Scientific Articles

International Conference

"AGRICULTURE AND FOOD FOR THE XXI CENTURY"

Celebrating the XXV Anniversary of Agronomy Higher Education in Sibiu

CONFERENCE SECTION

Food

FOOD DESIGN AND ENGINEERING
FOOD QUALITY CONTROL
CHEMISTRY AND FOOD SCIENCE
FOOD BIOTECHNOLOGY
ECONOMICS, ENERGY AND ECOLOGY IN FOOD INDUSTRY
FOOD MANUFACTURING, SERVICES AND PRODUCTS MANAGEMENT

May 11-13, 2017
Published in Romania by "Lucian Blaga" University Sibiu
ISSN 1843-0694

Scientific Articles of the International Conference
"AGRICULTURE AND FOOD FOR THE XXI CENTURY"
May 11-13, 2017, Sibiu, Romania

Sponsors:

PRIMĂRIA MUNICIPIULUI SIBIU
CONSILIUL JUDEȚEAN SIBIU
ASOCIAȚIA JUDEȚEANĂ DE TURISM SIBIU
S.C. APILIFE RO S.R.L.
S.C. AGROTRANSCOM-Ex S.R.L.
S.C.ANALYTIK JENA ROMÂNIA
S.C. BEST S.R.L.
S.C. DULCIURILE ANEI S.R.L.
S.C. SUN COOKIES S.R.L.
S.C.AMYLON S.A.
S.C.EXPERTAROM FOOD INGREDIENTS
S.C. GREWE
S.C. MINDENGINEERING
S.C. NATUR CARM S.R.L.
S.C. MOARA CIBIN S.A.
S.C. PEST AGRO PLANT S.R.L.
S.C. SARTOROM IMPEX S.R.L.
S.C. SYNGENTA AGRO S.RL
I.F. OPINCARIU LUCIAN "PUIUL TÂRGULUI"
I.F. STRĂULEA MIHAI
I.F. PAHOMI VASILE
P.F.A. MITREA ARIADNA

Dear colleagues and distinguished guests,

I have widely opened the time window and I've looked back. Where have already passed 25 years? How many of our beginning dreams have become reality and how many are still waiting to be transformed into reality? In mankind history, 25 years are only a blink of an eye, but for us they are years of teaching and thorough research, to put the name of Lucian Blaga University of Sibiu, by means of the Faculty of Agricultural Sciences, Food Industry and Environmental Protection on the map of agronomy higher education. Was it a hard way? We consider it wasn't, because when you work with pleasure, you are dedicated to live the feeling of spiritual fulfillment.

In a quite short period of time, a quarter of a century, the specialization "Montanology" of the Faculty of Agricultural Sciences, Food Industry and Environmental Protection has managed to define its role as trainer for professional elites. We have been continuously preoccupied by developing the professional skills and competences of our students, adapted to the demands of today's labor market and of the entrepreneurial society, constantly focusing on the modernization of teaching and research approaches, in order to provide the competences and to reach the performance standards for the future agriculture specialists, graduates of license and master programs.

The academic staff was always preoccupied by the harmonization of the teaching process with the national and international trends, and this was possible by developing partnership relations with similar universities, from Romania and Europe, by adapting our curricula and research areas to the national and abroad ones.

The visibility of our specialization is granted by the professional prestige of our 20 members of the teaching staff, whose concern towards the quality of the teaching is harmoniously combined with the results from their scientific research activity, these enabling our Faculty to be an excellence pole within the University, and not only.

The academic staff was involved during these 25 years in many research grants, has published more than 60 ISI papers and other 720 in prestigious journals from Romania and abroad. We are involved in many SOP-HRD projects, by which we provide our students with practical training, entrepreneurial formation, etc.

But the most we are proud because our students which, after being "polished" by the academic staff during four years, represent genuine "jewels", spread all over the country, making the expression "A good farmer makes a good farm" to be backed by reality. In mayoralties, agriculture boards, animal or vegetal farms, B&B houses, in education (primary, secondary or higher), in state or private jobs, our graduates have rapidly integrated into a very fluid and unpredictable labor market, and these celebrations represent a good opportunity to enjoy together the fact that the efforts of our teaching staff have resonated in the souls of our students, then graduates, that became not only good specialists, but true HUMANS.

From our best graduates, eight have completed their professional training by doctoral studies, obtained in prestigious universities from Romania and abroad.

Making a honest analysis of the achievements and flaws of all these years, we can state that we have sowed the "miraculous seed" of agricultural science and research in Sibiu, and we fully enjoy the results of our work. The efforts and endeavor of the whole "Montanology" staff during this quarter of a century enable us to look forward towards excellence, corresponding to the knowledge-based society, and to the prestige consolidation of the Faculty and of the Lucian Blaga University, worldwide. We have all the capabilities to focus the teaching process on new bases, making the agronomy higher education of Sibiu compatible with the Romanian and European one.

VIVAT, CRESCAT, FLOREAT!

With the highest consideration and warmest gratitude,

Dean,
Professor Camelia SAVA, Ph.D

Foreword of the Director of the Department of Agricultural Sciences and Food Engineering

In 2017 we celebrate with great honor the 25th anniversary of agricultural higher education at the Faculty of Agricultural Sciences, Food Industry and Environmental Protection – a "young" faculty founded in 1990 initially focused on food education of great significance for the overall evolution of the "Lucian Blaga" University of Sibiu.

The existing agricultural traditions and rural gastronomic culture in the Sibiu area have facilitated the development of agricultural studies at higher education, as well. The development of the faculty has been a successful one that has led to important achievements with the diversification of study programs in consensus with the scientific progress in the field, new technologies and challenges of the Romanian society.

It is worth noting not only the constant concern for the development of new curricula and the improvement of the existing infrastructure, but also the enthusiasm and the devotion of the academic staff who understood that the education of students must be done alongside with the scientific research through which students are guided to find their own way for professional evolution. For this, a lot of scientific publications, monographs, patents and research projects are testified.

These constant concerns have succeeded over the years in attracting many students, whose efforts during the studying years have been rewarded by their involvement in educational and scientific activities and projects together with the academic staff of the faculty and by the recognition of their professional skills through the employment in institutions and companies in the field; this, in an increasingly competitive labor market. Our graduate students have continued to join the faculty's efforts to integrate into a European educational system and to create strong partnerships between the academic community and the private corporations.

I would like to acknowledge each and every one of my colleagues from the Faculty of Agricultural Sciences, Food Industry and Environmental Protection for all the achievements so far and I wish them the best in on-going their works to make the agri-food higher education at Sibiu an attractive one at national and international level, both for students and for the academic society.

Sibiu, May 12, 2017

With the most kind thoughts and consideration,

DIRECTOR OF THE DEPARTMENT,
Professor Simona OANCEA, Ph.D

SUMMARY

VALORISATION OF SOME INDIGENOUS PLANT EXTRACTS FOR INDUSTRIAL APPLICATIONS

Mirabela Perju [1], Simona Oancea [2]

[1] *"Lucian Blaga" University of Sibiu, Faculty of Sciences, Applied Ecology Research Center,* [2] *"Lucian Blaga" University of Sibiu Sibiu, Faculty of Agricultural Science, Food Industry and Environmental Protection*

Abstract

A scientific literature review was made investigating bioactive compounds identified in flowers of Rhododendron kotschyi, Rosa canina, Paeonia peregrina and Crocus vernus. The survey showed that these species are not the subject of a substantial number of studies. In most cases, bioactive compounds from flowers were ignored, the main research being focused on extracts from the fruits of the above mentioned species. Such biological materials constitute excellent starting points for valorisation of their specific biochemical composition.

Keywords: Plant extracts, bioactive compounds, Rhododendron sp., Rosa sp., Paeonia sp., Crocus sp.

INTRODUCTION

Most of plants contain chlorophylls, an essential pigment, which is used for photosynthetic activity. Other pigments than green are as important to plant through creation of contrasting colors for the attraction of pollinators and seed dispersal animals.

Flavonoids are important bioactive compounds with good antioxidant, anti-inflammatory and anticarcinogenic character and ensure a better activity of the immune system. Extraction, especially from red or blue fruits, can provide some important information on their role in human nutrition; a healthy diet rich in flavonoids is associated with a reduced risk of cardiovascular disease, cancer, diabetes and neurodegenerative diseases.

Studies referring to concentrations of bioactive substances from the Romanian wild and cultivated flora are extremely scarce. Our target species in the present study, *Rhododendron kotschyi*, *Rosa canina*, *Paeonia peregrina* and *Crocus vernus* are not the subject of a substantial number of investigations, and, in most cases, bioactive compounds from flowers were ignored, the aims being especially the fruits of the plants, with higher concentrations of biochemical compounds, such as anthocyanins. *Rosa canina* hips, as raw

material for a large number of food and pharmaceutical products, has been the most important source for scientific analyses.

At international level, studies referring to bioactive substances concentrations from flower petals of the named species are also in reduced number and recently completed. From this point of view, the determination of bioactive compounds from flowers is an extremely important scientific subject, since these plants have a wide distribution on the Romanian territory and considerable populations.

Studies regarding Rhododendron kotschyi

Rhododendron kotschyi was never a quantitative study object for neither national nor international research, therefore the content of bioactive compounds from these species is still unknown. The only consistent research comes from Romanian one. Thus, Georgescu and Bratu (2006) performed a qualitative analysis regarding flavonic glycosides and phenylpropanic derivatives from the flowers, without reference to quantitative information on the bioactive compounds. The team used powder of flowers and leafs and analyzed the 5% methanolic extracts, in order to determine the pigment contents. After performing thin layer chromatographic (TLC) analysis, they made a photodensitometric evaluation of glycosides and phenylpropanic derivatives. The results showed that *Rhododendron* flowers contains hyperoside, chlorogenic acid, quercitroside, caffeic acid (Fig.1) and two unidentified compounds from flavonic glycoside family (Rf = 0.47 and Rf = 0.81).

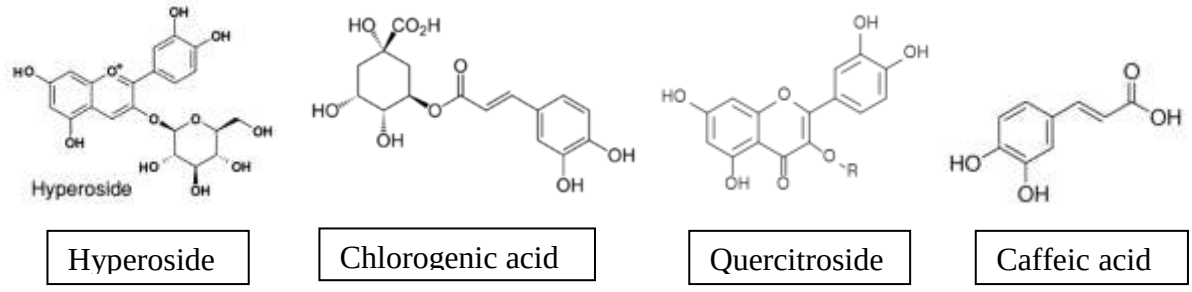

Fig. 1. Chemical formula of some constituents from *Rhododendron kotschy*.(Source: Wikipedia.com)

Qiang et al. (2011) made a review study regarding chemical and biological composition of the genus *Rhododendron*. The team considered all the research studies about phytochemical constituents and biological activity, made between 1990 and 2009. Kaempferol, quercetin isoquercitrin, quercitrin, hyperoside, everninic acid methyl ester,

caffeic acid, chlorogenic acid were found in *Rhododendron kotschyi* flowers from the investigated studies. This review study proved that this investigated genus is such an important source of natural bioactive compounds.

A short communication on triterpenoids from 16 *Rhododendron* species native to Russia is given by Fokina (1980). The researcher used leafy branches collected in the post-flowering phase, and performed quantitative determination for both ursolic and oleanolic acids, by either converting them into methyl esters, or with the help of a solution of diazomethane. Regarding *R. kotschyi*, the results showed that the second method is more effective than the first, the species showing the highest score for that method out of the species investigated, and the ursolic acid quantities were higher than those of oleanolic acid.

Studies regarding Rosa canina

An important study regarding *Rosa canina*, among other related species, was conducted by Adamczak et al. (2012), who investigated both the flavonoids and other organic compounds from 75 samples belonging to eleven species of rose hips (*Rosa* sp.). Using spectrophotometry for lyophilized plant material, in order to analyze flavonoids, and high performance liquid chromatography (HPLC) for freeze-dried and powdered rose hips, in order to extract citric and ascorbic acids, the authors showed that, among rose hips species, *R. canina* has the lowest amount of ascorbic acid (around five times less than the species with the highest values, *R. villosa* and *R. glauca*), one of the lowest amounts of flavonoids (half the values of *R. agrestis* and *R. rubiginosa*, the leading species in flavonoids concentrations), but one of the highest amounts of citric acid. Among the eleven species, *R. canina* seems to be most similar regarding bioactive composition to *R. jundzillii* and *R. zalana*.

Cunja et al. (2014) analyzed the presence of bioactive compounds from leaves and flowers of four wild rose hips species (*R. canina*, *R. glauca*, *R. rubiginosa* and *R. sempervirens*) and of three modern rose cultivars ((Rosarium Uetersen, Ulrich Brunner Fils and Schwanensee). The extraction was performed from a fine petal or leave powder with liquid nitrogen from which 1 g of powder was extracted, centrifuged and the supernatant was transferred to a HPLC system with elution solvents based on 0.1% formic acid. The results showed that phenolic constituents are found in large quantities both in flowers (seven anthocyanins and 31 flavonols) and in leaves (30 flavonols, 14 phenolic acids and their derivatives, 15 flavanols, as well as other 20 hydrolysable tannins). From the seven species and cultivars, *R. canina* has the highest concentrations of total flavonols, flavanols and

phenolic acids and their derivatives, being surpassed only in hydrolysable tannins by two other wild flora species.

The phytochemical substances from Portuguese *R. canina* were analyzed by Barros et al. (2011), in their investigation of exotic plants used in folk medicine. The authors used a variety of techniques to recover good concentrations of phytochemical substances: HPLC for determination of sugars and tocopherols, gas chromatography (GC) for fatty acids, and UV-VIS spectrophotometric techniques for the determination of phenolics, flavonoids, carotenoids, chlorophylls and ascorbic acid. The study pointed out that *R. canina* flowers are having the highest extraction yields of all plant's parts, one of the highest level of phenolics, alongside galls, and therefore, one of the highest antioxidant potential, consistently higher than the one of fruits. The flower petals also have the highest concentration of sugars, lycopene and tocopherols, but the lowest content of ascorbic acid.

An interesting study regarding dog-rose *(Rosa canina)* was made by Nowak and Gawlik-Dziki (2007) targeting the polyphenols from leaves extracts and their radical scavenging activity. The authors used as study material petals from 14 rose species, all grown in natural environments, and collected from Poland. Determination of high total phenolic contents was made by a colorimetric method, the absorption was measured at 660 nm on a UV-VIS spectophotometer on three replicates. The researchers measured the change in color at 515 nm on two replicates. The results showed that all investigated roses extracts have high total phenolic content ranging from 5.7% to 15.2% gallic acid equivalents (GAE) to dry weight. Low levels of phenolic contents were found in a few species, but *Rosa canina* contained 9.9 % GAE of dry weight.

Regarding the antioxidant activity, results showed high activity in all rose leave extracts, with values ranging amongst 83.4% and 95.7%; unfortunately, the study does not make a specific reference on *Rosa canina* petals antioxidant activity. Furthermore, the team showed a positive correlation between the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity and the phenolic content. According to this study, rose leaves have a consistent amount of total phenolic contents (Table 1).

Table 1. The quantity of some phenolics identified in *Rosa canina*, according to Nowak and Gawlik-Dziki (2007).

No.	Species	Track	Area	Quercitin Average area	Amount [μg g^{-1}]	Area	Kaempferol Average area	Amount [μg g^{-1}]
8	*R. canina*	1	0.708	0.776	7535.6±218	0.285	0.263	2380.4±130
		2	0.780			0.245		
		3	0.839			0.260		

A review study regarding *Rosa canina*-derived ingredients that are used in cosmetics (Bergfeld et al., 2016) reveals that derived ingredients from dog-rose are used in cosmetic industry, potentially in pharmaceutical industry. The scientific team found that dog-rose leaf extract contains a lot of bioactive compounds, such as saponins, glycosides and volatile oils. Derived ingredients from dog-rose extracts can be used to manufacture cosmetic products for skin and hair with 0.04% maximum concentration.

In folk medicine, the petals of *Rosa canina* are used to treat different diseases, such as common cold, flue and eczema. The research team described that rose-dog flower extract has also antimicrobial activity which was demonstrated *in vitro*. *Rosa canina* flowers, flower extracts and powder have some functions like fragrance ingredients, cosmetics astringents, anti-acne agents, skin-conditioning agent, as identified in the existing scientific literature.

Another interesting study was conducted on the antimicrobial effects of *Rosa canina* extracts (Shiota et al., 2000). The research team investigated the effects of rosehip petals extracts on methicillin-resistant *Staphylococcus aureus* treated with β-lactams. Dried *R. canina* petals were sampled, homogenized in 70% acetone, filtered, and the extract was then concentrated and extracted using three different solvents (diethyl ether, butanol and ethyl acetate). The active compounds were isolated using HPLC. The results showed that the *R. canina* extract significantly reduced the minimum concentration of β-lactams that creates an inhibitory effect on *S. aureus*. Two compounds were isolated and identified as responsible with the inhibitory effect, as follow: tellimagrandin I and rugosin B, the first compound having a stronger effect, especially in combination with tetracycline.

Studies regarding Crocus vernus

Our literature review showed that studies referring to concentrations of bioactive substances from *Crocus vernus*, either wild or cultivated, are extremely scarce in the

scientific literature. A consistent amount of studies are referring to the closely related saffron (*Crocus sativus*) because its economic importance as spice or test subject of medical researches.

Nørbæk et al. (2002) conducted a study regarding the pigment composition from flowers of *Crocus* species and cultivars that are used for a chemotaxonomic investigation, with the aim to identify anthocyanins and other flavonoids among different species, subspecies, cultivars and artificial hybrids of *Crocus.* They used flowers from 70 species, 43 cultivars and six hybrids for the analysis of pigments and two different gradient systems for the determination of the presence of anthocyanins. Using retention times and known UV-spectra, the research team identified 9 anthocyanins, used for the segregation of seven chemotypes, while chromatography investigation provided information about the contents of flavonoids, used for the segregation of four chemotypes. Six of the flavonoids appear to be specific for *Crocus* species. The gathered information proved useful for differentiation between different *Crocus* species, but several research are still needed for some species.

Studies regarding Paeonia peregrina

A small number of general studies are offering information about *Paeonia peregrina.* Wu et al. (2010) made a review regarding chemical constituents and bioactivities of plants from the entire *Paeonia* genus, finding that *Paeonia peregrina* has two of the principal bioactive components present in the flowers of the genus, paeoniflorigenone and paeoniflorin, used in traditional folk medicine. A lot of the components from *Paeonia* have biological and pharmacological activities but only a few species have been studied so far.

Another study investigated the immunological properties of flowers and root extracts from *Paeonia peregrina* (Nikolova and Ivanovska, 2015). The researchers showed that *Paeonia peregrina* extracts have anti-inflammatory effects, using powder of flowers and roots from wild population, with extractions made in 95% ethanol at room temperature, 3 times, 24 hours. For the test animals they used male mice, on which they proved a consistent anti-inflammatory effect of the *Paeonia* powder, an increase of the antibody response, a strong protective effect against infections with *Klebsiella pneumoniae* and even a suppressive activity against cobra venom.

In their review on biologically active compounds found in Bulgarian medicinal plants, Ivancheva et al. (2006) also discussed *P. peregrina*. The researchers indicated that the radix is used for its anticoagulant, anti-inflammatory, analgesic and sedative properties, as well as a

remedy for female genital diseases, since they are rich in benzoic and gallic acids, terpenoids, triterpenoids and tannins. Anthocyanidins and pro-anthocyanidins are also present in the flowers of Bulgarian exemplars, while benzoic and gallic acids are found in the leaves. The authors also indicated that *P. peregrina* is used by the Bulgarian folk medicine for the treatment of epilepsy.

Mobli et al. (2015) conducted a scientific review on plants indicated by the Iranian philosopher and physician Avicenna thousand years ago as medicinal remedies for abnormal uterine bleeding. In its book Canon, Avicenna indicated 24 plant species as useful for this particular affection, amongst them being *P. peregrina*. The researchers described that Avicenna's indication, although based on empirical data, has a scientific basis, since ethanol extracts from both flowers and roots of the plant have anti-inflammatory activity in acute episodes, and a glycoside extracted from *Paeonia* sp. root, called Paeoniflorin, has an anti-proliferative and also apoptotic effects on cancerous cells, therefore the plant is a good candidate for anti-inflammatory conditions.

Paeonia peregrina is, among other plants, the study subject of a research team investigating the antimicrobial effect of plant extracts from Turkey (Kunduhoglu et al., 2001). Twenty-two plant species were used in the experiment, air dried, powdered and extracted with a Soxhlet extractor using ethanol, acetone and ether. Dimethyl sulfoxide was used as solvent after the evaporation, and a filtering method as sterilizer. The obtained extracts were used as inhibitors for 14 microorganism taxons, and the results indicated that *P. peregrina* extracts, in different solvents, were efficient as inhibitors for 11 of the investigated taxons, the most important effect being observed, in the order of their intensity, on *Staphylococcus sp., Sarcina sp.* and *Bacillus sp*. The only species where the plant extracts have no effects are *Candida albicans, Pichia membranifaciens* and *Saccharomyces cerevisiae*, three species of yeast of the five investigated.

Rovná et al. (2015) conducted a relatively similar experiment regarding the antimicrobial effects of *P. peregrina* flower extracts, using different microorganism strains than the previous study, focusing on potential pathogens. The authors used a relatively similar methodology, exploiting air dried, powdered and ethanol extracted samples, but, unlike the previous study, they used ethanol and methanol as solvents. The results of the study pointed out that the effects of *P. peregrina* extracts were higher on the used bacterial strains (*Pseudomonas aeruginosa* and *Escherichia coli*) than on the microscopic fungi (*Aspergillus niger, Fusarium culmorum* and *Alternaria alternate*), somehow confirming the

findings of Shiota et al. (2000) and Kunduhoglu et al. (2001) on the importance of *P. peregrina* extracts as antibacterial substances, particularly in combination with specific antibiotics.

From the hereby investigated plants, it can be concluded that the most studied in the scientific literature was *Rosa canina*, followed by *Paeonia peregrina* and *Crocus* vernus, as presented in Figure 2.

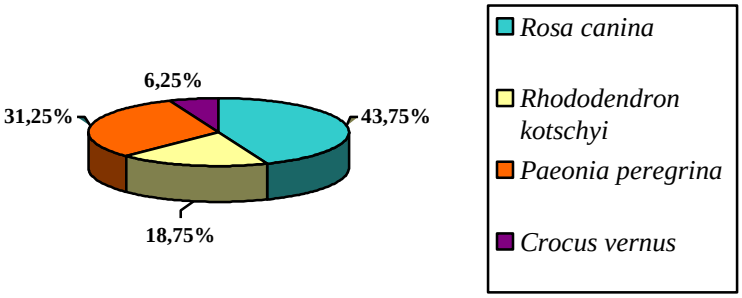

Fig. 2. The number of scientific studies on four plant species.

CONCLUSIONS

The investigated plant species in the present study have been the subject of a relatively small number of scientific studies, and, where the studies are present, they focused on the investigation of anthocyans-rich fruits of that species, overlooking other plant parts, especially flower petals.

As resulted from the present study on four plant species, the most representative in the scientific literature was *Rosa canina* (43.75%), followed by *Paeonia peregrina* (31.25%) and *Crocus vernus* (6.25%).

New approaches, such as the establishment of the correlation between the bioactive compounds level and various environmental factors become important issues for wild flora species to be tackled in the future. Such studies could provide useful information about the identification of high potential populations among species, and of potential optimal cultivation conditions for the species, with important economic advantages, respectively.

REFERENCES

Barros L., Carvalho A.M., Ferreira I.C.F.R, Exotic fruits as a source of important phytochemicals: Improving the traditional use of *Rosa canina* fruits in Portugal, *Food Research International*, 2011, 44, 2233–2236.

Bergfeld W.F., Belsito D.V., Hill R.A. et al. Safety assessment of *Rosa canina*-derived ingredients as used in cosmetics, *Cosmetic Ingredient Review Washington*, 2016.

Cunja V., Mikulic-Petkovsek M., Stampar F., Schmitzer V., Compound identification of selected rose species and cultivars: an insight to petal and leaf phenolic profiles, *Journal of the American Society for Horticultural Science*, 2014, 139(2), 157–166.

Fokina G. A., Triterpene acids of rhododendron plants of the flora of the USSR, *Chemistry of Natural Compounds*, 1979, 5, 734-735.

Georgescu C., Bratu I., Qualitative analysis of flavonic glycosides and phenylpropanic derivatives from Rhododendron kotschyi specie, *Journal of Agroalimentary Processes and Technologies*, 2006, 12, 1, 175-180.

Ivancheva S., Nikolova M., Tsvetkova R., Pharmacological activities and biologically active compounds of Bulgarian medicinal plants, *Phytochemistry*, 2006, 87-103.

Kunduhoğlu B., Pilatin S., Caliskan F., Antimicrobial screening of some medicinal plants collected from eskisehir, Turkey, *Fresenius Environmental Bulletin*, 2011, 20, 945-952.

Mobli M., Qaraaty M., Amin G., Haririan I., Hajimahmoodi M., Rahimi R., Scientific evaluation of medicinal plants used for the treatment of abnormal uterine bleeding by Avicenna, *Archives of Gynecology and Obstetrics*, 2015, 292, 21–35.

Nikolova P., Ivanovska N., Estimation of immunological proprierties of flower and rot extracts from Paeonia peregrine, *Journal of Herbs, Spices & Medicinal Plants*, 2015, 6:4, 1-9.

Nowaka R., Gawlik-Dzikib U., Polyphenols of Rosa L. leaves extracts and their radical scavenging activity, *Zeitschrift für Naturforschung ,* 2006, . 62 c, 32-38.

Nowak R., Tuzimski T., A solid-phase extraction–thin-layer chromatographic–fiber optical scanning densitometric method for determination of flavonol aglycones in extracts of rose leaves, *Journal of Planar Chromatography*,2005, 18, 437- 442.

Nørbæk R., Brandt K., Nielsen J.K., Ørgaard M., Jacobsen N., Flower pigment composition of *Crocus* species and cultivars used for a chemotaxonomic investigation, *Biochemical Systematics and Ecology*, 2002, 30, 763-791.

Rovná R., Petrová J., Terentjeva M., Černá J., Kačániová M., Antimicrobial activity of *Rosa canina* flowers against selected microorganisms, *Journal of Microbiology, Biotechnology and Food Sciences*, 2015, 4, 62-64.

Shiota S., Shimizu M., Mizusima T., Ito H., Hatano T., Yoshida T., Tsuchiya T., Restoration of e¡ectiveness of L-lactams on methicillin-resistant Staphylococcus aureus by tellimagrandin I from rose red, *FEMS Microbiology Letters*, 2000, 185, 135-138.

Winther K., Hansen A.S.V., Campbell-Tofte, J., Bioactive ingredients of rose hips (*Rosa canina* L) with special reference to antioxidative and anti-inflammatory properties: in vitro studies, *Botanics: Targets and Therapy*, 2016, 6, 11-23.

Wu S.H., Wu D.G., Chen Y.W., Chemical constituents and bioactivities of plants from genus *Paeonia*, *Chemistry & Biodivesity*, 2010, 7, 90-104.

THE ROMANIAN CUSTOMS FOR THE FISH AND FISHERY PRODUCTS CONSUMPTION COMPARED TO THE EU ONES

Carmen Georgeta Nicolae[1], Monica Paula Marin[1], Gratziela Victoria Bahaciu[1], Magda Ioana Nenciu[2]

[1] University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, 011464, Romania

[2] National Institute for Marine Research and Development "Grigore Antipa" Constanta, 300 Mamaia Blvd, Constanta 3, RO-900581, Romania

Abstract

Food is an integral part of the global trading system. At the international level it is considered that 77% of global production of fish and fishery products it used directly for food consumption. The fish represents about 16.6 percent of animal protein supply and 6.5 percent of all protein for human consumption. The food market is not uniform. There are some segments correlated with consumer incomes, tastes, needs, cultural or religious habits or thereof combinations. As markets are bigger and stronger, the requirements of consumers are more sophisticated. In this regard, a study based on questionnaires applied to consumers of fish and fish products in Romania was undertaken and the results were compared with those reported in EU. According to the results, 66% of consumers prefer fresh fish, bought from fishmonger or specialist shop (67%), and the product shelf life is important (12%). At EU level, only 35% of consumers prefer fresh fish, bought from the grocery store or super/hyper market (74%), and the product appearance is important (58%). The results of this study can serve as information material for the production, processing and distribution of fish and fishery products in achieving of traceability system. Implementing a traceability system significantly reduces the risk exposure of operators because it helps to identify, isolate and correct the occurred situation quickly and efficiently. This is a tool to guarantee food safety and quality and increase the confidence of consumer.

Keywords: consumer, food chain, quality, safety, traceability

INTRODUCTION

The distribution chain for fish products which includes all the links from the production (fishing or aquaculture) to final consumer must be realized by complying and assuring the quality and safety terms. The supply chain components vary according to geographic area, farm's type, transportation, information on the fishery market and management system (Nicolae et al., 2015). A successful distribution chain formula contains an equally mixed blend of competitive price, high quality products obtained in line with specific standards, modern logistics, strict specifications and efficient control. In fact, the

good results are confirmed by market studies that show consumer trust in a brand or a shop. If the system of traceability meets the demands of the stakeholders in the supply chain, it will be consider an efficient tool to guarantee the safety and quality of fish and fishery for consumers (Nicolae et al., 2014).

Companies in the fishery sector establish their marketing strategies based on consumers demands but also on the competitors ones. In the last years, publicity, diversification of the selling channels, globalisation and consumers travelling have determined the convergence of the international taste. Thus, the consumer needs continue to change and adapt, so producers, importers, exporters, traders of fish and fishery products must pay attention to those needs if they want to have the business going and make profit. In our country is an increasing of awareness stakeholders about the safety, environmental, social and legal issues associated with food, including fish and fishery products. This is an important challenge to the corporate social responsibility initiatives of companies (Moga et al., 2016).

MATERIAL AND METHODS

For data collecting it has used the questionnaire method. This questionnaire was focused on the issues that influence consumers habits of consumption and buying fish or/and fishery products. These issues were, in fact, key factors in implementing of an information system for quality traceability. The questionnaire has three sections: elements for respondents classification; consumers preferences (consumption, purchasing behaviour and important criteria for fish or/and fishery product purchasing) and factors related to product that might influence the buying decision (price, appearance, fish origin, brand of quality labels). The questionnaire respondents were asked to evaluate their answers on a five points Likert scale, from 0 to 5, which ranged from "totally disagree" to "totally agree".

The questionnaires were completed in 2015 by 734 consumers of fish and fishery products, with demographics and heterogeneous consuming behaviour, belonging to different regions of Romania. The results regarding Romanian habits for fish and fishery products consumption were compared with those obtained in 2014 by European Market Observatory for Fisheries and Aquaculture Products (EUFOMA), Annex 4 (https://www.eumofa.eu/eumofa-publications), in "EU consumer habits regarding fishery and aquaculture products", Final report. The results based on the data collected will be also use to

write specifications and to project an informatics system for fish and fishery products traceability.

RESULTS AND DISCUSSION

According to Annex 4 of "EU consumer habits regarding fishery and aquaculture products", in 2014, the percentage of employed people who eat fishery and aquaculture products at least once a month (regular consumers) was higher and it varies between 67-70% in Romania and 71-81% in EU (Fig. 1).

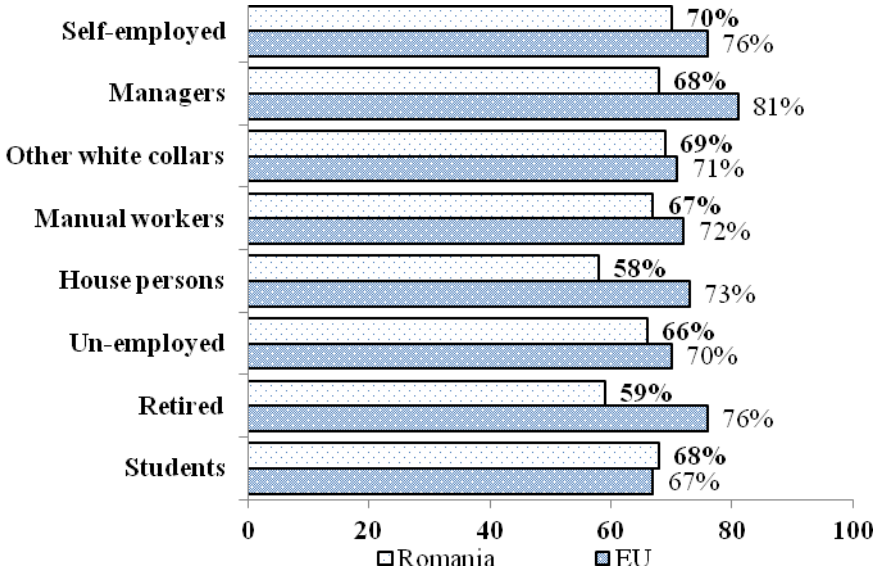

Fig. 1. Socio-professional category of regular consumers (Annex 4 - Country fiches_all MS)

In the study carried out in 2015, the highest percentage of respondents was represented by employed persons, 68% respectively (Fig. 2).

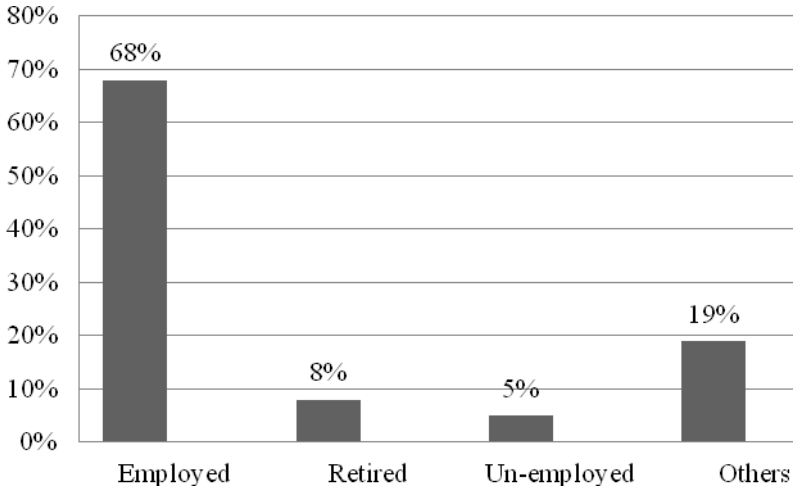

Fig. 2. Socio-professional category of respondents

In 2014, according to Annex 4, the highest percentages of the regular consumers were the people aged over 55 years in the EU (77%) and aged between 40-54 years in Romania (69%) (Fig. 3).

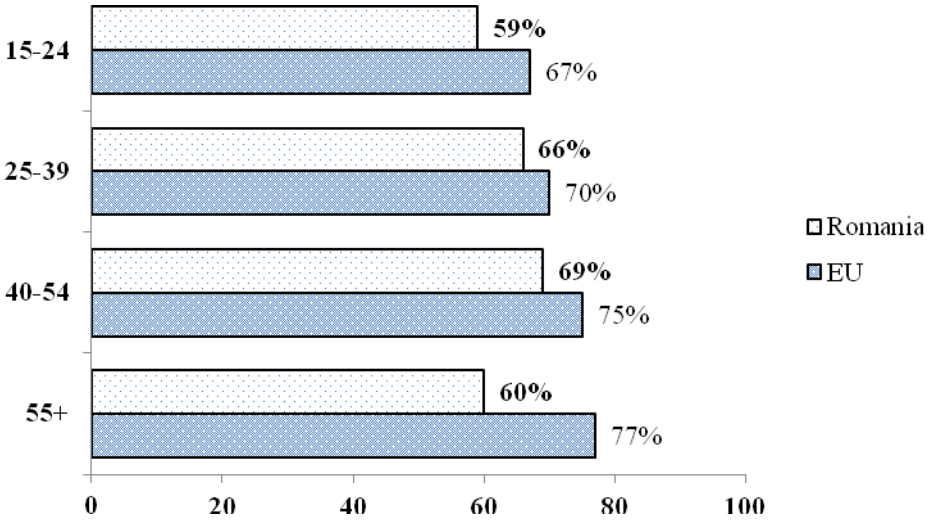

Fig. 3. Age categories of regular consumers (Annex 4 - Country fiches_all MS)

In our study, by analysing data collected in 2015, the highest percentages of respondents (37%) were the people aged between 40-55 years (Fig. 4).

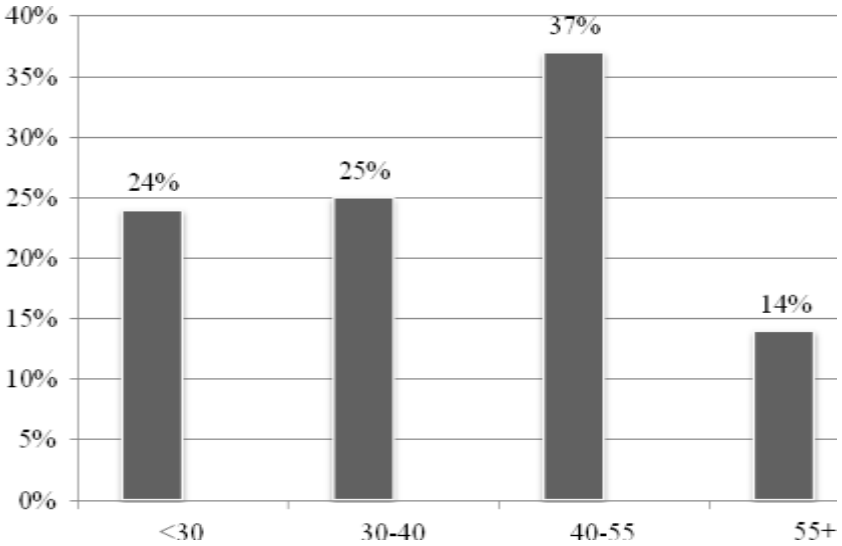

Fig. 4. Age categories of respondents

Regarding preferences about types of fishery products consumed, according to Annex 4, it is found that the highest proportion of Romanian and EU consumers prefer fresh fish. Compared to EU consumers, Romanian consumers prefer not to eat the breed fish (Fig. 5).

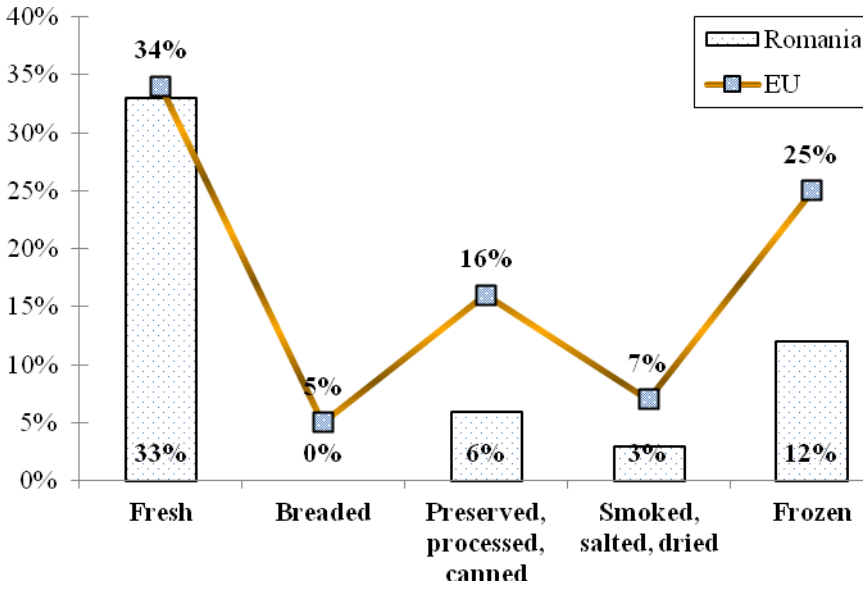

Fig. 5. Preference about types of products (Annex 4 - Country fiches_all MS)

In the poll conducted in 2015, the items considered influential on the consumption of fish and fishery products are related to some types of products: alive, fresh, canned, smoked

or salted, frozen. These items characterize the sale of fish products. Most of the respondents prefer alive (54%) and fresh fish (66% respectively), because it seems to fulfil all the quality conditions (sensorial characteristics, freshness, low microbiological load) (Fig. 6).

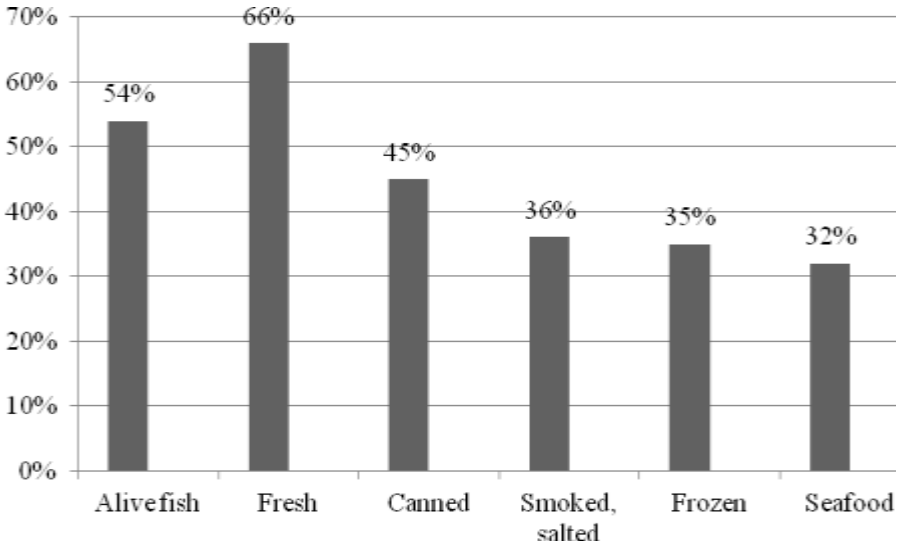

Fig. 6. Preference on types of products (2015)

In both studies, EU and Romanian consumers prefer to buy fish from super and hypermarket (Fig. 7 and 8).

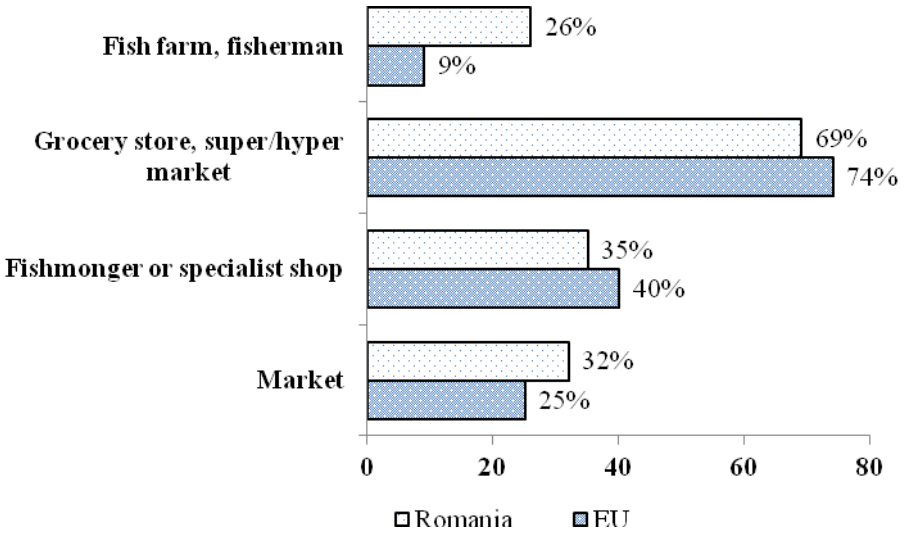

Fig. 7. Place of sale (Annex 4 - Country fiches_all MS)

The Romanian buying habits for fish and fishery products indicate that consumers prefer fishmonger or specialist shop (67%) more than super and hypermarket (66%) (Fig. 8). The reason for this choice may be represented by the fact that this type of store ensures criteria of freshness and quality of fish and fishery products.

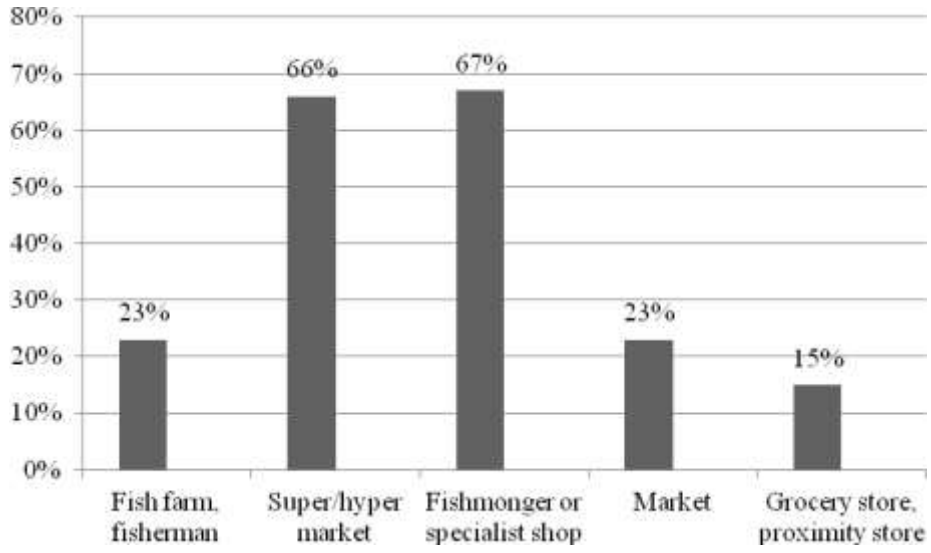

Fig. 8. Place of sale (2015)

In Annex 4, the main purchasing factor is the appearance of fish and fishery products: for Romanian consumers was 60% compared to EU consumers, 58% (Figure 9). For the Romanian consumers it is also important the brand of quality labels (Fig. 9 and 10).

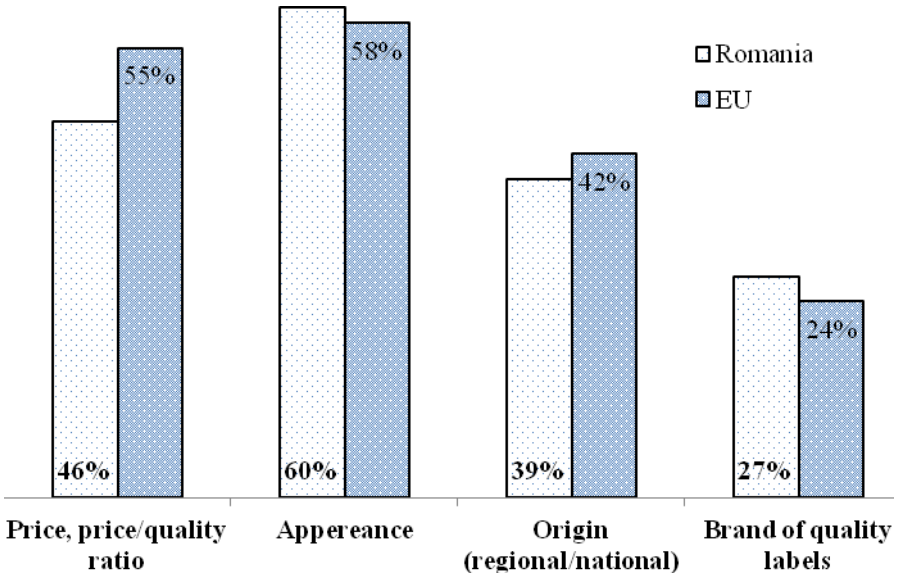

Fig. 9. Factors influencing consumer demand and purchasing behaviour (Annex 4 - Country fiches_all MS)

According to our study, for all respondents the price is less important in their fish and fishery products buying decision (Fig. 10). The quality of fish and fishery products is more important than its price.

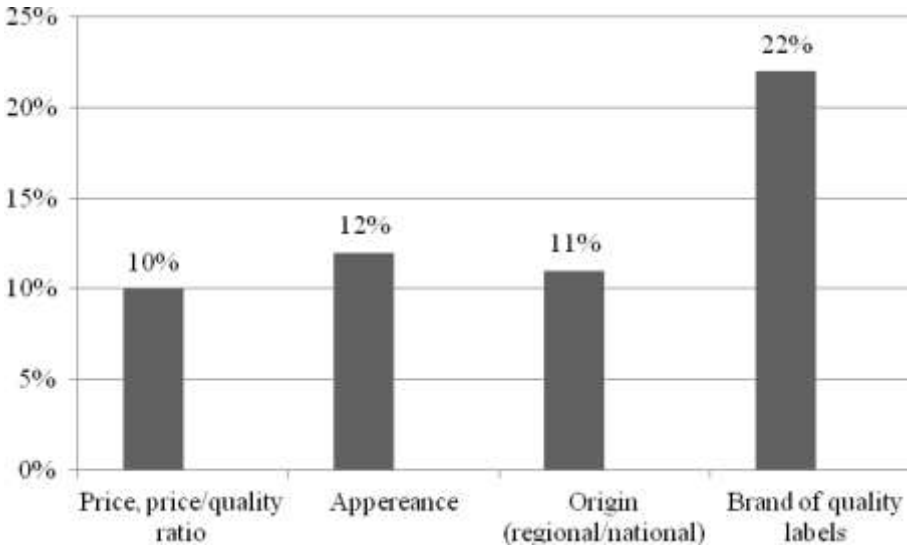

Fig. 10. Factors influencing consumer demand and purchasing behaviour (2015)

CONCLUSIONS

This study provides the elements to understand consumers needs on quality and safety of fish and fishery products. Most of Romanians habit consumption of the fish and fishery products is different compared with EU consumers. The Romanian consumers prefer to purchase alive and fresh fish than frozen or processed fish, provided from fishmonger or specialist shop, super and hypermarket. The price of fish and fishery products, for Romanian consumers is less important.

The Romanian and EU consumers will play a key role in the new Common Organisation of the Markets (CMO) in fish and fishery products.

Among the priorities of the European Maritime and Fisheries Fund (EMFF) it can be found the processing and marketing promotion, supporting and funding initiatives that improve conditions for fisheries and aquaculture marketing and promote the overall quality of products sold. In our country, this initiative should be supported by the implementation of traceability systems of fish and fishery products.

ACKNOWLEDGEMENTS

This research was financed by the Programme Partnerships in Priority Areas - National Plan for Research, Development and Innovation 2007 – 2013 (PN II), sponsored by the Ministry of National Education - Executive Agency for Higher Education, Research, Development and Innovation Funding (MEN - UEFISCDI), Project no. 167/2014, Cloud computing based traceability information system for fishery.

REFERENCES

Moga L.M., Dediu L., Zhang X., Nenciu M.I., Ududec Novac C., Gavrila S.P., Barriers of adopting traceability systems by Romanian fishery and aquaculture, *Journal of Environmental Protection and Ecology*, 2016, 17 (1), 284–290.

Nicolae C. G., Neculita M., Cristea D.S., Trends in the development of traceability systems for fish products, *Quality acces to success, Scientific Journal of Management Systems*, 2014, 15 (143), 95-97.

Nicolae C.G., Moga L.M., Nenciu M.I., Bahaciu G.V., Marin M.P., Particularities and management of the distribution chain for fish and fishery products, *Scientific Papers. Series "AgroLife Journal"*, 2015, 4 (1), 111-116.

https://www.eumofa.eu/eumofa-publications, accessed on February 19, 2017.

MONITORING OF OXIDATIVE-FERMENTATIVE PHYSIOLOGICAL STATES IN FED-BATCH *E. COLI* FERMENTATION

Anastasiya Zlatkova[1*], Velislava Lyubenova[1], Maya Ignatova[1]

[1] *Department "Mechatronic Bio/technological Systems", Institute of System Engineering and Robotics, Bulgarian Academy of Sciences, Acad. G. Bonchev str., P.O. Box 79, 1113 Sofia, Bulgaria*

[*] *corresponding author dr666chase@gmail.com, +359 884 779 741*

Abstract

A new method for monitoring of two physiological states - oxidative and oxidative-fermentative growth on glucose during fed-batch E. coli fermentation is proposed. The method is based on acetate kinetics accepted as reliable marker for recognizing the beginning of the each physiological state. A cascade scheme of software sensors for on-line monitoring of main kinetic parameters is developed with inputs on-line measurements of acetate and glucose concentrations. Tunings of the derived software sensors are realized. The efficiently of the proposed method is investigated by simulations using a new biochemical process model. Discussion about the accuracy of obtained estimates and their relationship with the values of tuning parameters is done. The proposed method could be applied for control algorithms design for each physiological state.

Keywords: monitoring, oxidative-fermentative growth, E. Coli fermentation, software sensors

INTRODUCTION

Escherichia coli is the most widely used host for recombinant proteins synthesis. Wide-spread and successfully employed strategy is high cell cultivation allowing production of various proteins with high yield [1,2].

The main targets of the development and optimization of these processes are production yields, product quality and purity. These can be enhanced at the biochemical [3, 4, 5] and cultivation levels [6-9]. The latter one is usually an object of different control strategies design [6-9].

One important condition for application of control strategies is the availability of on-line information for the main variables and kinetic parameters of the process. As it is well known with respect to biotechnological processes, this information is not fully accessible from hardware sensors. To obviate this problem, a widely applied method is based on derivation of so called software sensors (SS) [10]. These are combinations of hardware sensors with

estimation algorithms implemented to software used to provide on-line estimations of unmeasurable variables and parameters. A class of SS is based on operational models (models for control). They have to describe the main process phenomena with good accuracy and their structures have to be sufficiently simple.

A method for derivation of bioprocess's operational models is General Dynamical Model (GDM) of Bioreactors [11, 12]. It is based on the notion 'reaction scheme' - a set of biochemical reactions.

The reaction scheme of *E. coli* growth could be presented as follows:

- Oxidative growth on glucose, with specific growth rate μ_1 :

$$k_1 S + k_5 O \xrightarrow{\mu 1} X + k_8 C \quad (1)$$

- Fermentative growth on glucose, with specific growth rate μ_2:

$$k_2 S + k_6 O \xrightarrow{\mu 2} X + k_3 C + k_9 A \quad (2)$$

- Oxidative growth on acetate, with specific growth rate μ_3:

$$k_4 A + k_7 O \xrightarrow{\mu 3} X + k_{10} C \quad (3)$$

where *X, S, A, O* and *C* are respectively concentrations of biomass, glucose, acetate, dissolved oxygen and carbon dioxide in the culture broth, k_1-k_{10} – yield coefficients.

Due to the nonstationary nature of bioprocesses and the lack of experiment's reproducibility, the adaptive on-line monitoring and control algorithms are preferred. In the literature, several adaptive approaches based on SS for on-line estimation of partially known process kinetics and/or unmeasured state variables are proposed [13-15]. In [14], an approach for on-line monitoring of biomass concentration and three biomass growth rates is proposed. It is based on adaptive observer theory using on-line measurements of dissolved oxygen, carbon dioxide concentrations and laboratory measurements of biomass. The proposed software sensors estimate the biomass growth rates as unknown time-varying parameters. They are organized in a cascade scheme with large number of tuning parameters which is not favorable for used-friendly synthesis. In contrast to [14], the derived in [15] adaptive software sensors of the same growth rates are based on two sub-models describing oxidative-fermentative growth on glucose and oxidative one on glucose and acetate. The marker for switching the sub-models that describe current state is the critical value of glucose consumption rate obtained on the basis of experiments. In practice this value is not constant

during the process due to its dependence from the strain type and the lack of bioprocesses reproductively.

In this paper, a new method for on-line monitoring of main kinetic parameters related to oxidative and fermentative physiological states of fed-batch *E. Coli* fermentation is proposed. It is realized as three steps cascade scheme of software sensors. Inputs of the scheme are on-line measurements of acetate and glucose concentrations. As it is stated in the literature, [16, 17, 18] there exists hardware sensors for on-line measurements of these variables. The properties of the estimation algorithms are investigated by simulations under different tuning parameters' values using the biochemical model of the process proposed in [19]. In conclusions the applicability of the proposed method is discussed.

MATERIALS AND METHODS

The process under consideration - fed-batch cultivation of *E. coli* is realized in the fermentation laboratory of University of Minho, Braga, Portugal. The experimental materials and methods are described in detail in [6]. A biochemical model in [19] includes two sub-models describing the both physiological states: oxidative growth - sub-model (4) derived according reaction (1) and oxidative-fermentative growth - sub-model (5), derived according both reactions (1), (2). Sub-models are presented as follows:

$$\frac{d}{dt}\begin{bmatrix} X \\ S \\ A \end{bmatrix} = \begin{bmatrix} 1 \\ -k_1 \\ 0 \end{bmatrix} \mu_1 X - D\begin{bmatrix} X \\ S \\ A \end{bmatrix} + D\begin{bmatrix} 0 \\ S_{in} \\ 0 \end{bmatrix} \tag{4}$$

with $\mu_1 = q_s / \mathrm{k}_1$; $q_s = \mathrm{q}_{\mathrm{s,max}} S /(\mathrm{K}_\mathrm{S} + S)$

$$\frac{d}{dt}\begin{bmatrix} X \\ S \\ A \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ -k_1 & -k_2 \\ 0 & k_3 \end{bmatrix} \begin{bmatrix} \mu_1 \\ \mu_2 \end{bmatrix} X - D\begin{bmatrix} X \\ S \\ A \end{bmatrix} + D\begin{bmatrix} 0 \\ S_{in} \\ 0 \end{bmatrix} \tag{5}$$

with $\mu_1 = q_{s,crit} / \mathrm{k}_1$; $\mu_2 = (q_s - q_{s,crit}) / \mathrm{k}_2$; $q_{s,crit} = \frac{\mathrm{q}_{\mathrm{o,max}}}{\mathrm{k}_{\mathrm{os}}} \frac{\mathrm{K}_{\mathrm{i,o}}}{\mathrm{K}_{\mathrm{i,o}} + A}$

where *X, S, A* – concentrations of the main process variables – biomass, glucose and acetate; k_1-k_3 – yield coefficients; μ_1 and μ_2 – specific growth rates related to oxidative and oxidative-fermentative biomass growth respectively; q_s – specific glucose consumption rate; $q_{s,crit}$ – critical values of specific glucose consumption rate; $\mathrm{q}_{\mathrm{s,max}}$, K_s, $\mathrm{q}_{\mathrm{o,max}}$, k_{os}, $\mathrm{K}_{\mathrm{i,o}}$ – kinetic

constants; $D= F_{in,s}/W$ - dilution rate, $F_{in,s}$ – glucose feed rate, S_{in} – glucose concentration in the feed solution, W–weight of reactor.

A new marker for recognition each physiological state at the beginning is proposed.

$$R_{ac} = \frac{dA}{dt} + \frac{F_{in.s}}{W} \tag{6}$$

In fact, it presents the kinetics of acetate production rate, calculated using available on-line information for acetate concentration, glucose feed rate and reactor volume The marker is applied to switch on the sub-models (4) and (5) during the simulation investigations.

RESULTS AND DISCUSSION

Operational model of the process

The derivation of software sensors requires development of operational models corresponding to the biochemical sub-models given above. According [12], the growth rates R_{X1} and R_{X2} could be considered as unknown time-varying parameters that have to be estimated using available on-line information. The operational model is presented by eq. (7) and (8):

$$\frac{d}{dt}\begin{bmatrix} X \\ S \\ A \end{bmatrix} = \begin{bmatrix} 1 \\ -k_1 \\ 0 \end{bmatrix} \mu_1(t) X - D \begin{bmatrix} X \\ S \\ A \end{bmatrix} + \frac{F_{in,s}}{W} \begin{bmatrix} 0 \\ S_{in} \\ 0 \end{bmatrix} \tag{7}$$

$$\frac{d}{dt}\begin{bmatrix} X \\ S \\ A \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ -k_1 & -k_2 \\ 0 & k_3 \end{bmatrix} \begin{bmatrix} \mu_1(t) \\ \mu_2(t) \end{bmatrix} X - D \begin{bmatrix} X \\ S \\ A \end{bmatrix} + \frac{F_{in,s}}{W} \begin{bmatrix} 0 \\ S_{in} \\ 0 \end{bmatrix} \tag{8}$$

where $R_{X1}=\mu_1 X$ and $R_{X2}=\mu_2 X$.

Cascade software sensor – structure derivation and stability analysis

The general scheme of software sensors is presented in Figure 1. It is a three steps cascade scheme. As a first step, an observer-based estimator of acetate production rate is derived using on-line measurements of acetate concentration. This information is used as input for the second step where the fermentative biomass growth rate, R_{X2} is estimated. In the last step, the information for oxidative biomass growth rate, R_{X1} is received using as inputs glucose measurements and the output from second step.

As a result, on the basis of on-line measurements of acetate (*A*) and glucose (*S*) concentrations, on-line information for oxidative, $R_{X1,}$ and fermentative, R_{X2}, biomass growth rates is received.

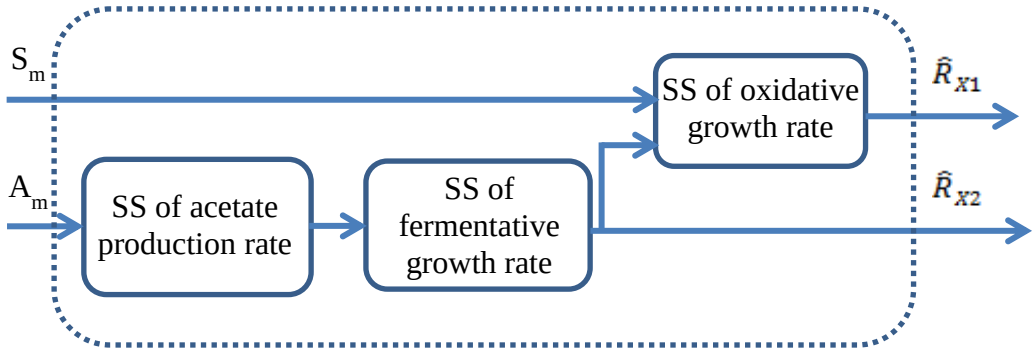

Fig. 1. Software sensor' scheme

On-line estimation of acetate production rate

According the approach proposed in [12], the estimator of the acetate production rate from on-line measurements of acetate concentration is described by the following system:

$$\frac{d\hat{A}}{dt} = \hat{R}_a - DA + w_1(A - \hat{A}) \tag{9a}$$

$$\frac{d\hat{R}_a}{dt} = w_2(A - \hat{A}) \tag{9b}$$

where $\hat{R}_a$ and $\hat{A}$ are the estimates of acetate production rate, R_a, and acetate concentration, *A*, respectively, *A* – the measured values of *A*; w_1 and w_2 - estimator' parameters which values have to satisfied stability conditions.

Stability analysis

Defining the errors $\tilde{A} = A - \hat{A}$ and $\tilde{R}_a = R_a - \hat{R}_a$, the following error system is derived from (9):

$$\frac{d\boldsymbol{x}}{dt} = \mathbf{A}\boldsymbol{x} + \boldsymbol{v}$$

$$\text{with } \boldsymbol{x} = \begin{bmatrix} \tilde{A} \\ \tilde{R}_a \end{bmatrix} \qquad \mathbf{A} = \begin{bmatrix} -w_1 & 1 \\ -w_2 & 0 \end{bmatrix} \qquad \boldsymbol{v} = \begin{bmatrix} 0 \\ \dfrac{dR_a}{dt} \end{bmatrix} \tag{10}$$

Let h_1 and h_2 are real eigenvalues of matrix **A** related by definition to w_1 and w_2 as follows:

$$w_1 = -(h_1 + h_2) \text{ and } w_2 = h_1 h_2 \tag{11a}$$

These real values avoid inducing of oscillations in the estimation values that do not correspond to any physical phenomenon.

If additionally the eigenvalues are equal $h_1 = h_2 = h$ with h – negative constant to be satisfied stability of the system (10), then the relationships (11a) is rewritten:

$$w_1 = -2h \qquad w_2 = w_1^2 / 4 \tag{11b}$$

The choice of a double eigenvalue has several advantages [1]:

(i) the degrees of freedom of the algorithm are reduced,

(ii) it allows an easy interpretation in terms of convergence and

(iii) the calculation of the tuning parameters is straightforward.

The tuning of the estimation algorithm is reduced to the choice of one design parameter, h, and is realized in section 'simulation investigation' as a compromise between estimate's convergence rate and their sensibility with respect to the time-derivative dR_a/dt as described in vector $\boldsymbol{\nu}$.

Depending on the value of $\hat{R}_a$ two cases are possible:

- ✓ $\hat{R}_a = 0$ – this is a sign that the process is in state of oxidative growth and the scheme presented in Fig. 1 is reduced to the third step of the cascade structure only.
- ✓ $\hat{R}_a > 0$ - it means that acetate production is started, the process is in the oxidative-fermentative growth and full scheme is active.

This gives us a reason the estimates of acetate production rate to be used as a marker for appearance of new physiological state.

In the case $\hat{R}_a > 0$, the following on-line information could be received:

On-line estimation of fermentative growth rate

The estimates of the fermentative biomass growth rate are obtained using the relationship between R_{X2} and acetate production rate, R_a, as follows:

$$\hat{R}_{X2} = \hat{R}_a / k_3 \tag{12}$$

where k_3 is the yield coefficient as presented in the model (8).

On-line estimation of oxidative growth rate

The third step of the scheme in the Fig. 1 includes following software sensor:

$$\frac{d\hat{S}}{dt} = -k_1\hat{R}_{X1} - k_2\hat{R}_{X2} - DS + DS_{in} + w_3(S - \hat{S}) \quad (13a)$$

$$\frac{d\hat{R}_{X1}}{dt} = w_4(S - \hat{S}) \quad (13b)$$

where $\hat{R}_{X2}$ are the estimates of R_{X2}, $\hat{R}_{X1}$ - the estimates of R_{X1}, w_3 and w_4 - estimator (13) parameters, which values are chosen according the procedure described by equations (8b). For SS (13) the relationships between w_3, w_4 and h are as follows:

$$w_3 = -2h \qquad w_4 = -w_3^{\,2}/4k_1 \quad (14)$$

In case $\hat{R}_a$=0, the received information is reduced to on-line estimation of oxidative growth only because $\hat{R}_{X2}$ =0 (see eq. (12)) and therefore the second term of eq. (13a) is rejected.

Simulation investigations

Simulation investigations of the proposed SS scheme are realized using the unstructured sub-models (4) and (5) with parameter's values as given in Table 1:

Table 1. Model parameter's values

Parameters	$q_{s,max}$	K_s	k_1	k_2	k_3	k_{os}	q_{omax}	K_{io}
Values	2.044	0.148	2.67	20.59	10.77	2.184	0.685	20.198

In Figure 2, the results from SS (9) are presented. In sub-figure 2a the model, estimated values and experimental data of acetate concentration are compared. As can be seen the model describes well experimental data and the model and estimates coincide. Till 18h there is no acetate and the microorganisms are in oxidative growth on glucose only. After that begins acetate production and the microorganism growths in oxidative-fermentative conditions.

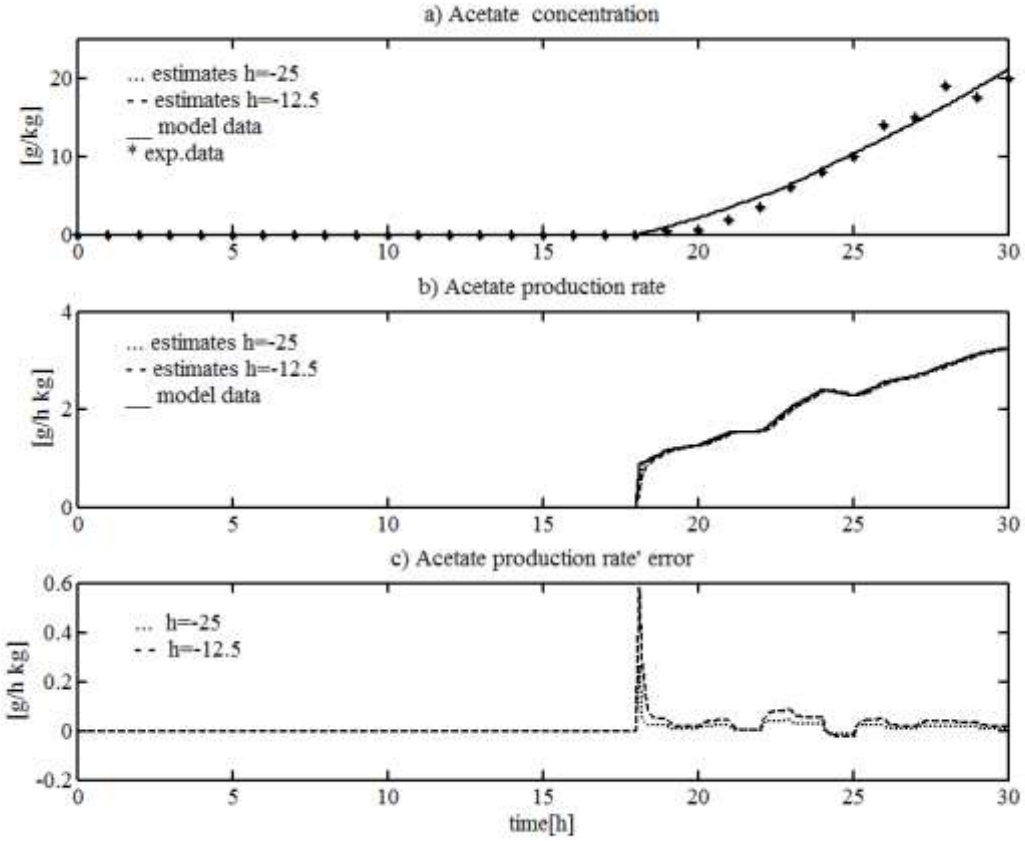

Fig. 2. Estimation of acetate production rate

For the SS' tuning, two eigenvalues are tested - with h= -25 (points) and h= -12.5 (dished line). This investigation compares the convergence of estimates and their sensibility with respect to time-derivative dR_a / dt. The results are with slightingly small differences of estimates of acetate production rate for the two cases as can be seen in sub-figure 2b. The estimate's errors presented in sub-figure 2c demonstrate better convergence and accuracy using higher eigenvalue especially in the beginning of the fermentative phase where the maximal relative error reaches 0.6 for h=-12.5 and 0.2 for h=-25.

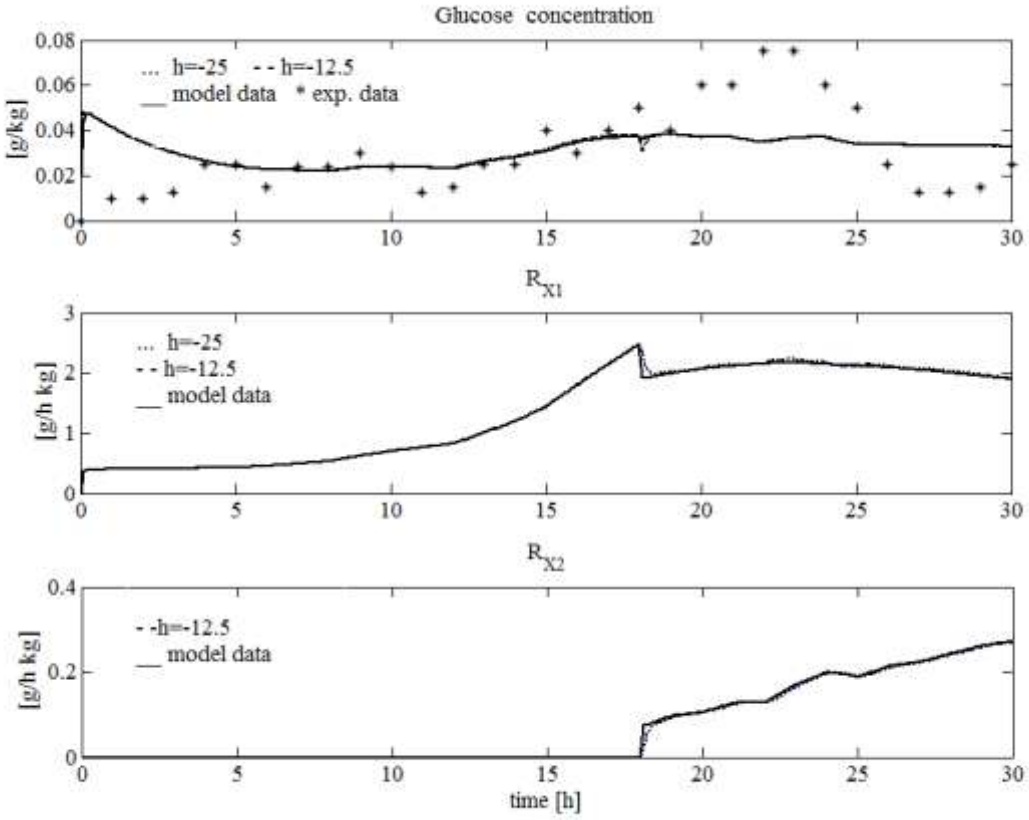

Fig. 3. Estimation of oxidative and fermentative growth rates

In Figure 3, the model and estimated values of glucose concentration (3a), oxidative growth rate R_{X1} (3b) and fermentative growth rate (3c) are compared. The observed in sub-figure 3a difference between the model and experimental data of glucose concentration is due to the inaccuracy of laboratory measurements at low values of glucose as is explained in [18]. Two eigenvalues for the estimator of R_{X1} and one eigenvalue for the estimator of R_a, i.e. R_{X2}, are tested. As can be remarked the estimation data are very closed to the model ones. Till 18 h, in the oxidative phase, biomass growths exponentially on glucose. After the beginning of acetate production, the oxidative growth keeps approximately constant value, while the fermentative growth increases linearly.

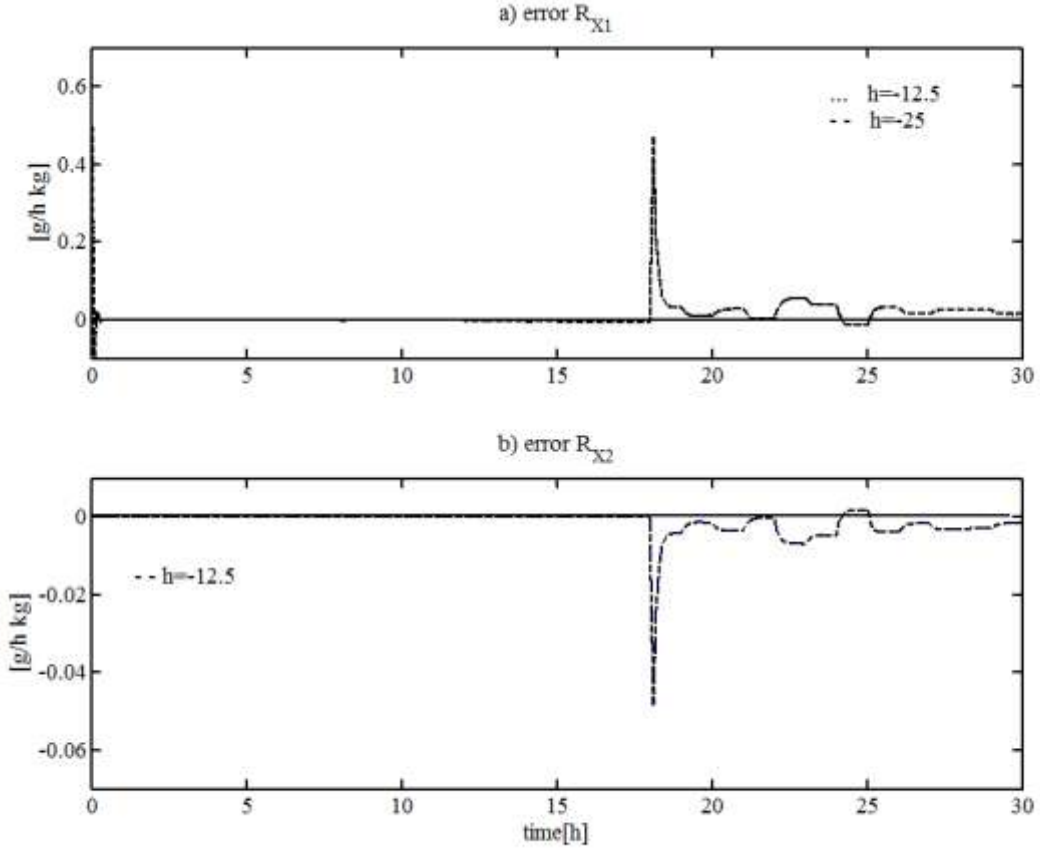

Fig. 4. Estimation errors of oxidative and fermentative growth rates

The estimation errors of both growth rates, R_{X1} and R_{X2}, from Fig. 3b and Fig. 3c are presented in Fig. 4a and 4b respectively. As can be seen in Fig. 4a, the estimates of R_{X1} with different values of the tuning parameter coincide. The biggest errors of both rates appear at 18 h of fermentation due to the jumps in their values and respectively in their time-derivatives. The maximal relative errors are 0.18 and 0.5 for R_{X1} and R_{X2} respectively.

CONCLUSIONS

The obtained results during simulation investigations of the proposed method for monitoring of fed-batch *E Coli* fermentation prove it's efficiently. The information for both biomass growth rates is good enough to be a basis for further investigations related to estimation of other physiological states inherent to *E Coli* cultivation as well as for monitoring of unmeasured main process variables. Such information will be useful for derivation of different control algorithms for each physiological state depending on the target product.

ACKNOWLEDGMENTS

This work was supported by Bulgarian Academy of Sciences under "Programme for assistance to the young scientists in BAS", project titled:"Innovative method for dynamics monitoring of *Escherichia coli* cultivation".

REFERENCES

1. Shiloach J., R. Fass (2005) Growing E. coli to high cell density—a historical perspective on method development. *Biotechnology advances*, 23(5), 345-357.
2. Soini J., K. Ukkonen, P. Neubauer (2008) High cell density media for Escherichia coli are generally designed for aerobic cultivations–consequences for large-scale bioprocesses and shake flask cultures. *Microbial Cell Factories*, 7(1), 1.
3. Todorova J., E. Pasheva (2015) The synthesis of recombinant HMGB1 protein in *E. Coli* is modulated by its c-terminus domain. *Comptes rendus de l'Academie bulgare des Sciences*, 68(10), 1247-1252.
4. Paunova-Krasteva T., S. Stoitsova, T. Topouzova-Hristova, E. Stephanova (2014) *Escherichia coli* O157: Effects of growth temperature on concanavalin A binding and the adherence to cultured cells. *Comptes rendus de l'Academie bulgare des Sciences*, 67(3).
5. Hannig G., S. Makrides (1998). Strategies for optimizing heterologous protein expression in Escherichia coli. *Trends in biotechnology*, 16(2), 54-60.
6. Rocha I. (2003) Model-based strategies for computer-aided operation of recombinant E. coli fermentation. *PhD Thesis*, University of Minho, Braga, Portugal.
7. Akesson M. (1999) Probing Control of Glucose Feeding in Escherichia coli Cultivation, *PhD thesis*, Department of Automatic Control, Lund Institute of Technology, Sweden
8. Santos L. O., L. Dewasme, D. Coutinho, A. Wouwer (2012) Nonlinear model predictive control of fed-batch cultures of micro-organisms exhibiting overflow metabolism: assessment and robustness. *Computers & Chemical Engineering*, 39, 143-151.
9. Pimentel G. A., M. Benavides, L. Dewasme, D. Coutinho, A. Wouwer (2015) An Observer-based Robust Control Strategy for Overflow Metabolism Cultures in Fed-Batch Bioreactors. *IFAC-PapersOnLine*, 48(8), 1081-1086.
10. Chéruy A. (1997). Software sensors in bioprocess engineering. *Journal of biotechnology*, *52*(3), 193-199.
11. Bastin G., D. Dochain (1990) On-line Estimation and Adaptive Control of Bioreactors. Amsterdam, Oxford, New York, Tokyo, Elsevier.
12. Ignatova M., V. Lyubenova (2011) Control of biotechnological processes - new formalization of kinetics: Theoretical aspects and applications. *LAP LAMBERT Academic Publishing*, GmbH & Co. Saarbrücken Germany, 120 pages
13. Veloso, A. C., Rocha, I., Ferreira, E. C. (2009). Monitoring of fed-batch E. coli fermentations with software sensors. *Bioprocess and biosystems engineering*, 32(3), 381-388.

14. Lubenova, V., Rocha, I., & Ferreira, E. C. (2003). Estimation of multiple biomass growth rates and biomass concentration in a class of bioprocesses. *Bioprocess and Biosystems Engineering*, *25*(6), 395-406.
15. Lyubenova V., I. Rocha, E. C. Ferreira (2003). Estimation of Biomass Concentration and Multiple Specific Growth Rates of Fed-Batch Fermentation of Recombinant E. coli. *Proceedings of International Conference Automatics and Informatics*, Sofia, Bulgaria, 1, 211-214.
16. Tamburini E., M. Marchetti and P. Pedrini (2014) Monitoring Key Parameters in Bioprocesses Using Near-Infrared Technology, *Sensors*, 14, 18941-18959.
17. Whitford W. et all. (2007) Bioreactors: Analytical Technology and PAT. *BioProcess Int.*, 5(1), 32–42.
18. Rocha I., E.C. Ferreira (2002) On-line simultaneous monitoring of glucose and acetate with FIA during high cell density fermentation of recombinant *E. coli*. *Analytica Chimica Acta*, 462, 293–304.
19. Zlatkova A., V. Lyubenova, S. Dudin, M. Ignatova (2016) Marker For Switching of Multiple Models Describing *E.Coli* Cultivation, *Comptes rendus de l'Académie bulgare des Sciences* (in press)

CONTRIBUTION CULINARY CHARACTERISTICS OF *SOLANUM TUBEROSUM*, AT SENSORY CHARACTERISTICS OF BREAD WITH POTATOS

***Maria Lidia Iancu*[1*]**

[1] *Faculty Agricultural Science, Food Industry and Environmental Protection*[*]*,"Lucian Blaga" University of Sibiu, 5-7, Dr. Ion Ratiu st ., România ,*

**Corresponding author: mirelaiancu68@yahoo.com*

Abstract

This study investigated the effect of pasta potatoes (PP), Impala(I),Orchestra (O), Laura (L), Lady Christl (L-CHR), Lady Claire(L-Cl) to flour in bread. These potato varieties were used to replace flour 550(F_1), flour type 650(F_2) with additives, wholemeal 1250 type flour(F_3) and brown flour type 1250 with additives(F_4) in different concentrations: 5%, 20%. The recipe of each dough(variant of work) were assesment using technique flour-graphics. The maxim value obtained from 5% add PP from white flour F_1 și F_2. According culinary characteristics, of potatoes and framing in classes, in combination with wheat flour of various extractions, bread crumb texture is influenced by starch fine structure(class A, B). The bread with 5% PP percentage of replacement irrespective of the type of potato and flour obtained the highest sensory score and was 19 point.

***Keywords**: potato, particulary cooking properties, potato bread, sensory characteristics*

INTRODUCTION

Romania is a country producing of potato. Făgăraș Basin is one of the most popular geographic areas where potatoes are growing in this country.

In the varietal composition, we have to take more account of varietal differences, especially in the carbohydrates content and in the options of processing to different products (Mareček *et al*., 2013). Potato tubers are characterized by a high proportion of water (75-80%) that is mainly represented by free water. The dry matter of tubers consists of starch (60-80% of dry matter) and the other major components (other saccharides, fiber, vitamins, nitrogenous substances, alkaloids and other). The dry matter content is a significant stabilizing factor of tubers. Its value is direct ly related to the dry matter components content and it also affects the taste properties of tubers (Pobereźny and Wszelaczyńska, 2011).

A higher starch content is typical for varieties of cooking type C and varieties for industrial use (Bárta et al., 2008; Mareček et al., 2015). In addition to the starch, other saccharides like sucrose, glucose, fructose (0.5-3.0%) have a significant impact on technological quality. They can affect the taste of tubers (sweetening) and the emergence of dark stains during the frying of chips (Maillard reaction). Currently, there is examination of the possibility of adding potato material to various food products (bakery products), the use of varieties with colored pulp and their nutritional potential, as well as changes in the material composition during storage (Martens and Thybo, 2000).

In this study purpose to determine influence of potato pulp varieties(culinary characteristics) on the quality bread (sensory properties). Potato was used to replace type 550 wheat flour(F_1), type 650 flour with additives(F_2), type 1250 flour(F_3), type 1250 whole flour with additive(F_4), in different concentrations: 5%, 20%. Potato variety was: Orchestra, Impala, Lady Claire, Laura, Lady Christl. Are also relevant, the sensory analysis of potato and potato bread and organoleptic properties.

MATERIALS AND METHODS

Used the flour type 550(*Mill Cibin Sibiu, Romania*), with the characteristics: glutenic index(SR 90:2007)(49); FN(s)(No.107/1, ICC Standard)(330s); titratable acidity (degree)(SR 90:07)(2,3); ashes(%)(No.104/1, ICC Stad, 1990)(0,549); hydration capacity(%) (ICC-Standard 179/1, 1998)(55,6 %), wet gluten(%) (No.106/1, ICC Standard 1976)(29%); deformation index(mm)(SR 90:07)(5,5); moisture(%)(ICC Stand. no.110/1)(14,5%), hydration capacity(%) (ICC-Standard 179/1, 1998) (55,6). Used same method like white flour wheat for the others wheat flour. So use flour type 650 with additives: wet gluten(32%); gluten deformation(4mm); gluten index(55,7); titratabile acidity(2,2 degree); water absorbtion (56,9 %), ashes (0.649%: moisture(13,9%); Falling Number (290-300s); brown flour wheat type 1250 with characteristics: moisture (13,9%); gluten wet (29,8%); deformation gluten(8 mm); Falling Number(230-300s); titrabile acidity(4 degree); water absorbtion from flour(60%), ashes(1.1%) gluten index(47,4); brown flour wheat type 1250 with additives with: humidity(12,9%); wet gluten(30%); deformation gluten(8 mm); index gluten(44,4); Falling Number(280s); titratabile acidity(2,1degree); water absorption from flour(61,5%), ashes(1.25%). Used the potato: yellow potato *Orchestra* variety with moisture PP, 81.3%(Thermobalance Method, AN ML-50 Moisture Analysis, A&D Company- Tokio), red potato *Laura* variety with moisture(73.5%), yellow potato *Lady Claire* variety with

moisture(79.3%), yellow potato *Lady Christl* variety with moisture(70.9%), yellow potato *Impala* variety with moisture(83.5%)(Institutul de dezvoltare și cercetare pentru cartof, România), the salt, yeast bakery with growth power(10 min).

PP(potato pasta) was obtained like in ather study (Iancu M.L., 2015).

Dough characteristics, establishing recipe and bread making process was obtained like in ather study(Iancu M.L., 2015)

Sensory analysis of bread - bread quality assessment based on assessment scores

Scoring scheme (Romanian variant) was used. Each of the properties analyzed given a number of points. For each characteristic the number of points was 20(maximum) and penalty for, without a maximum of 0.5-1 points of penality(Bordei, 2007). The elements are assessed by sensory analysis: crumb elasticity and porosity, size, crust structure and colour bread appearance, symmetry of shape, porosity, taste, smell. Girls and boys (a 15-member team) aged between 20 and 28, students of the SAIAPM Faculty (Sibiu) performed the sensory assessment of potato bread. In assessing the macroscopic characteristics of bread crumb used stereomicroscope Stemi 2000-C with stand C LED(made in Japan).

RESULTS AND DISCUSSION

The influence of potato pulp is the purpose of this study and the varieties on the physical characteristics of the bread. A recipe has been used as in another study (Iancu, et al., 2010).We evaluated five varieties of edible potatoes (*Solanum tuberosum*): Impala, Orchestra, Lady Claire, Lady Chistl and Laura. The culinary characteristics are influenced by potato variety (Ján Mareček J.,et al., 2015**).** Sensory evaluation of potato tubers can be realized by cooking type (A, B, C) determination according to the *European evaluation system*, where the tubers cooking ability: aspect, mealiness, taste, consistency, moisture, color, starch structure. Sensory quality is mostly determined by variety, chemical composition and by the cultivation conditions(soil, water precipitation). Taste of tubers is the most important sensory characteristic after their heat treatment. Consistency of tubers is very important for potatoes heat-treated only by steaming (Mareček et al., 2011).

According to the evaluations potatoes belonging to the following class.

Table 1. Appreciation of the culinary value of the boiled potatoes

Potato variety	Aspect (1-4)	Taste (1-4)	Crushing force to boiling (1-4)	Consistence (1-4)	Moisture (1-4)	Starch stucture (1-4)	Class	Content of starch %
Laura	2	3	3	3	3	2	A/B	13,5
Impala	1	1	2	1	4	1,5	A/B	15
Lady Claire	2,5	2	2	2	1,5	1,5	7BC	23
Lady Christl	2,5	2	2	2	2	2	8 A/A	18,5
Orchestra	2,5	2	2,5	2	2	2,5	7,5 A	17,5

Characteristics of traced values from 1(the lowest) to 4(the best)

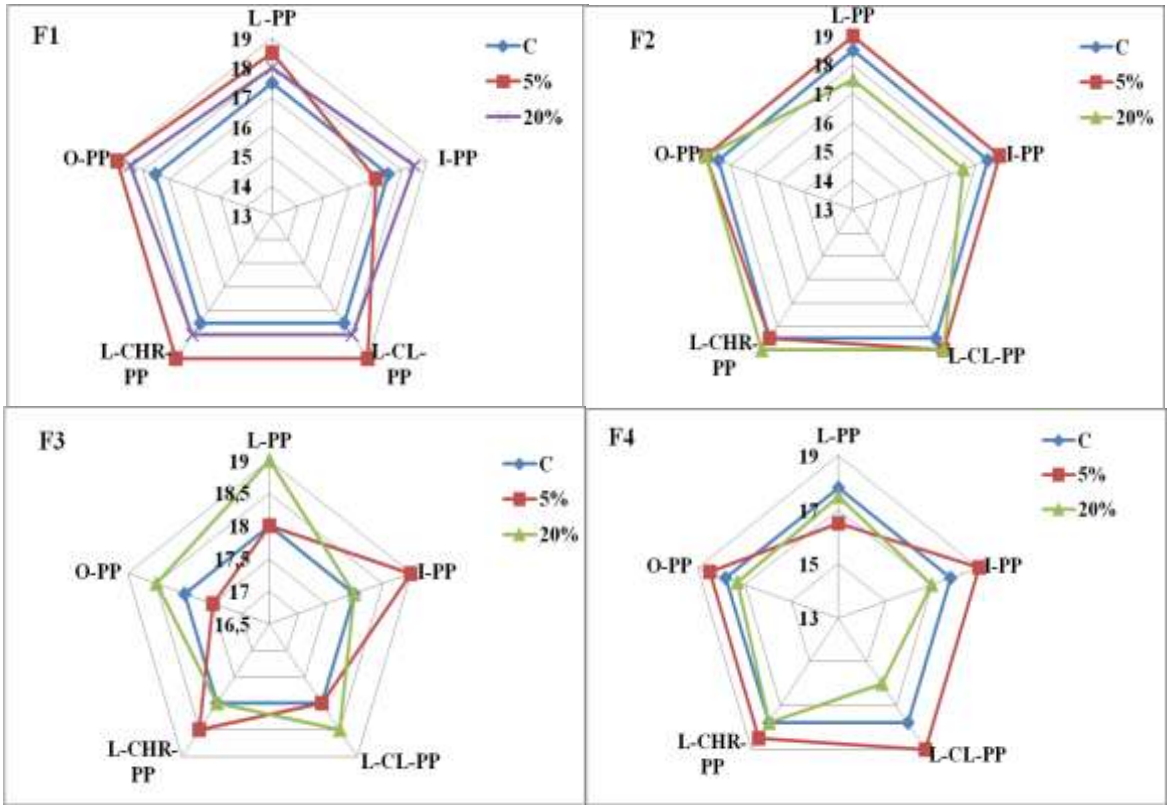

Fig. 1. Spider diagram with physical characteristics (sensory) of bread with potato bread

Sensory analysis

Evaluation bread with potato a 5%, 20% (sensory evaluation) is in shown in figure 1. Bread sample score O-PP-F_1 was the bighest, 18.5 points, at the 20% flour replacement with potato. The colour crust in bread with Orchestra potato is brown. Uniform porosity for bread crumb, the pore walls are thinner, the crumb is darker well-aerated, almost greyish, especially in samples with brown flour (F_3). The higher the potato content($\geq$ 20), the flavour and taste are enhanced. So also improved slicing properties. The score of analysis bread L –CL – PP - F_2 is most 19 points in the potato sample 20% (figure 2). The crust is reddish brown in the L-Cl potato sample. The properties organoleptic analysed is the same in all sample. For samples with 5% potato have the best results and the score was 19.Therefore, adding potato paste improves bread properties, especially with F_2 sample. According culinary characteristics, of potatoes and framing in classes, in combination with wheat flour of various extractions, bread crumb texture is influenced by starch fine structure(class A, B) (table 1).

Fig. 2. Attributes of bread with potatoes : white flour type 550(F_1) and brown flour type 1250 (F_3)

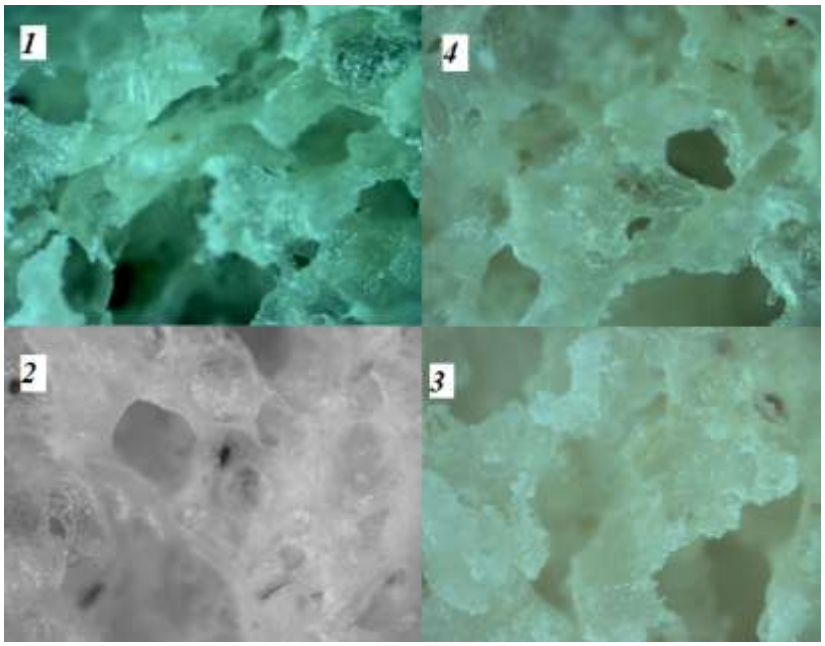

Fig. 3. Stereo microscope 3D image (5 x)with bread crumb: 1-Control sample(F_1), 2- L-PP-F_3(20%), 3- I-PP-F_3(5%), 4- Control sample(F_3)

It is shown from figure 3 stereomicroscope using the 3D image bread crumb porosity. In sample 1 and 4, pore walls are transparent if the system is not supplemented with potato starch. The transparency is given by the content of protein. With the increase the proportion of potato flour replacement (1;2 position in figure 3) the appearance translucent disappears. The structure is supported by the other macromolecular component of the mixture, the starch.

CONCLUSIONS

The quality of potato bread (the same amount, different varieties) proved to be good. It is provided by the other component, pregelatinized starch.

A potato addition of up to 20% improves the 10 sensory characteristics of the bread, investigated.

The bread with 5% PP percentage of replacement irrespective of the type of potato and flour obtained the highest sensory score and was 19 point.

The structure is supported by the other macromolecular component of the mixture, the starch and is demonstrated by the images obtained with stereomicroscop.

According culinary characteristics, of potatoes and framing in classes (*Lady Christl*-8A/A-table 1) in combination with wheat flour, in special white flour, bread characteristics sensorial was very good.

REFERENCES

Bárta, J., Cepl, J., Diviš, J., Hamouz, k., Jůzl, M., Vacek, J., Potatoes. In PRUGAR, J., Qualityplants products at the beginning of the third m illennium. Praha: VÚPaS, 2008, 241-257.

Bordei D., Controlul calității în industria panificației. Metode de analiză, Editura Academica, Galați, p: 502-504, 2007

Iancu M.L., Luiza M., Haubelt, G., Experimental model for the application of the flourgrafic technique to the study of the mixture of flour and raw potato, or hydro thermally processed potato, Journal of Agroalimentary Processes and Technologies, 2010, 16(1), 41-48.

MARIA LIDIA IANCU, EFFECT OF POTATO (Solanum tuberosum) ADDITION ON THE DOUGH PROPERTIES, SENSORY QUALITIES AND RESISTANT STARCH CONTENT OF BREAD, The Annals of the University Dunărea de Jos of Galaţi Fascicle VI – Food Technology (2015), 39(1), 93-10841% match (Internet from 10-Mar-2016)http://www.ann.ugal.ro/tpa/Anale%202015/8%20Iancu.pdf

Mareček J., Musilová J., Frančáková H, Andrea Mendelová, Krajčovič T., Labuda J., Ján Heldák, sensory and technological quality of slovak varieties of edible potatoeS, Journal of Microbiology, Biotechnology and Food Science, 2015,106-108

Mareček, J., Fikselová, M., Kačániová, M., Líšková, M., Content of saccharides and vitamin C in potato tubers stored under controlled atmosphere. Soil, plant and food interactions. Brno : MZLU, 2011, 268-272.

Mareček, J., Frančáková, H., Bojňanská, T., Fikselová, M., Mendelová, A., Ivanišová, E., Carbohydrates in varieties of stored potatoes and influence of storage on quality of fried products. Journal of Microbiology, Biotechnology and Food Sciences, 2(Sp. is.), 2013, 1744-1753.

Mart ENS, M., Thybo, A. K., Analysis of sensory assessors in texture profiling of potatoes by multivariate modelling. Food Quality and Preference, 2000, 11(4), 283-288.

Pobereźny, J., Wszelaczyńska, E., Effect of bioelements (N, K, Mg) and long-term storage of potato tubers on quantitative and qualitative losses. Part II. Content af dry mater and starch. Journal of Elem entology, 2011, 16(2), 237-246.

SR 90 : 2007, Wheat flour. Analytical methods

ICC Standard No.104/1,1990 Determination of Ash in Cereals and Cereal Products,

ICC Standard No.106/2, 1984, Working Method for the Determination of Wet Gluten in Wheat Flour

ICC Standard No.107/1, 1995, Determination of the "Falling Number" according to Hagberg - as a Measure of the Degree of Alph Amylase Activity in Grain and Flour Alpha-Amylase Activity (Falling Number)

ICC Standard No 179., 2010, for ICC procedure based Flourgraph E6, Determination of Water Absorption Capacity of Wheat Flours and Physical Properties of Wheat Flour Dough Using the Flourgraph E6 ("Farino"), ICC Method Book

RESEARCH ON SOME CHARACTERISTICS OF ACIDIC DAIRY PRODUCTS WITH THE ADDITION OF BEE PRODUCTS

Mihaela Adriana Tita[1], Ketney Otto[1]

[1] "Lucian Blaga" University of Sibiu, Faculty of Agricultural Sciences, Food Industry and Environmental Protection, Bulevardul Victoriei 10, Sibiu - 550024, Romania

Abstract

Functional aliments are foods. Bee pollen contains all vitamins, minerals and necessary acids for proper functioning of the body. The purpose of this work is to improve the yogurt properties using lyophilized pollen and to observe the degree of influence that pollen has on the yogurt properties at different pollen additions.

Keywords: pollen, yogurt, functional foods

INTRODUCTION

Functional aliments are foods or food components as well, that improve the health of consumers, avoid the risk of disease and improve physical or mental quality of life and the resilience after hard physical exercises and diseases as well. The concept of functional foods is considered to be published in Japan at the end of the 80's. However, functional foods actually have a long history [1]. However, the annual growth rate of functional foods market varied from 15% to 20% at the end of the 90's [2-4] to 10% the most recent estimate [1]. Bee pollen contains all vitamins [2], minerals and necessary acids [3] for proper functioning of the body. Also, the pollen and other bee products won an increased attention for their therapeutics properties, like antibacterial [4,5], antifungal, anticancer agents [6] and immunomodulatory effects [7]. Given it's nutritional composition, the dry bee pollen was used like aliment in human nutrition, offering a well being sensation and a functional balance. For maintaining his features, the pollen, must be dried in special ovens, at a max temperature of 50 ° C, until the humidity decreases between 5% and 8%, such that the pollen to be protected against fungal contamination [8].

The objective of this work is to improve the proprieties of acidic dairy products with the help of bee products by observing the degree of influence that the pollen has on yogurt proprieties.

MATERIALS AND METHODS

1. Samples subjected to analysis: For analyzes carried out on bee pollen yogurt 5 samples were taken:

- Sample 1: Simple yogurt 3% fat (blank sample)
- Sample 2: Yogurt 3% fat + 2% lyophilized pollen
- Sample 3: Yogurt 3% fat + 4% lyophilized pollen
- Sample 4: Yogurt 3% fat + 8% lyophilized pollen
- Sample 5: Yogurt 3% fat + 10% lyophilized pollen

3% fat yogurt was mixed with lyophilized pollen which has an humidity content of 0%. In order to perform the corresponding laboratory tests and titrations, were taken 10 ml for each sample and were diluted with 20 ml of distilled water. Pollen lyophilization was made for 100g of bee pollen with an lyophilizator (Model ALPHA 1-4 LDplus).

2. Sensorial analysis: The evaluation of each organoleptic characteristics was made in the described conditions of SR 6345 [12], by comparison with 0…5 points scoring scales and average score given by the panelists group

3. Acidity determination: It is made through the current titration method, according to described method in ISO/TS 11869:2012 [13], expressed in Thörner degree.

4. Humidity content determination: The percentage of water content is determined by oven-drying method [14], at a temperature of 102°C.

RESULTS AND DISCUTION

After tasting were conducted the following results were obtained and were centralized in figure 1:

The best result was obtained by sample 2 (the sample with 2% pollen) and the worst result was obtained by sample 5 (the sample with 10% pollen).

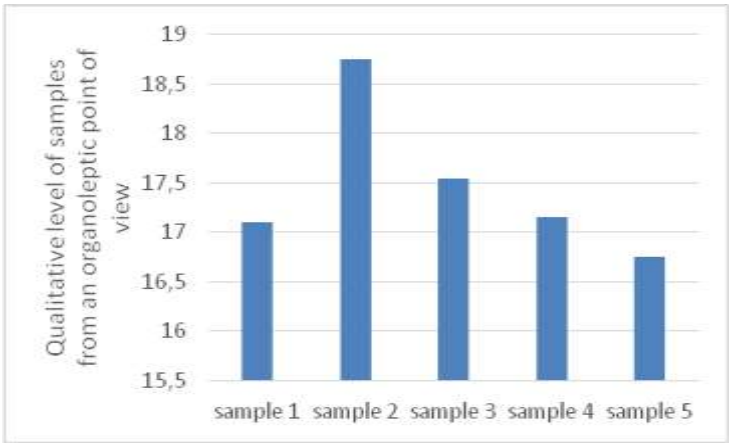

Fig. 1. The results of the sensory analysis

After performing the physical and chemical analyzes, the following results were obtained:

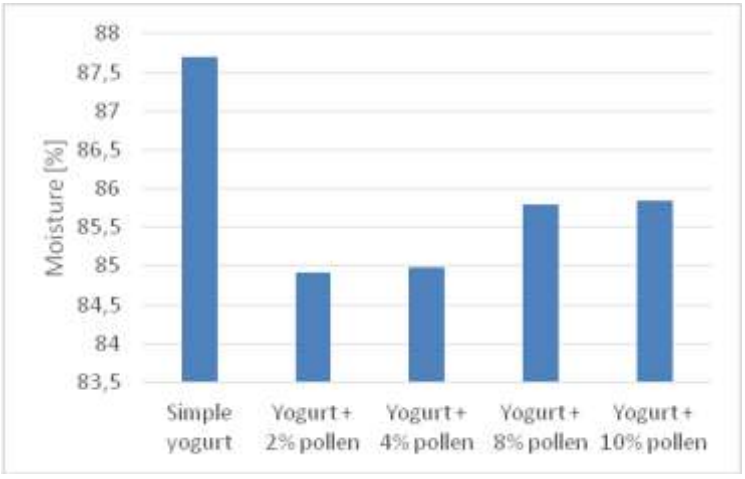

Fig. 2. The humidity contain from analyzed samples

The humidity of the product decreases with the addition of pollen, the blank sample having the biggest humidity. In the samples with pollen addition, the humidity increases with pollen content.

The acidity (figure 3) is directly proportional with the pollen quantity from the product. We can see that the acidity from sample 3 and blank sample decreases after 4 days and on the other samples the acidity increases.

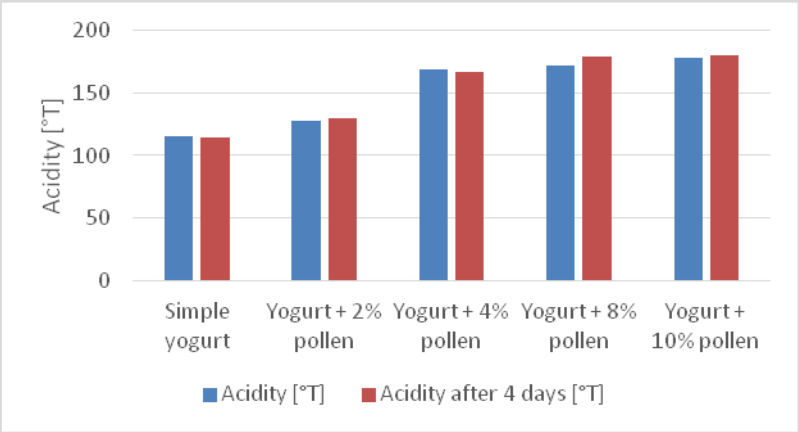

Fig. 3. The acidity level from analyzed samples

CONCLUSIONS

Following organoleptic research was observed that the best result was obtained by the yogurt sample with 2% pollen. The samples with 8% and 10% have obtained a lower score mostly because of the appearance. After mixing with pollen, the yogurt turns a yellow color that can give a less pleasant appearance to the product. The yogurt properties are improved by the pollen addition and all the physico-chemical properties of the product have increased with the added amount of pollen. An very important factor is that the sugar content increases with the added amount of pollen, which can provide a pleasant taste of the product.

REFERENCES

1.Weststrate, J., G. Van Poppel, and P. Verschuren, *Functional foods, trends and future.* British Journal of Nutrition, 2002. 88(S2): p. S233-S235.

2.De Arruda, V.A.S., et al., *Dried bee pollen: B complex vitamins, physicochemical and botanical composition* Journal of Food Composition and Analysis, 2013. 29(2): p. 100-105.

3.Loper, G., et al., *Biochemistry and microbiology of bee-collected almond (Prunus dulcis) pollen and bee bread. I. Fatty acids, sterols, vitamins and minerals.* Apidologie, 1980. 11(1): p. 63-73.

4.Garcia, M., et al., *Note. Pollen analysis and antibacterial activity of Spanish honeys.* Revista de Agaroquimica Tecnologia de Alimentos, 2001. 7(2): p. 155-158.

5.Proestos, C., et al., *RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of the antioxidant capacity and antimicrobial activity.* Journal of Agricultural and Food Chemistry, 2005. 53(4): p. 1190 1195.

6.Almas, K., A. Mahmoud, and A. Dahlan, *A comparative study of propolis and saline application on human dentin. A SEM study.* Indian journal of dental research: official publication of Indian Society for Dental Research, 2000. 12(1): p. 21-27.

7.Gebara, E.C., L.A. Lima, and M. Mayer, *Propolis antimicrobial activity against periodontopathic bacteria.*

Brazilian Journal of Microbiology, 2002. 33(4): p. 365-369.

8.Stanley, R.G. and H.F. Linskens, *Pollen: biology biochemistry management*. 2012: Springer Science & Business Media

VALORIZATION OF SECONDARY FLOWS FROM DAIRY INDUSTRY TO OBTAINING MICROALGAE BIOMASS

Sanda Velea[1], Florin Oancea[1], Ana-Maria Galan[1], Catalin Bogdan Matei[1], Alin Nicolae Cristian Vintila[1]

[1]National Research and Development Institute for Chemistry and Petrochemistry ICECHIM, Bucharest

Abstract

The present study investigates the possibility of integrating microalgae biomass production into dairy product industry. Whey, the by-product of cheese or casein production, is of relative importance in the dairy industry due to the large volumes produced and the nutritional composition; the production of 1–2 kg of cheese yields 8–9 kg of whey. Worldwide whey production is estimated at around 180 to 190×106 ton/year; of this amount only 50% is processed. The whey can be considered a valuable by-product with several applications in the food and pharmaceutical industries; however, it is often treated as a dairy wastewater (Baldasso et al., 2011). In a cheese factory for example, large amounts of whey are produced and filtrated, in order to separate the proteins from other constituents, leaving mainly lactose (Abreu et al., 2012). The remaining lactose concentrate is likewise free of contamination and could readily be used as a source of carbon (plus other nutrients) for microalgae cultivation. Has been demonstrated that mixotrophic growth, for many microalgae species, improve biomass productivity and respectively the oil content of microalgae biomass (Girard et al., 2014). The aim of this study is to grow two different microalgae species in mixotrophic conditions, using remaining lactose concentrate from dairy industry, in order to obtain microalgae biomass with a high content of PUFAs and antioxidants.

Keywords: cheese whey, microalgae, mixotrophic cultivation, lactose

INTRODUCTION

Microalgae are largely under investigation for their ability to produce oils and other valuable compounds for a variety of markets (Girard et al., 2014), for this reason microalgae cultivation has been carried out in order to produce biofuels, animal feed or high-value added products for cosmetics, pharmaceuticals and health supplements production (Abreu et al., 2012). The annual world production of microalgae biomass was estimated of about 5000–7500 t, generating average annual income of US $ 1.25 billion (Abomohra et al., 2016).

A significant advantage of use of microalgae biomass to produce biofuels and value compound is that microalgae can also be used for wastewater treatment, carbon dioxide (CO_2) mitigation (Girard et al., 2017).

Microalgae depend critically on a sufficient supply of carbon and light to carry out photosynthesis. However, they entertain more than one type of metabolism, i.e. heterotrophic, mixotrophic and photoheterotrophic besides photoautothrophic, and they undergo metabolic shifts in response to changes in environmental conditions(Amaro et al., 2012).

Taking in consideration this ability of microalgae to change their metabolism in response to environmental conditions, the recent original publications were focused on reduction of cost production of microalgae biomass by using waste products (co-products from dairy industry, biodiesel production etc.) in mixotrophic microalgae cultivation.

Whey is an important co-product of the cheese industry that possesses a substantial polluting power. This is due to its high dissolved organic carbon (DOC) content which is mainly lactose (>75% m/m of total solids) and no whey utilization strategy will succeed without suitable attention being paid to lactose. Whey proteins, which represent approximately 10% of total solids, are of high commercial value because of their nutritive qualities and health benefits (Girard et al., 2017). Among the major components of whey, the disaccharide lactose, which on hydrolysis yields glucose and galactose, is greatly responsible for its high Biochemical Oxygen Demand (BOD = 30000–50000 mg/L) and Chemical Oxygen Demand (COD = 60000–80000 mg/L). Exogenous sugars, such as glucose, galactose, mannose, fructose, sucrose and lactose have been commonly used for mixotrophic and heterotrophic cultivation of microalgae (Abreu et al., 2012).

Microalgae cultivation mode is one of the main factors restricting biomass production. Microalgae can be cultivated in four main cultivation mode: autotrophic, heterotrophic, mixotrophic and photoheterotrophic. Autotrophy is the most primitive way of cultivating microalgae. For autotrophic cultivation, microalgae generate organic matters and energy by fixing inorganic carbon, mainly using CO_2 as carbon source and sunlight as energy source (Zhan et al.). Compared with autotrophy, heterotrophy doesn't need light, microalgae growth depends on the metabolism of organics which provide the carbon source and energy. Photoheterotrophycally, also known as photo organitrophy, photo assimilation, photometabolism, describes the metabolism in which light is required to use organic compounds as carbon source (Zhan et al.).

Mixotrophic cultivation is a trophic culture method in which microalgae can drive both photoautotrophy and heterotrophy using both inorganic and organic carbon (C) sources. Inorganic carbon is fixed through photosynthesis, which is influenced by the conditions of illumination. Organic-C is assimilated through aerobic respiration which is affected by the

availability of organic carbon (Salati et al., 2017). Therefore, microorganisms cultivated under mixotrophic conditions synthesize compounds characteristic of both photosynthetic and heterotrophic metabolisms at high production rates (Abreu et al., 2012).

Recent studies suggested that the specific growth rate of microalgae under mixotrophic cultivation is approximately the sum of those under photoautotrophic and heterotrophic modes, whereas others believed that the specific growth rate in mixotrophy is not the simple combination of those in photoautotrophy and heterotrophy, and the two metabolic processes (i.e., photosynthesis for photoautotrophy and aerobic respiration for heterotrophy) affect each other under mixotrophic cultivation, which may contribute to synergistic effects and enhance biomass productivity (Wang et al., 2014).

Also, these studies confirm that mixotrophic cultivation of microalgae provides higher biomass productivity, and depending on the microalgae species used higher lipids, proteins or carbohydrates productivity. These advantage of mixotrophic cultivation, together with the cost reduction of growth media (in case of using carbohydrates from industrial and agricultural wastes) preparation and lower requirements for light intensities (because of dark:light illumination cycle) makes mixotrophic cultivation a feasible alternatives for the production of microalgae biomass.

Various organic compounds can be utilized by microalgae under mixotrophic cultivation. Among which, glucose and acetate are the two most efficient and most frequently adopted sources. Other types of organic compound such as glycerol, fructose, sucrose, and ethanol can also enhance productivities for specific microalgae strains (Wang et al., 2014).

In the context of using carbohydrates from industrial and agricultural wastes, crude glycerol from biodiesel production, acetate from anaerobic digestion, and carbohydrates from agricultural and industrial wastes offer great promise as inexpensive organic substrates for the cultivation of microalgae on mixotrophic mode (Abreu et al., 2012).

Stepan et al. performed mixotrophic cultivation of *Nannochloris sp.*, using 3g/L crude glycerol, and obtained a biomass productivity for the whole period of 380 mg/L/d. Also, Andruleviciute et al. reported for cultivation of *Nannochloris sp,* in growth medium with 2 g/L technical glycerol, a amount of triglycerides of 16.2% (Stepan et al., 2016). Shene et al. provide a study on mixotrophic cultivation of marine microalgae *Nannochloropsis oculata*. The results reveals that glycerol enhanced the lipid content of the biomass, but reduced the chlorophyll a content. Mixotrophic cultivation favored the production of lipids with a high percentage of saturated fatty acids (Shene et al., 2016). In case of mixotrophic growth of

microalgae *Chlorella vulgaris* and *Botryococcus terribilis* using domestic waste water (WW) amended with glycerol, the best results were obtained with a highest glycerol supplimentation (50 mM). For *Chlorella vulgaris* was obtained a biomass productivity of 118 mg/L/d and a lipid productivity of 18 mg/L/d. For *Botryoccocus terribilis,* the biomass productivity was 282 mg/L/d and lipid productivity 35 mg/L/d. Also, in the same study were estimated the productivities of the both strain, in case of 200 m^3/d of WW and 240 working days/y, thus, the biomass and lipid yield may be about 5,6 tons/y and 894.2 kg/y or 13.5 tons/y and 1.6 tons/y for *C. vulgaris* and *B. terribilis* (Cabanelas et al., 2013). The study performed by Gupta et al., for mixotrophic growth of Chlorella vulgaris using waste water and various carbon sources (glucose, glycerol, acetate), revealed Higher growth trend were observed in order Glucose > Glycerol > Acetate. Under optimized conditions, namely of 5 g/L glucose, *C. vulgaris* showed higher increments of biomass with 1.39 g/L dry cell weight achieving biomass productivity of 0.13 g/L/d. The biomass accumulated 19.29 ± 1.83% total lipid, 41.4 ± 1.46% carbohydrate, and 33.06 ± 1.87% proteins. Moreover, the cultivation of *Chlorella* sp. in glucose-supplemented wastewater removed 96.9% chemical oxygen demand, 65.3% total nitrogen, and 71.2% total phosphate (Gupta et al., 2016).

Zhan et al. presented in a recent review the advantages of mixotrophic mode in bioenergy production by considering the difference in growth, photosynthesis characteristic and bioenergy production, also this study present results published in the last years on mixotrophic cultivation of microalgae (Zhan et al.).

Few studies are available on mixotrophic cultivation of microalgae using organic carbon present in dairy wastes (cheese whey). Cheese whey was used as carbon source for Chlorella vulgaris growth. Mixotrophic microalgae showed higher specific growth rate, final biomass concentration and productivities of lipids, starch and proteins than microalgae cultivated under photoautotrophic conditions. Moreover, supplementation of the inorganic culture medium with hydrolyzed cheese whey powder solution led to a significant improvement in microalgal biomass production and carbohydrate utilization when compared with the culture enriched with a mixture of pure glucose and galactose, due to the presence of growth promoting nutrients in cheese whey (Abreu et al., 2012). Cheese whey (CW), wine lees and glycerol were used to produce Chlorella vulgaris. Was observed an increased biomass production compared with autotrophic conditions by 1.5–2 times, with the best results obtained for the CW substrate, i.e. 0.52 g /L/d of algal biomass vs. 0.24 g/ L/ d of algal biomass for autotrophic conditions, and protein content for both conditions adopted

close to 500 g/ kg dry biomass (Salati et al., 2017). Monadal et al. studied mixotrophic growth of *Chlorella sp*. BTA 9031 and *Clamydomonas sp.* BTA 9032 using various carbon sources. The highest biomass productivity of *Chlorella sp.* BTA 9031 and *Chlamydomonas sp*. BTA 9032 were 75 and 43 mg/L/d respectively, and was obtained using as carbon sources cheese whey permeate (CWP). The highest total lipid (38.6%) was obtained in *Chlamydomonas sp*. BTA 9032 using CWP as a carbon source (Mondal et al., 2016). Girard et al. present a research focused on mixotrophic growth of *Scenedesmus obliquus* on cheese whey permeate. Substituting 40% (v/v) of the culture medium with WP significantly stimulates *Scenedesmus obliquus* growth under mixotrophic (μmax= 1.083± 0.030 day−1) and heterotrophic (μmax= 0.702± 0.025 day^{-1}) conditions, compared to photoautotrophic control cultures (μmax= 0.267± 0.083 day^{-1})(Girard et al., 2014).

MATERIALS AND METHODS

Microalgae and culture medium

The microalgae species *Chlorella vulgaris* (AICB 311) was used in all the experiments. It was grown in a BBM medium, with the following composition (g/L): 2 $NaHCO_3$, 0.175 K_2HPO_4, 0.075 K_2HPO_4, 2 $NaNO_3$, 0.025 NaCl, 0.075 $MgSO_4.7H_2O$, 0.025 $CaCl_2.2H_2O$, 0.031 KOH 85%, 1 mL/L of trace metal solution, and 5 mL/L of chelated iron.

Cultivation conditions

Microalgae were cultivated in 250 mL Erlenmeyer flasks containing 230 mL BBM medium and 20 mL inoculum of *Chlorella vulgaris.* In order to achieve different concentrations of lactose in the culture medium (0.35, 0.50, 0.65, 0.80 g/L lactose), different volumes of BBM were replaced by deproteinized cheese whey with an initial lactose concentration of 4g/L. The flasks were incubated at constant temperature (25°C) and constant stirring rate (250 rpm), using an incubator Innova® 42 Incubator Shaker Series from New Brunswick Scientific. All the flasks were continuously aerated with synthetic gas mixture containing 7% CO_2, 14% O_2 and 79% N_2 (v/v) and illuminated using two fluorescent lamps at a light intensity of 250 μ$E/m^2/s$. The samples were incubated for 7 days until the cultures were reached in the stationary phase of growth. After reaching the stationary growth phase, the cells were collected and centrifuged at 8000 rpm for 20 minute, washed two times with distilled water and dried in oven at 60 °C until the constant weight was reached. Were

performed two experiments as follows: a) mixotrophic growth using continuous illumination of the culture; b) mixotrophic growth using a light:dark illumination cycle 12:12 h.

Cheese whey pretreatment

The cheese whey was procured from a local dairy farm. Before to be used for microalgae mixotrophic cultivation the cheese whey was deproteinized by heating at 90 °C for 30 min. The formed precipitate was removed by centrifugation at 8000 rpm for 30 min. The supernatant obtained was sterilized in autoclave at 120 °C for 20 min.

RESULTS AND DISCUSSION

The partial substitution of the BBM medium with different volumes of deproteinized cheese whey was performed, in order to obtain the following concentrations of the lactose in the culture medium: 0.35, 0.50, 0.65, 0.80 g/L. The results obtained for each value of the lactose concentration in case of a) continuous illumination and b) light: dark cycle are presented in the Table 1.

Our results revealed that the maximum biomass concentration (expresses as mg dry biomass/mL) was obtained using a continuous illumination. It was as observed an increase of the biomass concentration for all the samples regardless the lactose concentrations compare with the sample growth in photoautotrophic.

The maximum concentration of biomass was obtained for the samples with about 0.5 g/L concentration of lactose, both for continuous illumination and light : dark cycle. After this concentration the values obtained for biomass concentration present a stabilization or a slight decrease.

Table 1. Biomass concentration of Chlorella vulgaris growth in mixotrophic conditions using different cheese whey concentration and different illumination conditions

Lactose concentration, g/L	Dry microalgae biomass, mg/mL	
	Continuous illumination	Light : dark cycle
0 (photoautotrophic cultivation)	0,674	-
0.20	0,8264	0,5128
0.35	1,2360	0,8108
0,50	2,0696	1,0360
0.65	1,2580	1,0347
0.80	1,0864	1,0344

More work must to be done in order to establish the influence of mixotrophic growth using cheese whey as carbon sources in PUFA and antioxidants concentration in microalgae biomass.

CONCLUSIONS

The aim of the present study is the mixotrophic cultivation of microalgae *Chlorella vulgaris* using cheese whey as carbon source in order to increase PUFAs and antioxidants concentration, compare with the studies presented in the literature that aimed to increase the oil content of microalgae biomass for biodiesel production.

REFERENCES

Abomohra, A.E.-F., Jin, W., Tu, R., Han, S.-F., Eid, M., Eladel, H., Microalgal biomass production as a sustainable feedstock for biodiesel: Current status and perspectives. *Renew. Sust. Energ. Rev.*, 2016, 64, 596-606.

Abreu, A.P., Fernandes, B., Vicente, A.A., Teixeira, J., Dragone, G., Mixotrophic cultivation of Chlorella vulgaris using industrial dairy waste as organic carbon source. *Biores. Tech.*, 2012, 118, 61-66.

Amaro, H.M., Macedo, Â.C., Malcata, F.X. , Microalgae: An alternative as sustainable source of biofuels? *Energy*, 2012, 44(1), 158-166.

Baldasso, C., Barros, T.C., Tessaro, I.C. , Concentration and purification of whey proteins by ultrafiltration. *Desalination*, 2011, 278(1–3), 381-386.

Cabanelas, I.T.D., Arbib, Z., Chinalia, F.A., Souza, C.O., Perales, J.A., Almeida, P.F., Druzian, J.I., Nascimento, I.A., From waste to energy: Microalgae production in wastewater and glycerol. *Appl. Energ.*, 2013, 109, 283-290.

Girard, J.-M., Roy, M.-L., Hafsa, M.B., Gagnon, J., Faucheux, N., Heitz, M., Tremblay, R., Deschênes, J.-S., Mixotrophic cultivation of green microalgae Scenedesmus obliquus on cheese whey permeate for biodiesel production. *Algal Research*, 2014,5, 241-248.

Girard, J.-M., Tremblay, R., Faucheux, N., Heitz, M., Deschênes, J.-S., Phycoremediation of cheese whey permeate using directed commensalism between Scenedesmus obliquus and Chlorella protothecoides. *Algal Res.*, 2017, 22, 122-126.

Gupta, P.L., Choi, H.-J., Pawar, R.R., Jung, S.P., Lee, S.-M., Enhanced biomass production through optimization of carbon source and utilization of wastewater as a nutrient source. *J. Environ. Manage.*, 2016,184, Part 3, 585-595.

Mondal, M., Ghosh, A., Sharma, A.S., Tiwari, O.N., Gayen, K., Mandal, M.K., Halder, G.N., Mixotrophic cultivation of Chlorella sp. BTA 9031 and Chlamydomonas sp. BTA 9032 isolated from coal field using various carbon sources for biodiesel production. *Energ. Convers. Manage.*, 2016, 124, 297-304.

Salati, S., D'Imporzano, G., Menin, B., Veronesi, D., Scaglia, B., Abbruscato, P., Mariani, P., Adani, F., Mixotrophic cultivation of Chlorella for local protein production using agro-food by-products. *Biores. Tech.*, 2017, 230, 82-89.

Shene, C., Chisti, Y., Vergara, D., Burgos-Díaz, C., Rubilar, M., Bustamante, M., Production of eicosapentaenoic acid by Nannochloropsis oculata: Effects of carbon dioxide and glycerol. *J. Biotechnol.*, 2016, 239, 47-56.

Stepan, E., Enascuta, C.E., Oprescu, E.E., Radu, E., Radu, A., Galan, A.M., Vasilievici, G., Lavric, V., Velea, S.. Intermediates for synthetic paraffinic kerosene from microalgae. *Fuel*, 2016, 172, 29-36.

Wang, J., Yang, H., Wang, F., Mixotrophic Cultivation of Microalgae for Biodiesel Production: Status and Prospects. *App. Biochem. Biotech.*, 2014, 172(7), 3307-3329.

Zhan, J., Rong, J., Wang, Q. Mixotrophic cultivation, a preferable microalgae cultivation mode for biomass/bioenergy production, and bioremediation, advances and prospect. *Int. J. Hydrogen Energ.*- http://dx.doi.org/10.1016/j.ijhydene.2016.12.021

RESEARCH REGARDING THE ELECTRODEPOSITION OF ZINC

***Carmen Monica Cretu*[1]**

[1]*"Lucian Blaga" University of Sibiu, Faculty of Agricultural Sciences, Food Industry and Environment Protection, 7-9 Dr. Ioan Ratiu St., 550012 Sibiu, Romania , email: monica.cretu@ulbsibiu.ro*

Abstract

The paper shows the research regarding the electrodeposition of zinc on iron, using a low acid $ZnCl_2$ solution. The effects of $ZnCl_2$ concentration, current density and temperature on both the thickness and character of the deposited zinc and on current efficiency were studied. Optimum current density, temperature and $ZnCl_2$ concentration values were determined.

Keywords: electrodeposition, zinc, current efficiency, thickness

INTRODUCTION

The zinc electrodeposition on ferrous materials is done to protect against corrosion and for decorative purposes. In the iron–zinc couple, the base metal has a more positive electrochemical potential than the zinc. Therefore, when humidity is present, the galvanic couple formed within the pores will cause a gradual dissolution of the protective material, i.e. the zinc. This results in good protection of the ferrous materials against corrosion.

The galvanic deposition of zinc can be performed in acid [1, 2], cyanide [3] and alkaline [4, 5] electrolyte solutions. The alkaline electrolytes may be zincates or pyrophosphates. Among the acid electrolytes, the most important are those based on sulfates, chlorides and fluoro-borates [6, 7]. Acid and alkaline electrolytes are preferred, since cyanides are toxic.

This paper discusses the optimum conditions in the electrodeposition of zinc on iron from a low acid $ZnCl_2$ solution.

MATERIALS AND METHODS

The electrolyte solution used for this study of the electrodeposition of zinc is a low acid one, with the following concentrations: $ZnCl_2$ 35 - 50 g/L, KCl 145 g/L and H_3BO_3 30 g/L. The concentrations for the other addition agents used include: 30 ml/L for

alkylphenolethoxylate 5% and fatty alcohol ethoxylate 5%, 1ml/L for aromatic ketones 5 – 10%, isopropanol 10 – 25% and methanol 10 – 20% and 4 ml/L for salts of organic acids.

The electrodes were polished with abrasive paper and treated for electrochemical and chemical degreasing, chemical pickling and activation before the electrodeposition experiments. Trichloroethylene was used for chemical degreasing. For electrochemical degreasing an aqueous solution of sodium hydroxide 20 – 30%, sodium carbonate 30 – 50% and disodium trioxosilicate 30 – 50% was used. For chemical pickling, a 1:2 HCl solution and a solution formed by fatty alcohol ethoxylate 25 – 50%, fatty aminoxethylate 10 – 20 % and but -2-yne-1, 4-diol was used. A 1% HCl solution was then used for activation.

The solutions were obtained with distilled water, using AR grade chemicals.

The galvanic deposition of zinc on iron was achieved at room temperature of 20°C for 10 minutes. An Hull cell, with a volume of 269 cm^3, was employed. The purity of zinc strip anode was 99.99%. The cathode used was an iron strip. The ratio of surface for zinc/iron was 1:1. A magnetic bar was used for stirring. A Princeton galvanostat, type 185 was employed for the control of current density.

In order to determine the mass of the deposited zinc, so as to calculate an average thickness, each cathode was dried in an oven and weighed on an analytical balance after and before each test.

RESULTS AND DISCUSSION

The effect of current density

The influence of current density on the thickness and nature of the zinc layer and current efficiency was studied at 20 ^{0}C. The electrolyte solution consisted of: $ZnCl_2$ 35 g/L, KCl 145 g/L, H_3BO_3 30 g/L.

The relationship between current efficiency and current density is presented in Fig. 1.

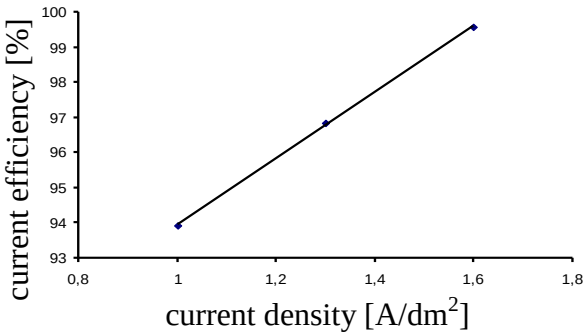

Fig. 1. The relationship between current efficiency and current density:
20 ^{o}C; pH = 5.2; concentrations: $ZnCl_2$ = 35 g/L; KCl = 145 g/L; H_3BO_3 = 30 g/L

The experimental values show an increase of current efficiency when the current density increases.

The variation of zinc deposit thickness versus current density is presented in Fig. 2.

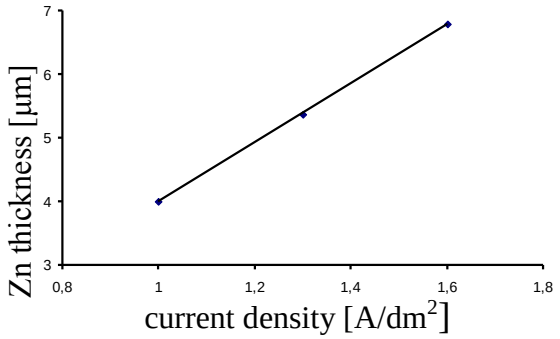

Fig. 2. Variation of zinc deposit thickness versus current density:
20 ^{o}C; pH = 5.2; concentrations: $ZnCl_2$ = 35 g/L; KCl = 145 g/L; H_3BO_3 = 30 g/L

When the current density increases, there is an increase of the zinc layer thickness However, at low current densities, the zinc film is bright, adherent and continuous, while at higher current densities it becomes dull. High current densities produce burnt zinc deposits.

The effect of $ZnCl_2$ concentration

The influence of $ZnCl_2$ concentration on current efficiency, as well as on the character and thickness of the zinc layer obtained, was followed at 20 ^{0}C and at a current density of 1 A/dm^2. Keeping constant the KCl and H_3BO_3 concentrations, the concentration of $ZnCl_2$ was in interval from 35 to 50 g/L.

The relationship between current efficiency and $ZnCl_2$ concentration is presented in Fig. 3.

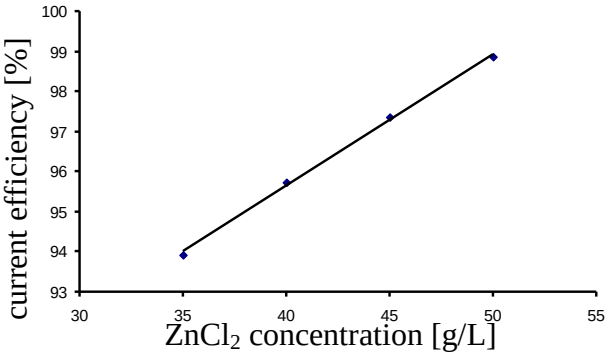

Fig. 3. The relationship between current efficiency and $ZnCl_2$ concentration:
20 ^oC; pH = 5.2; i = 1 A/dm^2; concentrations: KCl = 145 g/L; H_3BO_3 = 30 g/L.

Increasing the concentration of $ZnCl_2$ results in an increase of current efficiency.

The relationship between the resulting zinc layer thickness and character and the initial $ZnCl_2$ concentration is presented in Fig. 4.

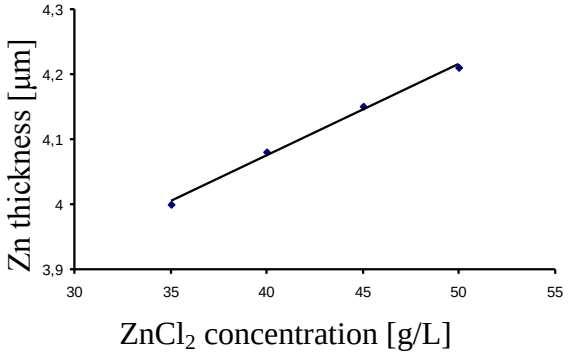

Fig. 4. Variation of zinc deposit thickness versus $ZnCl_2$ concentration:
20 ^oC; pH = 5.2; i = 1 A/dm^2; concentrations: KCl = 145 g/L; H_3BO_3 = 30 g/L.

An increase in concentration of $ZnCl_2$ leads to an increase of the zinc deposit thickness. Lower $ZnCl_2$ concentrations, i.e. 35 - 40 g/L, favor the forming of bright zinc deposits, adherent and with fine granulation. At $ZnCl_2$ concentrations above 40 g/L, the zinc deposits become dull or patchy dull. The optimum $ZnCl_2$ concentration for the electrodeposition of zinc is 35 g/L.

The effect of temperature

The influences of temperature on zinc layer thickness, quality and aspect and on current efficiency, were followed at a current density of 1 A/dm^2 and in an electrolyte solution consisting of: $ZnCl_2$ 35 g/L, KCl 145 g/L, H_3BO_3 30 g/L. The tested temperatures were in interval of 20 - 35 ^{0}C.

The effect of temperature on current efficiency is presented in Fig. 5. A temperature increase from 20 ^{0}C to 25 ^{0}C leads to an increase of current efficiency. The current efficiency decreases when the temperature increases above 25 ^{0}C. The variation of thickness zinc layer versus temperature is presented in Fig. 6.

The zinc layer becomes dull or patchy dull and its thickness decreases when the temperature is increased above 25 ^{0}C.

Therefore, the optimal temperature for the galvanic deposition of zinc, from a low acid $ZnCl_2$ electrolyte, is 25 ^{0}C. At this temperature, the zinc layer is bright and current efficiency is maximum.

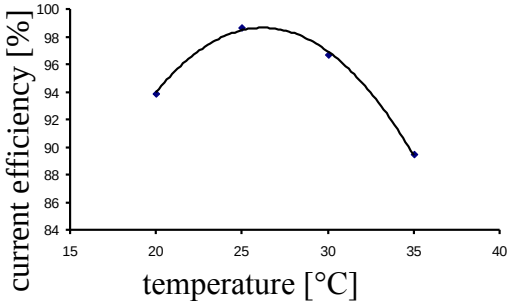

Fig. 5. The relationship between current efficiency and temperature:
pH = 5.2; i = 1 A/dm^2; concentrations:$ZnCl_2$ = 35 g/L; KCl = 145 g/L; H_3BO_3 = 30 g/L

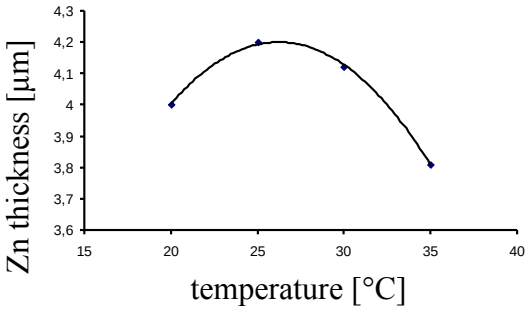

Fig. 6. Variation of zinc deposit thickness versus temperature:
pH = 5.2; i = 1 A/dm^2; concentrations: $ZnCl_2$ = 35 g/L; KCl = 145 g/L; H_3BO_3= 30 g/L

CONCLUSIONS

The research shows the optimum values which produce good quality zinc deposition, with anticorrosive properties and pleasant appearance aspects.

The test results, in a low acid $ZnCl_2$ electrolyte, show that:

- Increases in $ZnCl_2$ concentration and current density result in higher current efficiency and a greater zinc layer thickness, while still obtaining bright deposits. However too high a current density leads to dull or burnt zinc deposits.

- The preferred $ZnCl_2$ concentration, which favors bright zinc deposits on iron, is 35 g/L. If the $ZnCl_2$ concentration is greater than 40 g/L, dull and patchy dull zinc deposits are obtained.

- The optimum temperature for electrodeposition of the zinc metal is 25 °C. Temperatures above 25 °C cause a decrease of current efficiency and the zinc deposit becomes patchy dull. Below 25 °C current efficiency drops.

REFERENCES

1. Baik, D.S., Fray, D.J., Electrodeposition of Zinc From High Acid Zinc Chloride Solutions, *J. Appl. Electrochem*., 2001, 31, 1141-1145.

2. Mendoza-Huizar L.H., Rios-Reyes C.H., Gomez-Villegas M.G., Zinc Electrodeposition From Chloride Solutions Onto Glassy Carbon, *J. Mex. Chem. Soc.*, 2009, 53, 243-247.

3. Rezaite V., Vishomirskis R., Effect of Potassium Cyanide on Electrodeposition of Zinc From Zincate Solutions, *Prot. Metals*, 2001, 37, 25-30.

4. Naik, Y.A., Venkatesha, T.V., A New Brightener for Zinc Plating From Non Cyanide Alkaline Bath, *Rev. Indian J. Eng.&Mat. Sci.*, 2003, 10, 318-323.

5. Marcos F.C., Ivani A.C., Zinc Electrodeposition from Alkaline Solution Containing trisodium Nitrilotriacetic Added, *Electrochim. Acta*, 2013, 113, 229-239.

6. Naik, Y.A., Venkatesha, T.V., Nayak, P.V., Electroplating of Zinc From Sulphate-Chloride Bath, *Bull. Electrochem*., 2000, 16, 481-486.

7. Naik, Y.A., Venkatesha, T.V., Nayak, P.V., Electrodeposition of Zinc From Chloride Solution, *Turk. J. Chem*., 2002, 26, 725-733.

DETERMINATION OF CERTAIN NON-CONFORMITIES ON QUALITY CONTROL GOSTAT SALAMI

Valentina Gabriela Lazin [1], Madalina Jurcovan[1], FloareaN icolae [1], Daniela Fănuța –Mihăilă[1] , Catalin Galan [1]

[1]University "BIOTERRA" of Bucharest, , 81 Garlei Str., 013722, Bucharest, Romania

Abstract

In recent years occurred, also, made aware of a significant number of consumers regarding food safety problem, they have become increasingly interested not only for the quality but, also, the origin of food products consumed. It is appropriate, in those circumstances, the elimination of any deficiencies or weaknesses in the circuit traveled by food products from the farm gate to table. According to the European Union and the World Health Organization - food safety is everyone's responsibility, from their origin until they reach the end consumer. We must realize and the special role of trade in food safety since the food is sometimes found a significant period. Romania's alignment with European standards on foods implies first of all harmonization of national legislation with EU regulations.

Keywords: legislation, Gostat salami, physico-chemical parameters, quality control

INTRODUCTION

The cooked and cured meat products in the form of salami, represents a group of food products which occupies an important place in modern human diet. They provide a part of the necessary energy, protein and minerals specific to human metabolism.

Lately, we see a trend of increasing consumption of GOSTAT salami because the consumer is oriented towards the less processed meat products and low in additives. As is known in the market, the manufacturers policy of GOSTAT salami, is to promote products to satisfy this customer requirement. Thus, this salami do not contain thickening agents (Carrageenan), flavor enhancers (monosodium glutamate), starch, artificial colorings, artificial flavors and smoke flavoring. Also, in the manufacture of those preparations is using meat from different animal species (beef, pork), purchased domestically or imported, complying with national and European legislation.

This presentation brings to the fore the issue of compliance with the legislation in force, having as main objective determination of the fat and protein content in Gostat Salami,

for human consumption. Gostat Salami meets the requirements stipulated in the Ministerial Order 560/2006, approving the Norms regarding the marketing of meat products.[1]

MATERIALS AND METHODS

Materials and reagents: usual laboratory glassware (flasks, beakers, graduated pipettes, burettes); sulfuric acid (d = l, 84), free of nitrogen; 0.1N hydrochloric acid; 4% boric acid; copper sulfate and potassium sulfate p.a.; 33 % solution of sodium hydroxide free from nitrogen and carbonates and 0.1 N solution; Tashiro reagent alcoholic solution; dry and defatted filter cartridges; petroleum ether 40-60 °C; calcined sea sand; disodium phosphate or anhydrous sodium sulfate; wad fat free; boiling stones; weighing bottle with lid from glass or aluminum; desiccator with lid and hygroscopic substance (preferably $CaCl_2$); sea sand for laboratory analysis; enamel trays; spoons, spatula, celluloid cards.[7,8].

All reagents used were of analytical grade. Whole sale glassware was calibrated by the Romanian Bureau of Legal Metrology.

Facilities: mineralization system; distillation-titration system; extraction apparatus [2] Soxhlet-type (Scientific Velp) with 250 ml flask, 100 ml extractor and condenser / *Soxhterm extractor with extraction cups*; thermo-adjustable oven "Air Concept"; analytical balance Partner AC/220/C/2 accurate weighing 0,0001g.[10].

Moisture determination (water content): In a weighing bottle (from glass or aluminum) with a lid and glass rod are inserted 25 ... 30 g of sand and dried for 30 min. in the oven adjusted to a temperature of 103 ± 2 °C. After cooling in a desiccator to room temperature (approx. 30 min.), weighing bottle is weighed. Repeat the operations of heating, cooling, weighing until the difference between the two successive weighings of the weighing bottle with sand does not exceed 0.0002 g. The sand calcined like is used to bring the sample to the drying temperature in a time as low as possible and to ensure a uniform drying throughout the mass of the sample.

Then are inserted 3…5 g of sample (previously prepared for analysis) in the weighing bottle and weighed it accurate to 0,0001 g at the analytical balance. After weighing, is added to the weighing bottle approx. 5 cm^3 of 96% ethylic alcohol and homogenise well with the rod the mixture (the rod will stay all the time in the weighing bottle).

The lid next the weighing bottle containing the sand, the rod, the sample and 5 cm^3 ethylic alcohol are inserted in oven initially adjusted at 70-75 °C, where are maintained for

30 minutes, stirring from time to time with the rod to remove the alcohol. Then adjust the oven temperature at 150 °C and held for 1 hour, followed by the weighing.[20]

Determination of protein content: 0,5-2 g of sample, previously prepared, are weighing to analytical balance and the sample weighed is introduced in a Kjeldahl mineralization tube. Then are added 20 ml concentrated H_2SO_4, 1 g $CuSO_4$ and 5 g K_2SO_4 or *catalyst pellets. The tube is attached to the mineralization system. The mineralization system is heating gradually to avoid foaming. Initially the liquid* shows a blackish brown color and then is gradually clarifying. Mineralization is considered finished when the liquid becomes clear. From this point heating is continued for another 30 minutes. After cooling the mineralized has a bluish-green color. Typically mineralization lasts approximately 2 hours but the products with high fat content is mineralized harder. The digestion tubes is allowed to cool to 50-60 °C and added to each 50 ml of distilled water without ammonia. The cooled mineralized is passing in the distillation unit. Add 50-60 ml of 33% NaOH by means of automatic device and 25 ml H_3BO_3 in the manifold cup. The distillation is started and lasts maximum 7 min. (until 100 ml distillate is colected). The distillate is titrated with 0.1N HCl in the presence of Tashiro reagent from green to bluish-gray.[21]

Determination of fat content: 2-3 g of sample, previously prepared, are weighed in an extraction cartridge to the nearest 1 mg. The total mass with the extraction cartridge = wet sample weight. Is added a little sand to the weighed sample in cartridge and is mixed with a glass rod. The rod is cleaned with a skimmed cotton swab and the obtained mixture is added in the cartridge. The extraction cartridge is dried for 1 hour in a oven at 125 °C. Then the cartridge is removed from the oven and is allowed to cool in a dessicator. The glass cup extraction is weighed carefully with some boiling stones, to the nearest 1 mg. It is added 40 mL of petroleum ether. The cartridge with the sand and sample is attached to the extraction unit and then it is immersed for 30 min. in the boiling solvent; following 60 minutes of reflux washing with the raised cartridge and then is recovered the solvent. The cup containing the extract with the boiling stones is introduced in a oven at 103 °C for 30 minutes. To avoid fat oxidation during drying is recommended do not use temperatures higher than 103 C ± 2˚. Then the cup is allowed to cool in a dessicator. The weighing is made to the nearest 1 mg. The drying is repeating to constant weight.[22]

RESULTS AND DISCUSSION

We analyzed a set of 10 samples from different lots. These samples of Gostat Salami were crafted in double drawn at random on the Romanian market. The values obtained are listed in the table 1. [23, 24]

Tabel 1. The obtained values

No. Crt.	Sample	Fat, %	Protein, %	Water %	Sare, %	Nitriti, mg/100g
1	Gostat Salami 1	25	19	52	2,4	1,17
2	Gostat Salami 2	25	21	51	2,5	1,97
3	Gostat Salami 3	22	18	50	2,1	1,20
4	Gostat Salami 4	22	19	52	2,4	1,44
5	Gostat Salami 5	24	21	53	2,3	0,98
6	Gostat Salami 6	24	18	54	2,1	1,23
7	Gostat Salami 7	21	16	52	2,2	1,78
8	Gostat Salami 8	23	17	51	2,1	1,33
9	Gostat Salami 9	26	21	50	2,2	1,00
10	Gostat Salami 10	25	21	50	2,2	0,96

CONCLUSIONS

These measurements allow food quality control in accordance with the maximum allowable content of the chemical parameters and confirm the authenticity of these products. The methods used were relatively simple, quite fast compared to the classical methods and proved to be very sensitive, repeatable, reproducible. Applicability, repeatability, reproducibility of these methods were demonstrated by analyzing a large number of samples, here we present only several samples. For the determination of chemical parameters in the Gostat Salami with the methods presented in this article were made statistical calculations as well as development, validation and accreditation of these methods represent the subject of the next research topics.

Now when we think of "healthy fats" it take into account all factors: source, mode of preparation, the combination with other foods, integrating them into our lifestyle (diet, sports).

It is becoming increasingly clear the orientation trend of the Romanians to superior quality products, even if this means paying a higher price. Romanian consumer trust in brands, they are looking for on the shelves, do not buy products with unknown brand

precisely because the sausage is a "blamed" category because E's from the some food products. The amount consumed per capita decreases in turn increase product quality, in terms of consumer preferences.

All these trends of consumer and transformations of the class held by manufacturers through investing in new production technologies and communication are designed to revitalize an industry that has gone through many crises, arising from information published in the media over the year's sausage on unhealthy ingredients. Moreover, loss of consumer confidence is the biggest threat to growth category.

REFERENCES

[1] Order no. 560 of 16 August 2006 approving the Norms regarding the marketing of meat products

[2] E. Diacu - "Instrumental Methods of Analysis", Didactic and Pedagogical Bucharest, 2005.

[3] AB Bergamo, JA Fracassi da Silva Pereira de Jesus D. - "Food Chemistry", 124, vol 4, 2011, pp. 1714-1717;

[4] Richard C. Dorf , The Technology Management Handbook, CRC Press, 1999

[5] Judy S. Hannah PhD, Kathleen A. Jablonski PhD and David C. Robbins MD - " Journal of the American Dietetic Association ", Vol 98, September 1 , 1998, pp. A18 ;

[6] SR EN ISO 1443 - Determination of fat content. Meat and meat products.

[7] Savu C. - "Control veterinary food" CERES Publishing House, Bucharest, 1997.

[8] Popescu N., Meica S. - "Fundamentals of analytical chemistry and practical elements veterinary health" Diaconu Coresi Publishing , Bucharest , 1993;

[9] N. Popescu , Popa G. , Stanescu V. - " Physico -chemical laboratory for food of animal origin " , Ceres Publishing House , Bucharest , 1986;

[10] www.velp.com

[11] G. Rotaru, D. Borda , N. Sava , S. Stanciu, Quality Management in Food Industry , Galati , Academic Publishing , 2005

[12] Luca Ionescu , legislation and auditing quality systems Performantica Publishing House , Iaşi, 2007

[13] S. Apostu Food Quality Management , Ed Risoprint Cluj-Napoca, 2004

[14] Cecilia Pop, D.S Stef , I. M. Pop, Food Quality Management , Production Edict Publishing , Iași, 2009

[15] C. Banu, E. Barascu , A. Stoica , A. Nicolau , Sovereignty , Security and Food Safety
ASAB Publishing , Bucharest, 2010

[16] Gh Brădăţan , Food Safety , Ed "Ion Ionescu de la Brad" , Iaşi, 2007

[17] C. Banu, principles of food law , Agir Publishing House , Bucharest, 2003 July .

[18] Banu C. et al., 1999 - "Manual food engineer", Technical Publishing, Bucharest

[19] Nenitescu C. - "Organic Chemistry", Vol II;

[20] SR ISO 1442: 2010 - Determinarea umiditatii. Carne si produse de carne, conserve

[21] SR ISO 1443:2008 - Determinarea conţinutului de substante grase. Carne si produse din carne. (grasime totala) SR ISO 1444:2008 (grasime libera)

[22] SR ISO 937:20072 - Determinarea conţinutului de azot. Carne si produse din carne

[23] SR ISO 1841-2: 2000 PS-005 - Determinarea clorurii de sodiu. Carne si produse din carne

[24] SR EN 12014-3:2005 - Determinarea conţinutului de nitriţi. Carne si produse din carne

POLYPHENOLIC COMPOUNDS IN SEVERAL TYPES OF FERMENTED TEA PRODUCTS FROM *CAMELLIA SINENSIS*

Zin Mar Linn[1], C. Dumitru [2], Simona Oancea[3*]

[1]Department of Chemical Engineering, Yangon Technological University, Myanmar

[2]Department of Sciences, "Lucian Blaga" University of Sibiu, Romania

[3]Department of Agricultural Sciences and Food Engineering, "Lucian Blaga" University of Sibiu, Romania; simona.oancea@ulbsibiu.ro*

Abstract

Tea leaves are rich in phenolic compounds that are responsible for the antioxidant activity linked to the benefits of human health. The main objective of this study was to evaluate the quantity of phenolics, flavonoids and tannins content, as well as antioxidant activity of several types of commercial tea products from Romanian local market. The results were considerably variable between the different products regarding the amounts of phenolics (2140.228 to 1557.545 GAE mg/100 g), flavonoids (3393.768 to 1935.071 mg quercetin/ 100 g) and tannins (899.123 to 60.693 mg catechin/ 100 g) and also antioxidant activity. According to these results, we found that the content of tannin is directly linked to the antioxidant activity by FRAP method.

Keywords: commercial tea products, total phenol, flavonoids, tannin and antioxidants

INTRODUCTION

Tea (*Camellia sinensis*) is one of the most consumed drinks around the world. Despite that statistics show that Romania has not been ranked among countries with high tea consumption [1] there is a constant increase in national consumption mainly because of consumer awareness of its beneficial health effects [2].

The current six varieties of tea - green, yellow, dark, white, oolong and black, differentiate themselves through the type of processing from fresh leaves of *Camellia sinensis*. Black tea is a fully fermented product containing enzymatically derived pigments (theaflavins and thearubigins as complex condensed tannins) and catechin oxidation products resulted from the fermentation step [3].

Black tea may be considered an important source of pharmacologically active compounds. The non-volatile compounds in black tea represent the major constituents (such as polyphenols, flavonoids, organic acids, amino acids, alkaloids, carbohydrates, pigments,

minerals), while the volatile ones are found in minor quantities (such as hexanal, heptanal, ionones, linalool, geraniol, benzaldehydes, dimethyl sulphide), but with great contribution to the tea aroma [4][5]. Despite the known chemical composition, there are still uncharacterized polyphenols from black tea as result of the complex oxidative reactions following the fermentation process.

Scientific literature abounds in *in vitro* models, *in vivo* and epidemiological studies which demonstrate the health-promoting properties of tea and tea products [6] [3] [7]. The beneficial health effects of black tea products in particular linked to their antioxidant potential, may be resumed as follow: (1) prevention of atherosclerosis; (2) decrease of risk of cancer; (3) anti-inflammatory; (4) antimicrobial; (5) protection against UVB-induced erythema; (6) anti-aging; (7) improvement of neurologic function; (8) anti-obesity.

As tea production is limited to several geographical areas with specific requirements for this plant growth, the products of the six main varieties are imported by most markets related to their demand for tea. The aim of the present study was to select the commercial black teas from local markets and to comparatively evaluate their total contents of phenolics, flavonoids and tannins.

The addition of tannins can enhance the phenolic content of musts and wines more than the other processing methods. For the antioxidant activity test, the results are highly correlated to the applied analytical method. Regarding musts, the DPPH assay showed small differences among other technologies, whereas beta-carotene and ABTS assays showed the highest antioxidant activity with the addition of tannins. [8]

On the other hand, some authors demonstrated that the total phenolics content is correlated to the antioxidant activity, while the total content of flavonoids and tannins was not linked as an inhibition activity percentage in the scavenge free radical activity [9]. That is why we plan to study the relationship between the bioactive compounds and the antioxidant activities of various commercial black teas from local market by comparative evaluation of their total contents of phenolics, flavonoids and tannins.

MATERIALS AND METHODS

Sample preparation

The samples used in this investigation were various types of fermented tea products produced in different countries. The samples are listed in the Table 1 that all were collected

as a random choice from the Romanian local market. Bioactive compounds were extracted using 70% ethanol.

Table 1. List of black tea Samples

No.	Product name/type (packing)	Brand / manufacturer country
1	Black tea (bags)	Tea Party / Poland
2	English breakfast (bags)	Twinings / UK
3	Indian black tea (bags)	Belin / Poland
4	English leaf tea (bulk)	Pickwick / The Netherlands
5	High grown black tea (bags)	Princess Noori / Russia
6	Gold Flowery Tea – *Ceylon* (bags)	Dukat / Poland
7	Rich black Tea – *Assam* (bags)	Dukat / Poland
8	Black sense Ceylon (bags)	Loyd / Poland

Determination of Total Phenolic contents

The total phenolic contents of the ethanol extracts of all samples were observed by the Follin-Ciocalteu assay [10]. The results were determined based on a standard calibration curve of gallic acid and expressed as mg GAE/100g DM.

Determination of Flavonoids

The total flavonoids content of sample extracts were determined by the colorimetric method [11] by using the UV-Vis spectrophotometer with the absorbance at 510 nm. The results were expressed as mg of quercetin on the basis of dry matter (mg quercetin/100g DM)

Determination of total condensed tannin contents

The tannin contents or Proanthocyanidin were measured by the method of Broadhurst et al., 1978 [12], using catechin as a reference compound. The condensed tannin was expressed as g E.Catechin.100g -1DM.

Antioxidant activity assay

Ferric reducing antioxidant power (FRAP) assay

The antioxidant activity of samples were determined by ferric reducing antioxidant power (FRAP) assay according to Benzie and Strain (1996)[13].

Statistical analysis

All of the measurements were performed in triplicate. Results were calculated and expressed as mean ± standard deviation of triplicate determination.

RESULTS AND DISCUSSION

The results on the content of bioactive compounds such as total phenolic compounds and total flavonoids of the selected black tea products are shown in Fig 1.

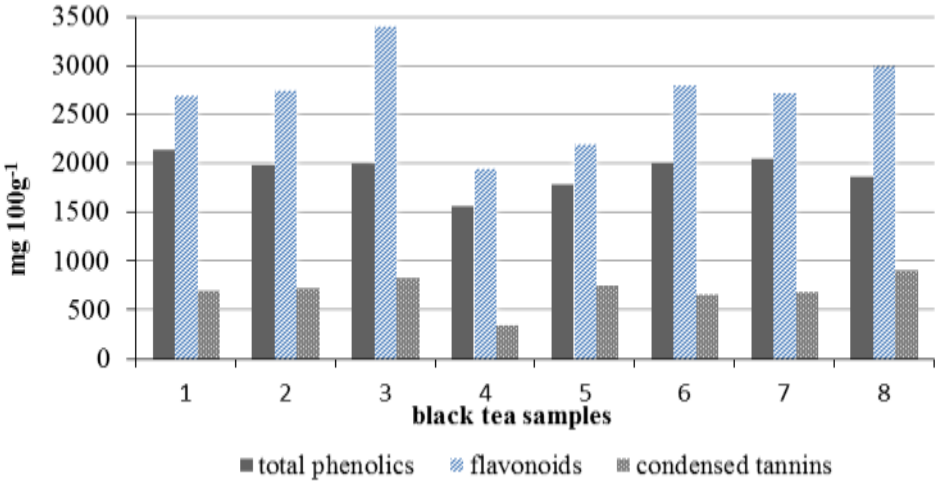

Fig. 1. The content of bioactive antioxidant compounds of various black tea products

The phenolic content in tea refers to the phenols and polyphenols such as catechins, theaflavins, tannins, and flavonoids, that are the natural plant compounds of tea, can affect the particular flavor and mouth-feeling and are recognized to provide potential health benefits. [14]

The results obtained in this study showed the significant high amount in most of the investigated products. This is of high significance, as phenolics are supposed to be the main bioactive compounds responsible for the health benefits of tea. Even the minimum amount was quite important 1557.545 GAE (mg/100g) for sample 4, while the highest one was 2140.228 GAE (mg/100g) for sample 1. Similar results were obtained by other authors [15-16] regarding the phenolic content of black tea from different geographical regions, ranging from 19 to 22 mg GAE/ g DW.

Another group of natural phenolics called the flavonoids are also of great interest because of their good influence on human health. Among other common food and beverage

products, tea is one of the famous for high content of flavonoids, especially catechins as the largest type of flavonoids in growing tea leaves. [17]

The total flavonoid contents of eight samples were showed in Figure 1. Among these results, sample 3 had the highest flavonoid content 3393.768 mg quercetin/ 100 g. However, we can assume that all of the tea products have a significant high amount of flavonoids.

Another important compound in tea is tannin that is naturally occurring compound, especially in black tea, to give a bitter flavor and astringent properties. In this study, we also found that the tannin content of all investigated samples were comparable to other reported research [18] which states that tannin content in black tea ranging from 11.76 to 15.14% with an average of 13.36% . Meanwhile in this study even the highest amount was just 899.123 mg catechin/100g that means only 0.9%.

The results regarding the antioxidant activity determined by FRAP method, are shown in Figure 2.

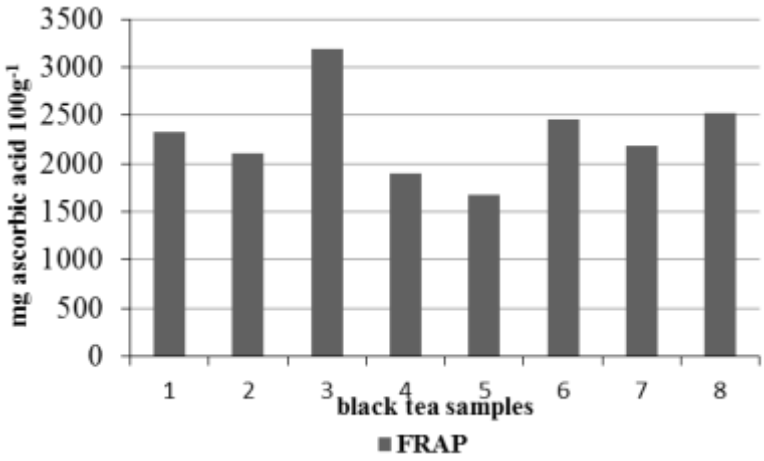

Fig. 2. The total antioxidant activity of various black tea products

In this study, the highest antioxidant activity was observed in sample 3 - which also indicated the highest flavonoids content and the second highest phenolic content as well as the second highest tannin content. On the other hand, sample 5 exhibited the lowest antioxidant activity but not the lowest content of phenolic, flavonoid and tannins. Other authors reported total antioxidant activity as measured by different assays (FRAP, DPPH). Based of significant differences on extraction solvent and analytical technique, it is difficult to have an accurate comparison of the results. Some authors reported antioxidant activity of black tea extracts as 0.38 mmol Fe/mL, varying between 1681 – 3190 mg ascorbic acid/100 g, or 365 μmol/g DW [15] [16].

CONCLUSIONS

The investigated black tea products studied in the present research showed high contents of bioactive compounds, in particular phenolics and flavonoids, and antioxidant activity. There were some variations in results among the eight samples, probably related to genetic, environmental and technological influences regarding the tea samples. The high content of antioxidant compounds and FRAP activity recommend such black tea products as significant for maintaining good health.

REFERENCES

1. Annual per capita tea consumption worldwide as of 2016, by leading countries (in pounds) https://www.statista.com/statistics/507950/global-per-capita-tea-consumption-by-country/
2. Euromonitor International, Tea in Romania, April 2016, http://www.reportlinker.com/p0190130-summary/Tea-Romania.html
3. C.-T. Ho, J.-K. Lin, F. Shahidi, *Tea and tea products: Chemistry and Health-Promoting Properties*, by CRC Press Taylor&Francis Group, 2008
4. Iarc working group, *IARC Monographs on the Evaluation of Carcinogenic Risk to Human, vol. 51, Tea: Chemical Composition*. 1991, pp. 217-223
5. Rawat R, Gulati A, Kiran Babu GD, et al. Characterization of volatile components of Kangra orthodox black tea by gas chromatography-mass spectrometry. *Food Chem*. 2007;105(1):229–235.
6. Y. Zhen, Tea: bioactivity and therapeutic potential, *Journal of Natural Products*, Taylor&Francis, 2002.
7. H. Pan, Y. Gao, Y. Tu, *Mechanisms of body weight reduction by black tea polyphenols*, Molecules 21, 2016.
8. Baiano A[1], Terracone C, Gambacorta G, La Notte E. Phenolic content and antioxidant activity of Primitivo wine: comparison among winemaking technologies. *J Food Sci.* 2009.
9. Ainnie Rahayu Abdullah, Nor Aziyah Bakhari , Hasnah Osman, Study on the Relationship of the Phenolic, Flavonoid and Tannin Content to the Antioxidant Activity of Garcinia Atroviridis, *Universal Journal of Applied Science* 1(3): 95-100, 2013
10. Singleton V.L., Rossi J.A. Jr., Colorymetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *Am. J. Enol. Vitic*. 16 (1965) 144−158.
11. G.C. Bag, P. Grihanjali Devi, Th. Bhaigyabati, *Int. J. Pharm*. Sci. Rev. Res., 30(1), 2015; 154-159
12. Broadhurst RB, jones WT. Analysis of condensed tannins using acidified vanillin. *Journal of the Science of Food and Agriculture*. 1978; 48(3): 788–794.
13. I.F.F. Benzie, J.J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power – the FRAP assay, *Analytical Biochemistry*, 239 (1996), pp. 70–76
14. https://en.wikipedia.org/wiki/Phenolic_content_in_tea.

15. Raghad Shakir Shayea Al-Obaidi, Dina Hamid Sahib "Determination of Antioxidants Activity in Tea Extract" *American Journal of Biochemistry*, 2015.
16. Alexander Yashin , Yakov Yashin , Boris Nemzer "Determination of Antioxidant Activity in Tea Extracts, and Their Total Antioxidant Content", *American Journal of Biomedical Sciences* ISSN: 1937-9080, September 2011
17. https://en.wikipedia.org/wiki/Phenolic_content_in_tea
18. Jyotismita Khasnabis , Chandan Rai and Arindam Roy "Determination of tannin content by titrimetric method from different types of tea" *Journal of Chemical and Pharmaceutical Research*, 2015, 7(6):238-241

CATALASE ACTIVITY OF SOILS AS INDICATOR OF CONTAMINATION OF SOME ROMANIAN REGIONS WITH HEAVY METALS

Alexandru Marian Ganea[1], Simona Oancea[2*]

[1]Department of Sciences, "Lucian Blaga" University of Sibiu, Romania

[2]Department of Agricultural Sciences and Food Engineering, "Lucian Blaga" University of Sibiu, Romania;*

alexandruganea@yahoo.com, simona.oancea@ulbsibiu.ro

Abstract

There is still a great concern for the contamination of soil with pollutants, such as heavy metals. These toxic compounds are known to impart adverse effects on human health. The present paper aimed at the evaluation of catalase activity of six samples of soils contaminated with various levels of heavy metals (lead, cadmium, zinc and copper), and one control sample (Poplaca forest). The results showed that catalase is very sensitive to increased concentration of heavy metals, in particular Pb, Cd and Zn. The concentration of heavy metals varies according to the sampling site. The highest values were recorded in three Copșa Mică sites which are situated near the source of pollution, while the lowest values were at Slimnic comparative to Poplaca forest, most remote sites from the pollution source. A negative correlation between the level of soil heavy metals and the catalase activity was found. The hereby presented results demonstrate that high levels of heavy metals negatively influence the enzymatic activity, and indirectly may affect foods and human health.

Keywords: soil, catalase, pollution, heavy metals

INTRODUCTION

Copșa Mică from Sibiu county, Romania has been one of the most polluted areas particularly with heavy metals, the main cause being the activity of two industrial enterprises. Until closed, one of the entreprise produced carbon black, which leads to major negative effect at all levels: plants, animals, humans, ecosystems and landscape. Due to the emissions of heavy metals, sulphur dioxide and solid particles in suspensions, the other enterprise was responsible for the decreased quality of life of the people in that area (5).

The pollutants produced by these factories caused a major industrial pollution, with high impact on the environment, affecting water, air and soil, and finally the health of local population.

As result of the contamination with various toxic compounds, mostly resulted from human activities, pollution leads to poor functioning of soil. This refers not only to the quantitative aspect but also the qualitative one, such as the change of the composition of biomass through retention of pollutants with adverse consequences for plant and animal species (3). A major problem of soil contamination is that plants have the capacity to accumulate polluants from soil and thus they pass on humans and animals body. (2)

Studies revealed that heavy metals as a consequence of soil contamination, were found in significant amounts in samples of different fruits, vegetables and animal products, representing a major risk for people health (4).

Enzymatic activity of soil, in particular of oxidoreductases, transferases and hydrolases, represents an indicator for its quality, together with microorganisms biomass, microorganism respiration and nitrification (1).

The present paper aimed to evaluat the catalase activity of soil samples from Copșa Mică region, and to correlate with the concentration of some heavy metalls (lead, cadmium, zinc, copper).

MATERIALS AND METHODS

Samples

For the present study, six soil samples were collected from Copșa Mică sites (Târnăvioara, Copșa Mică town hall, Copșa Mică village, Valea Viilor, Șeica Mare, Slimnic) and a control sample from Poplaca forest situated in Sibiu (non-polluted area), during October 2011. Sampling was done at soil depth of 0-10 cm soil using a sampler. Samples were stored under sterile conditions at 4^{o}C. To remove impurities, soil was passed through a sieve with a diameter of 0.5 mm.

Determination of heavy metals concentration

The investigated samples were digested and analyzed using atomic absorbtion spectometry and ICP-MS technique, at the Laboratory for physico-chemical analysis of the Environmental Protection Agency Sibiu.

Assay of catalse activity

The method used to determine soil catalase activity is based on the titrimetric technique (6), consisting in the decomposition of hydrogen peroxide by catalase and determination of the undecomposed H_2O_2 with a solution of $KMnO_4$.

RESULTS AND DISCUSSION

Soil catalase activity of the investigated samples (contaminated and control) are presented in Figure 1. It can be noted that the enzymatic activity of soil increases with the distance from the source of pollution. The highest value of soil enzymatic activity was obtained in the control sample, as expected. Among the investigated soil samples, the highest value for catalase activity was found in the sample from Slimnic, while the lowest activity was registered in the sample collected from Târnăvioara.

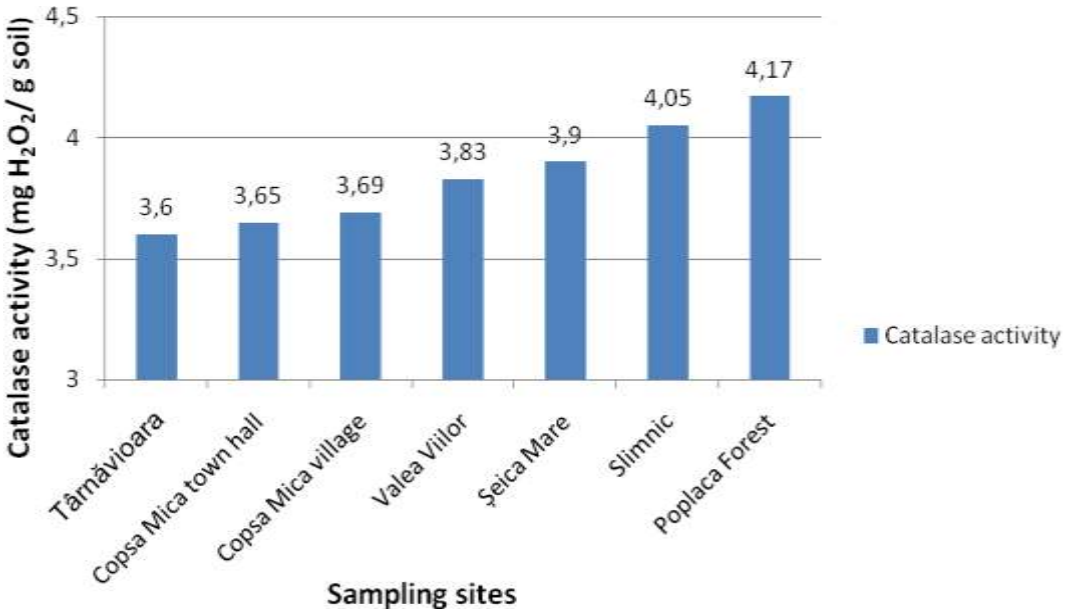

Fig. 1. Soil catalase activity in the investigated samples

The values of soil catalase activity varied between 3.60-4.17 mg H_2O_2/ g soil. Catalase activity of the soil registered lower values in samples collected near Sometra and high levels in samples collected from the Copșa Mică area. In sample collected from Slimnic there was no lead contamination of soil.

The content os some heavy metals (Pb, Cd, Zn and Cu) in the investigated samples are indicated in Table 1.

Table 1. Heavy metal concentrations in investigated soil samples

Sampling sites	Heavy metals (mg/kg DM)			
	Pb	**Cd**	**Zn**	**Cu**
CopșaMică-town hall	694	22	1801	120
Târnăvioara	794	22,5	1847	78,7
Valea Viilor	39,9	1,73	120	17,3
Copșa Mică village	413	12	1022	101
Șeica Mare	25,3	0,49	99,6	62,7
Slimnic	0	0	58,7	12
Poplaca Forest	37,01	0	58,46	17,65

According to the Government Decision no 756/1997 (7) the reference value of lead is 20 ppm, while the alert level in the sensitive area is 50 ppm. As observed from Table 1, three values of the level of heavy metals were above the alert level, while the other values were under this level. For cadmium, the reference value is 1 ppm, while the alert level is 3 ppm, this value being exceeded in three samples from Copșa Mică town Hall, Copșa Mică village and Târnăvioara. Values under the alert level were registered in samples from Valea Viilor and Șeica Mare, while in sample from Slimnic and Poplaca Forest, the cadmium was absent. In case of zinc, the reference value is 100 ppm and the alert level in the sensitive area is 300 ppm. Concentrations above the alert level were registered in the samples from Copșa Mică area. Reference value has been exceeded in the case of sample originated from Valea Viilor, but the concentration was under the alert level. For the other investigated sites, the concentrations of zinc situated under the alert level. The concentration of soil copper exceeded the alert level (100 ppm) in the two sites from Copșa Mică, while values were below this level in the other investigated sites.

The obtained data suggest that there is a negative correlation between the level of soil heavy metals and the soil catalase activity. Thus, samples originated from non-polluted area (Slimnic, Poplaca forest) having low or no heavy metals, showed high catalase activity.

CONCLUSIONS

The enzymatic activity of soil represents an useful indicator of its quality, as enzymes are very sensitive to changes caused by different amounts of toxic compounds.

The present investigation conducted on six samples of soil contaminated with heavy metals and one control sample (Poplaca forest) showed that catalase is very sensitive to increased concentration of heavy metals, in particular Pb, Cd and Zn. The concentration of heavy metals varies from one site to another according to a gradient represented by the distance from the source of pollution, high values being recorded in Copșa Mică and Târnăvioara (sites situated near the source of pollution), while the lowest values were at Slimnic and Poplaca Forest, most remote from the source of pollution.

There is a negative correlation between the level of soil heavy metals and the soil catalase activity.

REFERENCES

1. Castaldi S, Rutigliano, F.A., Virzo S.A. *Suitability of soil microbial parameters as indicators of heavy metal pollution, Water, Air, & Soil Pollution*, Napoli, Italy, 2002, 21-35

2.Lăcătușu R., C. Răută, S. Cârstea, I. Ghelase, *Soil-plant-man relationships in heavy metal polluted areas in Romania, in Applied Geochemistry*, 11 (1), 1996, 105-107

3. Micu, M.O. *Ecopedolgy and soil protection with elements of general pedology*, Alma Mater (Ed.) Sibiu, Romania, 2004

4. Muntean E., Muntean N., Mihăescu T.. *Cadmium and lead soil pollution in Copșa Mică area in relation with the food chain*, Research Journal of Agriculture Science, 42(3), 2010, 731-734

5 Muntean E., Muntean N., Duda M. *Heavy Metal Contamination of Soil in Copșa Mică Area*, *Pro-Environment* 6, 2013, 469-473

6. Johnson J.I., Temple K.L., *Some variables affecting the measurements of catalase activity in soil*, *Soil Sci. Soc. Am. Proc*. 28, 207-216, 1964

7. Goverment Decision no. 756/1997 for the approval of regulation regarding the environment pollution assessment, published in Romanian Monitor no 303 bis

THE INVESTIGATION OF THE PREBIOTIC ACTIVITY OF INULIN-TYPE FRUCTANS FROM *CICHORIUM INTYBUS* AND *TARAXACUM OFFICINALE*

S. Patriche[1], R. Costea[2], A.V. Dediu (Botezatu)[1], R.M. Dinica[1*]

[1] Chemistry, Physics and Environment Department, Faculty of Science and Environment, "Dunărea de Jos" University of Galati

[2] Faculty of Medicine and Pharmacy, "Dunărea de Jos" University of Galaţi

[*]E-mail address: rodica.dinica@ugal.ro

Abstract

Our main goal was to extract, isolate and characterize the inulin-type fructans from Cichorium intybus and Taraxacum officinale roots in order to evaluate the prebiotic activity of this polysaccharide. The spectrophotometric methods and HPTLC tehcnique were used to quantify the inulin content of these vegetable extracts. The highest content of inulin-type fructans was found in Cichorium intybus roots (33,07 µg/ml). The investigation of the prebiotic activity of inulin from these vegetable products was performed for both lyophilized and dried extractsin the presence of Lactobacillus plantarum strains, which showed a significant growth dynamics compared to the blank sample represented by a commercial product, Synergyn. The prebiotic activity of the lyophilized extracts was lower than to the one of the dried samples due to the sensitivity of inulin to the heat treatment, which caused the decomposition of the polysaccharide. In conclusion, Cichorium intybus and Taraxacum officinale roots due to their significant inulin content are characterized by an essential prebiotic potential and can be used as additives resources to improve the textural and organoleptic properties of various food products.

Keywords: prebiotic activity, inulin, HPTLC, spectrophotometric methods

INTRODUCTION

Inulin is a food ingredient that belongs to a class of carbohydrates known as fructans, being used as a bulking agent for use in fat replacement, textural modification and organoleptic improvement [1]. Furthermore, inulin can be used as prebiotic with various effects on structure, quality, sensory acceptance and glycemic response of gluten-free breads [2]. This fructan-type polysaccharide was found in more than 30000 vegetable products, such as: *Helianthus tuberosus* (Jerusalem artichoke), *Cichorium intybus* (chicory), *Dahliapinnata* (dahlia) and *Polymnia sonchifolia* (yacon) [3]. The goal of this study consists of the extraction, isolation and characterization of the inulin-type fructans from *Cichorium intybus*

and *Taraxacum officinale* roots in oder to evaluate the prebiototic activity of this polysaccharide.

MATERIALS AND METHODS

The plant material used in our study was collected from romanian spontaneous flora (Buzau area). The inulin was extracted from *Cichorium intybus* (chicory) and *Taraxacum officinale* (dandelion) roots. The content of this polysaccharide from the vegetable extracts was quantified using spectrophotometric methods and HPTLC technique. The structure of chemical compounds discovered in theses extracts was identified using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) and a commercial inulin, as standard solution. The prebiotic activity of inulin extracted from *Cichorium intybus* and *Taraxacum officinale* roots was performed in the presence of *Lactobacillus plantarum* strains and a commercial product, Synergyn.

RESULTS AND DISCUSSION

The highest content of inulin-type fructans was found in Cichorium intybus roots (33,07 μg/ml) compared to the aqueos lyophilized extract from *Taraxacum officinale* roots (10,76 μg/mL). Both the inulin standard solution and plant extracts showed similar functional groups (C-C: 1018÷1044 cm^{-1} and C-OH: 1106.06÷1114 cm^{-1}). Thus, the chemical structure of the compounds found in the plant extracts is almost similar to that of the standard solution of inulin. The dynamic growth of *Lactobacillus plantarum* in the presence of plant extracts with high content of inulin shows a maximum point developed after 48 hours of culture samples with the addition of the freeze-dried chicory and powdered oven dried dandelion. The lowest values were recorded for the samples of oven dried chicory powder and freeze-dried ethanol extract of dandelion. Both lyophilized and dried extracts in the presence of *Lactobacillus plantarum* strains showed a significant growth dynamics compared to the blank sample represented by a commercial product, Synergyn.

CONCLUSIONS

Prebiotics are food fibers or polysaccharides with partial hydrolysis, which are found in certain natural foods or added as additives. One of the polysaccharides with the greatest potential prebiotic is inulin, which has essential nutritional and functional properties. In our study, we have investigated the prebiotic activity of the inulin-type fructans extracted from

Cichorium intybus and *Taraxacum officinale* roots. The highest content of inulin-type fructans was found in *Cichorium intybus* roots. The investigation of the prebiotic activity in the presence of *Lactobacillus plantarum* strains, showed a significant growth dynamics compared to the blank sample represented by a commercial product, Synergyn.The prebiotic activity of the lyophilized extracts was lower than to the one of the dried samples due to the sensitivity of inulin to the heat treatment, which caused the decomposition of the polysaccharide. In conclusion, *Cichorium intybus* and *Taraxacum officinale* roots due to their significant inulin content are characterized by an essential prebiotic potential and can be used as additives resources to improve the textural and organoleptic properties of various food products.

REFERENCES

1. Karimi R., Azizi M.H., Ghasemlou M., Vaziri M., Application of inulin in cheese as prebiotic, fat replacer and texturizer: A review, *Carbohydrate Polymers*, 2015, 119, 85-100.

2. Capriles V.D., Arêas J.A.G., Effects of prebiotic inulin-type fructans on structure, quality, sensory acceptance and glycemic response of gluten-free breads, ***Food Funct., 2013,* 4,** 104-110.

3. Apolinário A.C., de Lima Damasceno B.P.G., de MacédoBeltrão N.E., Pessoa A., Converti A., de Silva J.A., Inulin-type fructans: a review on different aspects of biochemical and pharmaceutical technology *Carbohydrate Polymers*, 2014, 101, 368-378.

E-TONGUE FOR ROMANIAN HONEY AUTHENTICATION

Sorina Ropciuc[1], Mircea Oroian[1*], Sergiu Pădureț[1]

[1] *Faculty of Food Engineering, Stefan cel Mare University of Suceava*

Abstract

The aim of this study is to develop an e-tongue for Romanian honey authentication. For this reason it was used a cyclic voltammetric method based on three electrodes as: reference electrode (Ag/AgCl), counter electrode (glassy carbon electrode rod) and working electrode (silver). The honeys analysed were of four different botanical origins as: acacia, tilia, sunflower and polyfloral. The procedure consisted into a cyclic voltammetric test and the data obtained were submitted to Principal component analysis (PCA) and Linear discriminant analysis (LDA). It was observe that in the case of PCA scores the monofloral honeys are well separated into different groups, while the polyfloral honeys are near to sunflower and tilia honeys; this fact can be upon the great variability of the polyfloral honeys in terms of pollen grains. The LDA has reached a 100% correct classification of all the honey samples accordingly to their botanical origins.

Keywords: honey, authentication, e-tongue

INTRODUCTION

Food authentication is a process which verifies the information labelled and content of the product can. The information which is checked are: the origin (species, geographical or genetic), production method (conventional, organic, traditional procedures), or processing technologies (Danezis et al., 2016). Honey authentication has two major principles: (i) honey origin and (ii) the mode of production of honey. The origin of honey includes botanical and geographical origins, while production is related to the honey harvesting (Siddiqui et al., 2017).

The electronic tongues (e-tongues) are useful for the detection of chemical components. This device is replacing the human senses of taste. The e-tongue enables the possibility to gather information of the analysed solutions which can be related through chemometrics analysis as Principal Component Analysis of Linear Discriminant Analysis (Dong et al., 2017). The advantages of using the e-tongue methods based on electrochemical methods are: simplicity, rapidity and low costs (Apetrei & Apetrei 2016). The e-tongues have been used in the last years for the discrimination and quantification of different biological

active compounds in food matrix or for checking the freshness of different food products (Bougrini et al., 2016, Rudnitskaya et al., 2017, Dong et al., 2017).

The aim of this study is to evaluate the potential of a cyclic voltammetric e-tongue for honey authentication, for this purpose there was analysed 20 samples of honeys of four different botanical origins (acacia, tilia, polyfloral and sunflower); the potential of the e-tongue has been checked using Principal Component Analysis and Linear Discriminant Analysis.

MATERIALS AND METHODS

Materials

The honey samples have been purchased from local beekeepers located into Suceava county, in the North-East part of Romania. The honey samples were from different botanical origin as: acacia (five samples), tilia (five samples), polyfloral (five samples) and sunflower (five samples).

E-tongue

Electrodes

The electrodes used for the electrochemical measurement were: reference electrode (Ag/AgCl), counter electrode (Glassy Carbon Electrode Rod) and working electrode (Ag) (Metrohm, Germany).

Measurement system and experimental procedure

The measurement system consists in a PGSTAT 204 with FRA32M module (Metrohm, Germany), coupled to the electrodes presented above. The experimental data were recorded

120 using a NOVA 2.0 software (Metrohm, Germany). The voltage has been set between – 1V to + 1 V, with a scan rate of 0.5 V/s for the electrodes, and the frequency was set at 100 Hz. The electrodes were immersed in a vessel with 50 ml of honey solution (40 g honey completed to 200 ml with deionized water). To reach the electrochemical balance, the experimental data acquisition was made 10 minutes after the electrode has been placed into the honey solution. The experimental procedure was set for 45 s (1664 readings). All the samples were warmed to 25 °C before the experiment. To control the baseline drifts of the metallic electrodes, the electrodes were placed into a buffer solution at pH 7 before starting the measurements. The final values of current intensity were obtained subtracting their potential values from that of the buffer solution. Each experiment was made in triplicates.

Statistical analysis

Statistical analysis (Principal Component Analysis and Linear Discriminant Analysis) was performed using Unscrambler X 10.1 software system (Camo, Norway).

RESULTS AND DISCUSSION

In order to achieve the suitability of the e-tongue for the Romania honey authentication, we have analysed a number of 20 samples of different botanical origin (5 samples of acacia, tilia, sunflower and polyfloral respectively). The voltage was set between -1 V to 1V and the currents generated have been recorded. In the figure 1 are presented the typical cyclic voltammograms of the honeys analysed.

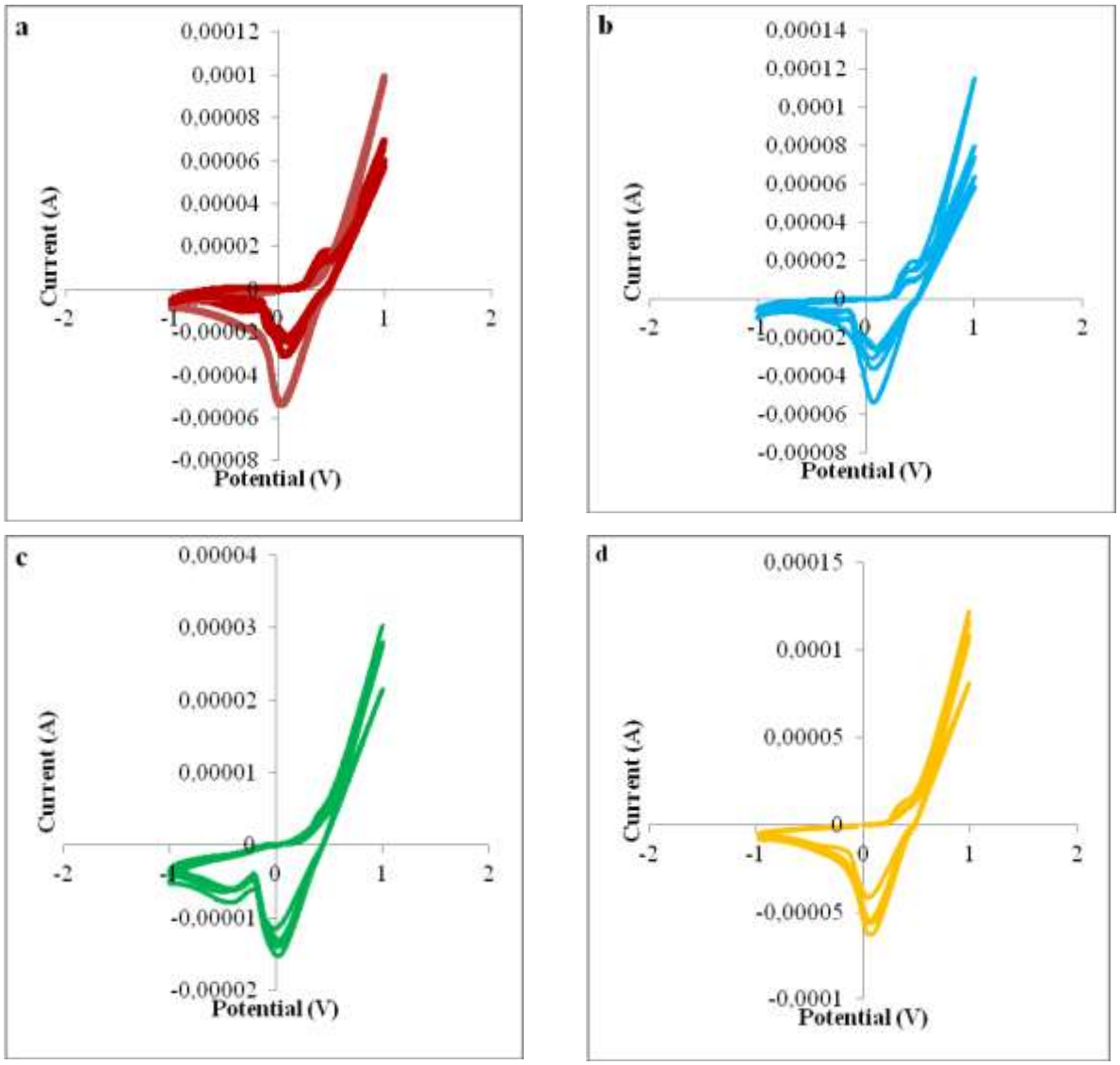

Fig. 1. Cyclic voltammetry of honey samples: a – sunflower, b – polyfloral, c – acacia and d – tilia

As it can be observed in the figure 1, the voltammograms of sunflower honeys and polyfloral are similar. The acacia honeys generate the lowest currents, while the tilia honeys generated the highest currents. The intensity of the current is influenced by the presence of the minerals dissolved and is known that tilia honeys have a higher mineral content than acacia, sunflower or polyfloral honeys (Oroian 2012).

Principal component analysis

The voltammetric data have been submitted to Principal Component Analysis in order to check the suitability of the data for honey authentication. In Figure 2 is presented the PCA scores of the honeys. It was found that the two principal components (PCs) explained 95.0% of variations in the data set. The first component explained 71% of the variations while the second component explained 24% of the variations, respectively. The PC1 divide the acacia and sunflower honeys by the tilia honeys, while the PC2 divides the acacia honeys by the sunflower, tilia and polyfloral honeys.

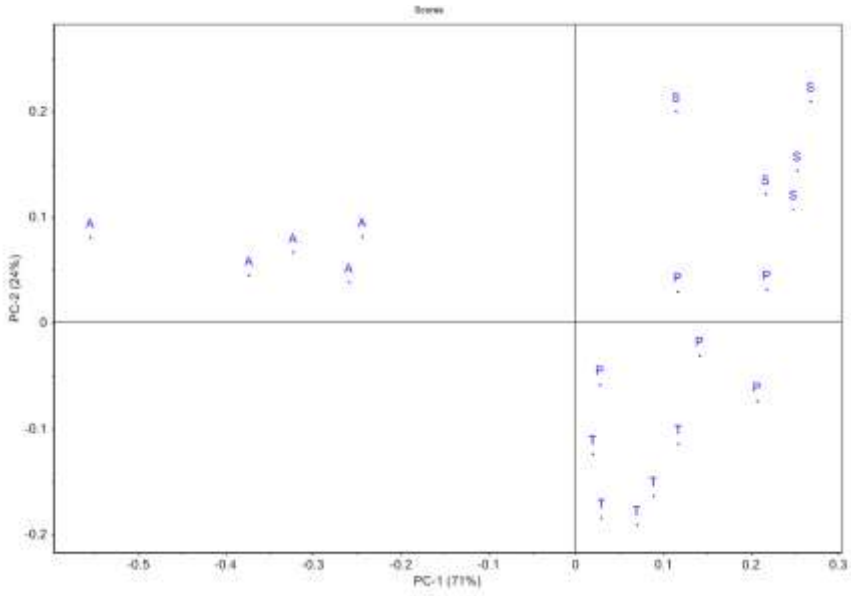

Fig. 2. Principal component analysis – scores: A-acacia, P-polyfloral, S-sunflower, T-tilia

Linear discriminant analysis

The e-tongue data where submitted to Linear discriminant analysis to check the suitability of them on honey classification. In the LDA every independent variable is entered or removed from the model. In order to check the model classification capacity, the percentage of samples classified correctly was considered as: original grouped (using all samples to estimate the classification model) and cross-validated grouped (leaving one out) to

estimate the robustness (Oroian et al., 2015). The LDA applied to the e-tongue data resulted into two canonical functions with the eigen values 3036116.5 and 2080.4. The function 1 explains 99.9% of the total variance, while the function 2 explains 0.01% of the total variance. In the table 1 is presented the classification results of the honey according to the botanical origin (original and cross validation), and it can be observed a 100% correctly classification of honeys accordingly to their botanical origin.

Table 1. Honey classification according to their botanical origin – original and cross validation

	Honey type				Total	% correct
Honey type	Acacia	Sunflower	Tilia	Polyfloral		
Acacia	5	0	0	0	5	100%
Sunflower	0	5	0	0	5	100%
Tilia	0	0	5	0	5	100%
Polyfloral	0	0	0	5	5	100%
Total	5	5	5	5	20	100%

In figure 3 is presented the linear discriminant analysis observations, there can be observed that each honey is concentrated in a zone of the projection each honey is in a different dial. The function 1 separates the sunflower and polyfloral honeys by acacia and tilia honeys, while function 2 separated the acacia and sunflower honeys by the tilia and polyfloral honeys.

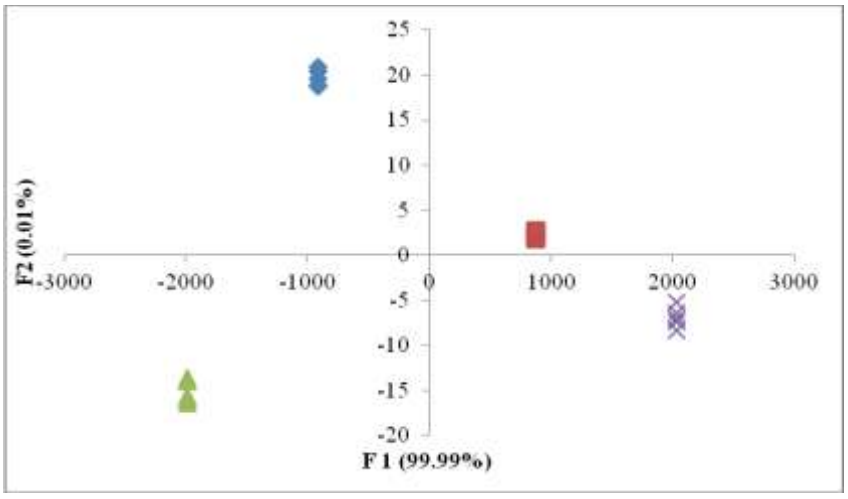

Fig. 3. Linear discriminant analysis observations: rhombus- sunflower, square-polyfloral, triangle-acacia, cross-tilia

CONCLUSIONS

The e-tongue is a suitable method for the honey authentication accordingly to their botanical origin (acacia, sunflower, polyfloral and tilis). The two statistical methods (PCA and LDA) applied proves the suitability of the e-tongues. The LDA reached a 100% correct classification of the honey according to their votanical origin.

ACKNOWLEDGEMENT

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS – UEFISCDI, project number PN-II-RU-TE-2014-4-0110.

REFERENCES

Apetrei, I. M., & Apetrei, C. (2016). Application of voltammetric e-tongue for the detection of ammonia and putrescine in beef products. *Sens. Act. B: Chem.*, *234*, 371-379.

Bougrini, M., Tahri, K., Saidi, T., El Hassani, N. E. A., Bouchikhi, B., & El Bari, N. (2016). Classification of honey according to geographical and botanical origins and detection of its adulteration using voltammetric electronic tongue. *Food Ana.l Met.*, *9*(8), 2161-2173.

Danezis, G. P., Tsagkaris, A. S., Camin, F., Brusic, V., & Georgiou, C. A. (2016). Food authentication: techniques, trends & emerging approaches. *TrAC Trends Anal. Chem.*, *85*, 123-132.

Dong, W., Zhao, J., Hu, R., Dong, Y., & Tan, L. (2017). Differentiation of Chinese robusta coffees according to species, using a combined electronic nose and tongue, with the aid of chemometrics. *Food Chem.*, *229*, 743-751.

Oroian, M. (2012). Physicochemical and rheological properties of Romanian honeys. *Food Bioph.*, *7*(4), 296-307.

Oroian, M., Amariei, S., Rosu, A., & Gutt, G. (2015). Classification of unifloral honeys using multivariate analysis. *J. Essent. Oil Res.*, *27*(6), 533-544.

Rudnitskaya, A., Schmidtke, L. M., Reis, A., Domingues, M. R. M., Delgadillo, I., Debus, B., Legin, A. (2017). Measurements of the effects of wine maceration with oak chips using an electronic tongue. *Food Chem.*, *229*, 20-27.

Siddiqui, A. J., Musharraf, S. G., & Choudhary, M. I. (2017). Application of analytical methods in authentication and adulteration of honey. *Food chem*, *217*, 687-698.

PSYLLIUM HUSKS AND CHIA SEEDS AS FAT REPLACERS IN MUFFINS

Roxana Tufeanu[1], Mihaela Tița[1], Ovidiu Tița[1]

[1] *Faculty of Agricultural Sciences, Food Industry and Environmental Protection, "Lucian Blaga" University of Sibiu, Victoriei Street, 10th, Sibiu, Romania*

Abstract

The objective of this research was to develop muffins with low fat content by using two fat replacers: chia flour and Psyllium husks. Eight muffin formulations with fat replacer were produced and compared with the full fat sample. Psyllium husks and chia seeds were used due to their rich composition in fibre, proteins, minerals, antioxidants, omega-3 and omega-6 fatty acids. The parameters measured were moisture, water activity, weight, volume, central height and sensory properties evaluated by nine trained assessors. The moisture and water activity were evaluated at storage days 1 and 5. The fat reduction induced the increase in moisture content values of samples. In weight of muffins with chia gel no significant differences were found. For muffins with 50 %, respectively 75% butter substitution with Psyllium gel, the weight is higher compared to control. The muffin formulated with 23∼47% replacement of butter with chia gel and with 15∼75% Psyllium gel received the most acceptable sensory scores.

Keywords: muffins, chia, Psyllium, fat replacers

INTRODUCTION

The dietary habits of the population influence the occurrence of obesity, atherosclerosis and coronary heart diseases. Diets rich in saturated and trans fats can have a negative impact on consumer health. Thus, reducing fat has become a concern for consumers, observed in the demand increase for reduced fat foods (Martínez-Cervera et al., 2011; Chugh et al., 2015; Tufeanu et al., 2016). Fat can be partially or totally replaced by a wide range of products, classified as substitutes or mimetics. Fat substitutes are synthetic compounds which usually have a chemical structure similar to fats. Fat mimetics are protein- or carbohydrate based natural compounds, which usually require a high amount of water to have the functionality of the replaced fat (Jones, 1996; Akoh, 1998).

Many researches are moving in the direction to design products which can have benefits on the health status. For example, in the field of sweet bakery products, reducing the fat content has been achieved by partial or total replacing with: peach fibre (Grigelmo-Miguel

et al., 2001), corn bran fibre (Jung et al., 2005), corn dextrins (Kim et al., 2001), β-glucan concentrates prepared from barley and oats (Kalinga and Mishra, 2009), cocoa fibre (Martínez-Cervera et al., 2011), papaya and mango (Paintsil, 2008); Yuja pectin (Lim et al., 2014).

Psyllium is very consumed in India and lately in USA and Europe. Since Food and Drug Administration (FDA) associated the Psyllium soluble fibre with reducing the risk of heart disease, the demand for this product has increased (Chugh et al., 2015; FDA, 2012). Psyllium is obtained from the seed of the *Plantago* plant and is an important source of soluble and insoluble fibre (Bijkerk et al., 2004).

Chia seeds have remarkable nutritional properties due to their high content in fatty acids (omega-3 and omega-6), protein, natural antioxidants, minerals and fibre (Bushway et al., 1981; Taga et al., 1984; Ayerza, 1995; Ixtaina et al., 2008; Reyes-Caudillo et al., 2008; Ayerza , 2010). Some of the most important benefits of the high fibre content are the regulation of intestinal transit and reduction of glycaemic index (Reyes-Caudillo et al., 2008; Vazquez-Ovando et al., 2009).

The objective of this research was to produce muffins with low content of fat and with improved nutritional qualities, acceptable flavour, texture, appearance by substitution of butter with chia powder and Psyllium husks.

MATERIALS AND METHODS

Ingredients for muffins preparation

The muffins ingredients were wheat flour, corn starch, semi skimmed milk, butter …, eggs, sugar, baking soda, salt, chia seeds and Psyllium husks. All the ingredients were procured from local markets.

Muffins preparation

Muffins were prepared by replacing butter with chia gel and Psyllium gel in different concentrations and compared with control muffins. Chia powder and Psyllium husks were hydrated with milk, in a ratio of 1:10, respectively 1:15 (for 30 minutes -1 hour) to obtain the gels. The butter, kept at room temperature for minimum 30 minutes before use, and/or the fat replacer were mixed with sugar using an electric hand mixer until the sugar was melted. Then egg and milk were added and the entire composition was mixed at speed 3 for few seconds. In the obtained mixture were added half of the quantity of flour, corn starch, baking

powder and salt. All the ingredients were mixed at speed 1 for 30 seconds and after that the remaining flour was added and all was manual homogenised. 40 g of batter were distributed in the moulds. Muffins were baked for 30 minutes at 180 °C in an electric oven preheated to this temperature for 10 minutes. After cooling for 10 min, muffins were removed from the moulds and were left to cool completely at room temperature for 1 h. The muffins were stored at 20°C±3°C for 5 days in polypropylene bags.

Table 1. Recipe for control muffins (CM), muffins with chia gel (MC) and muffins with Psyllium gel (MP)

Ingredients (g)	Samples								
	CM	**MC1**	**MC2**	**MC3**	**MC4**	**MP1**	**MP2**	**MP3**	**MP4**
Flour	90	90	90	90	90	90	90	90	90
Corn Starch	10	10	10	10	10	10	10	10	10
Eggs	60	60	60	60	60	60	60	60	60
Sugar	63	63	63	63	63	63	63	63	63
Butter	42	32	22	12	0	35,7	31,5	21	10,5
Milk	42	42	42	42	42	42	42	42	42
Baking Soda	3	3	3	3	3	3	3	3	3
Salt	1	1	1	1	1	1	1	1	1
Chia gel	0	10	20	30	42	0	0	0	0
Psyllium gel	0	0	0	0	0	6,3	10,5	21	31,5

Methods

Moisture content

The moisture content of muffins was determined by air-oven method. 5 g of sample were accurately weighed in empty moisture dish previously brought to constant mass. The dishes with the samples were then placed into the oven at 130 ± 2°C for 1 hour. The samples were dried to constant mass.

Moisture loss

The moisture loss during baking and after five days of storage was calculated by using the following equation:

$$\%ML = \frac{W_1 - W_2}{W_1} \cdot 100$$

Where W_1 is the weight of batter distributed in the moulds (40 g) and W_2 is the weight of baked muffin after 1 hour cooling and after 5 days of storage at room temperature.

Water activity (a_w)

The water activity of products was determined using Novasina Labmaster aw.

Volume and specific volume

The volume of muffin was determined by rapeseeds displacement method. By dividing the volume by muffin weight was obtained the specific volume.

Sensory evaluation

Muffins prepared were evaluated for sensory properties by 9 untrained panelists. They evaluated each sample for appearance, height, colour, sponginess, difficulty in chewing and swallowing, flavour and sweetness on 7-point scale, where 1=dislike extremely, 2=dislike moderately, 3=dislike slightly, 4=neither dislike or like, 5=like slightly, 6=like moderately and 7=like extremely.

The panellists received the muffin samples, coded, on a tray, a bottle of 500 mL with water and the evaluation sheet. Five samples for each formulation were tested in a random order at room temperature.

RESULTS AND DISCUSSION

Table 1 describes the physical characteristics of muffins with different levels of fat replacers. In weight of muffins with chia gel as fat replacers no significant differences were found. For muffins with Psyllium no trend was observed in weight, in reference to the control sample. But it was found that for muffins with 50 %, respectively 75% butter substitution, the weight is higher compared to control. This is due to the fibres that absorb more quantity of water and prevent the water loss during baking. Similar results were obtained by Bhise and Kaur (2015) by adding oat, Psyllium and barley fibres in muffins.

For the muffins with fat replacers was observed that volume and specific volume have higher values compared with the control sample.

Table 1. Physical characteristics of muffins

	CM	**MC1**	**MC2**	**MC3**	**MC4**
Weight (g)	33.69	33.59	33.65	33.57	33.92
Volume (cm^3/100g)	104.68	117.53	123.53	139.17	130.33
Specific volume (cm^3/g)	3.10	3.50	3.67	4.14	3.84
Central height (mm)	35	35	35	30	35
	CM	**MP1**	**MP2**	**MP3**	**MP4**
Weight (g)	33.69	32.94	33.63	34.13	34.92
Volume (cm^3/100g)	104.68	129.43	123.81	128.98	126.46
Specific volume (cm^3/g)	3.10	3.93	3.68	3.78	3.62
Central height (mm)	35	35	40	35	40

The moisture content values of the control sample were lower than those for the samples with fat replacers (Table 2). This result can be explained by moisture value of butter lower than the moisture of gels used as fat substitutes. Lower moisture content for control sample than the cookies prepared with Oatrim and avocado puree were observed by Wekwete and Navder (2007). Also, greater moisture content can be associated to the capacity of the fibres to retain water that can positively influence the sensory qualities of the product. Thus, can be obtained a moister product that is better accepted by consumers than a dry product, difficult to swallow. Grigelmo-Miguel et al. (2001) and Martínez-Cervera et al. (2011) have also reported increased moisture for muffins obtained by fat replacement with peach fibre, respectively cocoa fibre.

Water activity is an important parameter linked with shelf life of foods. It offers information about unbound water that influences the activity of enzymes and microorganisms (Wekwete and Navder, 20007; Andrade et al., 2016). In table 2 are presented the water activity values. For all formulation with fat replacers, the water activity was higher compared with the control. Wekwete and Navder (2007) also observed that water activity was higher in cookies prepared with avocado puree compared to control and Oatrim cookies. All samples showed a_w values greater than 0.7 - limit for moulds development (Andrade et al., 2016).

Table 2. Physical characteristics of muffins

	Moisture (%)		**Moisture loss (%)**	**Water activity**	
	1st day	5th day	**	1st day	5th day
CM	21.13	21.05	15.77	0.803	0.824
MC1	22.68	21.46	16.02	0.811	0.846
MC2	25.49	23.10	15.87	0.828	0.838
MC3	29.51	25.08	16.07	0.848	0.85
MC4	31.32	28.00	15.2	0.848	0.862
MP1	22.73	21.67	17.65	0.842	0.832
MP2	23.83	19.77	15.92	0.843	0.827
MP3	25.02	22.75	14.67	0.847	0.852
MP4	25.64	23.46	12.7	0.843	0.847

**after baking and 1 hour cooling at room temperature

Appearance, height, colour, sponginess, difficulty in chewing and swallowing, flavour and sweetness of control muffin and muffins with fat replacers were evaluated and the results are presented in figure 1 and figure 2. With regard to the appearance, control and MC1 were the most appreciated, followed by MC2. For MC3 and MC4 the scores were lower

approximately 4, and it was due to a yellow colour with light shades of grey from chia seeds. The appearance of muffins with Psyllium gel was between the range of 5.67- 6.00. Also, the stickiness and more intensive colour of the crust have affected the appearance of the samples with fat replacers. The scores for sweetness for all samples were between the range of 4.33-4.89, meaning that the sugar content used is considered not very pleasant, but neither unpleasant.

Significant differences were found in colour, sponginess, difficulty in chewing and swallowing for muffins prepared with chia gel as fat replacer. Only for MC3 and MC4 the sponginess and difficulty in chewing and swallowing were perceived as being slightly unpleasant.

The achieved scores, on the seven-point hedonic scale for MC1, MC2- (23.8% and 47.6% replacement of butter) and MP1, MP2, MP3, MP4 (15%, 25%, 50% and 75% replacement of butter) suggested that the use of gels is an optimal option.

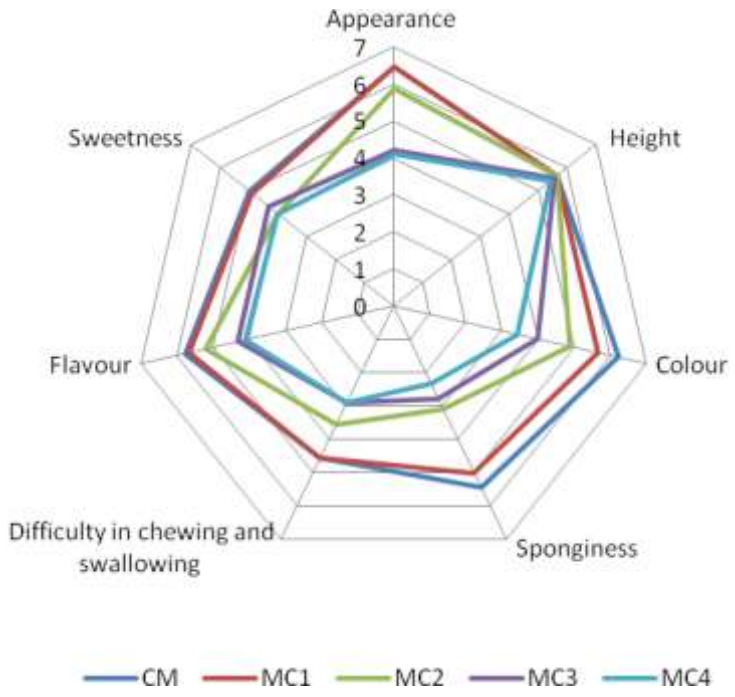

Fig. 1. Mean values for the sensory properties of muffins with chia gel as fat replacer (CM-control muffin; MC1- 23.8% replacement of butter; MC2- 47.6% replacement of butter; MC3- 71.4% replacement of butter; MC4- 100% replacement of butter)

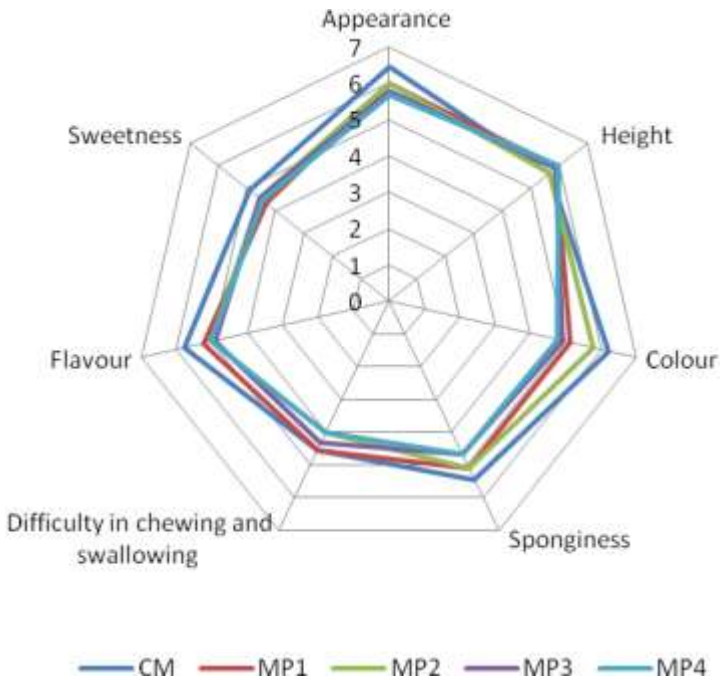

Fig. 2. Mean values for the sensory properties of muffins with Psyllium gel as fat replacer (CM-control muffin; MP1- 15% replacement of butter; MP2- 25% replacement of butter; MP3- 50% replacement of butter; MP4- 75% replacement of butter)

CONCLUSIONS

New formulations of muffins with chia gel and Psyllium gel were developed. Muffins with butter content reduction of 23~47% chia gel and with 15~75% Psyllium gel received the most acceptable sensory scores.

The results indicate that chia seeds and Psyllium husks are good options for replacing butter in muffin formulation and for improve the nutritional content.

REFERENCES

1. Akoh, C.C., Fat Replacers, *Food technology*, 1998, 52, 47-53.
2. Andrade F.J.E.T., Farias M.D.P., Vieira S.K.V., Sá D.M.A.T., Egito A.S., Carneiro-da-Cunha M.G., Fat content evaluation in physical properties and texture profile of cakes, *X CIGR Section IV International Technical Symposium*, Food: the tree that sustains life, 24th – 27 th October 2016, Gramado/RS, Brazil.
3. Ayerza R., Effects of seed color and growing locations on fatty acid content and composition of two chia (Salvia hispanica L.) genotypes, *J Am Oil Chem Soc*, 2010, 87, 1161–1165.
4. Ayerza R., Oil content and fatty-acid composition of chia (Salvia hispanica L.) from 5 northwestern locations in Argentina, *J Am Oil Chem Soc*, 1995, 72, 1079–1081.

5. Bhise S., Kaur A., Fortifying muffins with psyllium husk fibre, oat fiber and barley fibre to improve quality and shelf life, *Carpathian Journal of Food Science and Technology*, 2015, 7(2), 5-16.

6. Bijkerk C.J.,. Muris J.W.M, Knottnerus J.A., Hoes A.W., de Wit N.J., Systematic review: The role of different types of fiber in the treatment of irritable bowel syndrome, *Alimentary Pharmacology & Therapeutics*, 2004, 19, 245–251.

7. Bushway A.A., Belyea P.R., Bushway R.J., Chia seed as a source of oil, polysaccharide and protein, *J Food Sci*, 1981, 46, 1349–1350.

8. Chugh B., Singh G., Kumbhar B. K., Studies on the Optimization and Stability of Low-Fat Biscuit Using Carbohydrate-Based Fat Replacers, *International Journal of Food Properties*, 2015, 18, 1446–1459.

9. FDA (2012). CFR – code of federal regulations title 21. Retrieved June 5, 2016 from U.S. Food and Drug Administration web site. Web: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=101.81

10. Grigelmo-Miguel N., Carreras-Boladeras E., Martin-Belloso O., Influence of the addition of peach dietary fiber in composition, physical properties and acceptability of reduced-fat muffins, *Food Science and Technology International*, 2001, 7 (5), 425–431

11. H.Y.L. Kim, H.W. Yeom, H.S. Lim, S.T. Lim, Replacement of shortening in yellow layer cakes by corn dextrins, *Cereal Chemistry*, 2001, 78 (3), 267–271.

12. Ixtaina VY, Nolasco SM, Tomas MC Physical properties of chia (Salvia hispanica L.) seeds, *Ind Crop Prod*, 2008, 28,286–293.

13. Jung J.Y., Kim S.-A., Chung H.-J., Quality characteristics of low-fat muffin containing corn bran fiber *Journal of the Korean Society of Food Science and Nutrition*, 2005, 34 (5), 694–699.

14. Jones, S.A. *Issues in fat replacement*. In S. Roller & S. A. Jones (Eds.), Handbook of Fat Replacers, Boca Raton: CRC., 3–26, 1996.

15. Kalinga D., Mishra V.K., Rheological and physical properties of low fat cakes produced by addition of cereal β-glucan concentrates, *Journal of Food Processing and Preservation*, 2009, 33, 384–400.

16. Lim J., Ko S., and Lee S., Use of Yuja (Citrus junos) Pectin as a Fat Replacer in Baked Foods, *Food Sci. Biotechnol.*, 2014, 23(6), 1837-1841.

17. Martínez-Cervera S., A. Salvador, B. Muguerza, L. Moulay, S.M. Fiszman, Cocoa fibre and its application as a fat replacer in chocolate muffins, *LWT - Food Science and Technology*, 2011, 44 (3), 729–736.

18. Grigelmo-Miguel N., Carreras-Boladeras E., Martin-Belloso O., Influence of the addition of peach dietary fiber in composition, physical properties and acceptability of reduced-fat muffins, *Food Science and Technology International*, 2001, 7 (5), 425–431.

19. Paintsil Y.P., 2008, Sensory and rheological properties of reduced-fat rock buns and mango pie containing a papaya (carica papaya)-derived fat replacer, Teza Postdoctorat, Kwame Nkrumah University Of Science and Technology, Kumasi, Ghana.

20. Reyes-Caudillo E., Tecante A., Valdivia-Lopez M.A., Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (Salvia hispanica L.) seeds, *Food Chem*, 2008, 107, 656–663.

21. Taga M.S., Miller E.E., Pratt D.E., Chia seeds as a source of natural lipid antioxidants, *J Am Oil Chem Soc*, 1984, 61, 928–931.

22. Tufeanu R., Tița O., Possibilities to develop low-fat products: A Review, *Acta Universitatis Cibiniensis Series E: FOOD TECHNOLOGY*, 2016, 20 (1), 3-19.

23. Vazquez-Ovando A., Rosado-Rubio G., Chel-Guerrero L. Betancur-Ancona D., Physicochemical properties of a fibrous fraction from chia (Salvia hispanica L.), *Food Sci Technol Leb*, 2009, 42, 168–173.

24. Wekwete B., Navder K..P., Effects of avocado fruit puree and oatrim as fat replacers on the physical, textural and sensory properties of oatmeal cookies, *Journal of Food Quality*, 2008, 31, 131–141.

COPPER REMOVAL FROM AQUEOUS SOLUTIONS BY FLOTATION WITH ANIONIC COLLECTOR

***Adrian Turtureanu*[1]**

"Lucian Blaga" University of Sibiu, Romania, Faculty of Agricultural Sciences, Food Industries and Environmental Protection

Abstract

Copper removal from dilute aqueous systems is often studied for environmental protection and copper recovery. The paper presents the removal of copper from dilute aqueous solutions by flotation (dispersed-air-flotation) at a laboratory scale, using an anionic collector (oleic acid). The optimum values of the main parameters influencing this process were determined (pH of Cu(II) solutions, molar ratio collector:Cu(II), air flow rate, time, temperature and initial concentration of Cu(II) in sample. Using the optimal conditions, high removal degree (> 98%) of copper was obtained.

Keywords: copper, removal, flotation, oleic acid

INTRODUCTION

Heavy metals are considered as hazardous pollutants due to their toxicity even at low concentration, and non-biodegradable properties. The transportation and bioavailability of heavy metals in the aquatic system is affected greatly by their binding to surfaces of solid phases and complexion with the ligands in the water [1].

Copper is naturally in all animals and plants. At very low concentrations, copper is essential to all known forms of life, including humans. At higher concentrations of metal, copper is a highly toxic for all living things, with lethal effects.

More copper compounds are used in agriculture for the treatment of various diseases of the plants, water treatment as algaecides, as a preservative for wood, leather and fabrics.

The main sources of copper, in surface water, are wastewater from mining, non-ferrous metallurgical plants and galvanizing plants. Also, copper may reach in surface waters from domestic wastewater, from burning fossil fuels and waste, from wood processing industry, etc. [2].

It is, therefore, necessary to remove copper from aqueous industrial wastes before discharging it into the natural water stream. Various procedures have been reported for the

removal of heavy metals from aqueous media such as precipitation [3, 4], solvent extraction [5], ion exchangers [6-8], adsorption [1, 9-14] and flotation [15, 16].

MATERIALS AND METHODS

Chemicals:

- copper sulphate ($CuSO_4 \cdot 5H_2O$ p.a.) stock solution (2 g Cu(II)/dm^3) from which were prepared solution with 100 mg Cu(II)/dm^3;
- anionic collector, oleic acid, 1 M solution in ethanol;
- 15% and 1M NaOH solutions;
- 1M HNO_3 solution.

Apparatus:

- bench-scale equipment for dispersed-air flotation technique;
- pH-meter WTW 96;
- atomic absorption spectrophotometer PYE UNICAM model SP 1900.

Working procedure:

The flotation bench-scale equipment consist of compressor, cock for air flow rate adjustment, rotameter, 3,3 cm inner diameter glass flotation column (60 cm in height) with porous glass frit (porosity G_4).

From stock solution with 2 g Cu(II)/dm^3 there were prepared solutions with 100 mg Cu(II)/dm^3. The pH was adjusted to the desired value by adding NaOH or HNO_3 and than it was added the collector, oleic acid. The resulted solution was submitted to flotation.

The main parameters influencing this process were studied: pH of Cu(II) solutions, molar ratio oleic acid:Cu(II), air flow rate, time, temperature and initial concentration of Cu(II) in sample. An atomic absorption spectrophotometer PYE UNICAM model SP 1900 was used to determine the copper content of the solutions.

The copper removal efficiency, R%, was calculated with the relation:

$$R\% = (1 - \frac{C}{C_0}) \cdot 100$$

where: C – concentration of Cu(II) after flotation;

C_0 – initial concentration of Cu(II) in sample.

RESULTS AND DISCUSSION

The pH of the solution is one of the most important factors which influences the ion separation by flotation, as it determines the magnitude and sign of the charge on the ions and also the dissociation degree of the ionic groups of the surfactant molecules. Preliminary experiments, shown in Figure 1, were conducted in order to determine the pH effect on the copper removal efficiency, (R%) and on the copper concentration after flotation, (Cu(II) rez. [mg/dm^3]).

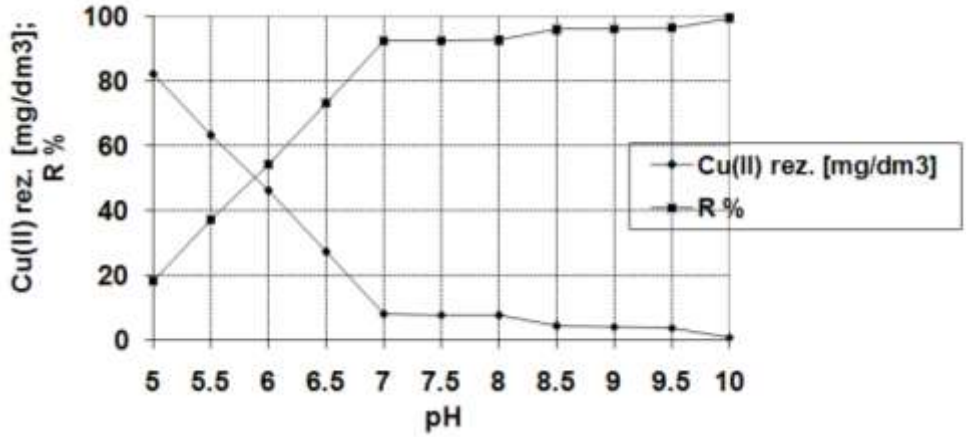

Fig. 1. Influence of pH on the copper removal efficiency
Molar ratio oleic acid:Cu(II)=1; air flow rate=15 dm^3/h, flotation time=15 min

It can be seen that the flotation of copper ions, with oleic acid, as collector, begins at pH = 5, but good separation efficiency is obtained at pH values greater than 7. At this pH values Cu(II) precipitates as hydroxide and flotation is a precipitate flotation process.

The second studied factor was the effect of the molar ratio oleic acid:Cu(II). As surfactant was used oleic acid 1 M solution in ethanol. Addition of ethanol as frother had the further advantage that the sizes of bubbles are smaller, because of the lower surface tension of the solution. The results are shown in Figure 2. The increase of the molar ratio oleic acid:Cu(II) determines a fast increase of removal efficiency.

The influence of air flow rate on Cu(II) removal efficiency is shown in Figure 3.

The increase of air flow rate from 5 up to 15 dm^3/h determines an increase of removal efficiency up to 98%.

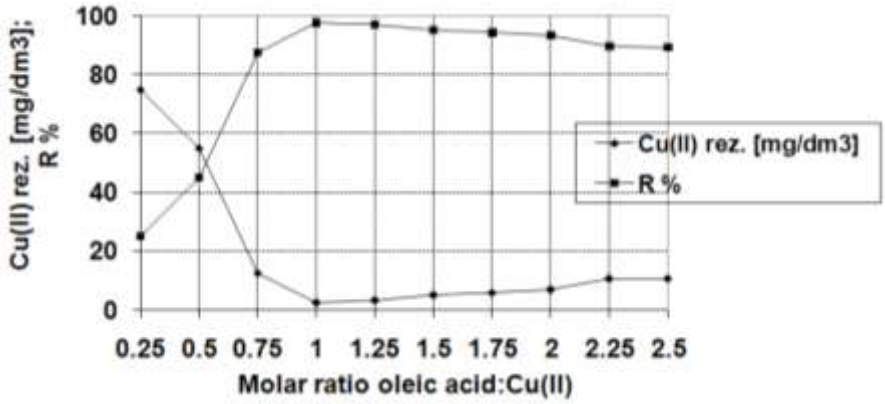

Fig. 2. Effect of molar ratio oleic acid:Cu(II) on the copper removal efficiency
pH=8,5; air flow rate=15 dm^3/h, flotation time=15 min

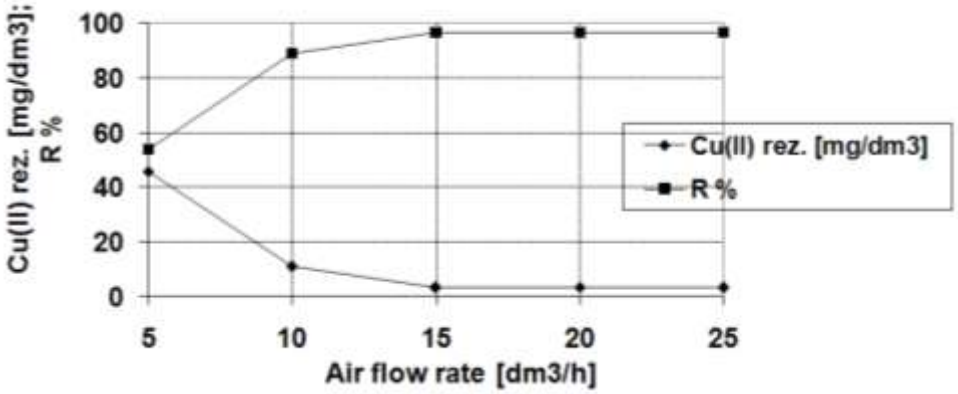

Fig. 3. Effect of air flow rate on the copper removal efficiency
pH=8,5; molar ratio oleic acid:Cu(II)=1, flotation time=15 min

The influence of flotation time on Cu(II) removal efficiency is shown in Figure 4. It can be seen that the separation process is very fast. In only 5 minutes is achieved a removal efficiency of 94%, and increasing the flotation time over 10 minutes the efficiency is more than 98%.

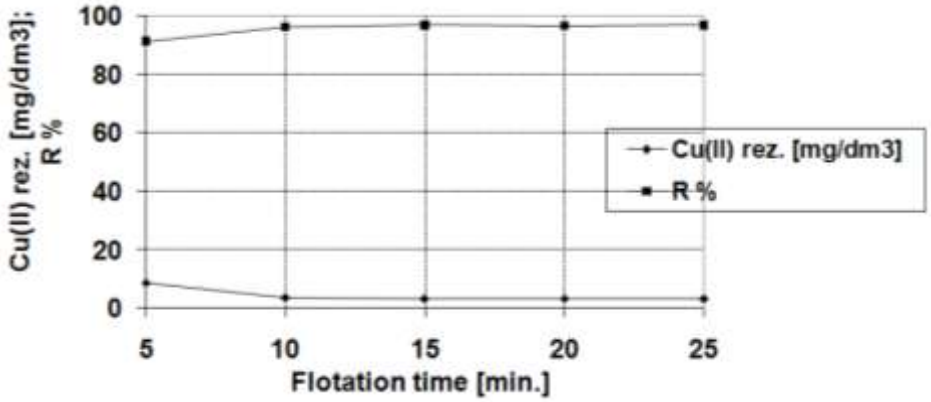

Fig. 4. Effect of flotation time on the copper removal efficiency
pH=8,5; molar ratio oleic acid:Cu(II)=1, air flow rate=15 dm^3/h

Another factor influencing the flotation process is the initial concentration of Cu(II) in solution. The results are shown in Figure 5. By decreasing Cu(II) concentration it can be observed a slow decrease of removal efficiency, until 80%.

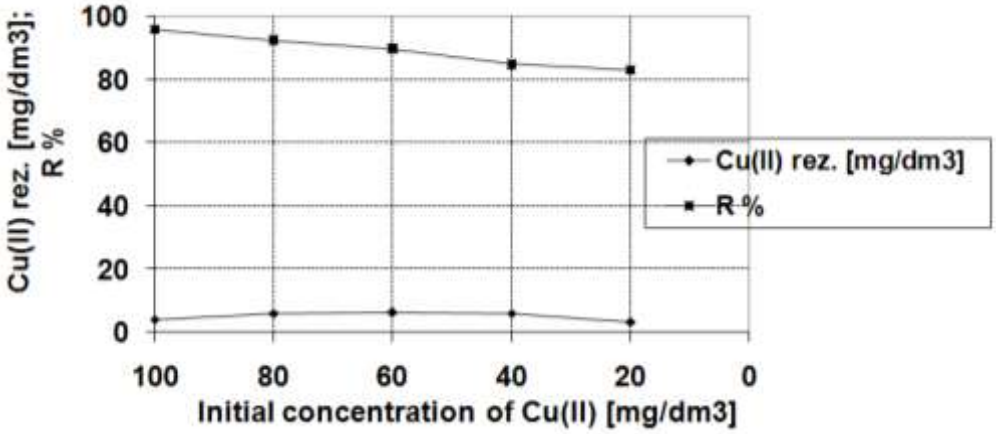

Fig. 5. Copper initial concentration effect on the removal efficiency.
pH=8,5; molar ratio oleic acid:Cu(II)=1, air flow rate=15 dm^3/h, flotation time=10 min

The temperature influence was also studied in interval 20 – 60^oC and we found out that the removal efficiency shown a decrease from 98% to 85%, at 40^oC, in the same separation condition. (Figure 6).

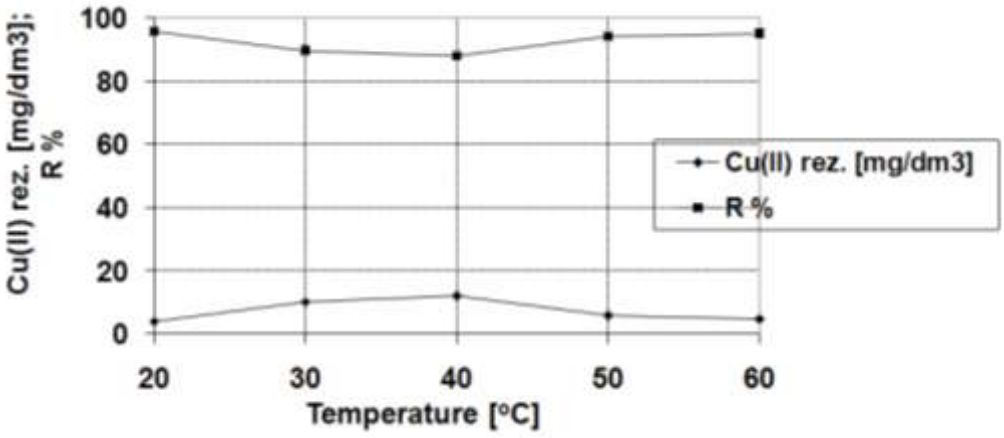

Fig. 6. Effect of temperature on the copper removal efficiency
pH=8,5; molar ratio oleic acid:Cu(II)=1, air flow rate=15 dm^3/h, flotation time=10 min

CONCLUSIONS

High removal efficiency (> 98%) of Cu(II) from dilute aqueous solutions has been obtained by applying the precipitate flotation process, at relatively shorter time than those used within other removal methods.

REFERENCES

1. Srivastava, V.C., Mall, I.D., Mishra, I.M., Modelling Individual and Competitive Adsorption of Cadmium(II) and Zinc(II) Metal Ions from Aqueous Solution onto Bagasse Fly Ash, *Sep. Sci. Technol*., 2006, 41, 2685-2710.
2. Turtureanu A., Epurarea unor ape reziduale cu conţinut de metale grele, *Teză de doctorat*, 2003, 16.
3. Islamoglu, S.,Yilmaz, L., Ozbelge, H.O., Development of a Precipitation Based Separation Scheme for Selective Removal and Recovery of Heavy Metals from Cadmium Rich Electroplating Industry Effluents, *Sep. Sci. Technol*., 2006, 41, 3367–3385.
4. Veeken, A.H.M., De Vries, S., Van der Mark, A., Rulkens, W.H., Selective Precipitation of Heavy Metals as Controlled by a Sulfide-Selective Electrode, *Sep. Sci. Technol*., 2003, 38(1), 1–19.
5. Khalid, N., Chaudhri, V., Shamim, A., Saeed, M.M., Ahmed, J., Separation and preconcentration of lead and cadmium with 4-(4-chlorophenyl)-2-phenyl-5-thiazoleacetic acid and its application in soil and seawater. *Sep. Sci. Technol*., 1996, 31(2), 229–239.
6. Cortes-Martinez, R., Martinez-Miranda, V., Solache-Rios, M., and I. Garcia-Sosa, I., Evaluation of Natural and Surfactant-Modified Zeolites in the Removal of Cadmium from Aqueous Solutions, *Sep. Sci. Technol*., 2004, 39(11), 2711–2730.
7. Kenduzler, E., Determination of Cadmium(II) in Water and Soil Samples after Preconcentration with a New Solid Phase Extractor, *Sep. Sci. Technol*., 2006, 41, 1645–1659.

8. Biskup, B., Subotic, B., Removal of Heavy Metal Ions from Solutions Using Zeolites. III. Influence of Sodium Ion Concentration in the Liquid Phase on the Kinetics of Exchange Processes Between Cadmium Ions from Solution and Sodium Ions from Zeolite A, *Sep. Sci. Technol*., 2004, 39(4), 925–940.
9. Qadeer, R., Khalid, N., Removal of Cadmium from Aqueous Solutions by Activated Charcoal, *Sep. Sci. Technol*., 2005, 40, 845–859.
10. Guo-Zhen, F., Jin, T., Xiu-Ping, Y., Synthesis and Evaluation of an Ion-Imprinted Functionalized Sorbent for Selective Separation of Cadmium Ion, *Sep. Sci. Technol*., 2005, 40, 1597–1608.
11. Mathialagan, T., Viraraghavan, T., Adsorption of Cadmium from Aqueous Solutions by Vermiculite, *Sep. Sci. Technol*., 2003, 38(1), 57–76.
12. Saito, T., Torii, S., Microcapsule for adsorption and recovery of cadmium (II) ion. *Sep. Sci. Technol*., 2002, 37 (1), 77–87.
13. Leyva-Ramos, R., Rangel-Mendez, J.R., Mendoza-Barron, J., Fuentes-Rubio, L., Guerrero-Coronado, R.M., Adsorption of cadmium(II) from aqueous solution onto activated carbon, *Water Sci. Technol.*, 1997, 35 (7), 205–211.
14. Mohan, D., Singh, K.P., Single- and multi-component adsorption of cadmium and zinc using activated carbon derived from bagasse—An agricultural waste, *Water Research*, 2002, 36(9), 2304–2318.
15. Pavlovska, G., Cundeva, K., Stafilov, T., Zendelovska, D., Flotation Method for Selective Separation of Silver, Cadmium, Chromium, Manganese, Thallium, and Zinc from Aragonite Before Atomic Absorption Spectrometric Determination, *Sep. Sci. Technol.*, 2003, 38(5), 1111–1124.
16. Kojuncu, Y., Bundalevska, J.M., Ay, U., Cundeva, K., Stafilov, T., Akcin, G., Atomic Absorption Spectrometry Determination of Cd, Cu, Fe, Ni, Pb, Zn, and Tl Traces in Seawater Following Flotation Separation, *Sep. Sci. Technol.*, 2004, 39(11), 2751–2765.

WHEAT PREPARATION BY CONDITIONING, RELATED TO THE GINDING RESISTANCE OF THE GRAIN

Cristina-Anca Danciu[1], Anca Tulbure[1]

[1]Lucian Blaga University of Sibiu, The Faculty of Agricultural Science, Food Industry and Environmental Protection

Abstract

In the milling process, a well wheat preparation by conditioning is at least half the battle toward mill balance, which results in the most favorable flour extraction and flour quality and also a lower energy consuming process. The conditioning is influenced by the physical and chemical state of the wheat. An optimum rest time in the wheat conditioning can provide a higher flour yield with 2-5%, a much more satisfactory flour colour and an reduced grinding energy consumption with 10-20%. The aim of this paper is to study the relevance of the conditoning parameters of wheat in the grinding process related to the grinding resistance of the wheat grain.

INTRODUCTION

In the Romanian mills, the wheat grain readiness is achieved without heat by adding cold water to the wheat and allowing the wheat to rest in bins (silos) until it reaches the optimum moisture distribution and kernel suitability for milling.

Usually, the optimum rest time for the moistened wheat is determined from diagrams (nomograms) containing the physical and chemical indicators determined in running laboratory (Edwards M.A. et al, 2007). Another method is using the laboratory mills (Brabender, Buhler, Miag, Chopin) for milling moistened wheat samples, in different period of time (from 0,5 to 1 hour), in few successive breaking steps and then sorting by sieving. Finally, the qualitative and quantitative analysis of these milling products can provide the establishment of the optimum rest time in the wheat conditioning (Banu I., 2010). But all these methods are long lasting proceedings with high working volume. It was state by the authors (Dexter J. E., 1988) that there is a correlation between the grinding energy and the technological properties of the wheat (related to the conditioning steps: addition of water and rest time). That is why we propose to assess the optimum rest time in the wheat conditioning, with the micromill designed to determine the grinding resistance of the wheat grain.

MATERIALS AND METHODS

The investigations were carried out on two Romanian winter wheat varieties (*Triticum aestivum, ssp. vulgare*) Dropia and Pegasus. The preparation of the samples colected carried out according to the chess-board pattern method, after cleaning with an Sadkiewicz Instruments Scourer. The physicochemical characteristics of the wheat were evaluated as follows: the moisture content using the SR ISO 712 : 2005; the wet gluten content, protein content using the NIR technique (Inframatic, model 8600, Perten Instruments AB); vitreous kernel using the STAS 6283-2/1984 (farinotom apparatus). The quality indices of the studied wheat varieties are depicted in Table 1.

Wheat preparation by conditioning means removal or, more often, addition of water followed by a rest period. The moistened wheat is then allowed to rest for a period of time, to let the added moisture penetrate evenly throughout the kernel parts. The objective is that all the wheat grains reach the same physical condition.

Table 1. Quality indices of the wheat varieties

Variety / **Indicator**	**Dropia**	**Pegasus**
Hectolitric weight [kg/hl]	77,6	77,4
Vitreousness [%]	18	62
Wet gluten content [%]	21	32,4
Moisture content [%]	13	12,3
Falling number [s]	260	333
Protein content [%]	10,8	15

RESULTS AND DISCUSSION

The optimum rest time value for the conditioning of Dropia variety is 5 hours. For this value it was obtained the lowest average resistant moment in the grinding process of this soft wheat variety, which it means also the lowest energy consumption. The lowest average resistant moment was obtained also for the Pegasus variety (hard wheat) for 9 hours rest time in the conditioning process. The resistant moment of the particles grounded between the rollers (measured by a tensometric cell, conected to a PC computer and managed with a software program) has been obtained for each period of rest time for the moistened wheat.

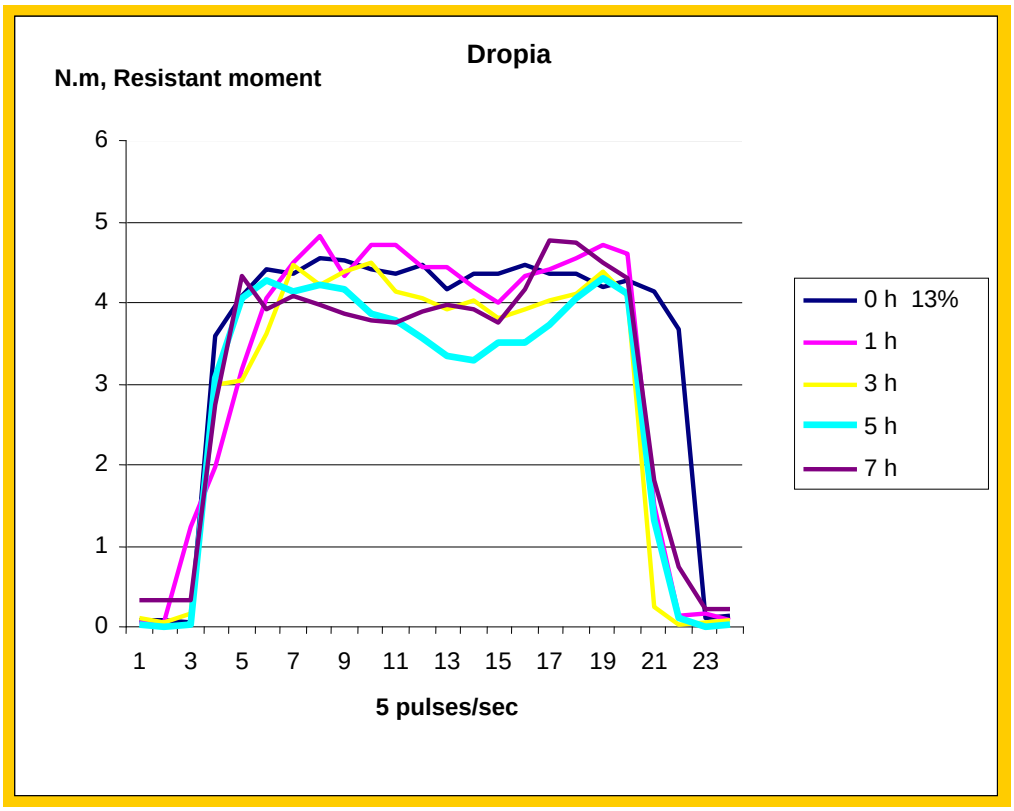

Fig. 1. Resistant moment for different rest time on Dropia variety

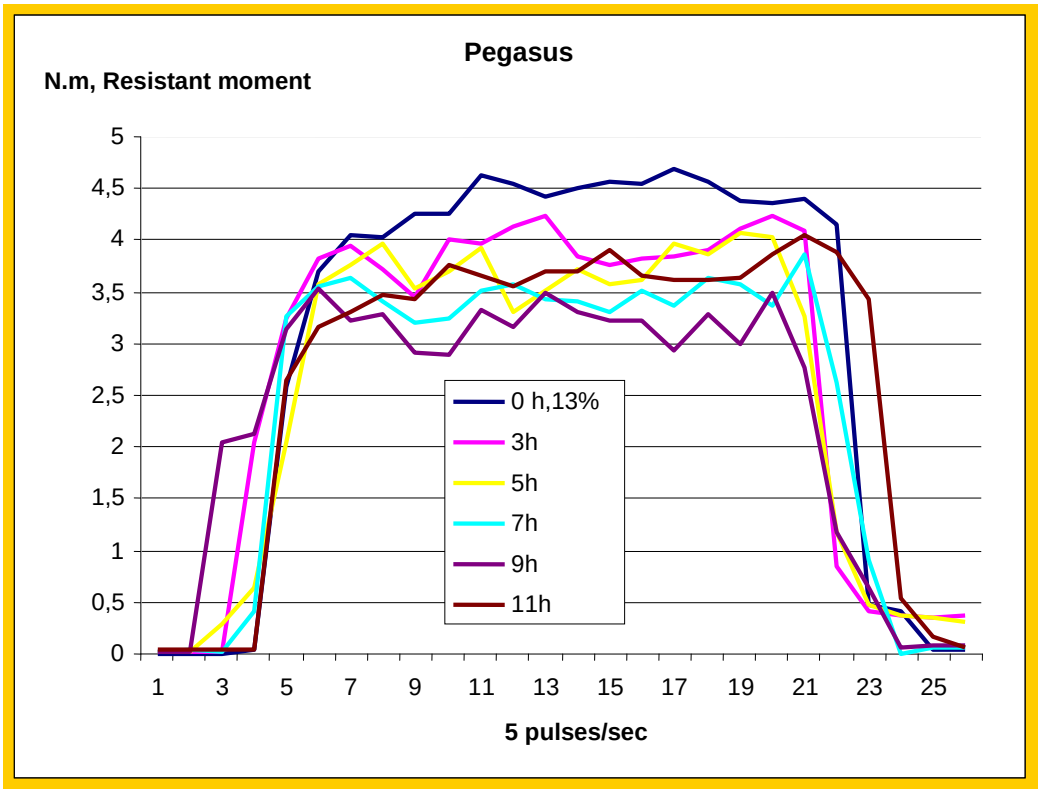

Fig. 2. Resistant moment for different rest time on Pegasus variety

The results are confirming that for the lowest resistant moment value, the optimum rest time is 5 hours for Dropia variety (Fig. 1) and 9 hours for Pegasus wheat variety (Fig. 2).

The method has the same accuracy as the classical one and has the advantage to be quicker and less demanding as work volume.

It is an alternative way to describe the optimum for the conditioning process and can be used in laboratory for the benefice of students as well in the milling industry.

CONCLUSIONS

The amount of water added depends on original moisture of the wheat and the relative humidity in the mill processing space.

This tempering process is probably the only stage at which the miller can modify the physical and chemical state of the wheat.

The assessment of the optimum parameters in the conditionning process can be made by the micromill designed to determine the grinding resistance of the cereals.

REFERENCES

Banu, I, *Procesarea cerealelor în industria morăritului*, Editura Galaţi University Press, 2010, ISBN 978-606-8008-67-7.

Dexter, J. E., Williams, P. C., Edwards, N. M., and Martin, D. G. *The relationships between durum wheat vitreousness, kernel hardness and processing quality*. J. Cereal Sci., 1988, 7:169-181.

Edwards, M.A., Osborne, B.G., Henry, R.J, *Investigation of the effect of conditioning on the fracture of hard and soft wheat grain by the single-kernel characterisation system: Acomparison with roller milling*, Journal of Cereal Science., 2007, 46, 64-74

FRESH & ENRICHED MIXTURES FRUITS JUICES ASSESSED BY ANTIOXIDANT CAPACITY VIA CHEMILUMINESCENCE ASSAY

***Neli Darie*[1]**

[1] *"Lucian Blaga" University of Sibiu, Faculty of Agricultural Sciences, Food Industry and Environmental Protection, 7-9 Dr.I. Ratiu Street, 550012 Sibiu, Romania, neli.darie@ulbsibiu.ro.*

Abstract

The total antioxidant capacity measurements, via chemiluminescence assay were made in eight fresh fruits juices, known as antioxidant rich, in different mixture, in order to find a nutritional health antioxidant beverage. Samples containing fresh single fruit juices of kiwi, local apple and blackcurrant berry were mixted in two distinguish ratio and also with pasteurized cow UHT milk. There obtained good total antioxidant capacity results and the best beverage was taken out from (kiwi, apple, blackcurrant berry fresh mixture fruits juice) with skimmed UHT milk.

Keywords: chemiluminescence assay, fresh juice, mixture antioxidant capacity, kiwi, apple, blackcurrant

INTRODUCTION

Fruits juices, mostly those fresh, are usually preferred in daily consumption for their nourishing contain in fibers, vitamins, sugars and especially for the healthy antioxidants compounds: as flavonoids, some vitamins (E and C), carotenoids, polyphenols, terpenoids and antioxidant enzymes [4].

The antioxidants (AOX), as species of much interest, both water and also lipophilic soluble, enzymatic or non-enzymatic, both endogenous and exogenous contribute to improve the biological systems resistance in their struggle with oxidative stress (OS) due to the free radicals (FR) shaping. The human oxidative stress, defined as "a body rusting from inside to outside" [9], appears in case of a balance lack between the reactive free radicals, especially reactive oxygen species (ROS) and the human biological system capacity to clean these noxious compounds or to repair the FR damage effects. That chemical event materializes by changing the normal cell redox potential [10]. By this way OS sets in, with a high concentration of peroxides and FR in human cells, which causes great human organs damages. The OS strength could produce deteriorate changes cells inside or outside and in

this way it could set in metabolic syndrome (MetS) with all the relative dysfunctions in human body and at all the levels also.

The natural exogenous AOX, intake of daily dietary nourishment, have some beneficial features which make them good parameters in the well working of all human organism cells (figure 1, adapted after [13]). Thus they:

□ must have a high bioavailability (when they will come from fresh and organic vegetables or fruits) and low calories

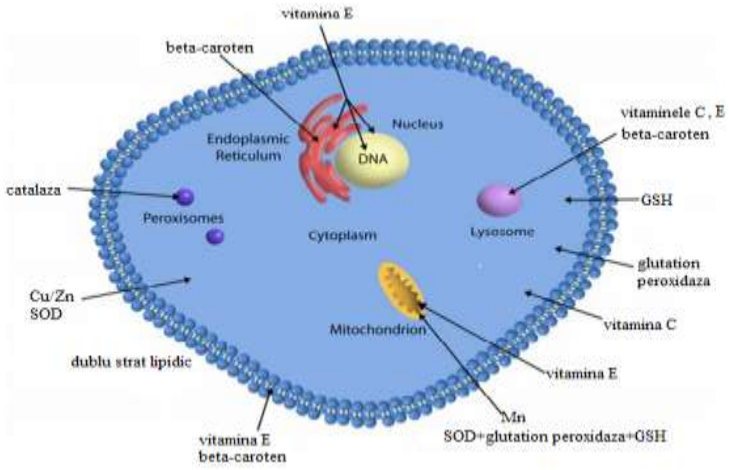

Fig. 1. Natural antioxidants involved in the cell defense systems

□ in certain pathological backgrounds, AOX intensify some drugs action and hurry up the cure by this way

□ they prevent the new deseases appearance, having a protector part against the degeneratin ones (like cancer, stroke, cardiovascular sickness, rheumatism, neurological, renal and liver disorders, auto-imun deficiency diseases, hypertension, inflammation, adult respiratory distress syndrome [1])

□ they also improve the human organism natural immunity.

The FR, having a short life in biological living systems, initiate theirselves chain reactions, involving many bio-macromolecules until they become also FR and the chain reaction is stopped [5]. In the biological structures the FR, having different reaction mechanisms types, can modify the unsaturated fatty acids in the lipid cells membranes, the proteins and also the DNA. In this reason the biological human systems have developed many anti-FR substances and protective mechanisms, respectively they enlarge antioxidative systems, enzymatic and non-enzymatic, in order to interrupt the FR chain reactions.

Therefore additional AOX amount is necessary in our daily diet in order to release antioxidant compounds. Quantitatively the main dietary antioxidants are: polyphenols compounds, vitamins and carotenoids. A reasonable and various AOX intake could be more beneficial than a great one AOX type diet intake.

In the present research it sets out from the idea that the antioxidant capacity (AC) of the fresh fruits juices could be more bio-accessible than the AC in the solid fruits. Several fruits fresh juices are known to be rich in AOX with sensible total antioxidant capacity (TAC) associated with hydrophilic and lipophilic AOX in extracts [3].

Using a chemiluminescence assay, TAC was measured for fresh single fruit juices and also for fresh mixture fruits juice, in order to establish an optimal mixture with a rich AC.

Since on the market, newly, there are available the mixtures beverages obtained from fruits juices and milk, in this research it has tried to evaluate the AC in this current mixtures. These have a double advantage and offer in a single juice both the AOX fruits (vitamins, phenolics, carotenoids) and the milk nutritive compounds as calcium and proteins [12].

MATERIALS AND METHODS

Samples

Three types of fruits juices were investigated and made ready in this work from three rich antioxidant fruits and from UHT milk (table 1).

A)the juices were obtained from following fruits purchased from a local Romanian market:

1) kiwi raw fruits (rich in vitamins C, A, E, lutein [14])
2) Romanian apple raw fruits (rich in catechin, vitamin C, antociane, fibers [10])
3) Romanian blackcurrant berry fruits (containing vitamins C, B1, B2 and B6, proteins, flavonoides, pectins, tannins [8])

Every sample fruit juice was prepared by mixing 70% (v/v) of pressed fruit and 30% (v/v) water. After a well homogenizing, the mixture was vortexed for 30-40 s and then each mixture was centrifuged 35 min, 7500 x g, at 25^0C. All fresh fruits juices have received a sample symbol in order to work well (from P1 to P3). The chemiluminescence measurements were carried out from the supernatant and these ones were diluated with methanol or with water, according to the measurement type (lipophile or hidrophile AOX).

Table 1. Analyzed beverages types from fruits juices and mixtures

Nr. crt.	Beverages types	Samples symbol	Sources/ /preparation from	Sample features
1.	fresh single fruit juice	P1	kivi fruit	ripe maturated pulp raw fruit, species *Actinidia deliciosa*
		P2	apple fruit	ripe maturated pulp raw fruit, species *Malus domestica*, sort *Jonathan*
		P3	blackcurrant berry fruits	ripe maturated raw berry fruits, species R*ibes nigrum*
2.	fresh mixture fruits juice	P4a P4b	kiwi, apple and black currant berry mixture	ratio 1:1:1 ratio 1:2:2
3.	mixture fresh single fruit juice and pasteurized cow milk	P5	kiwi juice & UHT milk	60% kiwi juice P1, 20% water, 20% skimmed UHT milk
		P6	apple juice & UHT milk	60% apple juice P2, 20% water, 20% skimmed UHT milk
		P7	blackcurrant berry juice & UHT milk	60% blackcurrant juice P3, 20% water, 20% skimmed UHT milk
4.	mixture fresh mixture fruits juice and pasteurized cow milk	P8	kiwi, apple, blackcurrant berry fresh mixture fruits juice and skimmed UHT milk	50% fresh mixture fruits juice P4b, 30% water, 20% skimmed UHT milk

B) sample P4 is a fresh mixture fruits juice, respecting the ratio kiwi:apple: blackcurrant as 1:1:1 (P4a) and 1:2:2 (P4b). Then, the mixtures P4a and P4b were got ready like samples P1-P3.

C) samples P5-P7 were prepared homogenizing 60% (v/v) mixture fresh single fruit juice fruit (from P1 at P3) with 20% (v/v) water and 20% (v/v) skimmed UHT milk,

purchased from local supermarket. Then, the mixtures P5-P7 were prepared as samples P1-P3.

D) sample P8 is a mixture fresh mixture fruits juice and pasteurized cow milk, with the feature from the table 1 and the above samples identically preparation, that means: 50% fresh mixture fruits juice P4a or P4b, 30% (v/v) water and 20% (v/v) skimmed UHT milk.

Chemicals and reagents

Trolox, reaction buffer, photo-sensitizer for water-soluble substances, detection reagent and kits were purchased from and Analytik Jena AG (Jena, Germany). Samples solvent, calibration standard for cuantification of water soluble antioxidants in equivalents of ascorbic acid were purchased from Merck (Darmstadt, Germany).

Photochemiluminescence assay

In this assay the FR are generated and those that aren't scavenged by AOX are then appreciably detected by photo-induced chemiluminescence when is used luminol (a chemiluminogenous substance). The AC can be measured at different concentrations, appreciate with a calibration curve and it is represented in Trolox units or ascorbic acid units. The AOX present in the fruits juices eliminate the FR in the measurement solution and they modify the chemiluminescence intensity. TAC was measured in samples P1-P8 in aqueous phases, following the chemiluminescence increase when it was used a photochem from Analytic Jena according to their analytical protocol [2]. For the device calibration was used an acid ascorbic standard solution. The measurements have done using 30 μL from each samples P1-P8.

RESULTS AND DISCUSSION

Total antioxidant capacity was first measured (in aquous phase) for fresh single fruit juices in samples P1 (kiwi fruit), P2 (apple fruit) and P3 (blackcurrant berry fruits). The obtained values are exhibited in equivalent acid ascorbic units (mM) in figure 1.

All experimental results are the mean of three measurements and expressed as mean ± SD.

In order to establish an optimal mixture with a rich AC, two mixture models with different ratio was estimated.

Thus for the fresh mixture fruits juice: sample P4a with a ratio of 1:1:1 and sample P4b with 1:2:2 ratio of the three fruits. The results are comparatively presented in figure 2.

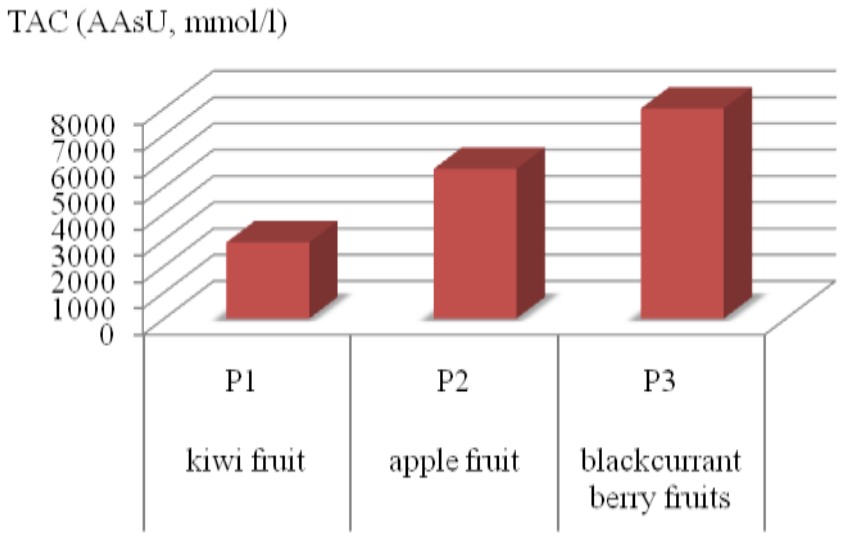

Fig. 1. Total antioxidant capacity (TAC), expressed as ascorbic acid units (AASU)/ml fresh single fruit juices

Although the kiwi fresh juice contains antioxidant vitamins and lutein, the TAC value was found smaller than for blackcurrant berry fruits and apples juices. Therefore in sample P4b (as fresh mixture fruits juice) has attempt an enriched ratio between fruits with high TAC, respectively one of 1:2:2 (kiwi:apple: blackcurrant). How was expected, the experimental results for TAC in sample P4b, enriched in apple and blackcurrant juices, were better values, that the optimal ration was used for sample P8.

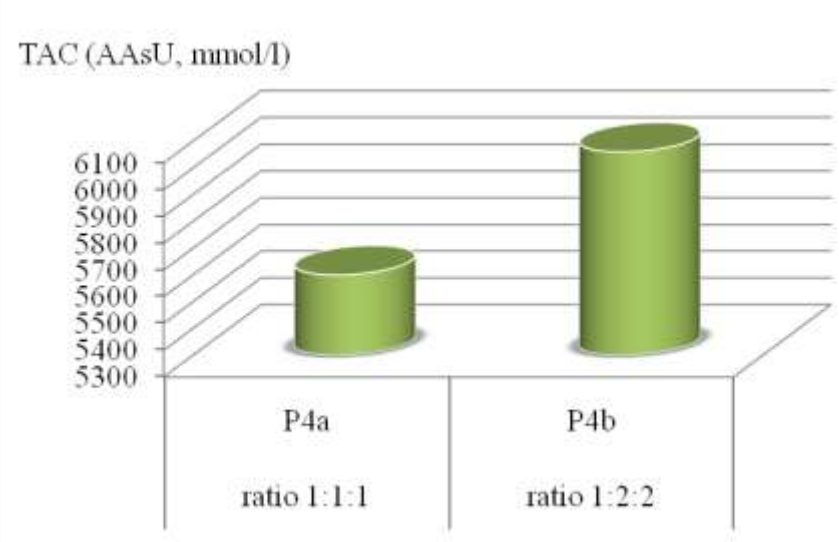

Fig. 2. Total antioxidant capacity (TAC), expressed as ascorbic acid units (AASU)/ml fresh mixture fruits juice

On the market it can find a great interesting beverages obtained from fruits juices and milk [12]. In the present research there were tried three samples of fruit fresh juice with skimmed UHT milk, in a receipt of 60% fruit juice Px, 20% water and 20% skimmed UHT milk. The results are shown in figure 3. The higher value for ascorbic acid units, as expression of AC has found in sample P7 (containing blackcurrant berry juice & UHT milk).

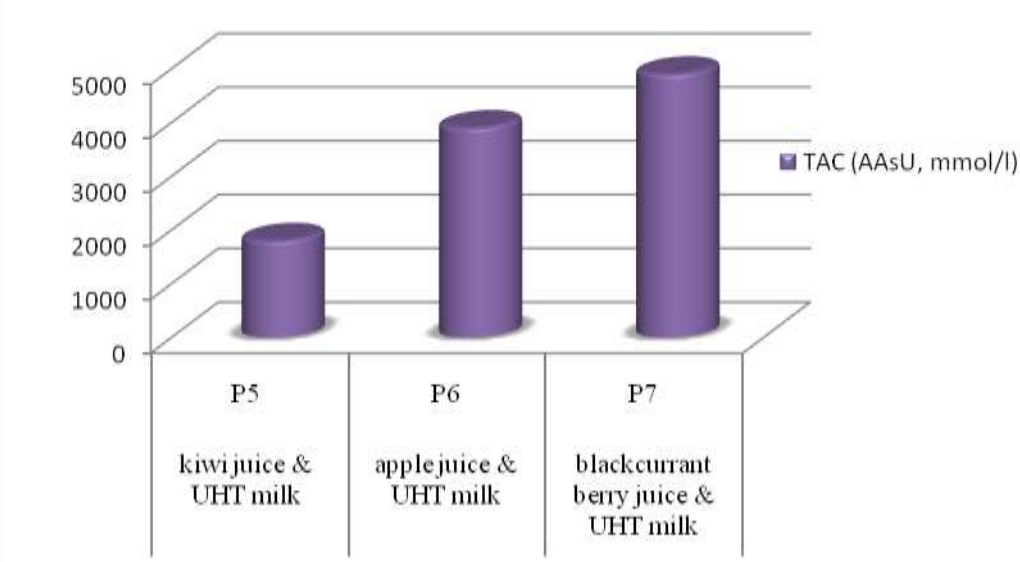

Fig. 3. Total antioxidant capacity expressed as ascorbic acid units (AASU)/ml mixture fresh single fruit juice&pasteurized cow milk

In sample P8 (kiwi, apple, blackcurrant berry fresh mixture fruits juice and skimmed UHT milk) was also estimated the antioxidant capacity in a receipt easy modified as against of sample P5-P7. TAC in P8 was found about 5200 AASU, the biggest value in experiments. All obtained results in all the experiments have been in a good shapes, the TAC values by chemiluminescence assay can be determinated by a good reproductibility (of CV<1,5%).

CONCLUSIONS

In the paper the TAC measurements, in some fresh fruits known as AOX rich in different mixture, were done in order to find a nutritional rich antioxidant beverage. Three samples containing fresh single fruit juices (of kiwi, apple and blackcurrant berry), twoo samples with fresh mixture fruits juice in two distinguish ratio and four samples of mixture fresh single fruit juice & pasteurized cow milk were investigated. There are good results for samples P3 (fresh single blackcurrant berry fruit juice), P4b (fresh mixture fruits juice of

kiwi, apple and black currant berry mixture in a ration of 1:2:2), P7 (blackcurrant berry single fresh & UHT milk) and P8 (kiwi, apple, blackcurrant berry fresh mixture fruits juice and skimmed UHT milk), all having a significant antioxidant capacity.

REFERENCES

1. Amer, J., Ghoti, H., Rachmilewitz, E., Koren, A., Levin, C., Ibach, E., Red blood cells, platelets and polymorphonuclear neutrophils of patients with sickle cell disease exhibit oxidative stress that can be ameliorated by antioxidants. *Brit J Haemat*, 2006, 132(1), 108-113.

2. Analytik Jena AG,User's Manual Photochem, 2003, 1-79.

3. Deladino, L., Schneider Teixeira, A., Reta, M., Molina García, A. D., Navarro, A. S., Martino, M. N., Major Phenolics in Yerba Mate Extracts (*Ilex paraguariensis*) and Their Contribution to the Total Antioxidant Capacity. *Food Nut Sci J*, 2013, 4(8A), 154-162.

4. Garzon, G.A., Riedl, K.M., Schwartz, S.J., Determination of antocyanins, total phenolic content and antioxidant activity in Anders berry (*Rubus glaucus Benth*). *J Food Sci*, 2009, 74, 227-232.

5. Hu, D., Pratt, D.A., Secondary orbital interactions in the propagation steps of lipid peroxidation. *Chem Commun* 2010, 46, 711-3713.

6. Mancas, M. ROMEDIC. Mere. http://dieta.romedic.ro/aliment/mere, 2017, [accessed at 17 April 2017].

7. Ristov, M., Schmeisser, S., Extending life span by increasing oxidative stress. *Free Rad Biol Med*, 2011, 51, 327.

8. Rotaru, P. Coacăzul negru (Ribes nigrum) - beneficii şi proprietăţi, http://www.csid.ro/plante-medicinale-fitoterapice-si-gemoterapice/coacazul-negru-ribes-nigrum-11850730, 2017, [accessed at 14 April 2017].

9. Suciu., L., Wilson, I.,Stresul Oxidativ. Available at: <http://www.drsuciu.com/?lang=ro>, 2016, [accessed at 28 noiembrie 2016].

10.Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M., Telser, J., Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*, 2007, 39(1), 44–84.

11.Zulueta, A., Esteve, M.J., Frigola, A., ORAC and TEAC assays comparison to measure the antioxidant capacity of food products, *Food Chem*, 2009, 114, 310-316.

12. Wu, X., Beecher, G.R., Holden, J.M., Haytowitz, D.B., Gebhardt, S.E., Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J Agri Food Chem*, 2004, 52, 4026-4037.

13. ***Antioxidant Enzyme Systems, 2015, Available at: <http://www.newsmedical.net/health/Antioxidant-Enzyme-Systems.aspx>, [accessed at 21 noiembrie 2016].

14.***Wikipedia. Free Encyclopedia, 2016, Available at: https://ro.wikipedia.org/wiki/Kiwi_(fruit), [accessed at 18 April 2017].

IDENTIFICATION AND QUANTIFICATION OF POLYPHENOLS IN TONIFYING BEVERAGES HARNESSING THE BIOACTIVE POTENTIAL OF CERTAIN INDIGENOUS PLANTS

***Daniela Maria Șandru*[1]**

[1] *"Lucian Blaga" University of Sibiu, e-mail: danielaraulea@yahoo.com*

Abstract

This paper aims at analysing the polyphenols in six tonifying beverages made of indigenous plant macerates and red wine, plants that scientific literature records as rich in bioactive compounds. We used the modified Folin-Ciocâlteu method to identify polyphenols, and built the standard scale with successive concentrations of gallic acid. The measurements revealed that all the tonifying drinks contained polyphenols, and their amounts were closely connected to the indigenous plants used to prepare the drinks under study.

Keywords: polyphenols, tonifying drinks, plant macerates

INTRODUCTION

Polyphenols are natural phytochemicals that occur in plant-based food products, such as fruits, vegetables, whole grains, cereals, tea, coffee, wine, and cocoa; more than 8000 polyphenolic compounds, including phenolic acids, flavonoids, stilbenes, lignans and polymeric lignans were identified in whole vegetable products (Neamţu, 1996). These compounds are secondary plant metabolites that act like a shield, protecting from UV radiations, oxidants, and pathogens. Polyphenols can be classified in several categories, depending on the number of phenol rings and structural elements linking these rings together (Pietta et al., 2003). Phenolic acids make up about a third of the polyphenolic compounds in our diet and include two main classes of hydroxybenzoic acid derivatives (protocatechuic acid, gallic acid, p-hydroxybenzoic acid) and hydroxycinnamic acid derivatives (caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, sinapic acid). Foods rich in phenolic acids include: berries, kiwi, cherries, apples, pears, chicory and coffee (Manach et al., 2004). Flavonoids are polyphenols that occur more often in human nutrition; over 4000 such compounds were identified. There are six flavonoid subclasses, including anthocyanins, flavonols, flavones, flavanones, flavones, and izoflavones; anthocyanins (cyanidin,

pelargonidin, delphinidin, malvidin) occur in berries, red wine, red cabbage, cherries, black grapes, and strawberries. Flavonols, including quercetin, kaempferol, and myricetin, were mainly found in onion, savoy cabbage, leek, broccoli, and blueberries. Izoflavones are the most important dietetic flavonoids; these include: daizenin, genistein, and glycitein. Soy and soy-products are the richest sources of such compounds, that are structured like estrogen (Madrigal and Sangronis, 2007). High concentrations of lignans were found in flaxseed and other cereals. Stilbenes occur in human nutrition in very small amounts; resveratrol, one of the most studied compound in this group, is mostly found in grapes and red wine (Manach et al., 2004; Adlercreutz, 2007).

MATERIALS AND METHODS

- Tonifying beverages noted I, II, III, IV, V, VI;
- The amount of polyphenols in the six beverages was determined using the Folin-Ciocâlteu method.

The Folin-Ciocâlteu method is employed to establish the amount of polyphenols. The method is based on the oxidation of polyphenols using molybdowolframat (Na_2WO_4/Na_2MoO_4); results are read with a UV-VIS equipment, at a wavelength of 750 nm.

This reaction results in O_2, which reacts with molybdate to form the ion (Mo^{4+}) (blue), whose absorbancy is measured spectrophotometrically in the interval 420-1000.

The reaction takes place in a highly alkaline environment. Gallic acid is the AO reference. The FC method is simple, specific, and quick. However, we need an acid pH, which results in slow and unspecific reactions.

RESULTS AND DISCUSSION

In the beverages under study, the amount of polyphenols falls between 5.620 mg/L in the case of beverage IV, and 25.760 mg/L in the case of beverage V. Values slightly lower than the maximum amount of polyphenols were recorded in the case of beverage III (24.040 mg/L), and beverage II (22.000 mg/L). Values slightly lower than those found in beverages II and III were determined in the case of beverage VI, reaching 19.460 mg/L, and beverage I, reaching 19.200 mg/L.

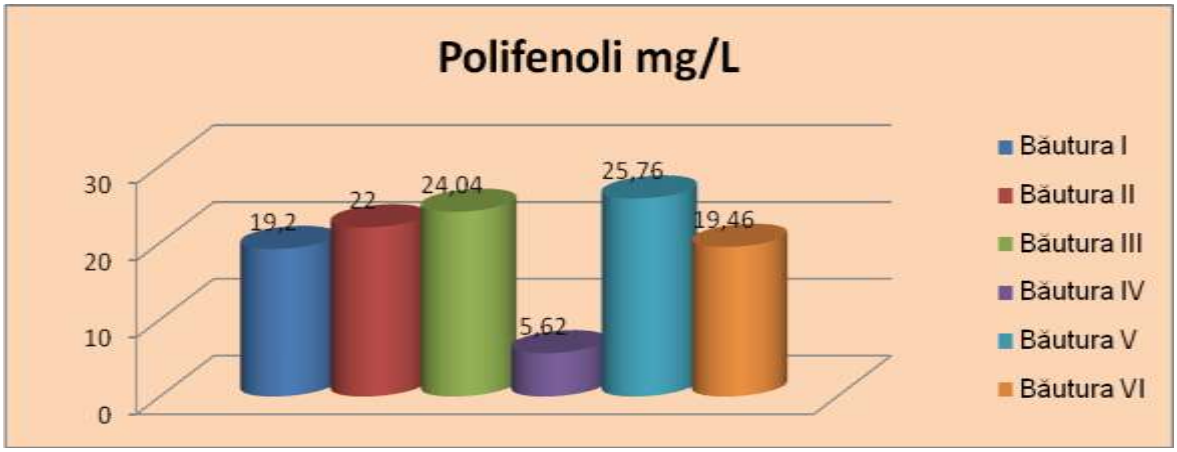

Fig. 1. Amount of polyphenols in plant beverages.

CONCLUSIONS

All the six beverages contain a significant amount of polyphenols. A maximum amount of polyphenols was identified in beverage V: 25.760 mg/L. Slightly lower values were found in beverage I (19.200 mg/L), while the lowest amount was found in beverage IV (5.620 mg/L).

REFERENCES

1. Pietta P., Minoggio M., Bramati L., 2003. Plant polyphenols: structure, occurrence and bioactivity. Stud Nat Pro Chem., 28:257–312.
2. Manach C., Scalbert A., Morand C., Rémésy C., Jiménez L., 2004. Polyphenols: food sources and bioavailability. Am J Clin Nutr., 79:727–747.
3. Adlercreutz H., 2007. Lignans and human health. Crit Rev Clin Lab Sci, 44:483–525.
4. Neamțu G., 1996. Substanţe naturale biologic active. Editura Cereş, Bucureşti.
5. Madrigal L., Sangronis E., 2007. Inulin and derivates as key ingredients in functional foods. Archivos Latinoamericanos de Nutricion. 57(4):387-96, Dec.

QUALITATIVE SCREENING OF PHYTOCHEMICALS FOUND IN AQUEOUS EXTRACT OF *PRUNUS DOMESTICA* STONE

Ana Alexandra Sorescu[1,2], Alexandrina Nuţă[1,3], Rodica Mariana Ion [1,2]

[1]National Research and Development Institute for Chemistry and Petrochemistry – ICECHIM, 202 Splaiul Independentei Street, district 6, 060021, Bucharest, Romania: e-mail: anaalexandrasorescu@yahoo.com

[2]Valahia University, Materials Dept., 13th Sinaia Alley, 130004, Targoviste, Romania

[3]The Romanian Academy "Stefan S. Nicolau" Institute of Virology, 285 Mihai Bravu Avenue, District 3, 030304, Bucharest, Romania

Abstract

Plums (Prunus domestica) are fruits full of vitamins, especially vitamins A and C, rich in minerals but also low in calories, carbohydrates and with a low glycemic index thus making these fruits extremely effective in many illnesses. Not much can be told about plum seed, largely known as stone, which are usually discarded when eating and are thrown away. In this paper, plum stone are studied by means of qualitative analyses and a phytochemical screening is carried out to determine what type of phytocomponents can be found in plum stones. Therefore, plum stones are dried and milled and three aqueous extracts are obtained: from plum stone, the shell of the plum stone and its core. By means of qualitative analyses, we were able to determine whether the three aqueous extracts contain saponins, flavonoids, terpenoids or other phytocomponents and the results are presented by comparison between the three extracts.

Keywords: plum stone, aqueous extracts, phytocomponents, qualitative determinations

INTRODUCTION

Plums are fruits part of the subgenus *Prunus* belonging to genus *Prunus* and can be easily distinguished from other fruits that belong to the same family by the fact that they present a dusty – white waxy coating that gives them a glaucous appearence [1]. Plum has numerous species and, depending on the taxonomist, all over the world between 19 and 40 different species of plum can be identified. However, from all these species only two are of worldwide commercial significance, namely the hexaploid European plum (*Prunus domestica*) and the diploid Japanese plum (*Prunus salicina* and hybrids) [2, 3].

The subgenus *Prunus* is divided into three different sections as follows:

- Section *Prunus* (Old World plums) with the following characteristics: leaves in bud rolled inwards, flowers 1-3 together, fruits are smooth, often wax-bloomed;

- Section *Prunocerasus* (New World plums) with leaves in bud folded inwards, flowers 3-5 together, smooth fruits, often wax-bloomed;
- Section *Armeniaca* (apricots): leaves in bud rolled inwards, flowers very short-stalked, velvety fruits; this type is often treated as a distinct subgenus by some authors.

From the nutrition point of view, raw plums consist of 87% water and other nutritive components such as carbohydrates (11%), proteins (1%) and less than 1% fat. Plums have also low caloric content, 100 g of raw plums providing only 46 calories. Besides that, plums are a moderate source of vitamin C (12% daily value) and small quantities of vitamin B, E and K can also be found thus making these fruits extremely effective in many illnesses [4].

Not much can be told about plum seeds, largely known as stone, which are usually discarded when eating and are thrown away. In recent years, scientists discovered the benefits of plum seed oil especially for different skin care problems and, therefore, this oil is now used in daily face creams and in body lotions, especially due to its emollient properties.

In this paper, plum stones are studied by means of qualitative analyses and a phytochemical screening is carried out to determine what type of phytocomponents can be found in plum stones. Therefore, plum stones are dried and milled and three aqueous extracts are obtained: from plum stone, the shell of the plum stone and its core. By means of qualitative analyses, we could determine whether the three aqueous extracts contain saponins, flavonoids, terpenoids or other phytocomponents and the results are presented by comparison between the three extracts.

MATERIALS AND METHODS

MATERIALS

Fresh plums were procured from the local market, thoroughly washed and the fruit pulp was gently removed from all the plum stones. Then, all the plum stones were once more washed twice with tap water and twice with distilled water to remove surface impurities and were left to dry for 10 days at room temperature and in the dark. The dried plum stones were divided into three parts: plum stones, plum stones' shell and plum stones' core in order to prepare three aqueous extracts.

PREPARATION OF SAMPLE EXTRACTS

25 grams of dried raw material (plum stone, plum stone shell and plum stone core) were accurately weighted, finely crushed using a blender and then dissolved in 100 ml

distilled water to obtain the aqueous extract using a "French press" type coffee filter (Figure 1). All the 3 extracts were left to incubate for 72 hours so that as much of the raw material as possible could be converted to the aqueous extracts. The obtained aqueous extracts were separated and once more vacuum filtered so that all impurities are removed and were kept in the refrigerator for further use.

Fig. 1. "French press" type coffee filters used to prepare the aqueous extracts

EXTRACTIVE VALUES OF FRUIT SAMPLES

The extractive value (yield percentage) of the fruit (plum stone, plum stone shell and plum stone core) samples were calculated before and after the preparation of the aqueous extracts using the formula below [5]:

$$\text{Extract yield \%} = [W_1/W_2] \times 100$$

where: W_1 represents the net powder weight (measured in grams) resulted after extraction and W_2 represents the total powder weight (measured in grams) used to prepare the aqueous extracts.

PHYTOCHEMICAL SCREENING – QUALITATIVE ANALYSIS

The aqueous extracts prepared from the three raw materials (plum stone, plum stone shell and plum stone core) were used for phytochemical screening carried out using standard phytochemical methods [6 - 8].

TEST FOR CARBOHYDRATES

Two standard phytochemical methods were used to test the presence of carbohydrates:

a) to 2 ml aqueous extract 1 ml of Molisch's reagent (α – naphthol in ethylic alcohol) and few drops of concentrated sulphuric acid were added. The presence of purple – reddish colour indicates the presence of carbohydrates;

b) to 1 ml of aqueous extract 5 ml Benedict's reagent were added and heated to boil. The presence of a blueish – green colour indicates the presence of carbohydrates.

TEST FOR TANNINS

To 1 ml aqueous extract 2 ml of 5% ferric chloride were added. The formation of a dark – blue or greenish – black colour indicates the presence of tannins.

TEST FOR SAPONINS

To 2 ml of aqueous extract 2 ml of distilled water were added in a graduated cylinder and shaken lengthwise for 15 minutes. The formation of a 1 cm foam layer indicates the presence of saponins.

TEST FOR FLAVONOIDS

To 2 ml aqueous extract 1 ml of 2N sodium hydroxide were added. A yellow colour indicates the presence of flavonoids.

TEST FOR ALKALOIDS

To 1 ml aqueous extract 1 ml of Wagner's reagent (iodine in potassium iodide solution) were added. The formation of a reddish – brown precipitate indicates the presence of alkaloids.

TEST FOR ANTHRAQUINONES

To 1 ml aqueous extract few drops of 10% ammonia solution was added. The formation of a pink colour precipitate indicates the presence of anthraquinones.

TEST FOR ANTHOCYANOSIDES

1 ml aqueous extract was mixed with 5 ml dilute hydrochloric acid (HCl). The appearance of a pink colour indicates the presence of anthocyanosides.

TEST FOR PROTEINS

To 1 ml aqueous extract 5 – 6 drops of Millon's reagent were added and a white precipitate appears. Upon heating, the white precipitate changes its colour to red indicating the presence of proteins.

TEST FOR STEROIDS

To 1 ml aqueous extract add 10 ml of chloroform and slowly, by the sides of the tube, 10 ml of sulphuric acid. Upper layer turns red and sulphuric acid layer showed yellow – greenish fluorescence.

TEST FOR TERPENOIDS

To 1 ml aqueous extract 2 ml of chloroform were added and then slowly few drops of concentrated sulphuric acid. An interface with a reddish – brown coloration indicates the presence of terpenoids.

RESULTS AND DISCUSSION

EXTRACTIVE VALUES

The initial quantity used to prepare all the three aqueous extracts (plum stone, plum stone shell and plum stone core) was 25 g. in Table 1 are given, on one hand, the amount of fruits (plum stone, plum stone shell and plum stone core) that resulted after the aqueous extracts were prepared and on the other hand the extractive values (%) for all three aqueous extracts.

Table 1. Extractive values of *Prunus domestica* stone aqueous extracts

Crt. No	Aqueous extract	Weight after extraction (g)	Yield (%)
1	Plum stone (*Prunus domestica)*	22,20	88,8
2	Plum stone shell (*Prunus domestica*)	24,74	98,96
3	Plum stone core (*Prunus domestica*)	18,70	74,8

From what it can be clearly seen in the table above, the highest extractive yield was found in the aqueous extract of plum stone shell.

QUALITATIVE PHYTOCHEMICAL SCREENING

The results obtained after qualitative screening the three aqueous extracts (plum stone, plum stone shell and plum stone core) are presented in Table 2.

Table 2. Qualitative phytochemical screening

Crt. No	Phytochemical test	Plum stone	Plum stone shell	Plum stone core
1	Carbohydrates - Molisch	-	-	-
2	Carbohydrates - Benedict	-	-	-
3	Tannins	-	+	-
4	Saponins	-	+	-
5	Flavonoids	-	-	-
6	Alkaloids	+	+	+
7	Anthraquinones	-	-	-
8	Anthocyanosides	-	-	-
9	Proteins	+	+	+
10	Steroids	-	-	-
11	Terpenoids	-	-	-

The results obtained from the qualitative phytochemical screening for all three aqueous extracts clearly show the presence of alkaloids and proteins in all three extracts (plum stone, plum stone shell and plum stone core). Additionally, in the case of plum stone shell, tannins and saponins were also found to be present in the aqueous extracts.

CONCLUSIONS

In this paper we have reported a simple and cheap method to prepare aqueous extracts from plum stones, plum stones' shell and plum stone' core in order to capitalize this not so used component of plums (*Prunus domestica*). All the 3 extracts were left to incubate for 72 hours, the obtained aqueous extracts were separated, vacuum filtered and were kept in the refrigerator for further use.

The highest extractive yield was found in the aqueous extract of plum stone shell. The qualitative phytochemical screening clearly showed the presence of alkaloids and proteins in all three extracts (plum stone, plum stone shell and plum stone core). Additionally, in the case of plum stone shell, tannins and saponins were also found to be present in the aqueous extracts.

REFERENCES

[1] Martinez-Espla A., Zapata P.J., Castillo S., Guillen F., Martinez-Romero F., Valero D., Serrano M., Preharvest application of methyl jasmonate (MeJA) in two plum cultivars. 1. Improvement of fruit growth and quality attributes at harvest, *Postharvest Biology and Technology*, 2014, 98, 98-105

[2] Sahamisirazi S., Moehring J., Claupein W., Greff – Hoenninger S., Quality assessment of 178 cultivars of plum regarding phenolic, anthocyanin and sugar content, *Food chemistry*, 2017, 214, 694-701

[3] Guerra M.E., Rodrigo J., Japanes plum pollination: A review, *Scientia Horticulturae*, 2015, 197, 674-686

[4] Contessa C., Mellano M.G., Beccaro M.G., Giusiano A., Botta R., Total antioxidant capacity and total phenolic and anthocyanin contents in fruit species grown in Northwest Italy, *Scientia Horticulturae*, 2013, 160, 351-357

[5] Thomas R.A., Krishnakumari S., Phytochemical profiling of *Myristica fragrans* seed extract with different organic solvents, *Journal of Pharmaceutical and Clinical Research*, 2015, 1, 304-307

[6] Caroling G., Vinodhini E., Mercy Ranjitham A., Shanti P., Biosynthesis of Copper Nanoparticles using Phyllanthus Embilica (Gooseberry) extract – Characterisation and study of antimicrobial effects, *International Jorunal of Nanomaterials and Chemistry*, 2015, 1, 53-63

[7] Veerachari U., Bopaiah A.K., Preliminary phyto-chemical evaluation of the leaf extract of five *Cassia Species*, *Journal of Chemical and Pharmaceutical Research*, 2011, 5, 574-583

[8] Periannan U., Pragakaran G., Studies on antibacterial activity and preliminary phytochemical analysisof *Aegle marmelos L.* (Beal), *International Journal of Current Sciences and Technology*, 2013, 2, 17-20

IMPACT OF THE UNIVERSITY EDUCATION IN GASTRONOMY, FOR BEING PREPARED TO SIBIU- GASTRONOMIC CAPITAL OF EUROPE IN 2019

***Cristina-Anca Danciu*[1], *Anca Tulbure*[1]**

[1]*Lucian Blaga University of Sibiu, The Faculty of Agricultural Science, Food Industry and Environmental Protection*

Abstract

Food, gastronomy and hospitality are becoming increasingly important as sources of growth, competitive advantage, cultural identity and creative experiences for Europe's regions. In this context, Romania's region of Sibiu was officially entitled as European Region of Gastronomy 2019, title received from the International Institute of Gastronomy, Culture, Art and Tourism (IGCAT) on January 25, in Athens. The aim of this paper is to reveal how the study of Gastronomy as part of the university curriculum, has relevance to the Sibiu Region on hospitality education and training, since such study underpins the advice which leads to the art or science of good eating.

Keywords: food, gastronomy, university education, students

INTRODUCTION

Regions play a key role in the gastronomic value chain, from agricultural food production to food processing, providing gastronomic experiences and hospitality in hotels and restaurants, and attracting visitors with regional gastronomy products.

Mărginimea Sibiului, Ţara Oltului, Valea Târnavelor, Valea Hârtibaciului and Ţara Secaşelor are the five ethnographic parts of the Sibiu County which have deposited and defined a cultural identity through the centuries, together with their own customs, traditional clothing and culinary specificity. Traditional cuisine in this part of the country was influenced by the Saxon and Hungarian population, which is why you will enjoy a special culinary experience in Sibiu. Sibiu already has the qualities of a complete touristic location because along with the touristic objectives, it offers an exceptional spiritual heritage, but also the beauty of its traditions and a special gastronomic experience. As a matter of fact, Sibiu is preparing to be the Gastronomic Capital of Europe in 2019, thus seeking to highlight this aspect of the region of Sibiu. The Sibiu region hosts more than 30 festivals of gastronomical

culture each year. Among these events, arranged in chronological order of organization, there are included the Lola Run (Fuga Lolelor) in Agnita (for the donuts that are offered on the street), the Cultural Days of the county, the Mountain Peony Festival in Gura Raului , Up On Jina Mountain Festival or the Christmas fairs in Sibiu and Medias. In the Sibiu region, there are currently 88 ecologically certified producers, 175 restaurants and 3 traditional certified producers (https://romaniatourstore.com/blog/romania-for-the-foodies).

Related to the 2019 big event, it is important to emphasis the Gastronomy studies as a trans-disciplinary perspective that does not replace, but complements, perspectives provided by the many disciplines studying food and culture, food and society, and food and marketing. It is also an answer to the urgent need for research that evaluates performances and identifies inadequacies, efficiencies and potential improvements in the gastronomic life of communities (Scarpato, 2001).

Also, Brillat-Savarin makes the following points clear:

a. the aim of gastronomy is "to obtain the preservation of man by means of the best possible nourishment";

b. its objective is "giving guidance, according to certain principles, to all who seek, provide, or prepare substances which may be turned into food";

c. according to him, "Gastronomy, in fact, is the motive force behind farmers, winegrowers, fishermen, and huntsmen, not to mention the great family of cooks, under whatever title they may disguise their employment as preparers of food" (Brillat-Savarin, 1994). In the words of Brillat-Savarin, "To entertain a guest is to make yourself responsible for his happiness so long as he is beneath your roof" (Brillat-Savarin, 1994).

Food and wine tourism (also referred to as gastronomic tourism, culinary tourism and cuisine tourism) has become a significant part of tourism in general, in the past few decades. Within cultural tourism, with its emphasis on "participating in" and "relating to" a culture and environment that is different to the "home" culture and environment, gastronomic tourism can take the form of a live-in cooking school, experiencing traditional gastronomic feasts and celebrations, helping with a grape harvest, visiting regional wineries and food producers along a Wine and Food Trail (Santich, 2004).

G. Richards points out that "Gastronomic holidays are therefore an important aspect of the emerging creative tourism sector, as tourists can learn to cook, can learn about the ingredients used, the way in which they are grown and appreciate how culinary traditions have come into existence" (Richards, 2002, pp. 16–17).

Since it is in the course of tourism and travel that visitors and strangers require and seek hospitality, the hospitality industry is very closely allied to gastronomy and with education in this field, through undergraduate or/and graduate classes. It is therefore important for education in Gastronomy to respond to new directions in travel and tourism.

MATERIALS AND METHODS

Food and gastronomic businesses provide employment. High qualified employers are an important part of the success in the hospitality industry.

The graduate students from Lucian Blaga University of Sibiu, with a major in Engineering and Management in Public Food and Agriturism are the future managers, able to **o**rganize major events that will use food and gastronomy as a means to stimulate innovation and showcase Sibiu's regional food cultures and identities.

Fig. 1. Gingerbread made by the Lucian Blaga University students
(with the five gastronomic regions of the Sibiu County)

In order to put on the spot this goals, the university developed programs for students for a greater awareness and knowledge about local and regional food, issues of nutrition, health and sustainability. Educational strategies stress the fact that graduates must not only possess a high level of knowledge, but also be able to contribute to innovation and entrepreneurship in order to be employable. That's why Lucian Blaga University signed

various partnership with private associations but also with the Sibiu's City Hall and City Council. It is about collaborative projects work, linked to both learning and employability. The project work is student directed and driven by engagement, mutual responsibility and a sense of ownership. Teachers act as advisors and consultants while also ensuring that student work progresses within the formal framework of the curriculum. In these entire project works, food was eaten, discussed, debated, grown, digested, and cooked. In other words, many different kinds of knowledge, understanding, and experience were exchanged. The students were involved in programs for the development of the quality food offer in the restaurants of Sibiu, by promoting the traceability of local ingredients and by reinterpreting traditional recipes in view of the new consumption trends (e.g. Adi Hadean Association – Cooking school, My Transylvania Association - Sibiu City Menu).

. Very often, graduates from problem-oriented programs are well employed. The most probable main reason for this is that problem-oriented learning activities meet society's demands for flexible and adaptive education and foster independent, critical thinking and creative graduates.

Given that the students of today are the hospitality managers of tomorrow who must face and respond to the challenges of the future, their ability to meet these challenges is largely dependent on their education and the content and quality of the current curriculum.

The quality of service, which includes being able to satisfy the visitor's requests for information and also having the knowledge that will enhance the visitor's experience, is an important factor in success. In order to respond to current trends in tourism, it is important that hospitality education include a significant and relevant gastronomy component, in addition to practical and business or management courses, so that students develop an understanding of the history and culture of food and drink, and in particular, the history, culture and traditions of the products of their particular region or country.

The key element to strengthen the gastronomic sector is the creation of highly skilled human resources -disciplinary and technically- in broader knowledge linked to the history, culture, production management, service innovation and food products; techniques and methodologies for the development, identification and reappraisal of the different cuisines of Sibiu region.

Gastronomy studies should be involved at all levels of analysis, planning and execution of plans related to gastronomic tourism or to the gastronomic part of any form of tourism. Professional individuals, working within the gastronomy studies framework, should

physically be involved in community policy making, training of tourism management and business planning Science-based cooking is closely associated with the design of stimulating and novel dishes that make guests feel an explosion of sensations. Chefs are expected to use high quality foods and thorough preparation techniques. But food science is not only texture and technology; it is also nutrition and health. From a nutritional point of view, science-based cooking may contribute to providing certain nutrients and other food components, which could confer healthy aspects to the dishes and menus. Chefs may then also consider nutritional aspects when designing dishes and menus.(Navarro et all, 2012).

RESULTS AND DISCUSSION

The *European Qualification Framework* (EQF) states that at the end of the second cycle (master's level), the graduate should be able to "manage and transform work or study contexts that are complex, unpredictable and require new strategic approaches, take responsibility for contributing to professional knowledge and practice and/or for reviewing the strategic performance of teams" (EQF level 7).

Fulfilling this aim, the number of Bachelor and Master Graduates that study the curriculum of Gastronomy in Lucian Blaga University increased with 50% since 2016, due the higher demands of the Hospitality industry from the Sibiu like an European Gastronomic Capital to be in 2019. The program should balance the development of practical management skills with development of a more general understanding of various social science disciplines.

Number of graduation theses and dissertation projects on the theme of gastronomy or related fields has also raised with 25%, for the last year.

The favorable context led to an increasing number of persons taking part in gastronomy educational programs developed by the university; in the same time were implemented new research-development- innovation projects.

CONCLUSIONS

The university curricula in Gastronomy should be capable of responding to industry needs at the same time as it produces graduates who can understand and manage the economic, social and cultural impacts of tourism on the residents of the 2019 Sibiu Gastronomic Capital.

University education provides high-quality services of initial formation, continuous training of human resource, dissemination of research-innovation results, and commitment to the social and cultural life of the community.

By including the study of Gastronomy within the general framework of hospitality education so that it complements the existing curriculum, educators will not only enhance the general knowledge of students, but also help equip them to fulfill more effectively their duties and responsibilities as host.

It is desirable to have professionals capable of carrying Sibiu regional cuisine at the highest level of recognition for all that brings in economic, cultural and social terms.

REFERENCES

Andersen Hanne Leth , *Education and Employability Research-based ways of working and real-world competences* , www.globalsciencia.com, GLOBALSCIENTIA, 2010, Issue 3

Brillat-Savarin, *The Physiology of Taste*, trans: A. Drayton, Harmondsworth: Penguin, 1994

Brillat-Savarin, J. A., *The Physiology of Taste*, trans: M.F.K. Fisher, Everyman's Library, 2009

Navarro Virginia, Gema Serrano, Dani Lasa, Andoni Luis Aduriz, Josune Ayo, *Cooking and nutritional science: Gastronomy goes further* ,International Journal of Gastronomy and Food Science 1, 2012, 37–45

Richards G., *Gastronomy: an essential ingredient in tourism production and consumption*, Routledge London, New York, 2002, 3-20.

Santich B., *The Study of gastronomy and its relevance to hospitality education and training*, Hospitality Management 23, 2004, 15–24.

Scarpato, Rosario, *Gastronomy, Academia and Hospitality: The missing articulation* – Paper presented at the Second International Conference of the Research Centre for the History of Food and Drink, Adelaide University, Australia, 2001.

IMMOBILISED DEHYDROGENASES LAYER EMPLOYED IN FAVOUR OF SOME WINEMAKING CONTROL POINTS

***Neli Darie*[1]**

[1] *"Lucian Blaga" University of Sibiu, Faculty of Agricultural Sciences, Food Industry and Environmental Protection, 7-9 Dr.I. Ratiu Street, 550012 Sibiu, Romania, neli.darie@ulbsibiu.ro.*

Abstract

In the immobilised dehydrogenases layer of D-lactate and L-malate biomarkers, the coupling of the main enzymatic reactions with an enzymatic NADH-cutback short the analytical response time, in red wine analysis. There were estmated optimal immobilisation patterns for two dehydrogenases scheme (D-LDH and L-MDH/D enzymes) with NAD-dextrane coenzyme also immobilised by attached on two preactivated poliamide membranes. Suitable L-malate and D-lactate spectrophotometer responses in wine were found for optimal smaller NAD^+-dextran concentration.

Keywords: immobilised enzymes, L-malate DH, red wine analysis, D-lactate DH, NAD-dextrane coenzyme

INTRODUCTION

In white and red winemaking quality control, especially in the fermentation processes there are reported some precise biochemical parameters which warn the winemakers on the possible undesirable events in the attained wines. Two such important biomarkers afford data on the certain kind stage winemaking [4].

One of them, L-malate as biomarker, calls attention in malolactic fermentation (MLF) development in order to manage the process and to obtain high-quality red wines, without wine taste alteration. The MLF finish moment must be accuracy known in order that it passes at a new operation, the wine sulphitation. In this way the wine technologist must be sure that before the sulphitation operation beginning, all L-malate has gone missing [9].

The other one, D-lactate biomarker is noticeable suddenly increased in wine lactic souring (an undesirable wine disease), in the wine MLF step. Unfortunately after the L-lactate synthesis this one remains as such and after bacteria attack. Its concentration growing affects especially the red wine quality since it stamps an unpleasant sweet-sour taste. When the wine technologists well know the L-lactate concentration in MLF they step in

winemaking technology to prevent the wine disease and to conduct through an alcoholic fermentation, by adding yeasts [7].

For both L-malate and D-lactate biomarkers more methods have been used, particullary the enzymatic ones because of their high substrat specificity [5].

In the present research there were used and performed two immobilised dehydrogenases layer enzymes adapted to the studied biomarkers D-lactate and L-malate, for laboratory terms detection in wine samples. The immobilisation preactivated membrane selectivity was improved using enzyme amplification event put by a second enzyme, diaphorase, in the immobilisation layer.

By enzymes immobilisation on an inert and insoluble support there are offered some advantages such as: done over again use of a enzyme batch, a good enzyme separation from the reactions products and also increased enzyme thermal and kinetic stability [2, 8]. The enzymes could be bound to an insoluble support (as membrane or silica gel) by different means. The covalent binding provides a intense enzyme-support interaction (figure 1).

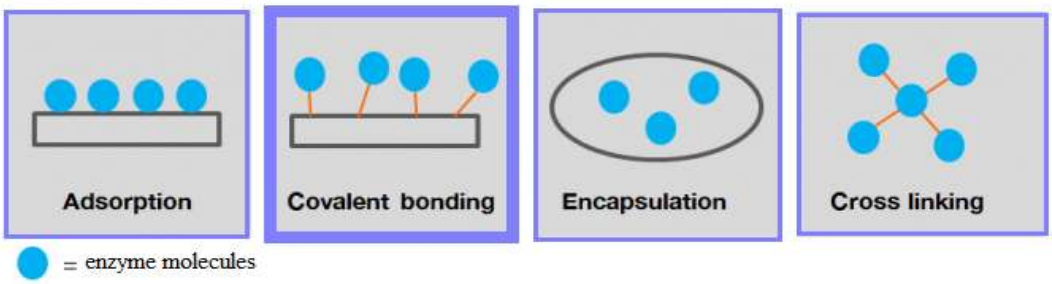

Fig. 1. Enzymes immobilisation types on an inert and insoluble support (adapted after [1]).

Since the adequate nicotinamide dinucleotide (NAD) coenzyme, required in each measurement is so soluble in the medium reaction, in order to assure and stabilize the analytical response, the NAD was enriched with its water soluble derivate, having a bigger molecular weight and stability.

MATERIALS AND METHODS

Samples

Some Romanian types red wines (*Cabernet, Merlot, Pinos noir, Feteasca Neagra*) from the fermentative medium were checked into for the two biomarkers, L-lactate and L-

malate. Each red wines sample was preliminary treated with active charcoal (optimal concentration of 40 g/l for 15 minutes contact with red wines) in order to be absorbed all the interferent colouring matters.

Chemicals and reagents

There were used the successive four immobilised enzymes on a preactivated poliamidic membrane, Immunodyne type (TR/Pall Industries): D-lactate dehydrogenase (D-LDH) - E.C. 1.1.1.28; L-malate dehydrogenase (L-MDH) - E.C. 1.1.1.37 glutamate oxaloacetate transaminase (GOT) - E.C. 2.6.1. and diaphorase (D) - E.C. 1.8.1.4. As NAD coenzyme were employed two NAD forms: light NAD^+- coenzyme and NAD^+-dextrane. All of them were supplied by Sigma.

The others chemicals employed were analytical grade; the solutions were prepared from them with distilled and deionized water. A buffered phosphate solution (20 mM) was used in return for remove the dehydrogenase balance towards the NADH shaping at a 9 pH value.

Enzymes immobilisation

On two preactivated poliamide membranes (Immunodyne type) there were applied enzymes solutions (20 μl volume): D-LDH+D, respectively L-MDH+TG+D. After 90 minutes the membranes were successively washed with solutions of sodium carbonate/potassium chloride (0,7M)/potassium carbonate (0,6M). All the supports containing immobilised enzymes were top covered by half-permeable cellophane membranes of optimal thickness (40-100 μm), supplied by Parafilm American National Can TM.

Enzymatic assays for L-malate and D-lactate

For the two biomarkers there were applied the Boehringer protocols [6, 3]. Later on, in order to short the analytical response time, the protocols were improved by the main enzymatic reactions coupling with an enzymatic NADH-cutback, respectively by consuming NADH in another reaction, diaphorase catalysted. The enzymatic systems have worked agreement with the bellow mechanisms: reactions r.1 and r.4 for D-lactate and r. 2; r.3; r.4 for L-malate. Since the L-MDH reaction balance lies on the side of L-malate, a trap-enzyme (GOT) was necessary to oxaloacetate.

The NADH coenzyme maked up in the dehydrogenase reactions was estimated by an amplification enzymatic event. The coenzyme was consumed in a hexacyanoferrate (III) reduction with diaphorase catalyst (r.4).

D-lactate + D-LDH-NAD^+ $\rightarrow\rightarrow\rightarrow$ pyruvate + NADH + H^+ (r.1.)

L-malate + L-MDH-NAD^+ $\rightarrow\rightarrow\rightarrow$ oxaloacetate + NADH + H^+ (r.2.)

oxaloacetate + L-glutamate + GOT $\rightarrow\rightarrow\rightarrow$ 2-oxoglutarate + L-aspartate (r.3.)

NADH + D + 2Fe $(CN)_6^{3-}$ $\rightarrow\rightarrow\rightarrow$ NAD^+ + 2Fe $(CN)_6^{4-}$ + H^+ (r.4.)

The hexacyanoferrate (III) disappearance was spectrophotometrical persued (at 420 nm, ἐ = 1,04 $cm^{-1}.mmol^{-1}.l$) and the absorbance variation was high straight proportionally with biomarkers concentrations.

RESULTS AND DISCUSSION

In this study there were employed two the immobilised dehydrogenase D-LDH and L-MDH, in this study of wine lactic souring and MLF control have as coenzyme a nicotinamide one, the NAD^+ as it was pointed in r. 1; r.2 and r.4 the hydrogen transport on makes through the pyridine nucleus. A technical protocol problem was the NAD coenzyme form. This one is soluble and also has a small-molecular-weight (about 700Da). In the biochemical reactions of the biomarkers substrates, the coenzyme is lost in reaction environment. As NAD^+ must be present in each measurement, the coenzyme was preferred immobilised beside the dehydrogenases, in the sensitive membranes layer.

In the modified Boehringer protocols, with small NAD mediator concentration at a standard temperature of 25^0C, in red wine samples the analytical responses had shown a dependence on the pyridine coenzyme concentration (figure 1) for the both biomarkers. The experiments have been repeated for different NAD concentrations, from 1 to 15 mM in red wine sample, in order to establish an optimal coenzyme amount in the immobilised enzymes layer. The spectrophotometric absorbance data for both D-lactate and L-malate were increased parallel with the NAD^+ growth concentrations, until a plate analytical response (for around of 8mM NAD^+ concentration in the immobilisation membranes).

Based on these values, in the same kinetic parameters, a NAD-dextrane coenzyme has brought in the research in order to take out the disadvantage of coenzyme missing and simultaneously spectrophotometer measurements were recorded for D-lactate and L-malate in red wine samples.

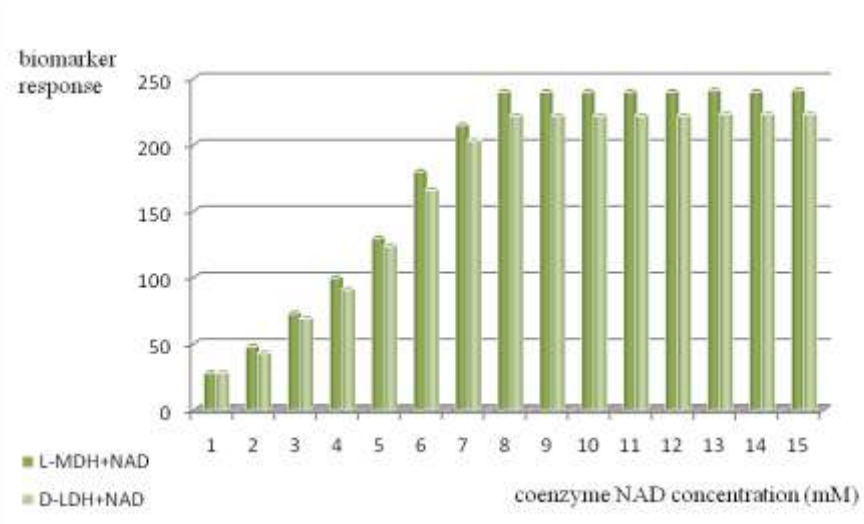

Fig. 1. Immobilised light NAD-coenzyme evolution in L-malate and D-lactate measurements (in red wine)

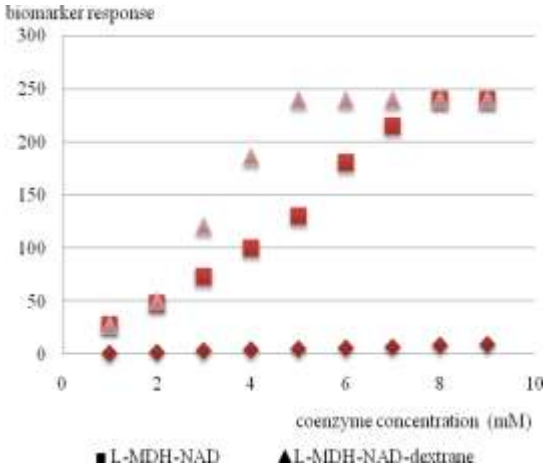

Fig. 2. – Immobilised L-malate response with NAD-dextrane and light NAD

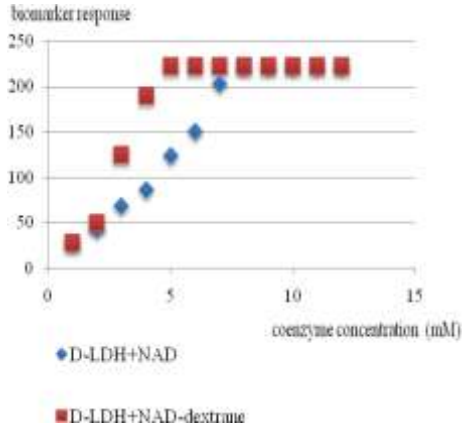

Fig. 3. Immobilised D-lactate with light and load NAD coenzyme

In NAD-dextrane the coenzyme is closely linked at the soluble dextrane, also has a high molecular weight and happily doesn't pass through the preactivated membranes. Experimental values have pointet out that simply smaller NAD-dextrane concentration (about 5 mM) is enough for the biomarkers analysis in the wine samples (figure 2 and 3) instead of about double with light NAD+, that meaning a low cost analysis. The optimal NAD-dextrane concentration value was used in the subsequent immobilised enzymes layer in favour of red wine samples control.

CONCLUSIONS

In the immobilised enzymes layer of D-lactate and L-malate biomarkers, the coupling of the main enzymatic reactions with an enzymatic NADH-cutback shorts the analytical response time, in red wine analysis. Optimal immobilisation pattern for the studied dehydrogenases was found being D-LDH and L-MDH/D enzymes with NAD-dextrane coenzyme, every single one, attached on two preactivated poliamide membranes. There were obtained similar L-malate and D-lactate spectrophotometer responses in wine, optimal for a smaller NAD^+-dextran concentration.

REFERENCES

1.Biocatalysts exceeding enzyme expectations, Does your process require an immobilised enzyme, Available at: h*ttp://www.biocatalysts.com*, 2016, [accessed at 12 April 2017].

2.Gray, C. J., Weissenborn, M. J., Eyers, C. E., Flitsch, S. L., Enzymatic reactions on immobilised substrates, *Chemical Society Reviews*, 2013, 42 (15), 6378.

3. BOEHRINGER MANNHEIM / R-BIOPHARM, Enzymatic BioAnalysis / Food Analysis L-Malic acid, UV-method for the determination of L-malic in foodstuffs and other materials, Cat. No. 10 139 068 035. Available at: *https://www.azmax.co.jp/wp-content/uploads/2015/11/Malic-acid_EN_10139068035_2013-03.pdf*, 2015, [accessed at 14 Mars 2017].

4. Cotea, V.D., Zanoaga, C., Tratat de oenochimie, 2009, *Editura Academiei Române*, București.

5. Jacobson, J., Introduction to Wine Laboratory Practices and Procedures, *Springer Science and Business Media*, 2010, New York, ISBN 978-1-4419-3732-2.

6. Office International de la Vigne et du Vin (OIV), *Recueil des Methodes Internationales d'Analyse des Vins et des Mouts*, OIV, Paris, 1990.

7. Mansell, T., Buttery bacteria: Malolactic fermentation and you, 2009, *Palate* Press.

8. Wu, H., Yanpeng, L., Jiafu, S., Xiaoli, W, Dong, Y., Zhongyi, J., Enhanced stability of catalase covalently immobilized on functionalized titania submicrospheres, *Materials Science and Engineering*, 2013, C. 33 (3), 1438–1445.

9. Zoecklein, B., Fugelsang, K., Gump, B., Nury, F., Wine Analysis and Production, *Kluwer Academic Publishers*, 1999, New York, ISBN 0834217015.

COMPARATIVE STUDIES REGARDING THE CONTENT OF POLYPHENOLIC BIOACTIVE COMPOUNDS IN *THEOBROMA CACAO* PRODUCTS

***Crina Nicolae* [1], *Simona Oancea* [2*]**

[1]*Department of Sciences, "Lucian Blaga" University of Sibiu, Romania*

[2*]*Department of Agricultural Sciences and Food Engineering, "Lucian Blaga" University of Sibiu, Romania; simona.oancea@ulbsibiu.ro*

Abstract

Cocoa (Theobroma cacao) products contain significant levels of phytochemicals in particular of phenolic structure. For the last decades, these compounds have been largely studied because of their potential chemopreventive properties based on their antioxidant activities. The present study aimed to comparatively investigate the total content of phenolics and tannins of twelf different cocoa samples from various local markets. Higher levels of total phenolics and tannins were found, in particular for Van cocoa Maspex-GMV and powder of bio cocoa samples. Such results are important for completion of data regarding content of bioactive compounds in various foods of plant origin. Consumption of such products results in beneficial effects on human health.

Keywords: cocoa powder, phenols, tannins, Theobroma cacao, catechin

INTRODUCTION

Cocoa (*Theobroma cacao*) is consumed worldwide at increasing rates in various forms, either as chocolate or cocoa powder. There are products, food and non-food that may incorporate cocoa, such as different beverages, cosmetics, pharmaceuticals and toiletries. (A., Tafuri, et al., 2004, G., Fenling et al., 2013)

Physico-chemical composition of cocoa is very complex, most of the time it depends on geographical area of tree variety, but depends largely on the process on cocoa beans. Cocoa powder contains high amounts of phenols and tannins which are regarded as very important in human health. Studies have shown that they have numerous beneficial properties. Phenolics exhibit strong antioxidant capacity, antiradical scavenging and numerous benefits for human health, such as antitumor, antimicrobial, anti-inflammatory, antiulcer, vasodilator, immunomodulatory effects. Some authors (K. Lee et al. in 2003)

demonstrated that cocoa is richer in phenols than teas and wine. Cocoa phenols show high concentrations of 12-18% calculated to total weight of dry beans. The main identified classes of phenolic compounds are simple phenols (phenolic acids, hydroxycinnamic acids, phenylacetic acids, flavonoids, benzoquinones, naphtoquinones, anthraquinones, acetophenones, phenylpropenes, coumarines, chromones, xanthones, stilbenes, lignans, lignins). From the main groups of cocoa phenols, catechin as (-)-epicatechin is found up to 35% of the total phenols content. Other important phenols found in significant amounts are pro-anthocyanidins (58%). The content and composition of cocoa phenols may be influenced by different extent by type of processing (Hii et al., 2009).

Another constituent of cocoa powder, present in much greater amounts than phenols, is the group of tannic substances. The chemical properties of these substances and their amounts may be considered for potential antinutritional properties of cocoa. Kuzmeski and Mueller analyzed eighteen samples of commercial cocoa powder for cacao-red, and found values ranging from 2.62 to 15.59 %. (W. Muller et al, 1946). Recent studies showed that tannins have antioxidant capacity and consequently numerous benefits for human health.

MATERIALS AND METHODS

Sample preparation

12 samples listed in table 1 and acquired from Romanian market were investigated in the present study.

Table 1. List of cocoa (*Theobroma cacao*) samples investigated in the present study and their fat content.

No.	Product name/type	Fat content (%)
1.	Cocoa (Dr. Oetker)	11
2.	Black cocoa (Dr. Oetker)	22
3.	Cocoan VAN (Maspex-GMV)	10-12
4.	Cocoa sweet	10-12
5.	Cocoa Schmidt (Randler)	20-22
6.	Cocoa	11
7.	Cocoa powder bio (Managis)	11
8.	Cocoa (Clever)	10-12
9.	Cocoa castello	10-12
10.	Cocoa belbake	20-22
11.	Cocoa powder	10
12.	Cocoa (Colin Daily)	10-12

Determination of total phenols

Total phenols were determined spectrophotometrically by the Folin-Ciocalteu method (Singleton V.L., 1965) on cocoa extracts in 70% ethanol solution. Values are expressed as gallic acid equivalents (GAE) per 100 grams of product.

Determination of tannins

Tannins were determined spectrophotometrically by vanillin method (Price et al. 1978). Values were expressed as catechin equivalents per 100 grams of product.

Statistical analysis

Mean values of total phenols and tannins were calculated. Correlation coefficients were evaluated.

Results and discussion

12 samples of cocoa were investigated for their total content of phenols and tannins. The nutritional composition (protein, fat, sugars, salt) as declared by manufacturere is presented in Figure 1.

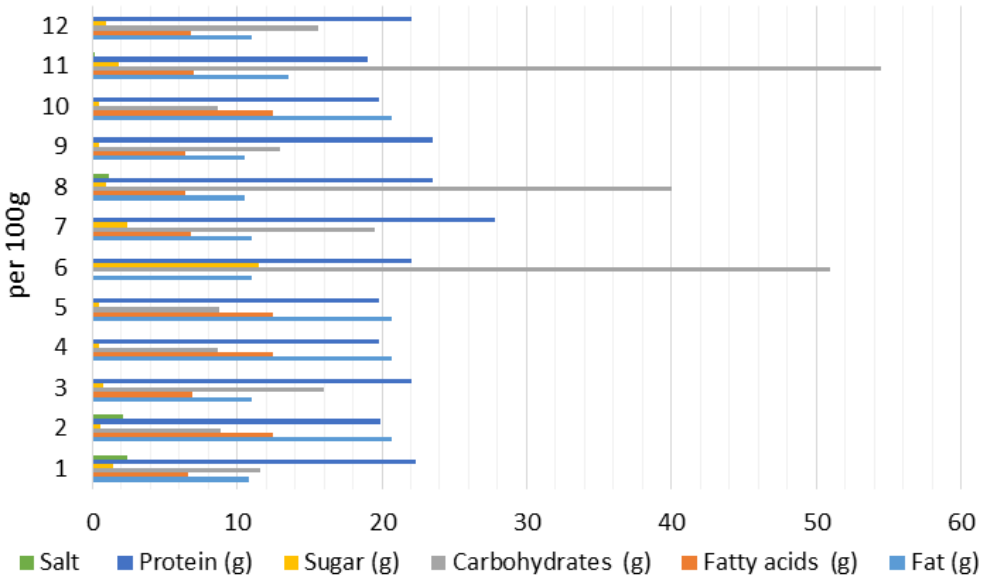

Fig. 1. Nutritional information of the investigated cocoa (*Theobroma cacao*) samples as declared by manufacturer (for 100 g product).

Total phenols were determined by the Folin-Ciocalteu method. The results on total phenolic contents are presented in Figure 2 . The highest concentration of total phenols was found for in sample **3** (693.504 mg/GAE 100g), followed by samples **7** (650.583 mg/GAE 100g) and **4** (624.720 mg/GAE 100g). Samples **1, 2, 5, 8, 9, 10**, showed mean values between 400 and 500 mg/GAE 100g, with slight variations. The lowest total phenols content was found in samples **6** (333.879 mg/GAE 100g), **11** (288.456 mg/GAE 100g), and **12** (230.783 mg/GAE 100g). The obtained results are in agreement with those from the literature, such the studies conducted by B. Miller, 2006, on several samples of cocoa powder or by K. W. Lee et al., 2003, which indicated similar. A slight increased concentration of total phenols from cocoa powder was obtained in the investigation conducted by S. Jolic, 2011.

The results regarding tannins in the hereby investigated 12 cocoa samples presented in Figure 3 showed that sample **7** (presents the greatest amount of tannins 4878.819 mg catechin /100 g) compared to the other samples exceeding limit with average much other evidence, followed by sample **3** (3469.228 mg catechin /100 g). The samples **1, 2, 4, 5, 9** showed mean values between 1000 and 2000 mg catechin /100 g. Apart sample 12 (264.047 mg catechin /100 g), which presented the lowest amount of tannin in the series, lying well below the average level, small amounts of tannins were found in the other samples, such as **6, 8, 10, 11**.

Compared to the study of J. Bonvehi et al., 1996, our results indicate similar results except samples **7** and **12** showing very high and very low concentration, respectively.

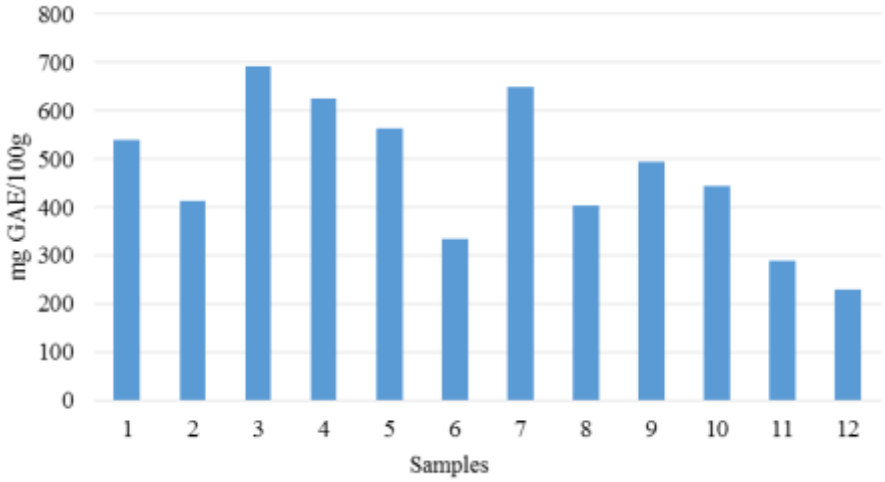

Fig. 2. Total phenols content of the investigated cocoa (*Theobroma cacao*) samples.

No statistically significant correlation was found between bioactive compounds content (phenols, tannins) and nutritional composition (lipids, fatty acids, carbohydrates, sugars, proteins and salt) of the investigated samples at $p < 0.05$.

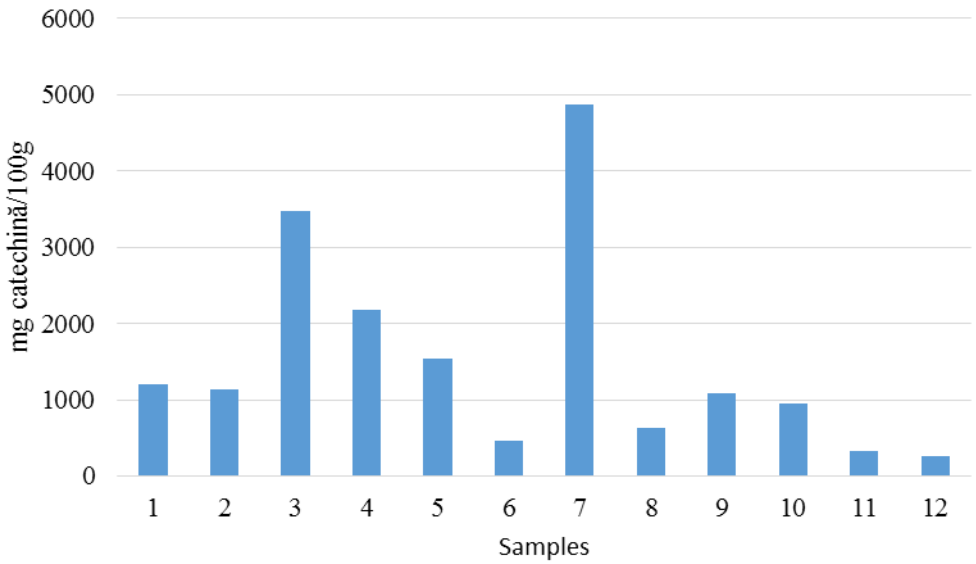

Fig. 3. Total tannins content of the investigated cocoa (*Theobroma cacao*) samples.

CONCLUSIONS

The results on bioactive content of 12 samples of cocoa (*Theobroma cacao*) powder products indicate values between 230.783 mg GAE/100 g and 693.504 mg GAE/100 g for phenols and between 264.047 mg catechin/100 g and 4878.819 mg catechin/100 g for tannins, respectively. The highest content of phenols and tannins was found for VAN cocoa Maspex-GMV and powder bio cocoa samples. Such bioactive polyphenolic compounds provide positive health effects based on their strong antioxidant potential. No statistically significant correlation was found between bioactive compounds content (phenols, tannins) and nutritional composition (fat, proteins, sugars, salt).

Such results are important for completion of data regarding content of bioactive compounds in various foods of plant origin.

REFERENCES

Baba, S., Osakabe, N., Kato, Y. et al., Continuous intake of polyphenolic compounds containing cocoa powder reduces LDL oxidative susceptibility and has beneficial effects on plasma HDL cholesterol concentrations in humans, *American Journal of Clinical Nutrition*, 2007, 85, 709–717.

Bonvehi, J., Coll, F.V., Evaluation of bitterness and astringency of polyphenolic compounds in cocoa powder, *Agricultural and Food Laboratory*, 1996, 60, 365-370.

Gu F., Tan L., Wu H., Fang Y., Xu F., Chu Z., Wang Q., Comparison of Cocoa Beans from China, Indonesia and Papua New Guinea, *Journals Foods*, 2013, 2304-8158 .

Hii, C. L., Law C. L., , Misnawi S., Cloke M, Polyphenols in cocoa (Theobroma cacao L.), *Asian Journal of Food and Agro-Industries*, 2009, 1906-3040.

Jolic S. M., Redovnikovic I. R., Markovic K., Sipusic D. I., Delonga K., Changes of phenolic compounds and antioxidant capacity in cocoa beans processing, *International Journal of Food Science and Technology*, 2011, 46, 1793-1800.

Jolic. S. M., Redovnikovic, I. R., Markovic. K., Durdica. S. and Karmela, D. 2011. Changes of phenolic compounds and antioxidant capacity in cocoa beans processing, *Journal of Food Science and Technology*, 2011, 46: 1793-1800

Lee, W.K.; Kim, J.Y.; Lee, J.H.; Lee, Y.C. Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine, *Journal of Agricultural and Food Chemistry*, 2003, 51, 7292–7295.

Miller K. B., Hurst W. Y., Flannigan N., Ou B., C. Lee Y., Smith N., Stuart D. A. Survey of commercially available chocolate- and cocoacontaining products in the United States. 2. Comparison of flavan-3-ol content with nonfat cocoa solids, total polyphenols, and percent cacao, *Journal of Agricultural and Food Chemistry*, 2009, 57, 9169-9180.

Miller, K.B., Hurst, W.J., Payne, M.J., Impact of alkalization on the antioxidant and flavanol content of commercial cocoa powders, *Journal of Agriculture and Food Chemistry*, 2008, 56, 8527–8533.

Miller, K.B., Stuart, D., A., Smith, N.L. Lee C. Y., McHale, N. L., Flanagan, J., A., Hurst, W. J., Antioxidant Activity and Polyphenol and Procyanidin Contents of Selected Commercially Available Cocoa-Containing and Chocolate Products in the United States, *Journal of Agricultural and Food Chemistry*, 2006, 57, 9169-9180.

Ortega N., Romero M. P., Macia A., Reguant J., Angles N., Morello J. R. & Motilva M. J. Obtention and characterization of phenolic extracts from different cocoa sources, *Journal of Agricultural and Food Chemistry*, 2008, 56, 9621- 9627.

Price, M.L., van Scoyoc, S., Butler, L.G., A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain *Journal of Agricultural and Food Chemistry*, 1978, 26, 1214-1218.

Serra Bonvehl, J. & Ventura Coil, F., Parameters affecting the quality of processed cocoa powder:acidity fraction. Z. Lebensm. Unters. Forsch., 1997, 204 (in press).

Tafuri A., Ferracane, R., Ritieni, A. Ochratoxin A., in Italian marketed cocoa products, *Journal of Agricultural and Food Chemistry* , 2004, 88, 487–494.

Wong, M. K., Dimick, P. S., Hammerstedt, R. H., Extraction and high performance liquid chromatography enrichment of polyphenol oxidase from Theobroma cacao seeds, *International Journal of Food Science and Technology*, 1990, 55, 1108-1111.

APPLICATION OF MICROENCAPSULATED PROBIOTICS IN FRESH JUICES

Camelia Rovinaru[1], Diana Pasarin[1]

[1]Institutul National de Cercetare-Dezvoltare pentru Chimie si Petrochimie – ICECHIM

Abstract

Probiotic bacteria provide numerous health benefits including control of intestinal infections, improvement of lactose utilization, influence the immune system, lower serum cholesterol levels and consumer demand for products containing such beneficial microbes had increased. The most important probiotics known to have beneficial effects on the human gastro-intestinal tract are Lactobacillus and Bifidobacterium species. Fruit juices are an alternative vehicle for the delivery of probiotics, because they are rich in sugars, minerals and vitamins which stimulate microorganisms growth and do not contain starter cultures that compete for nutrients with probiotics. Probiotic-fortified fruit juices could be used as a functional healthy beverage to promote health to the population. In order to confer benefits to the consumer, probiotic bacteria have to be present in significant numbers (at least 10^7 CFU/mL) and remain viable in the fresh juices until the time of consumption. Improvement of their viability is obtained by microencapsulation into coating materials, technique which protect probiotics at the passage through the stomach and small intestine, but also make a controlled release at the level of the distal intestinal tract. Currently, microencapsulation can be achieved by methods divided in chemical, physical-mechanical and physicochemical processes and using a wide range of encapsulated materials. The aim of this study is to test the interaction between probiotics viability and fruit juice matrix and to microencapsulate probiotic bacteria that enables prolonged viability in fresh juices, by dispersion methods, using coating materials selected from a wide variety of natural ones, in order to obtain stable microcapsules, resistant to gastric juice and to provide controlled release of probiotics in the gut.

Keywords: apple juice, Lactobacillus acidophilus, Lactobacillus plantarum, viability, microencapsulation

INTRODUCTION

Probiotics are "live microorganisms which when administered in sufficient amounts confer a health benefit balancing microbial flora of the host" (FAO/WHO definition, 2001). Today, the definition of probiotics is "non-pathogenic living microbes that are taken singly or in combination by humans or animals promoting the host health via increasing the number of beneficial intestinal bacteria" (Yoon KY, 2005). According to this definition many

microorganisms are considered as probiotics (table 1) (Burgain J, 2011), but only lactic acid bacteria are of importance for food and nutrition (Sophorn Hap, 2010).

Table 1. Recognized probiotic strains

Lactobacillus species	***Bifidobacterium species***	**Other lactic acid bacteria**	**Non-lactic acid bacteria**
L.acidophilus	*B.lactis*	*Enterococcus faecium*	*Bacillus cereus*
L.casei	*B.bifidum*	*Streptococcus thermophilus*	*Saccharomyces boulardii*
L.paracasei	*B.breve*	*Lactococcus lactis*	*Saccharomyces cerevisiae*
L.rhamnosus	*B.longum*		
L.plantarum			
L.reuteri			
L.fermentum			

Probiotic bacteria must have some health promoting properties for the consumers to be recognized as functional food components: non-pathogenic and non-toxic to the host, acid- and bile-stability, resistance to digestive enzymes, adhesion to intestine surface, antagonistic activity against human pathogens, maintenance of mucosal integrity, reducing cancer and cardiovascular disease risk, stimulation of immunity (Nagpal R, 2012).

Probiotics are usually added to dairy fermented products, but in recent years, development of functional beverages containing functional microorganisms is an ascending consumer trend (Heenan CN, 2002). Fruit juices are considered a suitable carrier for probiotics being appreciated as a healthy food product with high content of vitamins, minerals, dietary fibers and consumed by a large segment of the population (Tuorila H, 2002).

From technological point of view, probiotic microorganisms must have viability during the processing and storage, facility of application in food products, maintenance of important properties and low cost. The most important properties of probiotic food products are the ability to provide and maintain viable bacteria at a concentration of minimum 10^6 CFU/mL of intestinal fluid (Bourlioux P, 2003) and the inhibition of pathogens (Sophorn Hap, 2010). The protection and viability of probiotics during processing, storage and passage of the probiotic product through gastrointestinal tract can occur by microencapsulation.

In this context, the aim of this study is to test the interaction between probiotics viability and fruit juice matrix and to microencapsulate probiotic bacteria to prolonge viability in fresh juices, using coating materials of natural origin, in order to obtain stable microcapsules, resistant to gastric juice and to provide their controlled release in the gut.

MATERIALS AND METHODS

*Raw material***:** apple juice was obtained from SC ANNABELLA FABRICA DE CONSERVE RAURENI SRL, Valcea

*Bacterial strains***:** *Lactobacillus acidophilus LA5* was obtained from CHR. HANSEN. *Lactobacillus plantarum* NCIMB 11974 was obtained from National Collection of Industrial, Food and Marine Bacteria, UK. The bacterial strains stock cultures were maintained on MRS agar slants medium at 4^0C until required.

*Inoculum preparation***.** Inoculum for the two probiotic bacteria was obtained from fresh MRS agar culture taking out a colony with a sterile loop and transferred into individually Erlenmeyer flasks containing 100 mL of sterile MRS broth and incubated **aerobically** at 37^0C for 48 h. After incubation, from respective broth, second subcultures were performed and incubated in the same way assuring the adaptation of microorganisms to the new broth media.

Determination of free probiotic bacteria viability in apple juice.

Bacterial inoculum for viability tests, prepared by incubating the individually cultures at 37^0C in MRS broth for 18 h, was standardized to a concentration of 10^9CFU/mL. Sterile Erlenmeyer flasks containing 100 mL pasteurized apple juice were inoculated, under aseptic conditions, with 1% culture from each of the two bacterial strains and refrigerated at 4^0C during four weeks and their viability monitored. Every week, 10 mL apple juice from each flask was tested for pH.

The pH was measured using a pH-meter (Multi 340i SET- Multiparameter Instruments) after proper calibration.

Probiotics viable cell count (CFU/mL) was determined by plate count methodology in plates containing MRS agar, after 48 h incubation at 37°C.

Microencapsulation of probiotics

Bacterial strains were microencapsulated by extrusion method, using polymers of natural origin, in order to obtain stable microcapsules. *L.acidophilus* and *L.plantarum* were encapsulating in matrices comprising sodium alginate alone or in combination with other polymers in different ratios (data not shown).

RESULTS AND DISCUSSION

Apple juices have a high acid content and low pH and microorganisms incorporated as probiotic in order to confer benefits to the consumer, have to be present in significant

numbers (at least 10^7CFU/mL) and remain viable both in the fresh juices until the time of consumption and in the gut, resisting to the acidic conditions in the stomach or intestine (Champagne, 2005). Acids affect cells acidifying cytoplasm, increasing energy consumption required for maintenance of intracellular pH, inhibiting enzymatic reactions (Shabala, 2006).

When pasteurized apple juice was inoculated with *L.acidophilus* and *L.plantarum* a decrease of pH was observed in both cultures, comparative with control sample, where the modification of pH was insignificant, during 4 weeks storage in 4^0C. The initial pH 3.48 was reduced to 3 and 3.2 by *L.acidophilus* and *L.plantarum*, respectively (figure 1).

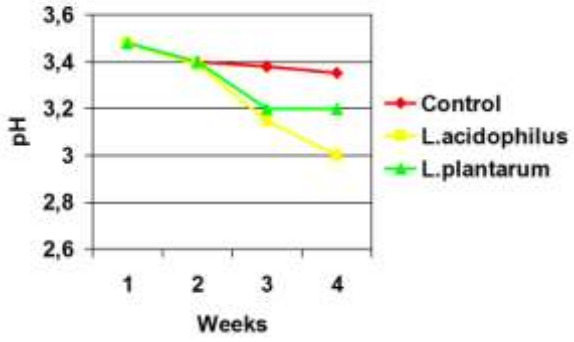

Fig. 1. Changes in pH of functional apple juice during storage

These results are in agreement with investigation of Nagpal R (2012) who fortified orange, grape and tomatoes juices with both *Lactobacillus* species, and have observed a decrease of pH during 72h incubation at 30^0C.

The ability of the two selected bacteria to survive in apple juice was also tested. The viability of free probiotic bacteria declines over four weeks of refrigerated storage, because of acidity in apple juice. The initial pH=3.48 changed to the final pH of 3, bacteria utilizing carbohydrates with accumulation of metabolic end products (organic acids, diacetyl, acetylaldehyde) which lower the pH of the juice and reduce culture viability (Hood SK, 1988). The initial cell count was $8.5x10^9$ and $8x10^9$ CFU/mL for *L.acidophilus* and *L.plantarum*, respectively. After 4 weeks of storage at 4^0C, the cell counts of the two bacteria decreased. The probiotic viability was shown to be strain-dependent. *Lactobacillus acidophilus* has high resistance to acidic environment and presented a greater number of living cells (~10^4 CFU/mL) after 4 weeks (figure 2).

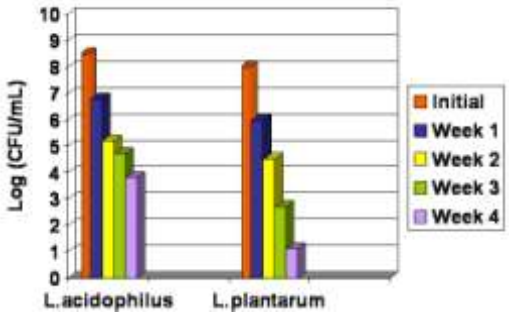

Fig. 2. Free probiotic bacteria viability in apple juice

Probiotic viability in fruit juice is influenced not only by strain, but also by method of culture preparation, state of the cells inoculated, storage temperature (Champagne, 2008).

Because of the bad effect of the low pH environment (<pH 4.0), it is very difficult to maintain the viability of probiotics in fruit juices without protection (Shah NP, 2010). A strategy to enhance the survival of probiotic bacteria in low pH is microencapsulation.

Microencapsulated probiotic bacteria in alginate capsules showed better survival vs. free cells in the same conditions of storage, encapsulation protecting probiotics against low pH of the apple juice, providing a physical barrier and > 10^5 CFU/mL were still present after four weeks of storage (figure 3).

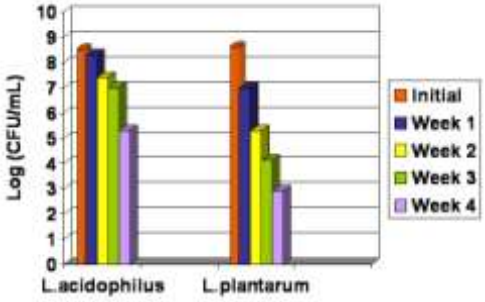

Fig. 3. Microencapsulated probiotic bacteria viability in apple juice

Our data are consistent with results obtained by Chandramouli V (2004), who observed that the viability of *L.acidophilus* CSCC 2400 cells in the sodium alginate microcapsules increased with alginate capsule size and concentration of the gel.

Encapsulation of probiotics in sodium alginate is a promising technology to improve the viability and stability of these bacteria in apple juices both during storage and passage through the gastrointestinal tract.

CONCLUSIONS

Both *Lactobacillus* species are able to survive in apple juice which is proper as carrier for probiotic bacteria. It must be mentioned that viability of free probiotics decreased due to juice low pH, but microencaspsulation protect the cells from the acidic environment, improving their survival in juices. *Lactobacillus acidophilus* is more tolerant to acid and has potential for being used as probiotic in fruit juices.

An important technological challenge to obtain microcapsules with functional properties is to reduce the particle size, because influence textural and sensorial properties of the juices.

ACKNOWLEDGEMENT

The authors would like to thank the UEFISCDI that support Eureka 92/2016 Project.

REFERENCES

Bourlioux P., Koletzko B., Guamer F., Braesco V., The intestine and its microflora are partners for the protection of the host, *The American Journal of Clinical Nutrition*, 2003, 78(4), 675-683.

Burgain J., Gaiani C., Scher J., Encapsulation of probiotic living cells: From laboratory scale to industrial applications, *Journal of Food Engineering*, 2011,104 (4), 467–483.

Champagne, CP., Gardner, NJ., Roy, D., Challenges in the addition of probiotic cultures to foods, *Critical Review in Food Science and Nutrition*, 2005, 45, 61–84.

Champagne, CP., Raymond, Y., Gagnon, R., Viability of *Lactobacillus rhamnosus* in an apple-based fruit juice under simulated storage conditions at the consumer level, *Journal of Food Science*, 2008, 73(5), M221–226.

Chandramouli V., Kailasapathy K., Peiris P., Jones M., An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions, *Journal of Microbiological Methods*, 2004, 56(1), 27-35.

Heenan, CN., Adams, MC., Hosken, RW., Fleet, GH., Growth medium for culturing probiotic bacteria for applications in vegetarian food products, *Lebensmittel-Wissenschaft und Technologie*, 2002, 35,171–176.

Hood SK, Zottola ML., Effect of low pH on the ability of *Lactobacillus acidophilus* to survive and adhere to human intestinal cell, *J Food Sci*, 1988, 5, 1514–1516.

Nagpal R, Fortification and fermentation of fruit juices with probiotic lactobacilli, *Ann Microbiol*, 2012, 62, 1573–1578.

Shabala, L., McMeekin, T., Budde, BB., Siegumfeldt, H., Listeria innocula and *Lactobacillus delbrueckii* subsp. *bulgaricus* employ different strategies to cope with acid stress, *International Journal of Food Microbiology*, 2006, 110, 1–7.

Shah, NP., Ding, WK., Fallourd, MJ., Leyer, G., Improving the stability of probiotic bacteria in model fruit juices using vitamins and antioxidants, *Journal of Food Science*, 2010, 75(5), M278–282.

Sophorn Hap, Functional properties of aqueous fruit extracts towards probiotic and pathogenic bacteria, *Thesis submitted to Auckland University of Technology*, 2010.

Tuorila H., Cardello AV., Consumer responses to an off/flavor in juice in the presence of specific health claims, *Food Quality and Preference*, 2002, 13(7-8), 561-569.

Yoon, KY., Woodams, EE., Hang, YD., Fermentation of beet juice by beneficial lactic acid bacteria, *Journal of Lebensm-Wiss. U-Technol*, 2005, 36, 73-75.