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CHARACTERIZATION AND ENCAPSULATION OF POLYPHENOLS AND XYLOOLIGOSACCHARIDES FROM OAT BRAN IN WHEY PROTEIN-MALTODEXTRIN COMPLEX COACERVATES

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Abstract

The trend for investigation of bioactive compounds, especially polyphenols and xylooligosaccharides (XOS) from cereals has increased in modern. These ingredients have proved to be effective for inhibiting or preventing different human ailments such as coronary, artery and cardiovascular diseases, several types of cancer due to their antiradical, antioxidant and anti-inflammatory properties. In this study, novel biotechnological approaches were suggested for the processing of grain by-products (oat bran) into functional food ingredients with antioxidant and prebiotic properties.

It is recognized that encapsulation is an effective technology in protecting the bioactive ingredients during processing and storage, and it prevents possible interaction with other food constituents. In order to obtain devices for controlled delivery of bioactive compounds, this investigation utilized whey protein as a wall material in combination with maltodextrin to form amorphous glassy matrices during the encapsulation process. The processed material was oat bran. The technology of its biotransformation was based on the ultrasound processing and enzymatic hydrolysis. The amount of protein was determined using the Kjeldahl method, carbohydrates and ash were determined according to the standard methods, XOS - using the thin layer chromatography. Extracts were examined for their scavenging effect on the diphenyl-2-picrylhydrazyl (DPPH) free-radical activity. The study of prebiotic activity was performed by cultivating *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on standard nutrient media with the addition of skim milk with XOS and skim milk with a mixture of biologically active substances (XOS and polyphenols) at a temperature of (37 ± 1) ^{0}C for 72 hours. Separation and quantitative determination of extract were followed using HPLC apparatus "Stayer" (Akvilon, Russia) system column Phenomenex Luna 5u C18(2) (250 x 4.6 mm). Total phenolic content was measured by the modified Folin-Ciocalteu method. To prepare microcapsules, whey protein concentrate (WPC) and maltodextrin (MD) solutions were mixed at ratios 6 : 4, 4 : 6 and 5 : 5 by gentle magnetic stirring for 1 h, and extracts (10% w/w) was then added and mixed for 15 min. Then, mixtures were treated by ultrasonication, and 10% w/w of guar gum solution as double wall material was added to each mixture under stirring. Finally, the microcapsules were dried to produce solid or powder microcapsules. The encapsulation efficiency (EE) was determined as a ratio of encapsulated phenolic content to total phenolic content. A digestion protocol that simulates conditions of the human gastric and intestinal tract was designed to investigate the effect of the structural characteristics of capsules on release kinetics of extracts.

Obtained data indicates a high antioxidant activity of polyphenols extracts. It is established that ultrasonic treatment improves the kinetics of extraction and yield of polyphenols with an increase in antioxidant activity. The results of the study on the change in the antioxidant activity of polyphenol concentrate during storage did not reveal changes within 8 months at a temperature of 20 ± 1 ^{0}C and a relative humidity of 70 ± 5%. The study of the growth dynamics of *L. acidophilus* and *B. bifidum* confirm the presence of prebiotic properties of XOS and their selectivity. It is noted that the accumulation of biomass of prebiotic

cultures occurs faster with the use of XOS and lactulose as compared to milk. In order to protect sensitive bioactive compounds the capsules were prepared with a studying their properties depending on a wall material. Thus, there were no significant changes in EE, however the highest EE of 95.28% was recorded at WPC : MD ratio of 60 : 40. The release percent of polyphenols coated in capsule during enzymatic hydrolysis *in vitro* was ranged between 70 and 83% after 2 h of digestion process.

Thus, the feasibility of biotechnology for transforming oat bran into functional ingredients has been confirmed, which will further allow them to be used in new technological solutions with bifidogenic properties. It is also proved that utilization of WPC which considers as a waste product of cheese manufacture can be beneficial for polyphenols encapsulation as a wall material.

***Key words**: Polyphenols, Xylooligosaccharides, Encapsulation, Whey protein.*

1. Introduction

The modern concept of a healthy diet assumes increasing of the food products biological value by introducing natural physiologically functional ingredients, which become sources of important biologically active substances. Balanced functional food products included in the diet not only provide human body with plastic material and energy, but also control specific physiological functions and contribute to the maintaining of health by reducing the risk of diseases [17 - 20]. Secondary products of grain processing are rich sources of physiologically functional ingredients, biotransformation of which made it possible to obtain a number of biologically active substances of different chemical nature with a wide range of physiological effects [1 - 2, and 16]. The antioxidant components in grains are mainly phenolic compounds including ferulic, protocatechuic, sinapinic, vanillic, p-hydroxybenzoic, and p-coumaric acids distributed in the bran fractions. The most of phenolics in bran are insoluble and are bound by ester and ether linkages with polysaccharides, such as arabinoxylan and lignin, in the cell wall [3 - 8, 14 - 15, and 21 - 22].

Due to their antioxidant properties, polyphenols are capable of prevent the spread of free radicals arising due to inflammation and radiation. Efficiency of phenolic antioxidants most active in cancer of stomach. Polyphenols by binding free iron dramatically reduce the amount of iron involved in oxidative reactions. So, in the intestines may contain a large number of potentially active iron ions (especially in the case of the predominance of meat products in the nutrition of the population). The presence of phenolic compounds acts as an inhibitory agent and reduces activity of iron ions, thus affecting the rate of carcinogenesis [5].

It was found that the effect of magnesium oxide on tumor growth in rats is suppressed under the action of phenolic compounds. Enabling high polyphenols in the feed did not have a significant adverse effect on animals even after 6 months of continuous feeding. The results of studies by foreign scientists also show that phenolic compounds can suppress active iron ions reactions with blood cells. In some hemoglobinopathies such as sickle cell anemia, excessive activation of peroxidation cell membranes with active iron leads to accelerated destruction red blood cells and a deep violation of their structure and functions. Antioxidants able to inhibit the destruction of red blood cells due to binding iron [10-12]. The antioxidant effects of polyphenols have been recognized in various experimental models of heart attack, pneumonia, and ulcers the stomach. The protective role of phenolic compounds against peroxidation colon lipids associated with high levels of active iron, has been proven through experimental studies in rats, mice and pigs [29].

Milk proteins have several unique and diversified functional properties. Nowadays, using milk proteins as the delivery vehicle for bioactive compounds become a new trend that received much attention [5]. Whey proteins are a mixture of globular proteins of variable composition and functional properties. β-lactoglobulin is the major whey protein and the functional properties of whey protein isolates (WPI) and WPC, are largely controlled by it. The β-lactoglobulin and whey protein preparations have been used as a delivery system for bioactive compounds [30]. To avoid the drawbacks of using whey proteins for the delivery vehicle of bioactive compounds which are finding wide applications nowadays. The core/shell nanoparticles are nanostructures that have a core made from a material coated with another material [19]. Different kinds from materials have been used for microencapsulation, namely polysaccharides, lipids (mono- and diglycerides) and proteins (casein, whey proteins and gelatin) in different encapsulation methods [32]. The one of most commonly used coating materials in the food industry was the MD of different dextrose equivalents. Maltodextrin is forming amorphous glassy matrices during the encapsulation process to protect the encapsulated material from oxidation [23, 33].

Therefore the aspects of encapsulation of phenols from grains in the combination of whey protein and maltodextrin is under the research over the word. The issue of chemical stability in the human gastrointestinal tract is of concern, and this work aims to adapt encapsulation protocols for phenolic extracts oat bran ns following three distinct extraction methods. It will

then evaluate the stability of these extracts *in-vitro* and identify the relationship between structural properties of the polymeric carrier and bioactive compound release on the basis of classical diffusion theory.

2. Materials and Methods

2.1 Materials

To obtain oat bran the "Tyumen golozerniy" variety of oat was selected. The oat bran used in the experiment was evaluated according to the regulatory and technical documents for their quality.

Enzyme preparations were supplied by Sigma Aldrich: α-amylase from *Bacillus subtilis* (2000 U/g), glucoamylase from *Aspergillus awamori* (6000 U/g), protease from *Bacillus subtilis* (70 U/g), Viscozyme L cell wall degrading enzyme complex from *Aspergillus* spp., lysing Enzyme from *Aspergillus* spp. with a number of activities (β-glucanase - 100 U/g, xylanase - 50 U/g, cellulase - 70 U/g, pektinesterase - 40 U/g, and feruloesterase).

Pure cultures of *Bifidobacterium bifidum and Lactobacillus acidophilus* were obtained from the museum of Department of Microbiology and Biotechnology of Saratov State Agrarian University.

Analytical reagents included sodium tetraborate, sodium dodecyl sulfate, calcium chloride, sodium chloride, hydrochloric acid and monobasic potassium phosphate were obtained from BDH Chemicals (Poole, England). All reagents were used without further purification. Bile salts, pancreatin (6,000 U) and pepsin (3,600 U) were purchased from Aventis Farm Ltd (India).

2.2 Experimental analysis

2.2.1 Phenolic determination

Total phenolic content (TPC) was estimated with the Folin-Ciocalteu method with some modifications [4]. Briefly, 0.2 mL of each sample was added to 0.8 mL of a freshly prepared Folin-Ciocalteu reagent (diluted to 1 : 10 v/v with milliQ water) for oxidation. After incubation for 10 minutes, 2 mL sodium carbonate (7.5 g/100 mL) was added to neutralize the solution. Next, 7 mL milliQ water was added, vortex mixed, and allowed to stand for 2 hr at ambient temperature in the absence of light. A UV-vis spectrophotometer (Lambda 35, Perkin Elmer, Singapore) was used to record the sample absorption at 720 nm. The content of polyphenol compounds in the extracts was calculated from a calibration curve which was constructed with series of water concentrations of a tannin standard obtained from Sigma Aldrich (CAS 1401-55-4). Prior to analysis, the calibration curve was constructed using quercetin.

2.2.2 Surface phenolic content (SPC)

For the determination of SPC, the method of Saenz *et al*., [36 - 37], was used. An amount of 100 mg of microcapsules were dispersed with 1 mL of ethanol and methanol mixture (1 : 1, v/v) for 1 min. The amounts of surface phenolic compounds were measured and quantified with the same method described in TPC section.

2.2.3 Encapsulation efficiency (EE)

The EE is the ratio of encapsulated phenolic content to total phenolic content. Encapsulated phenolic content (EPC) is determined by taking the difference of TPC and SPC. Encapsulation efficiency of microcapsules was calculated according to equation:

$$EE = \left(\frac{TPC - SPC}{TPC}\right) \times 100 \quad (1)$$

2.2.4 Antioxidant activity determination

Extracts were examined for their scavenging effect on the diphenyl-2-picrylhydrazyl (DPPH) free-radical activity. In doing so, ethanol solution of DPPH (0.05 mM) (300 mL) was added to 40 mL of extract at different concentrations (0.02 - 2 mg/mL). Ethanol 96% (2.7 mL) was added and the mixture was shaken vigorously. It was left to stand for 5 minutes and absorbance was measured spectrophotometrically at 517 nm. Ethanol was used to set the absorbance to zero. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. The radical scavenging activity of the tested samples, expressed as percentage of inhibition, was calculated according to the following equation [4]:

$$\text{Percent (\%) inhibition of DPPH activity} = [(AB - AA) / AB] \times 100 \quad (2)$$

Where: AA and AB are the absorbance values of the test and of the blank sample, respectively. A percent inhibition *versus* concentration curve was plotted and the concentration of each test solution required for 50% inhibition was determined and represented as the IC50 value.

2.2.5 Prebiotic activity determination

This determination was carried out using *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. The preparation of the inoculum of the microorganism *L. acidophilus* was carried out by introducing dry biomass into sterile skimmed milk and cultured for 48 hours at a temperature of 37 ± 1 ^{0}C for biomass accumulation. To obtain the inoculum of *B. bifidum* culture was placed on MRS which contains 10 g of peptone, 20 g of yeast extract, 20 g glucose, 1 g twin 80, 2 g of

potassium phosphate dibasic, 5 g sodium acetate, 2 g of triammonium citrate, 0.2 g of magnesium sulfate, 0.05 g of manganese sulfate ($MnSO_4 \times 4H_2O$), and topped with meat water in a volumetric flask with a capacity of 1000 cm^3. The components were dissolved by heating in a water bath and the pH was adjusted to 6.2 ± 0.1 at 25 ^{0}C. Then the medium was sterilized at a temperature of 121 ± 1 ^{0}C for 15 minutes. The tubes with the nutrient medium were stored at a temperature of 4 ± 1 ^{0}C for no more than 30 days. Next, the strains of microorganisms were sown on the medium and left for 48 hours at a temperature of 37 ± 1 ^{0}C for biomass accumulation. Cultivation of prebiotic cultures was carried out on standard media with the addition of the studied prebiotics (XOS and a mixture of biologically active substances consisting of xylooligosaccharides and polyphenols) at the rate of 2% mass fraction in the culture fluid for 72 hours. Standard nutrient medium with the addition of lactulose and skim milk was used as a control. The mass fraction of starter culture was 2%. Cultivation was carried out at a temperature of 37 ± 1 ^{0}C.

To determine the number of microbial cells the dilutions of *L. acidophilus* were prepared up to 10^{15}. From each of the last three dilutions, 1 cm^3 was added to two parallel tubes with sterile skim milk and cultured at 37 ± 1 ^{0}C for 72 hours. At the expiration of time in the test tubes, the formation of a clot was observed which indicates that the maximum accumulation of biomass was attained, and the colonies were counted. The number of microbial cells of *B. bifidum* was determined by sowing 1 cm^3 of ten-fold dilutions from 10^8 to 10^{13} of product in test tubes with semi-liquid thioglycolic medium of the following composition (g/L): casein peptone - 15; yeast extract - 5; glucose - 5.5; L-cystine 0.5; sodium chloride - 2.5; sodium thioglycolate - 0.5; resazurin sodium - 0.001; and agar-agar - 0.75. After incubating the cultures at a temperature of 37 ± 1 ^{0}C for 72 hours the colonies were counted as "cloves" in test tubes [3].

2.2.6 Moisture content

The moisture content was determined using a moisture analyser Evlas 2M (Sigagropribor, Krasnoobsk, Russia), Loss-On-Drying Methods. Three grams of sample was loaded on an aluminium plate and heated to 103 ^{0}C for 40 minutes. The averages of three values of moisture content were reported.

2.2.7 Protein, fat, soluble and insoluble dietary fibers content

Protein content was determined using a Kjeldahl method, and fat with a Soxhlet extraction method. Determination of soluble and insoluble dietary fiber was done by using the enzymatic method [3].

2.2.8 Xylooligosaccharides determination (XOS)

Qualitative and quantitative composition of xylooligosaccharides (XOS) was determined using a thin layer chromatography on plates Sorbfil, size 10 x 15 cm with silica gel CTX - 1A. The mobile phase, n - propanol : ethyl acetate : distilled water as 6 : 1 : 3. The plates after elution were treated with a developer, a 50% aqueous solution of sulfuric acid and dried at a temperature of 120 ± 1 ^{0}C for 5 min. Quantitative determination of XOS was performed using spectrophotometric method. For this, the areas of the sorbent corresponding to the zones of the carbohydrates under study were transferred to test tubes, 0.5 cm of aniline phthalate reagent was added and heated (105 - 110 ^{0}C, 1 hour). The reagent was prepared: 1.66 g of o-phthalic acid and 0.91 cm of aniline were dissolved in a mixture of 48 cm n-butanol, 48 cm of ether and 4 cm of water. The colored solution was cooled in a test tube and solid particles were dissolved, 4 cm of a mixture of concentrated hydrochloric acid and acetone (1 : 25) were added, kept for 1 h, centrifuged (8,000 rpm, 15 min.) and photometricized at a wavelength of 520 nm. The carbohydrate content in the sample was determined by the calibration curve constructed previously [3].

2.2.9 Microcapsules investigations

Surface morphology of the microcapsules was investigated using a Zeiss Libra 120 transmission electron microscope.

For *in-vitro* digestion studies, capsules were rinsed with deionized water to remove excessive surface oil. They were stored in a container with fresh 12 mol·L calcium chloride solution at 5 ^{0}C for 22 h before characterization [4, 24]. A digestion protocol that simulates conditions of the human gastric and intestinal tract was designed in order to investigate the effect of the structural characteristics of alginate capsules on release kinetics of fish oil.

In the gastric stage, we used a simulated gastric fluid (SGF) containing 2.0 g/100 g NaCl solution in Millipore water, 3600 U·mL^{-1} pepsin, with pH being adjusted to 2.0 with 1 mmol·L HCl. Twenty five grams of gel capsules were added to 500 mL of pre-warmed (37 ^{0}C) SGF. They were incubated in a water bath at the same temperature under constant shaking (100 horizontal strokes per minute) for predetermined periods of time. After 15, 30, 45, 60, 75, 90, 10,5 and 120 minutes of incubation, alginate capsules were washed with deionized water and 200 mg were taken at each stage

of the simulated gastric digestion for analysis of fatty-acid content.

In the intestinal stage, initial digestion of the oil-loaded alginate capsules was conducted in SGF for 2 h at 37 ^{0}C. Then, 20 g of the capsules were mixed with 400 mL of a simulated intestinal fluid (SIF) comprising 0.7 g/100 g monobasic potassium phosphate, 0.1 g/100 g bile salts and 0.4 g/100 g pancreatin. The pH of SIF was adjusted to 7.5 with 0.5 mmol·L NaOH. Samples were incubated at 37 ^{0}C under constant shaking (100 horizontal strokes per minute) for twenty minutes. In the end, 200 mg of alginate capsules were washed with Millipore water and analyzed for oil content as before [4].

Separation and quantitative determination of extract were followed using HPLC apparatus "Stayer" (Akvilon, Russia) system column Phenomenex Luna 5u C18(2) (250 x 4.6 mm).

2.2.10 Statistical analysis

Statistical analysis was performed in triplicate and mean values are reported with a standard deviation. Data were evaluated by a two-way analysis of variance (ANOVA). The level of significance ($p < 0.05$) was determined through Tukey test using IBM SPSS Statistic 23 software (IBM Corporation, Somers, NY).

3. Results and Discussion

3.1 Study of functional properties of oat bran extracts

A significant part (up to 80%) of oat polyphenols are contained in the bound state with a biopolymer matrix. As a result, drastic measures are needed to destroy this complex of biopolymers to extract biologically active components. Hydrothermal methods of exposure, chemical reagents and enzyme preparations in combination with the optimal choice of treatment mode can lead to the separation of fragments of oligomers of various degrees of polymerization and release due to the destruction of ether bonds, molecules of ferulic and coumaric acids. The biomodification of oat bran according to the developed technology includes thermal and mechanical pretreatment followed by the application of ultrasound, chemicals and stepwise enzymatic hydrolysis during the extraction. Thus, the step by step procedure was carried out as reported earlier on [3, 13, 27, and 38].

The resulting biologically active substances (BAS) concentrates, in addition to polyphenols, contain: protein from 5.9% to 6.7%, carbohydrates from 80.7% to 82.6% including XOS up to 71.5% and ash from 10.3% to 10.9% depending on the extraction method (Table 1).

To obtain polyphenols and XOS concentrates, the BAS concentrate was treated with ethanol (98%) in a ratio of concentrate/ethanol 1 : 3. Due to the effect of ethanol on the syrup, a separation of fractions, dissolution of phenolic compounds and precipitation of XOS were occurred. Centrifugation at 5,000 rpm for 25 minutes allows to fully separate the fractions in two independent media. The supernatant that consists phenolic compounds was concentrated to a final moisture content of 30% (w/w) and then dried a final humidity of 8 ± 1% (w\w). The polyphenol concentrate was a crystalline powder of light - yellow or light - brown color with vanilla-grain odour. It contained up to 91% of polyphenols, 8% of carbohydrates, and 2% of protein. It is shown in Figure 1 that the main component of polyphenols preparation was the ferulic acid (92 for ultrasound, 86% for the chemical extraction and 90% for the extraction with enzymatic hydrolysis). Based on published data, ferulic acid is the main phenolic acid of a polyphenolic composition in oat grain, exceeding by orders of magnitude coumaric, chlorogenic, gallic, and protocatechuic acids.

The antiradical activity of oat bran polyphenols of this study was determined by the DPPH method based on the reaction of a stable free radical of 2,2'-diphenylpycrylhydrazine with a mobile hydrogen atom or electron in an alcohol solution of the test substance. This is further corroborated in Figure 2 that summarizes the antioxidant activity of polyphenols following application of our experimental protocol. All the polyphenol preparation have a high antioxidant activity which can be compared with the literature [4, 18, 27, and 28]. However, the polyphenols obtained by chemical extraction reveal lower antioxidant activity (965.2 u.e.a./cm^3) as compared to the polyphenols of

Table 1. The chemical composition of BAS concentrates from oat bran [3]

Method of extraction	Moisture, %	Protein in dry matter, %	Ash in dry matter, %	Carbohydrates in a dry matter, %		Polyphenols in a dry matter, %
				XOS	Residue carbohydrates	
Physical extraction	31.2	6.7	10.9	35.3	45.3	1.0
Chemical extraction	30.6	6.3	10.5	60.4	22.2	0.7
Enzymatic hydrolysis	29.4	5.9	10.3	71.5	11.0	0.8

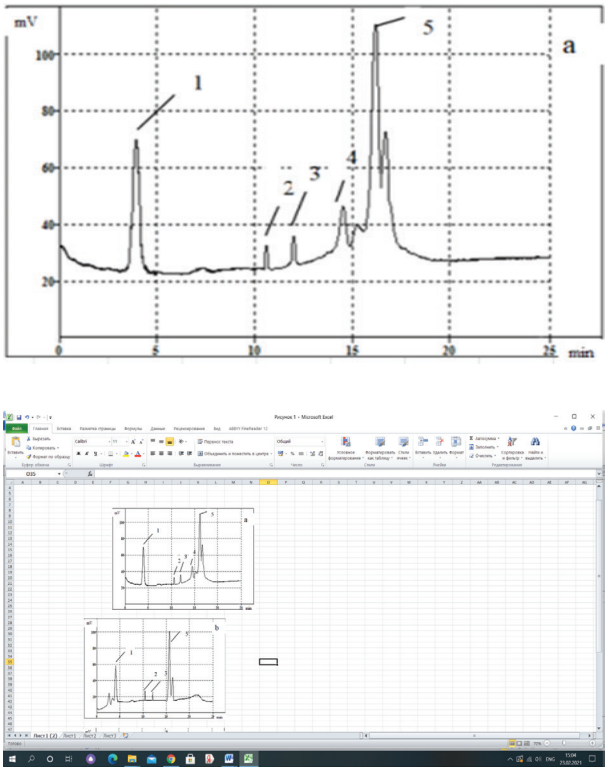

Figure 1. HPLC analysis of oat bran polyphenols obtained using physical (a), chemical (b) and enzymatic hydrolysis (c) extraction methods: 1 - gallic, 2 - 4-hydroxybenzoic, 3 - chlorogenic, 4 - p-coumaric, 5 - ferulic acids

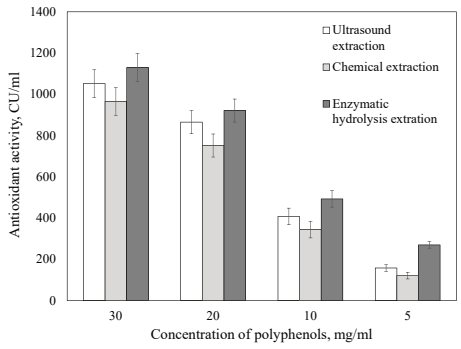

Figure 2. Antioxidant activity of extracted polyphenols obtained with different methods depending on their concentration

ultrasound (1052.2 u.e.a./cm^3) or enzymatic hydrolysis (11302 u.e.a./cm^3) methods of production. It is known that under the influence of ultrasonic vibrations there is a rapid and active destruction of intracellular tissues of plant materials, which leads to an intensification of the extraction process and an increase in the content of biologically active compounds in solution. The greater degree of extraction with the ultrasound treatment leads to a higher antioxidant activity, as compared to chemical treatment. The results of the study did not reveal changes in the anti-radical activity within 8 months at a temperature of 20 ± 1 ^{0}C, humidity 70 ± 5% [3].

The XOS precipitated with ethanol are likewise subjected to drying to a final moisture content of 8 ± 1% (w/w). This was a finely dispersed powder of light brown color with a slight grainy odour. It was revealed that the quantity of arabinose and xylose in XOS concentrate obtained using enzymatic hydrolysis exceeds on 2% as compared to XOS obtained using other methods. The data indicate the predominance of xylotriosis and xylotetrosis in XOS preparations from oat bran in a total amount of up to 42.4% [7]. It is known from the literature that xylan carbohydrate oligomers exhibit a significant prebiotic effect among other xylooligosaccharides, which makes them an object of interest in terms of its use as a component for food products [3, 8, 25 - 26, 30, and 33 - 34].

As can be seen in Figure 3, the active growth of of *L. acidophilus* and *B. bifidum* was observed in the first 48 hours of cultivation. In the next 24 hours, there was

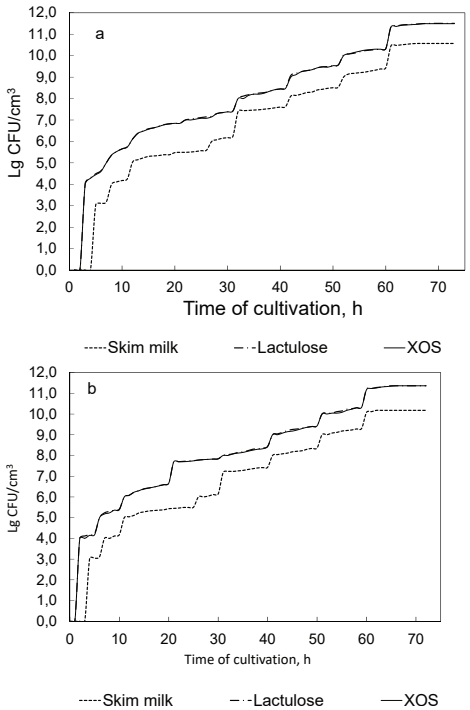

Figure 3. The growth dynamics of (a) *B. bifidum* and (b) *L. acidophilus* when cultivated on skim milk, lactulose and XOS obtained using enzymatic hydrolysis

a slight accumulation of microbial biomass, which reflects to the onset of stationary phase of growth of lactic acid microorganisms. According to the data in Figure 3, the number of *L. acidophilus* cells on the medium with the addition of the KOS preparation was 2.8×10^{11} CFU/cm^3 on the third day of cultivation. This was on 2.4×10^{11} CFU/cm^3 more than the number of colonies grown on skim milk. With further cultivation, the number of *L. acidophilus* cells did not increase. The number of colonies of *B. bifidum* on the third day of cultivation on the medium with the addition of XOS was 1.9×10^{11} CFU/cm^3, which exceeds on 1.7×10^{11} the number of colonies grown on skim milk (Figure 2). Further cultivation did not lead to an increase in the biomass of *B. bifidum*.

It is noted that the accumulation of biomass of prebiotic cultures occurs faster when using nutrient media with the XOS and lactulose as compared to milk. Thus, oat bran xylooligosaccharides have a significant stimulating effect on the growth of prebiotic cultures of *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. At the same time, the number of *L. acidophilus* and *B. bifidum* cells grown on the medium with the addition of XOS was similar lactulose, a well-known prebiotic.

Application of the stepwise treatments allowed to fully separate all the resulting products, thus greatly facilitating the advanced processing of oat bran. Upon consumption, there is an interest on encapsulated prebiotic and antioxidant activities of the concentrates, and this will be examined in the next session.

3.2 Encapsulation of polyphenols and xylooligosaccharides from oat bran in whey protein-maltodextrin complex coacervates

Microencapsulation is one famous of the techniques that are used for enhancing the stability and shelf life of phenolic compounds. When a nutraceutical encapsulated with a biopolymer, it is protected from light, oxygen, water or other environmental conditions [15]. Although the benefits from phenolic compounds and it is crucial to use it, there are susceptible to oxidize, light, heat [13], pH, water and enzymatic activities [9]. Encapsulation is an effective technology in protecting the bioactive ingredients during processing and storage, prevention possible interaction with other food constituents. Further, a real benefit of encapsulation is the ability to sustained release of the incorporated ingredients and to deliver them to a specific target at the required time and condition. In the biomedical and pharmaceutical sector have been used successfully many synthetic polymers as delivery systems [37 - 38]. However, these polymers not applicable in food industries that require food grade.

Transmission electron microscopy (TEM) was used to appear complex formation between WPI and MD. Also it shows whether it the ration WPC/MD works as a wall material for the encapsulation and properties of the resultant polyphenol capsules (Figure 4).

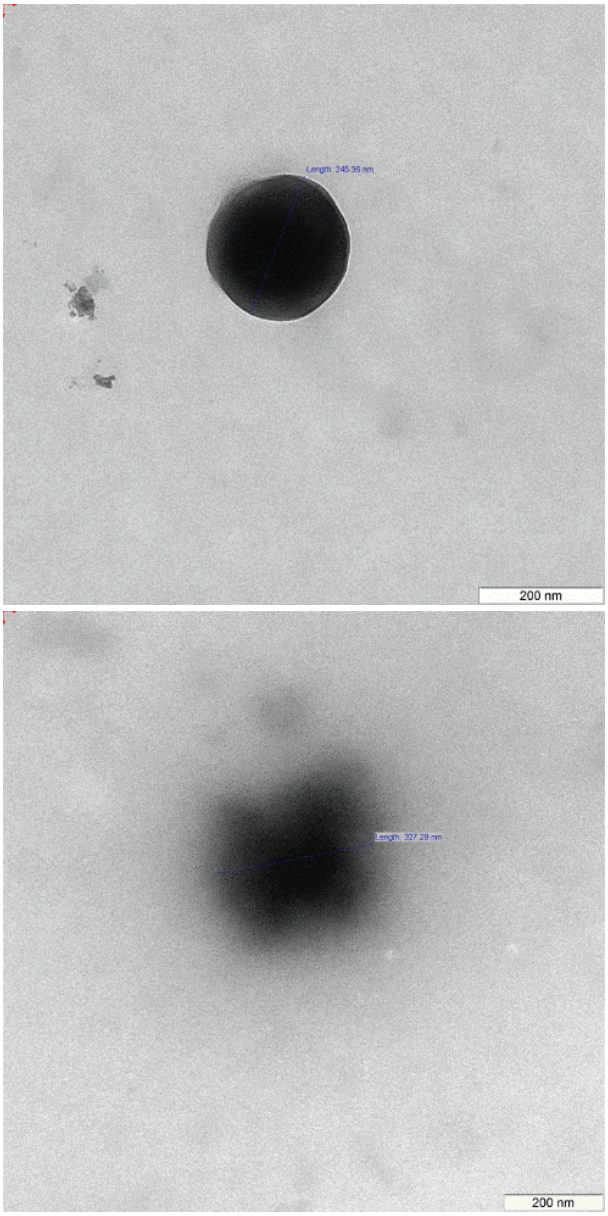

Figure 4. TEM micrographs of freeze dried microcapsules at WPC/MD ratios as a wall material being (a) WPC : MD 60 : 40, (b) WPC : MD 40 : 60

The image topography of the capsules particles indicates that there were no cracks appeared and had a capsules regular shape. Moreover it could be observed from TEM that microcapsules at ratio of 60 : 40 WPC : MD as wall material showed smallest particle diameter ranged from 321 - 338 nm. The results indicated that the use of MD with WPC as wall material was improved microencapsulation particle process and this results agreement with finding before [31, 35 - 36].

Microencapsulation efficiency as affected by WPC/MD as a wall material ratios is presented in Figure 5. Thus, there were no significant changes in EE, however the highest EE of 95.28% was recorded at WPC : MD ratio of 60 : 40. The release percent of polyphenols coated in capsule during enzymatic hydrolysis in vitro was ranged between 70 and 83% after 2 h of digestion process. In a high efficient encapsulation process, smaller amount of polyphenol content remains on the surface27. There was a significant ($p > 0.05$) difference between the surface polyphenol content of the mixture having wall material mainly WPC. This means that the presence of MD with WPC improved the WPC efficiency as a carrier of polyphenols constituents.

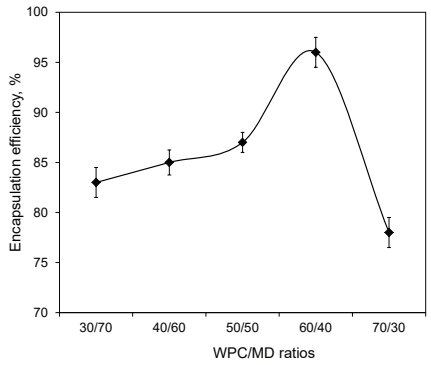

Figure 5. Effect of WPC/MD ratios on encapsulation efficiency of microcapsules

As observed in Figure 6, the release of all bioactives from the capsules was the least pronounced, i.e. higher amounts of bioactives remained in the capsules, due to their multilayer nature that enhances the ability to withstand the harsh gastric environment. Preparations of WPC/MD being 60 : 40 the least stable keeping the lowest amount of bioactives within the matrix. The figure indicated that maximum release was achieved in simulated intestinal conditions, i.e. after 120 minutes of experimentation, further underlining the structural stability of all capsules in the gastric stage.

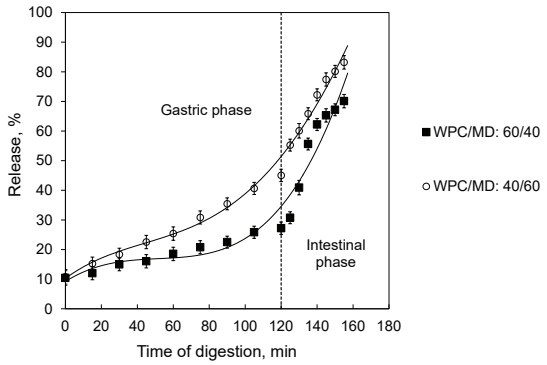

Figure 6. Polyphenols percentage released from microcapsules coated with different ratios of WPC : MD as wall material during enzymatic hydrolysis *in vitro*

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4. Conclusions

- This study has taken advantage of the potential application of secondary grain raw materials as a source of bioactive compounds, such as polyphenols and xylooligosarides. This research discovers the utilization of WPC produced from sweet whey which consider as a waste product of cheese manufacture that can be beneficial for polyphenols encapsulation as wall material.

- Our study will help the researcher to uncover the critical areas of incorporation of MD with WPC was improved EE up to a ratio of 60 : 40 WPC : MD that many researchers were not able to explore. Thus, a new theory on increased the portion of WPC in the coated wall material gave highest release percent after 120 minutes of digestion.

- The effect of ultrasound assisted extraction as well as the enzymatic hydrolysis treatment as compared to conventional chemical extraction method on the extraction process was described. Thus, the feasibility of biotechnology for transforming of oat bran into functional ingredients has been confirmed, which will further allow them to be used in novel formulation strategies with bifidogenic properties.

- In the future, of course, the issue of relationships between the structural components of matrixes should be taken into account.

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