CEFood Congress

Novi Sad, Serbia
23 - 26 May, 2012

PROCEEDINGS

of 6th Central European Congress on Food

IUFoST International Union of Food Science and Technology

EFFoST European Federation of Food Science and Technology

CEI Central European Initiative

ISBN 978-86-7994-027-8

6TH CENTRAL EUROPEAN CONGRESS ON FOOD, Novi Sad 2012, SERBIA

Publisher

University of Novi Sad, Institute of Food Technology
Bulevar cara Lazara 1.
21000 Novi Sad, Serbia

Main editor
Dr. Jovanka Lević

Editors
Prof. Dr. Viktor Nedović
Dr. Nebojša Ilić
Dr. Vesna Tumbas
Ana Kalušević, dipl. ing.

Abstract/Paper Review
All abstracts and papers are reviewed by the International Board of Reviewers

Technical editors
Bojana Kokić
Miona Belović
Dubravka Jambrec
Nataša Nedeljković
Olivera Đuragić
Tanja Radusin
Ana Kalušević
Tamara Dapčević
Tatjana Tasić
Jovana Vučković
Tamara Sarafijanović

Cover
Boris Bartula, BIS, Novi Sad, Serbia

Printed by
“Futura” – Novi Sad, Serbia

Number of copies
600 copies

CIP – Каталогизација у публикацији
Библиотека Матице српске, Нови Сад

338.439.4(082)
663/664(082)

CENTRAL European Congress on Food (6; 2012; Novi Sad)
Proceedings of 6th Central European Congress on Food, Novi Sad, Serbia, 23-26 May, 2012 / [editor Jovanka Lević]. – Novi Sad : Institute of Food Technology, 2012 (Novi Sad : Futura). – 1712 str. : ilustr. ; 30 cm

Tiraž 600. – Bibliografija uz svaki rad.

ISBN 978-86-7994-027-8

a) Храна – Производња – Зборници

COBISS.SR-ID 271466759

MICROBIAL POLYSACCHARIDES: BETWEEN OIL WELLS, FOOD AND DRUGS

Jovana R. Stefanović[1,2], Miroslav M. Vrvić[1,2*]

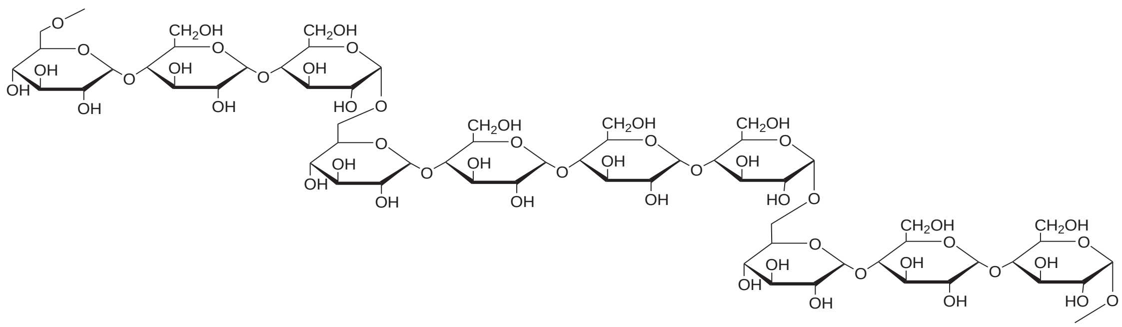
[1]Faculty of Chemistry, University of Belgrade, 11158 Belgrade, Studentski trg 16, POB 51
[2]Department of Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, 11158 Belgrade, Njegoševa 12, POB 473, Serbia

*Corresponding author:
Phone: +381112637273
Fax: +381112636061
E-mail address: mmvchem@sezampro.rs

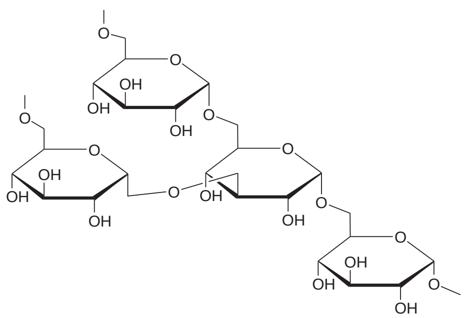
ABSTRACT: Microbial exopolysaccharides (MPSs) such as xanthan, dextran, gellan or pullulan have been commercially used, in their natural or modified state, for many years.
A large number of these natural polymer applications is a consequence of their excellent physical and chemical properties, based on their capacity to alter the basic properties of water (e.g. thickening or gelling). In addition, these polymers have related secondary functions, such as emulsification, suspension, stabilization, encapsulation, flocculation, film forming, binding and coating.
MPSs, and particularly exopolysaccharides have many other novel properties to offer, and discovery of immune modulation and bifidogenic effect of some of them should provide other applications.
This work focuses on the more recent developments in the extent of application of microbial polysaccharides in the various fields what makes these polymers promising and versatile materials in future, and also on our investigations within these natural products.
Key words*: microbial polysaccharides (MPSs), properties, material, application, industry*

BASIC KNOWLEDGE AND APPLIED ASPECTS

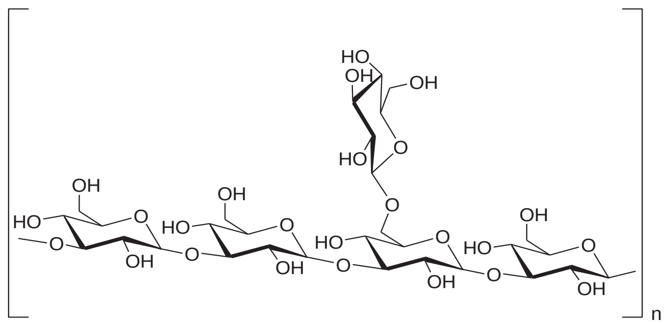
The ability to produce polysaccharides is widely found among different species, but despite many sources of these biopolymers, those from algae and higher plants are dominant on the world market. Polysaccharides derived from microorganisms, including bacteria, yeasts and moulds, are still not exploited enough. The main reasons for that are linked to the costs of production, because of specific substrate requirements in certain cases, bioreactors demands or obtaining aseptic conditions. Nevertheless, polysaccharide production from microorganisms has many advantages: it takes much less time compared to plants; in the case of some algae species it is more energy efficient because of use of solar energy for production; and a lot of industrial wastes and raw materials can be used as carbon sources, which is probably the greatest advantage of all (Donot et al., 2012).
MPSs are synthesized and accumulated mostly after the growth phase, and, in regard to their location in the cell, they can be divided into three main groups. Inside the cell, as carbon and energy sources are cytosolic endopolysaccharides. The second group is made of those that make up the cell wall. Polysaccharides exuded into the extracellular environment are known as exopolysaccharides (EPSs), and they appear in the form of capsules or slime. They are also involved in biofilm formation, where they have many significant roles: participation in attachment to a surface, formation and stabilization of biofilm structure, enhancement of resistance to environmental biotic and abiotic stresses and antimicrobial agents, preventing of desiccation and assumption of nutrients (Shia and Zhua, 2009).
MPSs are divided into two groups: homopolysaccharides, made up of a single type of monosaccharide (e.g. pullulan,dextran.or levan) and heteropolysaccharides, made up of several types of monosaccharide, with complex structures (e.g. xanthan or gellan). Structures of some MPSs are shown in Figure 1. In any case, they are mainly composed of



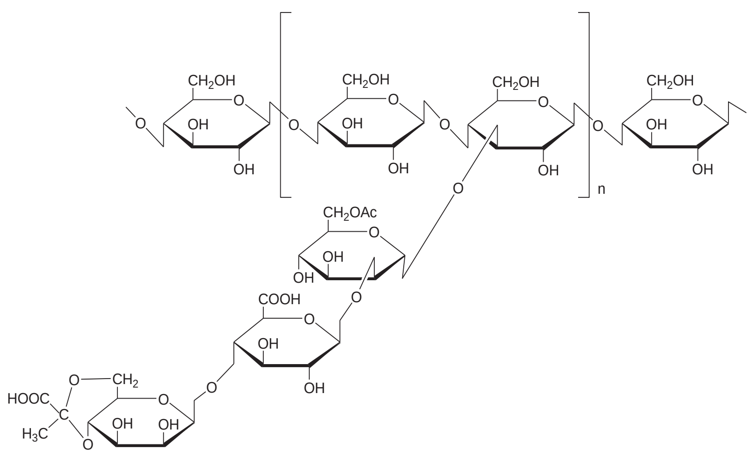
1.1.



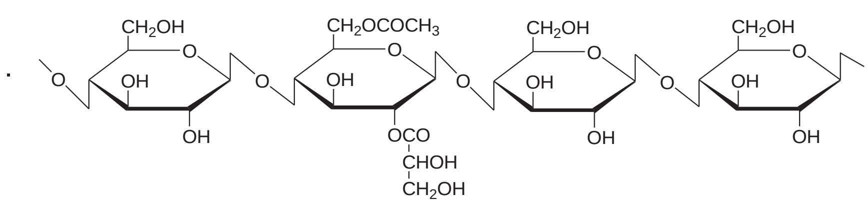
1.2



1.3..



1.4.



1.5

Figure 1. Structure of some widespread MPSs.
Homopolysaccharides: 1.1. Pullulan; 1.2. Dextran and 1.3. β-glucan (frequently component of cell wall-endopolysaccharide). Heteropolysaccharides: 1.4. Xantan and 1.5. Gellan.

glucose, galactose and mannose, but many other neutral, amino sugars and uronic acids are often present, too. Also they can contain some organic ester – linked substituents and pyruvate ketals, which give them anionic character and increase their lipophilicity (Freitas et al., 2011). Despite of differences in monosaccharide composition, they differ in charge, type and configuration of glycosidic linkages (which affects the rigidity of molecule), molecular weight, length and frequency of branches (from which rheological properties depends) (Duboc and Mollet, 2001).

EPSs have many various functions for microbial cells. Their role is mainly protective, against competition or extreme environmental conditions. On the other hand, their specific functional physicochemical properties are also the main reason for great interest for their application in various fields of industry – food, cosmetics, pharmaceutical, as well as in biomedicine and ecology. Despite that, only several have been industrially used since today (Freitas et al., 2011). Since they are involved in biofilm formation, they can make some serious problems (e.g. in food, process and paper industry, cooling water systems), because of causing food and water reservoirs spoiling, toxin production, pipes blockage, corrosion of the process equipment, etc. (Shia and Zhua, 2009).

One of the most important applications of microbial polysaccharides in industry is in drilling fluids for oil recovery, sequestration of toxic compounds, activating of sludge settling, suppressing of gasoline vaporization and chemical absorption of carbon dioxide. They are also used as depollution agents, and in processes of biofloculation, settling and dewatering of activated sludge (Neyens et al., 2004). Due to their texturing properties, microbial polysaccharides (such as xanthan, galactomannans and native or modified starch), independent or in combination with some other polymers, are widely applied in controlling the rheological properties and stabilizing oil-in-water emulsions (Desplanques et al., 2012).

The special rheological properties of xanthan, polysaccharide isolated from *Xanthomonas campestris* makes this polysaccharide very suitable for application in many industries, but especially in oil industry, for ‘enhanced oil recovery’ applications. In petroleum industry, it is used for oil drilling, fracturing, pipeline cleaning, and also in micellar – polymer flooding as a tertiary recovery operation (Palaniraj and Jayaraman, 2011). Emulsan, a lipopolysaccharide from *Acinetobacter calcoaceticus* is also able to stabilize oil-in-water emulsions, just like some other complexes made of polysaccharides, proteins and lipids (Lang, 2002).

Cyanobacterial EPSs, which are complex heteropolysaccharides, have a strong anionic character due to high content of uronic acids. Because of large number of negative charges, these polymers can effectively remove heavy metal ions from water solutions, and therefore can be used in remediation of polluted aqueous environments (De Philippis et al., 2011).

In food industry, microbial polysaccharides are mostly used as thickening, stabilizing, emulsifying, binding, structure creating and gelling agents, because of their high viscosity in aqueous media (Freitas et al., 2011). They need to have physicochemical properties that can satisfy some food processing conditions – variations in pH, temperature, ionic strength, influence of other food components etc. Many of EPSs have such properties, however, only two are allowed for use as additives in the food industry in Europe and United States: xantan and gellan (Donot et al., 2012).

Certain strains of lactic acid bacteria synthesize EPSs, which participate in production of fermented milk products: yoghurt, cheese, fermented cream, kefir, etc. They are very important for final texture (as biothickening agents they improve the reology of product – viscosity which makes them slimy and fluid, and elasticity which gives them firmness and gum-like properties), taste and stability of products (they bind water and limit syneresis). EPSs from lactic acid bacteria don’t have only technological utility, but also have some health benefits on consumers. Their viscosity increases time that fermented product spends in gastrointestinal tract, which helps its colonization with probiotic bacteria. Also they can be metabolized by the colon microorganisms to short-chain fatty acids (acetate, propionate, butyrate), and those can not only provide energy to epithelial cells, but also play role in the prevention of colon cancer (Duboc and Mollet, 2001). Because of this property, some microbial polysaccharides are defined as prebiotics – “non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a

limited number of bacteria in the colon, and thus improve host health" (Scantlebury Manning and Gibson, 2004).
Microbial exopolysaccharides can be shaped into micro/nanoparticles, scaffolds and hidrogels, which can be applied in biomedicine for drug delivery, encapsulation of bioactive compounds, imaging, tissue engineering and wound dressing. Some of those EPSs (xanthan, sulfated dextran, sulfated curdlan) posses some biological activity too, so their use is very important for design of such pharmaceuticals. Also, some formulations that contain fucose and oligosaccharides obtained by its hydrolysis are known as anti-cancerogenic, anti-inflammatory and anti-aging agents (Freitas et al., 2011). After adequate chemical modifications, they may serve as covalent carrier for drugs, e.g. antibiotics, facilitating their solubility in water while decreasing citotoxicity and retaining drug activity. The main advantages for use of such polymers in this kind of formulations are their biocompatibility, nontoxicity and biodegradability (Zhanga et al., 2011).
β-Glucans are structural polysaccharides of the cell wall of fungi, yeast, some bacteria and cereals. Those polysaccharides are non-digestible and can be, in some degree, fermented by intestinal microbial flora. They have the ability to stimulate immune system in the defense against some viruses, bacteria, protozoa and fungi, but also have anti-tumor activity even thou they have no direct cytotoxic effects. Therefore they are known as immunomodulators and anti-cancer agents. They participate in activation of macrofages, neutrophils, natural killer cells and lymphocytes. The main advantage of these supplements over conventional immune therapies is in possibility of oral administration, less costs and fewer side effects, but their independent use as therapeutics is still not enough (Chan et al., 2009; Murphy et al., 2010).
Bacterial cellulose, which has identical chemical composition as those from plants, has unique fibrillar nanostructure. It is used as component of high quality audio membranes, electronic paper, membranes of fuel cells and biomedical material (Weia et al., 2011). One of the most interesting uses is in treatment of chronic wounds, such as venous leg and diabetic ulcers, bedsores and burns, where this polysaccharide, in the form of film, provides necessary moist environment. It also helps in eliminating pain symptoms by isolating the nerve ending, provides good barrier against infection, and decreases healing time (Czaja et al., 2006). However, cellulose does not have antibacterial properties, but those can be obtained by impregnation with silver nanoparticles (Maneerung et al., 2008) or benzalkonium chloride (Weia et al., 2011).
To date, polysaccharides isolated from microorganisms are of great interest in the overall hydrocolloid market, even thou they are not represented enough. Research interest in its production is exponentially growing, especially because of possibilities of using low-cost substrates in their production and improving downstream processing, as well as possibility of metabolic engineering which allows controlled production of polymers with exact, fine-tuned properties. By altering conditions of some biotechnological process for obtaining microbial polysaccharides, such as nutrient media for growth of polysaccharide producing microorganism, carbon and nitrogen content, temperature, pH, aeration, stress conditions etc., polymers with various chemical composition, structure and consequently properties, can be obtained. In the next few years, significant increase in number of different products and technologies based on microbial polysaccharides can be expected.

REVIEW OF THE SOME OUR ESSENTIAL RESULTS IN THE MPSs RESEARCH

Our research group, Group of microbial chemistry, from Faculty of Chemistry, University of Belgrade and Department of Chemistry, Institute of Chemistry, Technology, and Metallurgy (IChTM), University of Belgrade, for several years is engaged in studying the microbial polysaccharides: dextran (in collaboration with the Faculty of Technology in Leskovac), pullulan (in collaboration with the Faculty of Technology in Leskovac) and β-glucan from the cell wall of baker's yeast (in cooperation with the fermentation industry Fermin, now Alltech-Serbia from Senta). These microbial polysaccharides are well known and have been commercial products.

Dextran is a glycan which can be characterized by its main structural feature, a backbone chain of α-(1→6)-glycosidic linked D-glucose units and *branch* points at *position 2*, 3 or 4. Strains of Leuconostoc *mesenteroides* are involved in *the production of this glucan*. Fractions of dextrans have application in medicine, biochemistry and biotechnology. One of the objects of our interest, in respect of this polysaccharide, was influence of process conditions on effects in biotechnological production of dextran by *Leuconostoc mesenteroides* (Lazic et al., 1993).
Pullulan is extracellular microbial polysaccharide which is produced by the yeast-like fungus *Aureobasidium pullulans*, strain CH-1 (IChTM, Collection of Microorganisms). This polysaccharide can be described as a linear α-D-glucan that consits of α-1,6-linked a regular repeating maltotriosyl units having about 7% of maltotetraosyl units randomly distributed in the polysaccharide chain (Jakovljevic et al., 2001.). *A. pullulans* CH-1 exhibited two beneficial effects, i.e., a biosorption of metal pollution and the production of pullulan (Radulovic et al., 2008). The choice of pullulan for object of our interest is based on the its properties such as non-toxicity, plasticity, lower permatibility of oxygen gas, and so on make it widely used in food packing, pharmacy, environmental protection and other industries.
In the last years β-glucans from cell wall many microorganisms attracted much attention due to their antitumor activity. Particular interest is devoted to β-glucans that isolated from cell wall of *Saccharomyces cerevisiae*. In addition to antitumor activity, these polysaccharides posses anti-inflammatory properties and manifest as nonspecific immunomodulators, as well as other, less significant and examined characteristics associated with their biological activity. Biological activity of the yeast cell wall glucan is the main reason for interest of our group for this polysaccharide (Zekovic et al., 2005). In this sense we perfected methods for isolation and purification of cell wall glucan from commercially available active dry yeast (Zlatkovic et al., 2003). By mild Pfitzner-Moffat oxidation of this glucan keto-aldehydo polymer was obtained (Zekovic et al., 2006). Bifidogenic properties of samples of β-glucans with different degrees of purity was investigated recently (Laugier et al., 2012).
The obtained results, as a result of interesting of our group for chemistry and applications of microbial polysaccharides should be a guideline for further work towards the study of biochemical properties of microbial polysaccharides, which are the subject of great interest in the world and in our laboratories.

ACKNOWLEDGEMENTS

Part of this paper is a result of the research within the Grant No. III 43004 (“Simultaneous Bioremediation and Soilification of Degraded Areas to Preserve Natural Resources of Biologically Active Substances, and Development and Production of Biomaterials and Dietetic Products”), supported by the Ministry of Education and Science of the Republic of Serbia.

REFERENCES

1. Chan, G. C. F., Chan, W. K., Sze, D. M. Y. (2009). The effects of β-glucan on human immune and cancer cells. *Journal of Hematology & Oncology*, 2 (25), 1-11.
2. Czaja, W., Krystynowicza, A., Bieleckia, S., Brown Jr., R. M. (2006). Microbial cellulose—the natural power to heal wounds. *Biomaterials, 27,* 145–151.
3. De Philippis, R., Colica, G., Micheletti, E. (2011). Exopolysaccharide-producing cyanobacteria in heavy metal removal from water: molecular basis and practical applicability of the biosorption process. *Applied Microbiology and Biotechnology, 92,* 697–708.
4. Desplanques, S., Renou, F., Grisel, M., Malhiac, C. (2012). Impact of chemical composition of xanthan and acacia gums on the emulsification and stability of oil-in-water emulsions. *Food Hydrocolloids*, 27, 401-410.
5. Donot, F., Fontana, A., Baccou, J. C., Schorr-Galindo, S. (2012). Microbial exopolysaccharides: Main examples of synthesis, excretion, genetics and extraction. *Carbohydrate Polymers, 87,* 951-962.
6. Duboc, P., Mollet, B. (2001). Applications of exopolysaccharides in the dairy industry. *International Dairy Journal, 11,* 759–768.
7. Freitas, F., Alves, V. D., Reis, M. A. M. (2011). Advances in bacterial exopolysaccharides: from production to biotechnological applications. *Trends in Biotechnology, 29 (8),* 388-398.

8. Lang, S. (2002). Biological amphiphiles (microbial biosurfactants). *Current Opinion in Colloid & Interface Science, 7,* 12-20.
9. Maneerung, T., Tokura, S., Rujiravanit, R. (2008). Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing. *Carbohydrate Polymers, 72,* 43–51.
10. Murphy, E. A., Davis, J. M., Carmichael, M. D. (2010). Immune modulating effects of b-glucan*. Current Opinion in Clinical Nutrition and Metabolic Care, 13,* 656–661.
11. Neyens, E., Baeyens, J., Dewil, R., De Heyder, B. (2004). Advanced sludge treatment affects extracellular polymeric substances to improve activated sludge dewatering. *Journal of Hazardous Materials, 106B*, 83–92.
12. Palaniraj, A., Jayaraman, V. (2011). Production, recovery and applications of xanthan gum by *Xanthomonas campestris*. *Journal of Food Engineering, 106,* 1–12.
13. Scantlebury Manning, T., Gibson, G. R. (2004). Prebiotics*. Best Practice & Research Clinical Gastroenterology, 18 (2),* 287–298.
14. Shia, X., Zhua, X. (2009). Biofilm formation and food safety in food industries. *Trends in Food Science & Technology, 20,* 407-413.
15. Weia, B., Yanga, G., Honga, F. (2011). Preparation and evaluation of a kind of bacterial cellulose dry films with antibacterial properties. *Carbohydrate Polymers, 84,* 533–538.
16. Zhanga, H., Li, F., Yic, J., Gub, C., Fana, L., Qiaoa, Y., Taoa, Y., Chenga, C., Wua, H. (2011). Folate-decorated maleilated pullulan–doxorubicin conjugate for active tumor-targeted drug delivery*. European Journal of Pharmaceutical Sciences, 42,* 517–526.
17. Lazic, M.L., Veljkovic, V.B., Vucetic, J.I., Vrvic, M.M. (1993). Effect of pH and aeration on dextran production by Leuconostoc mesenteroides. *Enzyme and Microbial Technology* 15 (4) , 334-338.
18. Jakovljević, D., Vrvić, M.M., Radulović, M., Hranisavljević-Jakovljević, M., (2001). Fine structural analysis of the fungal polysaccharide pullulan elaborated by Aureobasidium pullulans, CH-1 strain *Journal of the Serbian Chemical Society* 66 (6) 377-383.
19. Radulović, M. Đ., Cvetković, O. G., Nikolić, S. D., Đordević, D. S., Jakovljević, D. M., Vrvić, M. M., (2008). Simultaneous production of pullulan and biosorption of metals by *Aureobasidium pullulans* strain CH-1 on peat hydrolysate, Bioresource Technology **99** (14) 6673-6677.
20. Zeković, Dj., Kwiatkowski, S., Vrvić, M.M., Jakovljević, D., Moran, C.A., (2005) Natural and Modified (1$\rightarrow$3)-β-D-Glucans in Health Promotion and Disease Alleviation, Crit. Rev.Biotechnol. **25** 205-230.
21. Zlatković, D., Jakovljević, D., Zeković, D., Vrvić, M.M., (2003). A glucan from active dry baker's yeast (Saccharomyces cerevisiae): A chemical and enzymatic investigation of the structure, *Journal of the Serbian Chemical Society* 68 (11) , pp. 805-809.
22. Zeković, Dj., Radulović, M., Nastasović, A., Vrvić, M. M., Jakovljević, D., Kogan, G., (2006). Mild Pfitzer-Moffat Oxidation of the (1$\rightarrow$3)-β-D-Glucan from *Saccharomyces cerevisiae*, Chem. Pap.- Chem. Zvesti **60** (3) 243-248.
23. Laugier, O.B., Spasic, S. D., Mandic, V., Jakovljevic, D., Vrvic, Miroslav M., (2012). The effects of repetitive alkaline/acid extractions of Saccharomyces cerevisiae cell wall on antioxidative and bifidogenic efficacy. International Journal of Food Science and Technology, 47(2), 369-375.