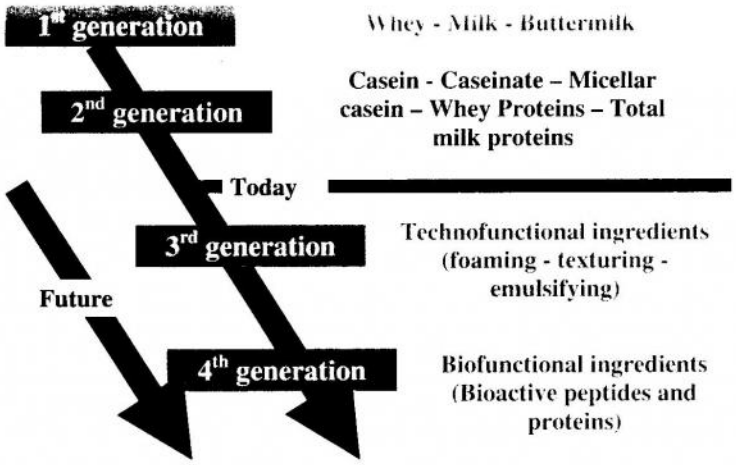

Figure 7: Different types of milk powders

The applications of dairy powders are multiple such as:

- Dairy products
- Dietetic foods
- Infant foods
- Foods for health
- Confectionery
- Cooking products
- Pharmaceutical products

2.4 Cheese Manufacture

Cheese is a universal food consumed in various parts of the world and constitutes an ancestral form of conservation of proteins and fat of milk. More than around 4000 different cheeses are known in the world over out of which 400 originated from France. In general, cheese is manufactured in several steps shown in the Fig. 8.

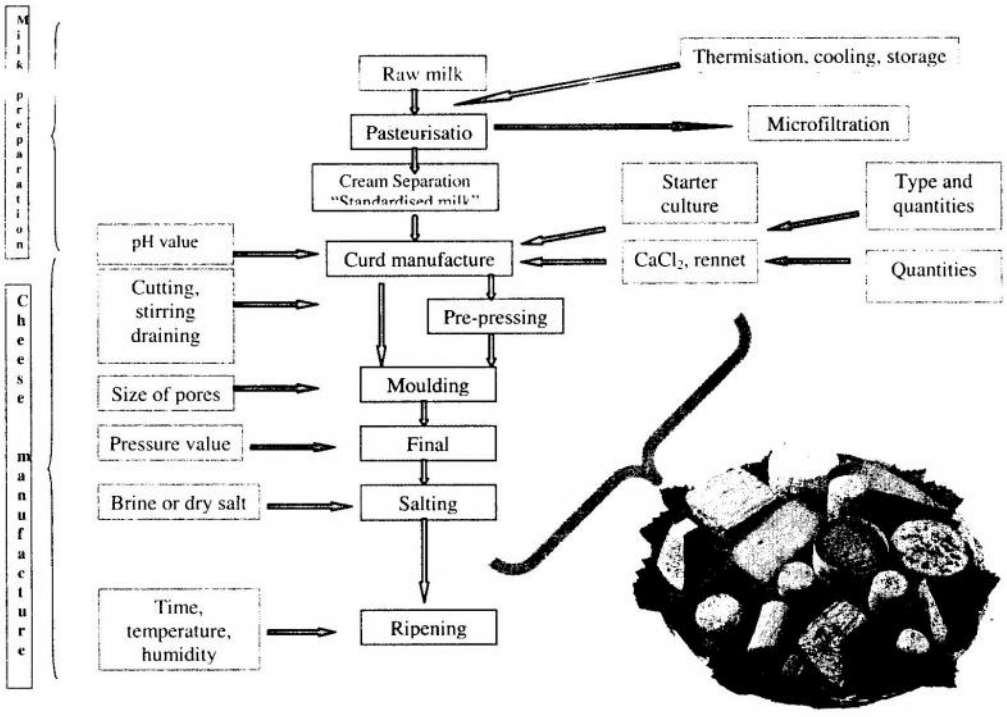

Figure 8: Manufacture of cheese

The different parameters of milk preparation and cheese manufacture, as shown in Figure 8, are the basis of variations in composition, texture and taste. Figure 9 shows different types of cheese commonly consumed in different parts of the world.

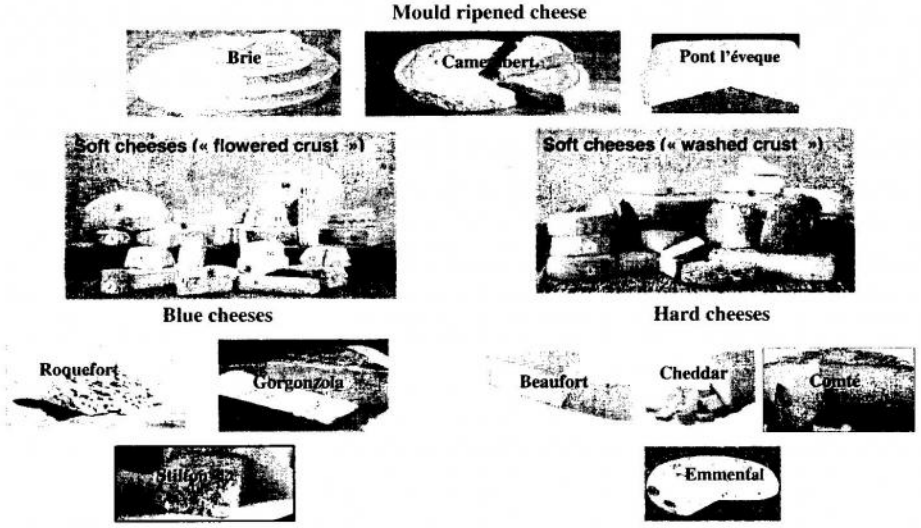

Figure 9: Diversity of cheeses

3. TRENDS IN DAIRY PRODUCTS WITH BIOLOGICAL ACTIVITIES

Today, it is possible to manufacture dairy products with specific biological activities; these specific activities can be related to :

- constituents of milk like calcium, bioactive peptides and proteins;
- externaly added components like minerals, vitamins, phytosterol,
- added specific bacteria such as probiotic bacteria.

Recent results show that these types of dairy products could have several beneficial effects on health. Positive effects on digestion, thrombosis, blood pressure, immunity, inflammation, antimicrobial and anticancer are the effects most studied.

Proceedings:
International Symposium on
Emerging Trends in Food Science and Technology
5th to 8th November, 2007
National Institute of Food Science and Technology

TRENDS IN MEAT SCIENCE AND TECHNOLOGY

Prof. Dr Eric Dufour
Foodstuffs Typicality Research Unit, ENITA Clermont, site de Marmilhat, BP 35, F-63370 Lempdes, France.E-mail: dufour@enitac.fr

INTRODUCTION

Mankind began domestication of animals at least 10000 years ago and has been modifying species and agricultural practices ever since. Cattle was domesticated from *urus* and *aurochs* for meat first and, about 6000 years ago, humans start milking cows. Considering total cattle in the world, 35% is in Asia (2002), 23% in South America, 17% in Africa, 12% in North and Central America and 10% in Europe. In 2003, the largest beef producer in the world was USA (about 20%) followed by European Union (12%), Brazil (~ 10%) and China (~ 9%). However, Australia was the country that exported the most beef on the world market in 2003. From an economical point of view, livestock production has to face the reality of the free world trade and the progressive removal of import barriers.

The major objective of agriculture has always been to satisfy the needs of humanity quantitatively and qualitatively. It has been achieved at least in developed countries and, following the economic development such as in Asia region, the consumption of meat and meat products has increased in these countries during the last decade. The highest consumption of beef is in Argentina (57.4 kg per capita in 2003), followed by the USA (37 kg per capita) and then the European Union (20 kg per capita). Meat and meat products constitute some of the most important foods in Western societies. Meat quality can be defined in terms of consumer appreciation of texture and flavour, nutritional profile and safety, which includes both the health implications and microbial contamination. These quality factors can be influenced by genetic makeup of the individuals, by environmental factors such as feeding and management of the animal during their growth, and by post-slaughter handling and processing.

It is quite clear that world demographic development predicts more than 8 billion inhabitants for the year 2020. But, whatever the increase in total population, the main cause in increasing demand of food will be growth in per capita consumption mainly from developing countries: beef consumption is forecast to increase by 2.1 fold between 1993 and 2020 (Delgado et al 1999). On the other hand, several food crises (BSE, foot-and-mouth disease) have decreased European consumers confidence in meat products. Some of the consumers do not accept some of the current agricultural practices. Therefore, consumers in the developed countries will demand the meat products of high quality and scientists must develop socially acceptable food production systems (Gibbon et al 1999). In addition, urban consumers ask for convenience foods (Issanchou, 1996) and this is another important feature to integrate when considering the future of livestock. The future of livestock is thus uncertain, but it is likely that quality of meat products, the evolution of consumer demands, as well as economical factors, will play a major role in the future.

THE CHALLENGE OF QUALITY

The concept of quality

The concept of quality has been defined in many ways, and differs between countries. For Northern European countries, quality refers to health and hygiene aspects and to any other public norms. In the Southern European countries, the concept of quality is much wider, including sensorial traits, the geographical and human environments, any link to a specific region or to any specific method of production. In this paper, quality will be considered as all the characteristics of food products valued by the consumer. It is therefore a holistic and multifaceted concept integrating safety, sensorial and nutritional traits, traceability, or social considerations (public interest in environment management, animal welfare). Safety and health are however the two major expectations of consumers in industrialised countries. Safety is the absence of any contamination in food products. It is not linked to the characteristics of the product itself, except in some specific cases (BSE for example). It is often linked to the introduction of external pathogens at any stage of the food chain.

Nutritional value of food depends more on the product composition. It includes the type and amount of protein, carbohydrate, lipid, vitamins and minerals. Consumers in industrialised countries have displayed an aversion to a fatty diet and therefore to food products with high fat contents. On the other hand, contents in proteins, vitamins, and micro-nutrients may be positive indicators of the nutritional value of meat. Sensory quality can be defined as texture, flavour, taste and visual aspect. These traits depend directly on the product characteristics in terms of structure and constitution. Social considerations arise from changes in consumers' attitudes towards food and ethical views of animal and environmental management. This is especially true for cattle, which are supposed to be reared at pasture. In addition, unlike monogastrics and human beings, ruminants are able to convert renewable resources (uncultivated land, crop residues, by-products) into humanly-edible food. Therefore, ruminants play a key role in sustainable livestock production systems. The perception of those phenomena by consumers is also important.

Traceability is the ability to trace the food products back to the farm. In other words, the agri-food sector is able to guarantee consumers the origin of the food products, including in terms of animal breed, farm where animals were reared, nutrition programme, etc. This concept differs from that of transparency (e.g. knowing that food products are produced without growth promoters, in respect of animal welfare, etc.). Traceability or transparency often means quality for at least some consumers, however, this is not always true because the origin of the product or its production conditions do not necessarily mean that it is of high quality. In addition, consumers' demands for low prices conflict with demands for high-quality products. In beef, demands for flavoured meat tend to conflict with demands for low fat.

It is important to underline that the consumer's expectations vary with consumer categories and also with time. It varies depending on incomes, country, culture, consumer's age and habits. For instance, the price of beef has not declined as much as those for other meats during the last two decades; consequently, beef consumption is more and more correlated to incomes. The concept of quality, and how it will be incorporated in the beef industries, will however change depending on the future, which is itself subject to controversial issues. What will be the public attitude and wishes? What will be the nature of these wishes? What impact might international companies promoting new technologies have? How will human societies meet the challenges of environmental issues and sustainable development? What is the future of local breeds and organic agriculture? None of these questions is simple to answer and therefore any forecasting is

hazardous. One point, which is obvious, is that consumers' needs and expectations must be satisfied.

How can quality traits be optimised and guaranteed to consumers?

Quality traits depend first of all on factors relative to live animals (genetic factors, nutritional factors and husbandry, tissue biology and characteristics). These factors affect the quality of muscle for beef. They include breeding and rearing strategies, especially nutritional management. Genetic improvement is quite effective because it is permanent and cumulative: indeed improvements made in one generation are passed to the next ones. Emphasis is nowadays put on genetics and genomics due to the sequencing of genomes from different species, the bovine one being available in the very short-term future (Lewin 2003). This will have direct applications in genetic improvement and traceability with the help of DNA-based techniques. On the other hand, functional genomic is likely to impact our knowledge of ruminant physiology. Genomic is indeed a new science which aims to better understand how biological traits are determined from genes. Genomic is changing the scientific paradigm, because the global expression of genes in cells and tissues will generate new biological hypotheses.

Husbandry may also affect the quality of the products depending on growth paths, growth rate, nutritional level and the nature of food given to the animals. During the last decade, emphasis has been put on nutritional factors, due especially to the BSE crisis. Nowadays, consumers are thus increasingly involved in how cattle are fed. Besides, the development of genomic tools now provides a powerful way to understand how production systems interact with genetics. Quality also depends on processing of raw carcasses into meat products. In the case of beef, the conditions of animal slaughtering and carcass chilling play a major role in the determination of tenderness. Methods of muscle stretching have therefore been given increasing attention (Sorheim and Hildrum, 2002). More generally, it is important to develop control systems and techniques for monitoring processes for high quality added-value products. The idea is indeed to anticipate the final properties of food products by any method based on physics, biochemistry or anything else. In this way, emerging technologies (for example, fluorescence and infrared spectroscopies), and their on-line applications, are expected to play a key role in predicting quality, and in providing the development of large scale industrial routine methods.

Because consumers cannot spontaneously assess the quality of food products, the value of those products is recognised through "quality labels". Brand name is the archetype of such labels. Others are official labels such as Protected Denomination of Origin (PDO), Protected Geographic Identification (PGI), Traditional Speciality Guaranteed (TSG) or Organic Farming. The credibility of those quality labels is based on promises for the future quality of food products. This implies the definition of quality conventions, which specify rules to characterise the products. These quality conventions may include quality traits based on origin or animal breeds, on production methods, or on specific supply-chain structure. Quality conventions may also involve typicality (connection with a given land), especially in France and Italy (Sylvander *et al* 2003). The market attractiveness, the economical factors and good co-operation within a supply chain are important parameters to ensure the practical success of such conventions.

Quality of meat products is a very complex concept, which needs to be better defined. Regarding the consumers, we must also face various types of consumers with different types of demands. It is necessary to distinguish perceived quality i) prior to purchase (mainly determined by beliefs and attitudes), ii) at the time of purchase (mainly determined by the product characteristics in interaction with its price) and iii) upon consumption. Different scientific disciplines such as genetics, genomics, physiology,

nutrition, husbandry, physics, biochemistry, food engineering, should help to better define the different dimensions of quality. They are also essential in predicting quality, i.e. in anticipating the different quality traits of meat products demanded by the consumers.

GENE TECHNOLOGY FOR MEAT QUALITY

Skeletal muscle is a tissue of major economic importance for meat production. Therefore, in the last century, research was conducted in some countries to increase the growth rate of cattle and to produce lean carcasses with the ultimate objective of increasing production of meat at the expense of external fat. Similarly, research was also successful with respect to adequately feeding animals so that they could fully express their higher genetic potential.

Genetic and environmental factors profoundly alter muscle characteristics, and hence beef quality. The advent of high throughput sequence analysis, DNA chip technology and protein analysis (proteomics) has revolutionised the approach of muscle physiology. For instance, global gene expression profiling at the mRNA or protein level will provide a better understanding of the mechanisms that underlie myogenesis and its control by nutrition. A powerful strategy is based on genomics in combination with classic biochemical and physiological studies. This is facilitated by the use of statistical tools that allow to understand the control of a wide variety of phenotypes from genetic, environmental or nutritional origins. One of the main challenges will be to solve the problems posed by the analysis and interpretation of the large amount of data that will become available from structural (QTL, SNP) and functional genomics (mRNA, protein levels). Applications are envisaged such as (a) the identification of new predictors of meat quality traits, (b) the monitoring of beef quality through the production systems for beef authentication, and (c) the improvement of animal selection (markers assisted selection) by including quality traits. These new information on muscle characteristics will be useful in selecting animals to ensure good quality meat for consumers.

Genomics: a new and promising scientific field

The advent of high-throughput analyses of gene sequence and expression has revolutionised the scientific approach to genetics and physiology, and in particular to muscle physiology (Eggen and Hocquette 2004). Firstly, improvements in the efficiency of DNA sequencing have allowed the recent full sequencing of several genomes. This allows for a better understanding of the genome structure. Secondly, finding single nucleotide polymorphisms is much easier than previously possible since they can be considered as a by-product of genome sequencing projects. This will help to identify genetic markers which will be useful for animal breeding.

High-throughput techniques have arisen as a result of manual procedures having been replaced by robotics, and automation/miniaturization has increased the speed of laboratory analysis. For example, the expression of thousands of genes can be studied simultaneously by quantifying the levels of their transcripts with microarrays (Jordan, 1998). This large-scale analysis of mRNA levels is called transcriptomics. So, genomics addresses the structure and function of genes, and is only possible by large-scale analyses of gene characteristics at the DNA and mRNA levels. Furthermore, proteomics allows the analysis of hundreds of proteins simultaneously. Initially defined as the effort to catalogue proteins expressed within tissues, the definition of proteomics has evolved since it now includes the systemic study of protein cellular location, post-translational modifications and interactions between proteins.

Genomics applied to bovine muscle tissue

One of the application of genomics has been the identification of genes specifically expressed in red or white muscles. It was a new attempt to understand, on a genomic

scale, the expression profiles that underline the skeletal muscle classes or allotypes. In mice, microarray analysis identified 49 mRNA sequences that were differentially expressed between white and red skeletal muscles, including newly identified genes. These genes can be classified into different biological functions: energy metabolism (14 genes), transcription factors and regulators (10 genes), contractile structure (7 genes), Ca^{2+} homeostasis and signalling (4 genes) (Campbell *et al* 2001). Not surprisingly, the majority of the differentially expressed genes were classified as metabolic genes, which confirms that differences in the ATP-generating process are important between muscle types.

As genetic improvement is a permanent and cumulative procedure, the objective of breeders is to exploit the variability among animals by selecting those with a superior genetic potential for the trait of interest. One of the problems with this approach is the high number of criteria, which have to be considered in order to improve beef quality. Unfortunately, no routinely used biochemical methods are available to breeders to assess those meat quality traits. Taking all the difficulties into account, research is currently being conducted in order to identify genes that influence quality traits, and therefore that can be used to select animals. Allelic variation, the genetic mechanism inducing variability in phenotypes, is a promising approach. The traditional approach to genetics is to map quantitative trait loci (QTL) to a chromosomal region using linkage and linkage disequilibrium. The region of interest will then be fine mapped by using new genetic markers in order to reduce the confidence interval that contains the genes of interest. The final step is the identification of the gene itself, which determines variability in the studied phenotype and of the causal mutation (Goddard 2003). This strategy takes advantage of the structural knowledge of the genome based on cytogenetics, genetics, radiation hybrids, physical and comparative maps of various genomes from different species (Eggen and Hocquette 2004).

Expression profiling at the mRNA or protein levels has already provided valuable data sets in the field of muscle biology in various species with different objectives including characterisation of muscle types. All these genes or proteins, that are important for muscle biology, are potentially interesting candidates for improving the understanding of bovine muscle biology. However, muscle tissue is a complex tissue with different cell types (multi-nucleated fibres, adipocytes, etc) in various proportions within the same muscle. This may induce a high sample-to-sample variability. The development of genomics and proteomics brings a huge potential for improving meat quality. Long-term applications in cattle breeding, husbandry and nutrition have to be considered. It can be anticipated that, in the future, individual prediction of beef quality could be achieved based on muscle-specific genomic data (e.g. the gene or protein expression signature) according to the production systems.

Spectroscopic techniques for online monitoring of meat quality

Spectroscopy is the study of the interaction between electromagnetic radiation and atoms, molecules or other chemical species. The use of spectroscopy in food science has increased tremendously in the last couple of decades as it has appeared that detection and estimation of a number of food constituents and properties may be achieved by measuring the amounts of this radiation that is either absorbed or emitted at different wavelengths. Absorption spectroscopy is now widely used in food analysis, including the estimation of proteins, carbohydrates, mineral elements, vitamins and many additives. Emission spectroscopy (or fluorescence spectroscopy) has increased much in importance in the last decade, and is presently in wide use estimation of fat oxidation, collagen, and certain elements.

Fluorescence offers several inherent advantages for the characterization of molecular interactions and reactions. First, it is 100-1000 times more sensitive than other spectrophotometric techniques. Second, fluorescent compounds are extremely sensitive to their environment. For example, tryptophan residues that are buried in the hydrophobic interior of a protein have different fluorescent properties than residues that are on a hydrophilic surface. This environmental sensitivity enables characterisation of conformational changes such as those attributable to the thermal, solvent or surface denaturation of proteins, as well as the interactions of proteins with other food components. Third, most fluorescence methods are relatively rapid and a spectrum is recorded in less than 1 second with a CCD detector. In meat, the intrinsic fluorophores include the aromatic amino-acids tryptophan, tyrosine and phenylalanine, structural proteins such as elastin and collagen, the enzymes and coenzymes NADH, FAD and NADPH, the vitamins A, K and D, derivatives of pyridoxal, porphyrins, phospholipids and the lipid pigments lipofuscin and ceroids. Riboflavin is another prominent fluorophore that occurs in meat products.

Tenderness and muscle types as evaluated by fluorescence spectroscopy

When there is a substantial range in collagen levels within a muscle or between muscles, there is a significant relationship between collagen content and the variation in tenderness [49]. Consequently, fluorescence can probably be used to estimate tenderness in such muscles. Determination of tenderness in a muscle like *LD* based on fluorescence emission spectroscopy (excitation between 332nm and 380nm) has, however, proven to be difficult[4]. The collagen content in *LD* is low and stable. In addition, variation in sarcomere length and other factors, which are not picked up by fluorescence, reduces the ability to obtain reliable calibration models for tenderness. A combination of fluorescence and measurement of light scattering by, for instance, NIR could improve the feasibility, but preliminary work suggests that there is not much to gain.

The tryptophan fluorescence spectra from meat have also been evaluated for tenderness measurement and for discrimination between different muscle types. Dufour and Frencia (2001) measured emission spectra of protein tryptophan residues for the meat samples from *Longissimus thoracis* (LT) and *Infraspinatus* (IS) at 2 and 14 days post-mortem. The maximum emission was observed at about 336 nm and shifted slightly as a function of meat sample and ageing. Based on the spectral profiles, it was possible to discriminate between the muscles. Frencia *et al.* (2003) continued this work by recording the tryptophan fluorescence spectra on 5 muscle types (*Tensor fasciae latae* (TFL), *Longissimus thoracis* (LT), *Semi-tendinosus* (ST), *infraspinatus* (IS) and *Triceps brachii* (TB) at 2 times (2 and 14 days) of ageing. By discriminant analysis, 82 % of the samples were correctly classified. It was concluded that tryptophan fluorescence spectra are characteristic fingerprints allowing a relatively good identification of muscle type at 2 and 14 days postmortem. These results indicate the possibility of classification of muscle type by fluorescence spectroscopy.

Preliminary studies also suggest that the tryptophan spectra might contain information related to rheology and sensory variables related to tenderness. The fluorescence spectra (spectrometer with a front-face device or coupled to a fibre optic), the mechanical properties and the sensory characteristics were recorded at 2, 6 and 11 days post-mortem on 3 muscles (*Longissimus thoracis*, *Semi-tendinosus* and *Triceps brachii*) sampled on 2 bovine carcasses. As the 3 methods were able to discriminate between the samples, the correlation between the results obtained with sensory analysis, rheology and fluorescence spectroscopy were investigated by canonical correlation analysis (CCA). Table 1 shows strong correlations between the different data tables.

Considering sensory and spectral data, the canonical coefficient for the canonical variates 1 was 0.95.

Table 1: Canonical correlation coefficients (R) for the first canonical variates of CCA performed on the sensory, rheology and spectral data

	R
Sensory analysis / Spectroscopy with optic fiber	0.93
Sensory analysis / Spectroscopy with front face device	0.95
Texturometer / Spectroscopy with front face device	0.95
Spectroscopy with front face device / with optic fiber	0.96

Lebecque et al. (2003) designed a sample set of *Semitendinosus* muscles of 25 Charolais carcasses of different sex and age (30 months to 8 years), which spanned a wide range of meat tenderness. Sensory analysis (8 texture attributes) and fluorescence analysis (tryptophan residues) were performed after 7 and 12 days of ageing. Canonical correlation analysis performed on the sensory and fluorescence data showed that the first two canonical variates were correlated with squared canonical correlation coefficient equal to 0.57 (level of confidence, $p < 0.001$). This result indicates that the texture attributes of meat may be derived from the fluorescence spectra of protein tryptophans. Allais et al. (2004) also showed that similar results could be obtained on meat emulsions and frankfurters. A laptop compatible spectrofluorimeter that may be used in the abattoir for texture measurements is currently under development.

CONCLUSIONS

ENITA Clermont is a member of the InnoViandes Competitiveness Cluster (http://www.innoviandes.org/) located in Clermont-Ferrand (France). It groups French resources together from public research laboratories (INRA), technical centres (ADIV) and institutes, universities (ENITA Clermont; University of Clermont-Ferrand) and companies in the meat and meat products sector. The partners in the Competitiveness Cluster represent 75% of resources available in France for research in this sector and 80% of resources available for transfer in terms of development, innovation and adult training. The research and development personnel of the Cluster's partners working in the meat and meat products sector represents approximately the equivalent of 200 full time workers. The goal of the Competitiveness Cluster is to provide research and development, innovation and training solutions for companies in the meat and meat products sector (slaughtering, cutting, transformation and distribution) and related sectors.

InnoViandes competitiveness cluster leads three missions:

- to make sure that companies in the sector express their needs regarding research, development, innovation and training;
- to connect up the needs of companies with the competencies of appropriate partners;
- to optimize the possibilities of funding projects created through this consultation with the award of a label and help for project bearers to find more appropriate sources of funding.
- Several phenomena are currently creating difficult conditions for the meat and meat products sector:

- generally in France, the drop in the consumption of meat joints;
- the repercussions of successive sanitary crises concerning cattle, pig, sheep and poultry which have resulted in new requirements with high costs;
- the evolution of consumer expectations regarding products, technologies and animal production methods;
- competition from countries which have lower production costs.
- In this context, the competitiveness of the French industry must be reinforced by:
- cost reduction thanks to an improvement in processes, an increase in productivity, reduction and added value for waste;
- the availability of products that stand out as being of high quality and technically superior, ensuring they can be distinguished from international competition.
- Following a survey conducted with 350 companies, 8 strategic fields have been defined as the key components of their competitiveness:

Hygiene and safety mastering the methods and habits that ensure there is respect for the rules and disciplines related to the organisation of sanitary safety in the meat and meat products sector, and a reduction in the costs of managing traceability within the industry.

Product innovation fully exploit the wealth of resources in France (breeding methods, production or cooking recipes etc) in order to offer new products which are mastered technically and nutritionally.

Environment, by-products, water, energy minimize the cost of disposal procedures used for waste and by-products, which remain highly prohibitive, by finding solutions to value them either in the form of energy or ingredients; save energy and water; limit environmental impacts especially from used water effluents.

Production and quality factors mastering production factors which have an impact on the quality of meat and meat products (tenderness, processing abilities, nutritional quality).

Mechanization-robotization-automation get round the lack of qualified workforce, particularly in the slaughtering sector, by mechanizing some tasks and creating new processes combining industrial automation and robotics.

The image and acceptability of meat products and technologies by the consumer-citizen define tools with which, from the representation the consumers have of meat products, from technologies used and breeding methods, it will be possible to predict the crisis and foresee the reactions of the consumers about new products.

Nutritional qualities better understanding and improvement in the nutritional quality of meat and meat products.

Training meeting company requirements regarding training of the workforce by designing programmes adapted to their needs.

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