Encapsulation of gallic acid on pectin: antioxidant activity, α-amylase and α-glucosidase inhibitory activity of complexes

IVANA BULJETA – JOSIPA VUKOJA – ANITA PICHLER – JOSIP ŠIMUNOVIĆ – MIRELA KOPJAR

Summary
Gallic acid is a phenolic acid with reported health-promoting properties such as antioxidant, antimicrobial, anticancer, antihyperglycaemic activities. Bioactive compounds, such as polyphenols, are prone to degradation during food processing, but their preservation could be achieved by encapsulation. The main objective of the present study was to encapsulate gallic acid by freeze-drying, using pectin as wall material. Various concentrations of pectin (25 $g \cdot l^{-1}$, 50 $g \cdot l^{-1}$, 75 $g \cdot l^{-1}$ and 100 $g \cdot l^{-1}$) were used for sample preparation. Additionally, the influence of complexation duration (10 min or 30 min) was also evaluated. The highest content of gallic acid (3.633 $g \cdot kg^{-1}$) was achieved in the complex where 25 $g \cdot l^{-1}$ of pectin was used with complexation of 30 min. The same complex possessed the highest antioxidant activities determined by 2,2'-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid) radical-scavenging assay, 2,2-diphenyl-1-picrylhydrazyl scavenging capacity assay, cupric reducing antioxidant capacity and ferric reducing antioxidant power assays, as well as capability of inhibition of α-amylase and α-glucosidase. Concentrations of pectin higher than 25 $g \cdot l^{-1}$, used for sample preparation, negatively affected gallic acid adsorption. Fourier transform infrared with attenuated total reflection spectroscopy analysis was conducted in order to confirm the binding of gallic acid onto pectin. From infrared spectra, it could be observed that band intensity changed and shift of bands occurred when gallic acid was present in the complex.

Keywords
gallic acid; pectin; freeze-drying; encapsulation; antioxidant activity; enzyme inhibition, infrared spectra

Gallic acid (3,4,5-trihydroxybenzoic acid) is a phenolic acid that belongs to a group of hydroxybenzoic acids. It is biosynthesized from precursors of the shikimate pathway by enzyme dehydroshikimate [1, 2]. It is found in a variety of plants, and particularly high content was found in berries, citrus fruits, tea, cereals, wine and herbs [2]. Since gallic acid possesses antioxidant, antimicrobial, anticarcinogenic, antimutagenic and antihyperglycemic properties, it was the subject of many scientific studies [3]. However, gallic acid has disadvantages such as sensitivity to extreme temperatures, oxygen and light [2]. Since these conditions in food processing often cannot be avoided, it is necessary to provide protection for sensitive compounds. That could be achieved by encapsulation, where the bioactive compound is incorporated into wall material. Choosing an encapsulation method as well as wall material is a key step for achieving efficient encapsulation. Among various encapsulation methods, such as spray drying, spray chilling or cooling, extrusion, coacervation or fluid bed coating, freeze-drying has great potential as a suitable method for gallic acid [2, 4].

Wall materials used in the food industry have some restrictions. They must be of food grade with low viscosity at high contents, good emulsifying properties, should contain a high percentage of solids, have biodegradable properties and low price [5, 6]. Polysaccharides, such as maltodextrins, β-cyclodextrins, pectin or modified starches, are common materials for encapsulation and, besides them, proteins, lipids, synthetic polymers and their combinations can be also used. Pectin is widely used in the food industry as a gelling, thickening, emulsifying and stabilizing agent. Those proper-

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ties depend on its chemical structure, especially carboxyl functional groups that determine its ability to interact with other compounds through coordination, electrophilic addition, esterification and transesterification reactions [7]. Pectin is known for its various beneficial effects on human health. Its consumption is connected with an increase in gut microbiota, production of short-chain fatty acids and gases. Intestinal fermentation products of pectin possess a positive effect on the treatment of Crohn's disease, ulcerative colitis, high blood cholesterol, diarrhea, high blood pressure and obesity [8, 9]. Pectin also demonstrated a positive effect in the reduction of blood glucose and, due to this reason, was proposed as a composite material in the delivery system for insulin [10]. Previous studies examined encapsulation of various polyphenols with various polysaccharides as wall materials such as maltodextrin, pectin, ι-carrageenan, gum arabic, maize starch, cellulose, citrus fibres, β-cyclodextrin, xanthan and chitosan alone or in combination [2, 11–18]. The literature data state that polyphenols can bind to polysaccharides through hydrogen bonds, hydrophobic interactions and van der Walls forces [16].

The aim of this study was to encapsulate gallic acid on pectin by freeze-drying. The impact of different concentrations of pectin (25 $g \cdot l^{-1}$, 50 $g \cdot l^{-1}$, 75 $g \cdot l^{-1}$ and 100 $g \cdot l^{-1}$) on gallic acid adsorption was examined. Complexation was performed during 10 min and 30 min. The obtained complexes were evaluated for gallic acid content by high-performance liquid chromatography (HPLC) analysis and by determination of the antioxidant activity and inhibition of α-amylase and α-glucosidase. Additionally, infrared (IR) spectra were recorded to confirm encapsulation.

MATERIALS AND METHODS

Materials

GENU pectin type LM-5 CS was obtained from CP Kelco (Atlanta, Georgia, USA). Gallic acid, 2,2'-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), α-amylase (from the porcine pancreas) and α-glucosidase (from *Saccharomyces cerevisiae*) were obtained from Sigma Aldrich (St. Louis, Missouri, USA). Potassium persulfate and sodium carbonate were obtained from Kemika (Zagreb, Croatia). Neocuproine, 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ) and copper (II) chloride were obtained from Acros Organics (Geel, Belgium). Orthophosphoric acid (HPLC grade > 85 %) was from Fisher Scientific (Waltham, Massachusetts, USA) and methanol (HPLC grade) was from J. T. Baker (Deventer, Netherlands). Iron (III) chloride hexahydrate, sodium acetate, ethanol and ammonium acetate were obtained from Gram-mol (Zagreb, Croatia). 4-Nitrophenyl-α-D-glucopyranoside and 3,5-dinitrosalicylic acid were obtained from Alfa Aesar (Kandel, Germany). Potassium dihydrogen phosphate was from BDH Prolabo (Poole, United Kingdom).

Preparation of complexes

In 50 ml of gallic acid solution (10 $g \cdot l^{-1}$), 25 $g \cdot l^{-1}$, 50 $g \cdot l^{-1}$, 75 $g \cdot l^{-1}$ and 100 $g \cdot l^{-1}$ of low-methoxylated pectin were added and mixed using a magnetic stirrer Stuart US152 (Buch and Holm, Hervel, Denmark) for 10 min or 30 min at room temperature (25 °C). After mixing, the mixtures were centrifuged for 15 min at 2 500 ×*g*. The supernatant was discarded and the obtained precipitate was frozen at –18 °C for 24 h and then freeze-dried in a freeze dryer Alpha 1-4 (Christ, Osterode am Harz, Germany). The freezing temperature was –55 °C, while the temperature of sublimation was in the range from –35 °C to 0 °C. Vacuum level was adapted at 22 Pa. The isothermal desorption temperature ranged from 0 °C to 21 °C under vacuum (6 Pa).

Extraction of gallic acid from complexes

Approximately 0.1 g of the complex was subjected to extraction with 5 ml of acidified methanol (0.12 $mol \cdot l^{-1}$ HCl in methanol). The extraction was performed at 25 °C for 24 h and then the mixture was filtered through filter paper. The extracts were used for HPLC determination of gallic acid content and defined spectrophotometric evaluation.

HPLC evaluation of gallic acid content

Agilent HPLC system 1260 Infinity II (Agilent Technologies, Santa Clara, California, USA) equipped with a quaternary pump, a diode array detector (DAD), a vial sampler and a Poroshell 120 EC C-18 column (4.6 mm × 100 mm, particle size 2.7 μm; Agilent Technologies) was used for determination of gallic acid content in complexes. Prior to injection, 1 ml of extract was filtered through a polytetrafluorenethylene syringe filter Chromafil Xtra PTFE-20/13, pore size 0.2 μm (Macherey-Nagel, Düren, Germany). Chromatographic separation was performed with 0.1% orthophosphoric acid as mobile phase A and 100% methanol as mobile phase B. For isocratic elution, 10 % of mobile phase A and 90 % of mo-

bile phase B for 6 min were used. Operating conditions were as follows: column temperature 27 °C, flow rate 1 ml·min^{-1} and injection volume 10 μl. Stock solution of gallic acid in 100% methanol was used for the construction of gallic acid calibration curve in concentrations ranging from 25 mg·l^{-1} to 100 mg·l^{-1} (r^2 = 0.9967). Areas of peaks were read at 270 nm. Measurements were conducted in duplicates.

Determination of antioxidant activity

Antioxidant activity was evaluated by four assays: 2,2'-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid) radical-scavenging assay (ABTS), 2,2-diphenyl-1-picrylhydrazyl scavenging capacity assay (DPPH), cupric reducing antioxidant capacity (CUPRAC) and ferric reducing antioxidant power (FRAP). Protocols of all assays were previously described by Buljeta et al. [18]. Assays were performed in triplicate and results were expressed as micromoles of Trolox equivalent per kilogram of sample. Measurements were conducted in triplicates.

Inhibition of α-amylase

A volume of 0.2 ml of α-amylase solution (0.5 mg·ml^{-1}) was mixed with 0.2 ml of sample and incubated for 10 min. Then, 0.2 ml of starch solution (10 g·l^{-1}) was added in the mixture and incubated at the same conditions. To stop the reaction, 1 ml of 3,5-dinitrosallicylic acid (DNS) reagent [19] was added and the mixture was boiled for 5 min. The mixture was cooled down to 25 °C in an ice bath and 10 ml of distilled water was added. Absorbance was measured at 540 nm. Inhibition (I) was calculated according to the Eq. 1 and expressed in percent.

$$I = \frac{A_c - (A_e - A_b)}{A_c} \times 100 \quad (1)$$

where A_e is absorbance of the enzyme treated with the extract, A_b is absorbance of the extract with substrate (no enzyme present) and A_c is absorbance of the uninhibited enzyme.

Inhibition of α-glucosidase

Solution of α-glucosidase (0.2 mg·ml^{-1}) was prepared in phosphate buffer (0.1 mol·l^{-1}, pH 6.8). A mixture consisting of 0.15 ml of sample and 0.3 ml of the enzyme solution was incubated for 10 min. Afterwards, 0.15 ml of substrate (4-nitrophenyl-α-D-glucopyranoside – 1 mmol·l^{-1}) was added. The mixture was incubated for 5 min, 1.1 ml of buffer was added and absorbance was read at 405 nm. Inhibition was calculated according to the Eq. 1 and expressed in percent.

FTIR-ATR spectroscopy

Infrared (IR) spectra were recorded for all complexes as well as for pure pectin by Fourier transform infrared with attenuated total reflection spectroscopy (FTIR-ATR spectroscopy). For that purpose, Cary 630 spectrometer (Agilent Technologies) equipped with software MicroLab Expert (Agilent Technologies) was used. Samples were screened in the range from 4 000 cm^{-1} to 600 cm^{-1}.

Statistical analysis

Software program Statistica 13.1 (StatSoft, Tulsa, Oklahoma, USA) was used for statistical evaluation of results by analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test, with the significance defined at $p < 0.05$. Additionally, cluster analysis was performed on the obtained results.

RESULTS AND DISCUSSION

Gallic acid content in complexes

Four different pectin concentrations and two different complexation durations (10 min and 30 min) were used for gallic acid adsorption in order to determine the most effective formulation. Tab. 1 presents the contents of gallic acid in complexes determined by HPLC. Contents ranged from 3.264 g·kg^{-1} to 3.633 g·kg^{-1}. The highest gallic acid content was in a complex where 25 g·l^{-1} of pectin was used and a complexation duration of 30 min. Also, it can be observed that complexes where 75 g·l^{-1} and 100 g·l^{-1} of pectin were used and complexation of 30 min had the lowest content of gallic acid (no statistical difference was between these two samples). In both cases (10 min and 30 min of complexation), higher concentrations of pectin for sample preparation had no positive impact on gallic acid adsorption. Furthermore, it was noticed that a combination of shorter complexation duration and higher concentrations of pectin

Tab. 1. Gallic acid content in complexes after different time of complexation.

Time of complexation	10 min	30 min
Pectin [g·l^{-1}]	Gallic acid [g·kg^{-1}]	
25	3.539 ± 0.002^{c}	3.633 ± 0.002^{c}
50	3.487 ± 0.006^{b}	3.610 ± 0.002^{b}
75	3.482 ± 0.004^{b}	3.264 ± 0.007^{a}
100	3.457 ± 0.002^{a}	3.276 ± 0.003^{a}

Within each column, means followed by different superscript letters are significantly different at $p \leq 0.05$ (ANOVA, Fisher's least significant difference test).

(50 g·l^{-1}, 75 g·l^{-1} and 100 g·l^{-1}) was more efficient than prolonged complexation with equal amounts of pectin.

PHAN et al. [16] examined molecular interactions between gallic acid, ferulic acid, chlorogenic acid, (+/–) catechin, cyanidin-3-glucoside and cellulose. The authors stated that interactions of cellulose and polyphenols occurred within 1 min of contact, increased within 30 min while no significant increase was observed after 2 h. Study of interactions between pectin and four phenolic acids (chlorogenic, gallic, vanillic and protocatechuic acids) showed that gallic acid binds most to pectin. Interestingly, the hydroxyl group at position five in gallic acid is one of the key factors of interaction with pectin. Protocatechuic acid was the least bound to pectin and the reason could be the non-existence of hydroxyl group at position five as opposed to gallic acid [20]. Hydrogen bonds and hydrophobic interactions have been hypothesized to be responsible for the interactions between pectin and polyphenols [20, 21]. In a study by VUKOJA et al. [17] raspberry polyphenols were encapsulated with cellulose. The higher contents of total polyphenols and monomeric anthocyanins were observed in complex with a lower amount of cellulose, while an increased amount of cellulose led to a decrease in polyphenols adsorption. They also examined the impact of complexation duration. It was observed that the 15 min complexation was more favourable compared to 60 min of complexation. By evaluation of total polyphenols and proanthocyanidins content as well as individual polyphenols content in citrus fibre/blackberry juice complexes, it was observed that those with lower fibre amounts (1 %) had higher values compared with those with higher fibre amounts (2 % and 4 %) [18]. When maltodextrin was used together with pectin for encapsulation of saffron petal polyphenols it was concluded that encapsulation was higher on pectin-containing samples than on pure maltodextrin [14]. Research of encapsulation of gallic acid on β-cyclodextrin, xanthan or chitosan was conducted by DA ROSA et al. [2] and authors concluded that gallic acid had the highest affinity for chitosan, then for β-cyclodextrin and the lowest for xanthan. Even though, gallic acid has low solubility in water it can form hydrogen bonds since it has polarizable hydroxyl of phenolic and carboxylic groups both intramolecular as well as intermolecular [2]. Thus, it was concluded that the interaction between gallic acid and chitosan or xanthan were hydrogen bonds. However, for β-cyclodextrin, formation of inclusion complexes was the probable mechanism of their interactions. In a study by DADI et al. [22] bioactive products from *Moringa stenopetala* leaves extract were encapsulated in maltodextrin with and without high methoxyl pectin by freeze-drying and spray-drying methods. Higher values of antioxidant activity, total flavonoids and polyphenol content, and lower encapsulation efficiency of freeze-dried samples were associated with higher content of surface polyphenols in obtained samples. Presence of high methoxyl pectin as core material in freeze-drying process resulted in higher encapsulation efficiency.

Antioxidant activity of complexes

Tab. 2 presents antioxidant activities of complexes evaluated by ABTS, DPPH, CUPRAC and FRAP assays. The results of ABTS assay ranged from 717.5 μmol·kg^{-1} to 931.5 μmol·kg^{-1}. The highest values were observed in complexes where 25 g·l^{-1} and 50 g·l^{-1} of pectin were used and prolonged complexation of 30 min (without a statistical difference between samples). These concentrations of pectin (25 g·l^{-1} and 50 g·l^{-1}) and the shorter time of complexation (10 min) resulted in lower values (917.4 μmol·kg^{-1} and 888.5 μmol·kg^{-1}, respectively). Regardless of the complexation duration, the higher concentrations of pectin (75 g·l^{-1} and 100 g·l^{-1}) caused a decrease in the antioxidant activities of complexes. That decrease was more pronounced at prolonged complexation.

Observing results from DPPH assay, it was noticed that the highest antioxidant activity was achieved in complexes where 25 g·l^{-1} (1 018.1 μmol kg^{-1}) and 50 g l^{-1} (1 016.6 μmol·kg^{-1}) of pectin were used and a prolonged complexation of 30 min. The higher concentrations of pectin caused a decrease in antioxidant activity when prolonged complexation was used. In complexes prepared with a shorter time of complexation, there was no statistical difference when 25 g·l^{-1} (998.0 μmol·kg^{-1}) or 50 g·l^{-1} (982.7 μmol·kg^{-1}), or 75 g·l^{-1} (977.2 μmol·kg^{-1}) of pectin were used.

Results of CUPRAC assay indicated that when 10 min complexation was applied, the highest antioxidant activity had the complex prepared with 25 g·l^{-1} of pectin (1 991.7 μmol·kg^{-1}). Further increase of pectin amount caused a reduction in antioxidant activity. On the other hand, 30 min of complexation showed a positive impact on antioxidant activity of the complex prepared with 25 g·l^{-1} of pectin (2 177.4 μmol·kg^{-1}), while the complex prepared with the highest concentration of pectin (100 g·l^{-1}) had the lowest value (1 786.6 μmol·kg^{-1}) compared to all complexes.

The results from FRAP assay showed the same trend as other assays and results ranged from 104.3 μmol·kg^{-1} to 179.2 μmol·kg^{-1}.

Tab. 2. Antioxidant activities of gallic acid plus pectin complexes determined by different methods.

Time of complexation	10 min	30 min
Pectin [g·l^{-1}]	Antioxidant activity [μmol·kg^{-1}]	
ABTS		
25	917.4 ± 0.6^{d}	931.5 ± 6.3^{c}
50	888.5 ± 3.8^{c}	936.0 ± 4.0^{c}
75	856.6 ± 1.0^{b}	772.2 ± 0.8^{b}
100	803.2 ± 7.9^{a}	717.5 ± 2.3^{a}
DPPH		
25	998.0 ± 1.4^{b}	1 018.1 ± 6.6^{b}
50	982.7 ± 6.4^{b}	1 016.6 ± 3.5^{b}
75	977.2 ± 8.1^{b}	952.1 ± 4.8^{a}
100	942.0 ± 3.2^{a}	934.5 ± 2.1^{a}
CUPRAC		
25	1 991.7 ± 10.4^{b}	2 177.4 ± 7.4^{d}
50	1 905.5 ± 6.4^{a}	2 125.3 ± 2.1^{c}
75	1 873.9 ± 20.2^{a}	1 833.1 ± 7.8^{b}
100	1 856.6 ± 10.8^{a}	1 786.6 ± 8.5^{a}
FRAP		
25	168.5 ± 2.4^{c}	179.2 ± 3.8^{c}
50	145.7 ± 1.8^{b}	154.0 ± 5.5^{b}
75	145.4 ± 0.9^{b}	103.7 ± 3.5^{a}
100	116.5 ± 0.9^{a}	104.3 ± 3.8^{a}

For each method, means followed by different superscript letters within column are significantly different at $p \leq 0.05$ (ANOVA, Fisher's least significant difference test).
ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical-scavenging assay, DPPH – 2,2-diphenyl-1-picrylhydrazyl scavenging capacity assay, CUPRAC – cupric reducing antioxidant capacity, FRAP – ferric reducing antioxidant power.

Tab. 3. Inhibition of α-glucosidase and α-amylase by pectin plus gallic acid complexes.

Time of complexation	10 min	30 min
Pectin [g·l^{-1}]	Inhibition [%]	
α-Amylase		
25	27.0 ± 0.2^{c}	35.6 ± 0.2^{c}
50	24.9 ± 0.2^{b}	34.4 ± 0.2^{b}
75	24.8 ± 0.2^{b}	28.5 ± 0.2^{a}
100	24.4 ± 0.0^{a}	28.5 ± 0.1^{a}
α-Glucosidase		
25	47.1 ± 0.0^{c}	48.9 ± 0.2^{b}
50	45.7 ± 0.3^{b}	48.9 ± 0.3^{b}
75	45.7 ± 0.1^{b}	31.5 ± 0.3^{a}
100	43.1 ± 0.0^{a}	31.4 ± 0.1^{a}

Within each column, means followed by different superscript letters are significantly different at $p \leq 0.05$ (ANOVA, Fisher's least significant difference test).

Da Rosa et al. [2] evaluated the antioxidant activity, by DPPH assay, of capsules prepared with gallic acid and β-cyclodextrin, xanthan or chitosan. They observed that there were no statistical differences between samples regardless of the used polymer for encapsulation. In study of Buljeta et al. [18] where blackberry juice polyphenols were encapsulated with citrus fibres, evaluation of antioxidant activity showed higher results when lower amounts of fibre were used. An increase in fibre amount had a negative effect on antioxidant activity which was consistent with this study. Antioxidant activities of cellulose plus raspberry encapsulates, determined by FRAP and CUPRAC assays decreased when the higher cellulose amounts (5 %, 7.5 %, or 10 %) were used [17]. Furthermore, results of antioxidant activity in this study were in accordance with gallic acid content in complexes; higher contents of gallic acid in complexes resulted in higher antioxidant activity. Milea et al. [23] encapsulated flavonoids from extracts of yellow onions skin in different core materials (maltodextrin, pectin and whey protein isolate) using the freeze-drying method. Powder with higher pectin content had a significantly higher total flavonoid content and that sample possessed the highest antioxidant activity assessed by the DPPH assay. Sivam et al. [24, 25] examined the effect of the addition of blackcurrant, kiwifruit and apple polyphenols extracts and pectin in model bread systems and observed that bread with added polyphenols extracts and pectin had greater antioxidant activity and higher extractable polyphenols content. Hence, properly formulated complexes obtained in this study can be considered for further investigation as food additives for increasing antioxidant activity.

Inhibition of α-amylase and α-glucosidase

Inhibition of α-amylase and α-glucosidase of the obtained complexes was also evaluated and results are presented in Tab. 3. Results showed that pectin plus gallic acid complexes inhibited α-glucosidase more than α-amylase. Complexes that were prepared by prolonged complexation of 30 min showed stronger inhibition of both enzymes. The trend of inhibition followed the trend of gallic acid content in complexes. Consequently, the highest inhibition was obtained with complexes prepared with 25 g·l^{-1} and 50 g·l^{-1} of pectin and inhibition was 48.9 % of α-glucosidase, while 35.6 % and 34.4 % of α-amylase, respectively. Studies showed that interactions between enzyme and phenolic acids are the reason for inhibition of starch digestive enzymes and usually those interactions are non-covalent [26]. It was pointed

out that hydrogen binding, cation-ϖ interactions, salt bridge interactions or electrostatic forces are responsible for interactions between chlorogenic, caffeic, *p*-coumaric, vanillic and syringic acids and the enzyme, causing enzyme inhibition [27]. Inhibition of α-amylase and α-glucosidase by natural inhibitors has a beneficial impact on diabetes treatment, while synthetic inhibitors can cause gastrointestinal disorders. Therefore, these results contribute to better insight into the positive effects of the obtained complexes.

As it was in our case, ALEIXANDRE et al. [28] also determined that gallic acid had a higher inhibition capability for the starch digestive enzymes α-glucosidase than for α-amylase. The same authors also investigated whether inhibition of enzymes was due to the interaction of phenolic acids with enzyme or starch so they incubated the enzyme with phenolic acids and starch with phenolic acids. Regarding gallic acid, they concluded that inhibition of both enzymes was consequence of its interaction with enzymes. For the inhibition of α-glucosidase they determined no difference in half maximal inhibitory concentration (IC_{50}) for these two incubation types. On the other hand, when gallic acid was incubated with α-amylase lower IC_{50} was achieved in comparison to its incubation with starch. Since phenolic acids can interact with amylopectin or amylose chains that was consequence of these results [28, 29]. However, inhibition of α-amylase was achieved with higher contents, so non-inclusion starch/gallic acid complexes were formed through weaker binding forces such as hydrogen bonds, hydrophobic interactions and/or electrostatic and ionic interactions [28, 30], after breaking of these forces gallic acid was capable to interact with the enzyme.

Based on the results of gallic acid content, antioxidant activities and inhibition of the two enzymes, cluster analysis of complexes was conducted. The obtained dendrogram (Fig. 1) describes the similarity of complexes based on the obtained results. Complexes were grouped into three main clusters. In the first cluster, complexes obtained with 50 g·l^{-1} and 10 g·l^{-1} of pectin, which were complexed for 10 min, were grouped. Complexes that were prepared by complexation for 30 min were divided into two clusters; one cluster consisted of complexes prepared with 25 g·l^{-1} and 50 g·l^{-1} of pectin and another one consisted of complexes prepared with 75 g·l^{-1} and 10 g·l^{-1} of pectin.

FTIR-ATR spectroscopic analysis of complexes

FTIR-ATR spectroscopic technique was used for screening of pectin and its complexes in order to evaluate changes caused by the adsorption of gallic acid. Fig. 2 presents the IR spectra with observed changes between pectin and pectin plus gallic acid complex. Only one complex is shown here due to the identical differences between all of them. In the region from 3 500 cm^{-1} to 3 000 cm^{-1}, which is assigned to symmetric stretching of O-H [31, 32], an increase in intensity was observed after adsorption of gallic acid. Additionally, a shift of a band at 2 937 cm^{-1} of pectin to 2 944 cm^{-1} in pectin plus gallic acid complex occurred indicating changes in asymmetric stretching of CH_3 [31, 32]. BICHARA et al. [31] reported that pectin has two intense bands in the region from 1 800 cm^{-1} to 1 500 cm^{-1} with approximate separation between them of 200 cm^{-1}, which was also observed with our pectin. These bands were attributed to COO^- groups with different moieties linked to them (-OH and -O-CH_3). The band at 1 602 cm^{-1} of pectin (assigned to asymmetric stretching of COO^- groups of pectin) shifted to 1 587 cm^{-1} when gallic acid was incorporated, indicating the formation of C-C stretch on phenyl ring [32]. That change was also accompanied by a decrease in the band intensity. Also, a shoulder at 1 543 cm^{-1} in the IR spectrum of pectin plus gallic acid complex was observed. Bands at 1 409 cm^{-1} and 895 cm^{-1} in pectin IR spectra shifted to 1 405 cm^{-1} and 890 cm^{-1}, respectively, when gallic acid was present causing the C-H rocking and C-O and C-C stretching [31]. In the IR spectrum of gallic acid plus pectin complex, the band at 917 cm^{-1} appeared and this

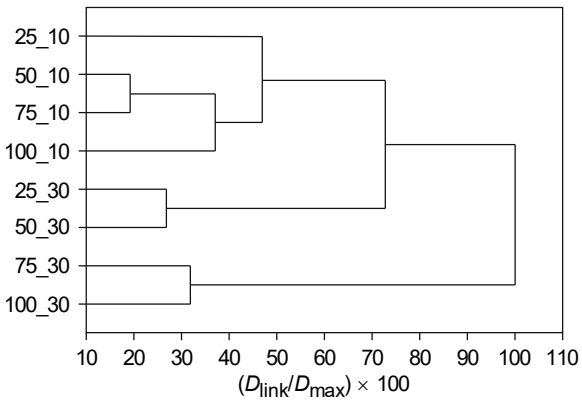

Fig. 1. Grouping by cluster analysis of the complexes based on gallic acid content, antioxidant activities as well as inhibition of α-glucosidase and α-amylase.

Sample codes: numbers 25, 50, 75 and 100 represent concentrations of pectin input for complexation (in grams per litre), numbers 10 and 30 represent time of complexation (in minutes).
D_{link} and D_{max} are distance for current and final clusters, respectively.

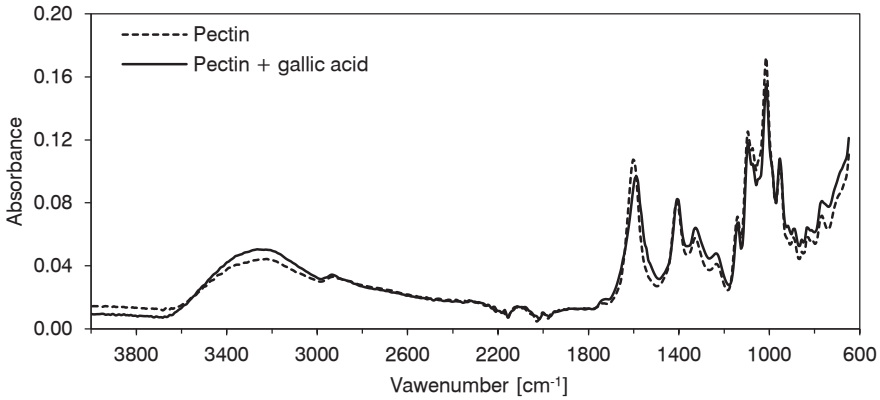

Fig. 2. Infrared spectra of pectin and pectin plus gallic acid complex.

could be assigned to C-O stretching [31]. Changes in band intensities can be noticed not only in the region from 3 500 cm^{-1} to 3 000 cm^{-1}, but also in the regions from 1 430 cm^{-1} to 1 200 cm^{-1} and from 920 cm^{-1} to 630 cm^{-1}. In those regions, the intensity of bands increased in pectin plus gallic acid complexes. Considering all changes observed in IR spectrum, it can be concluded that binding of gallic acid onto pectin occurred.

CONCLUSIONS

Pectin was chosen as a wall material for gallic acid encapsulation in order to produce a functional ingredient for possible applications in the food industry. Results presented in this study show the impact of wall material amounts and duration of complexation on gallic acid encapsulation. Favourable conditions were 30 min of complexation and 25 $g \cdot l^{-1}$ of pectin for complex preparation. Higher amounts of pectin caused a decrease in gallic acid adsorption. The results of antioxidant activities of complexes were in accordance with gallic acid content as higher gallic acid contents in complexes resulted in higher antioxidant activity of complexes. Also, the same complexes had a higher capability of inhibition of α-amylase and α-glucosidase. FTIR-ATR spectroscopic analysis confirmed the binding of gallic acid onto pectin. Future studies will concentrate on the examination of the impact of these ingredients on food products quality.

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