Article

Effect of Chemical Degradation of Sodium Alginate on Capsaicin Encapsulation

Dominika Kulig , Łukasz Bobak , Andrzej Jarmoluk , Aleksandra Szmaja , Żaneta Król-Kilińska , Anna Zimoch-Korzycka *

Posted Date: 2 November 2023

doi: 10.20944/preprints202311.0090.v1

Keywords: sodium alginate; depolymerization; oligosaccharides; encapsulation; capsaicin

Article

Effect of Chemical Degradation of Sodium Alginate on Capsaicin Encapsulation

Dominika Kulig, Łukasz Bobak, Andrzej Jarmoluk, Aleksandra Szmaja, Żaneta Król – Kilińska and Anna Zimoch-Korzycka *

Department of Functional Food Products Development, Faculty of Biotechnology and Food Science, Wroclaw University of Environmental and Life Sciences, Chelmonskiego 37, 51-630 Wroclaw, Poland; dominika.kulig@upwr.edu.pl (D.K.), lukasz.bobak@upwr.edu.pl (Ł.B.), andrzej.jarmoluk@upwr.edu.pl (A.J.), aleksandra.szmaja@upwr.edu.pl, (A.S.), zaneta.krol-kilinska@upwr.edu.pl (Z.K. – K.) anna.zimoch-korzycka@upwr.edu.pl (A.Z.-K.)

* Correspondence: anna.zimoch-korzycka@upwr.edu.pl

Abstract: The effect of chemical degradation of a sodium alginate solution and the possibility of encapsulation of paprika oleoresin were investigated. To produce sodium alginate oligomers, a depolymerization process was performed using hydrogen peroxide as a decomposition factor. The characteristics of the samples were obtained by measuring the contact angle of the surface, the surface tension of solutions, as well as thermal decomposition in thermo - gravimetric analysis. The obtained solution of alginate oligomers served as the carrier material for immobilization of capsaicin. Capsules were prepared by ionic gelation using a calcium chloride solution as a crosslinking agent. In this way, capsules without and with the core (capsaicin) were prepared and their ability to scavenge free radicals (DPPH) and iron-reducing properties (FRAP) were determined. The stability of the capsules was examined under conditions of the gastric and small intestine. It was found that alginate oligomers can be used in the encapsulation of bioactive compounds and the efficiency is above 80%. Capsule production from alginate oligomers affected their thermal stability. The use of alginate derivatives as a carrier increases the antioxidant properties of the finished product, as well as its ability to reduce iron ions. The use of alginate oligomers as a coating material prevents the active substance from being released too early in the conditions of the small intestine, prolongs the stability of the capsules, and supports their durability in gastric conditions.

Keywords: sodium alginate, depolymerization, oligosaccharides, encapsulation, capsaicin

1. Introduction

Because of its structure, alginate has the ability to gel, which is directly related to the number of G blocks in the chain. This is due to the ability to bind divalent metal cations. The calcium atom binds to the carboxyl groups of guluronic acid through ionic crosslinking. Such a gelation mechanism is called the "egg box" model [1]. The viscosity of the alginate increases as the pH of the solution decreases and reaches a maximum value in the range of pH 3.0-3.5. The molecular weight of alginates used on an industrial scale oscillates between 32,000 and 400,000 g/mol. Modifications that reduce the molecular weight improve the physical properties of the obtained alginate gels. On the other hand, an alginate solution obtained from a high molecular weight polymer has high viscosity, which is a problematic parameter during further processing [2, 3]. Therefore, sodium alginate can be degraded by various methods: irradiation (microwave, UV, X-ray, and gamma radiation), ultrasound, mechanical or thermal decomposition, oxidative degradation, and enzymatic or chemical hydrolysis. These treatment is most often performed to change the physical properties of alginate, primarily, the decrease in molecular weight, which significantly improves its functional properties. Changing the spatial arrangement of alginate affects its mechanical strength, physiological and rheological properties, eg stability, viscosity, solubility in solutions, hydrophobicity, and can also lead to an improvement in the reactive properties of the compound [4, 5]. The sodium alginate oligosaccharides formed in the process of depolymerization do not have or have very weak ability to form gels, but are characterized by other important biological activities. They affect the growth and

 doi:10.20944/preprints202311.0090.v1

rapid development of human cells, including epidermal cells, and prevent oxidative stress. They favor the growth of plants such as rice, lettuce, wheat, and tobacco. Sodium alginate oligomers have antimicrobial properties and have a destructive effect on multidrug-resistant bacteria, which is why they are used in the production of antibiotics. They contribute to *Aspergillus* and *Candida* fungi [6]. Based on our ponder study, it was recognized that the degradation with H_2O_2 is the most effective chemical method for the obtained bioactive alginate oligosaccharides [7].

There are chemical, physical, and physicochemical methods for the encapsulation of active substances. Chemical methods include polycondensation, polymerization, and interfacial condensation. Physical coating mechanisms include spray drying, spheronization, centrifugal extrusion, while physicochemical mechanisms include ionic gelation, coacervation, and extrusion [8]. The most popular way to form capsules is through the process of ionic gelation. This mechanism is based on interactions between polymers with different charges or the interaction of the polymer with a polycationic or polyanionic compound. Ion gelation consists of dropping the capsule solution into a water bath, thanks to which, through ionic interaction, the sol turns into a gel. When creating capsules, materials of natural origin, natural polymers such as agar, carrageenan, alginate, or chitosan are increasingly used [9]. It is recommended that the coating solution has the appropriate viscosity and concentration. By analyzing the available scientific articles, the coating material is most often present in a concentration of 0.5% to 2%. Because of its nontoxicity, biodegradability, and biocompatibility, alginate has become one of the main sources of coatings in the ion gel method. The advantage of using alginate for immobilization processes is its ability to form a gel in interaction with divalent metal ions, for example Ca^{2+}. The whole process takes place in a gentle and safe way, using room temperature. The simplest way to produce capsules is to dissolve or disperse the core substance in a solution containing the shell material, e.g. capsaicin in alginate, and then drop it into the cross-linking aqueous medium. In the case of alginate, the preferred water bath is a solution of calcium chloride, in which the solution solidifies and capsules are formed. Another way is to form core-shell microcapsules. The process involves a separate dosing of the matrix and core material through the nozzle. The active substance is coated with the polymer solution immediately before contact with the cross-linking solution. As a result of this treatment, a capsule is obtained containing a separate part of the core (usually liquid) and the shell [10, 11]. The concentration of calcium chloride in which the transition of alginate to gel form is observed is 0.1 M. The higher the concentration of ions in the crosslinking bath, the more Ca^{2+} ions pass into the capsule material during the mixing process. The final properties of the capsules are affected by the type of cross-linking agent, its concentration, the pH of the capsule formation reaction, and the temperature [9].

Capsaicin, as a chemical compound, has antimicrobial and antioxidant properties. Extracts from plants of the genus Capsicum inhibit the development of microorganisms such as: *Bacillus cereus*, *Bacillus subtilis*, *Clostridium sporogenes*, *Clostridium tetani,* and *Streptococcus pyogenes*. Its destructive effect has also been proven on some yeast species, e.g. *Saccharomyces cerevisiae*. The range of action of the alkaloid on microorganisms depends mainly on the concentration of the extract treated in a given colony and the resistance of the strain [12]. The antioxidant properties of capsaicin are used in the food industry to prevent reactions of fat oxidation, thus slow down the degree of degradation of food products. Its influence on thermoregulatory processes, analgesic, anti-inflammatory, anti-carcinogenic properties, and supporting the process of fighting obesity is used in medicine [12]. In the era of increasing consumer awareness and the search for alternative sources of food additives, capsaicin can be used to enrich food products with a natural bioactive substance.

The aim of the work was to produce sodium alginate oligomers and use them as a carrier in the encapsulation process of paprika oleoresin as the source of the bioactive substance, which is capsaicin.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 November 2023 doi:10.20944/preprints202311.0090.v1

2. Results and discussion

2.1.1. Contact Angle

Table 1 presents the results of the statistical analysis of the influence of hydrolysis time on the value of the liquid contact angle (CA). It was observed that the hydrolysis time had a significant impact on the wetting properties of the surfaces of all the analyzed samples. As hydrolysis progresses, the value of the contact angle decreases (Table 2). The highest angle value measured after 1 s is for the 1% sodium alginate solution (78.230°), which means that it has the ability to wet the surface, but to the smallest extent compared to the other samples. The alginate solution has the best wetting properties on the Teflon surface after 24 hours of oxidative degradation. The value of the measured parameter after hydrolysis is 56.397°.

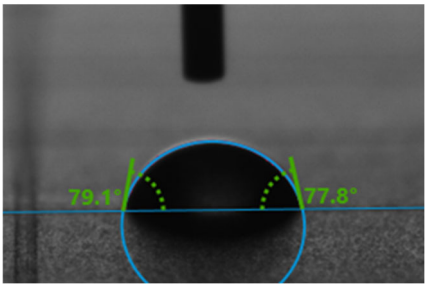
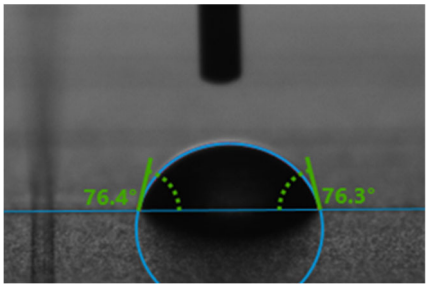
The value of the contact angle is a measure of the wettability of the surface. The contact angle is defined as the angle between the plane of the solid surface and the tangent to the liquid surface drawn at the point of contact of the three phases. If the liquid has the ability to completely wet the surface of a solid, this angle takes the value 0. If the angle is less than 90°, the liquid has the ability to wet the surface, but it does not wet the surface in the range of 90°-180°. The hydrolysis process increases the surface wetting property of the sodium alginate solution. After repeating the measurement after 10 s, a decrease in the contact angle value was observed. Statistical analysis showed that the contact angle values measured after 10 s are no longer significant within the hydrolysis times of 0 - 2 h, 2 - 3 h and 3 - 24 h. Because of the chemical degradation reaction, the polymer chains were shortened, which resulted in a decrease in the viscosity of the solution and, consequently, a reduction in the contact angle. Surface wettability has no effect on duration inertial wetting, but the viscosity of the liquid has [13]. Therefore, an increase in the liquid viscosity will increase the inertial wetting time, which confirms our observations.

Table 1. Effect of hydrolysis time: 0, 2, 3, 4,and 24 h on the contact angle of alginate sodium (A) solutions after 1 s and 10 s from the drop deposition.

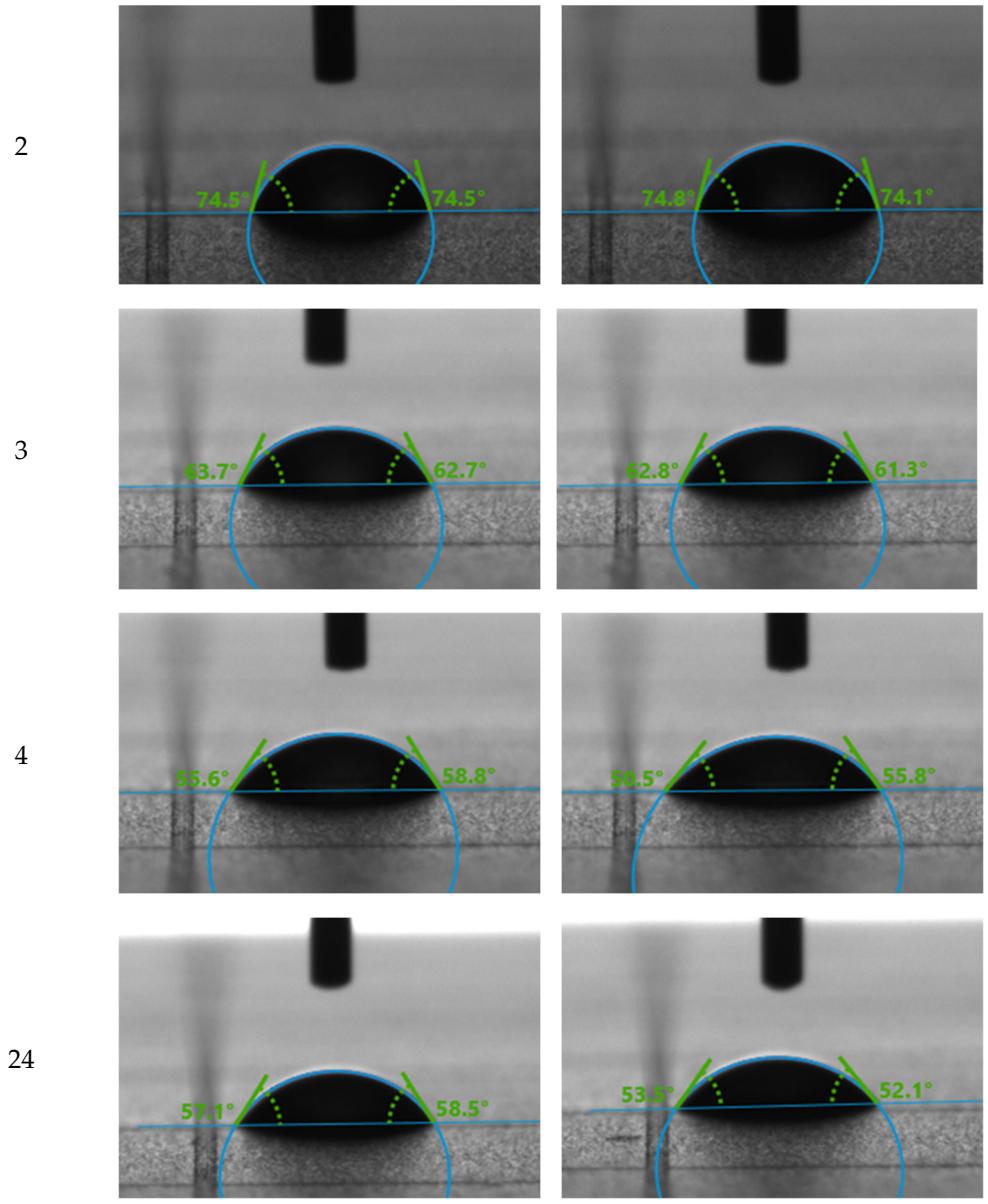
Hydrolysis time [h]	CA 1s [°]	CA 10s [°]
0	78.230 ^{a} ±0.288	73.167 ^{a} ±2.810
2	75.010 ^{b} ±0.713	67.087 ^{ab} ±7.178
3	63.560 ^{c} ±0.624	61.390 ^{bc} ±2.388
4	58.637 ^{d} ±1.262	56.473 ^{c} ±3.436
24	56.397 ^{e} ±1.281	54.053 ^{c} ±2.373

$^{a–e}$ values with different letters within the same column differ significantly ($p < 0.05$).

Table 2. Pictures of the measurement of the contact angle using the sessile drop method for individual alginate (A) solutions after 0, 2, 3, 4 and 24 h of hydrolysis.

Hydrolysis time [h]	CA 1s [°]	CA 10s [°]
0		

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 November 2023 doi:10.20944/preprints202311.0090.v1



2.1.2. Surface Tension

The effect of hydrolysis time on surface tension (SFT) using the pendant drop method is shown in Table 3. The highest surface tension was found for a 1% sodium alginate solution at 78.297 mN/m. As hydrolysis progresses, the surface tension value decreases and in its final phase it amounts to 69.133 mN/m. The hydrolysis time significantly affects the surface tension of the pendant drop. The essence of the method for determining the surface tension of liquids is the relationship between the shape of the liquid drop formed at the tip of the needle and its weight, as well as the corresponding equilibrium surface tension forces of the liquid. The acting surface tension forces lead to the droplet becoming spherical, but under the influence of gravity, the liquid droplet becomes elongated. The surface tension depends on the molecular weight and viscosity. The higher the molecular weight, the higher surface tension [14].

Table 3. Effect of hydrolysis time: 0, 2, 3, 4,and 24 h on the surface tension of alginate (A) solutions.

Hydrolysis time [h]	SFT [mN/m]
0	78.297 ^{a} ±0.176
2	74.680 ^{b} ±0.278

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 November 2023 doi:10.20944/preprints202311.0090.v1

3	71.187 [c] ±0.240
4	70.510 [d] ±0.392
24	69.133 [e] ±0.450

[a–e] values with different letters within the same column differ significantly ($p < 0.05$).

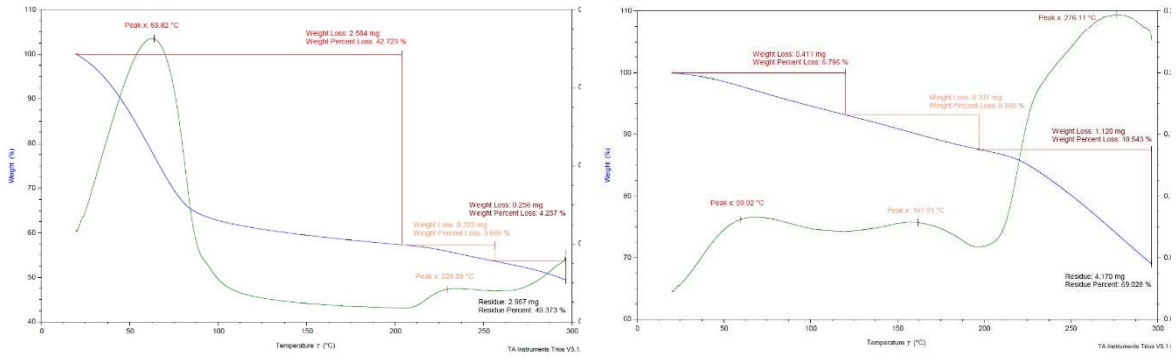
2.2. Capsule Characteristics

2.2.1. Encapsulation Efficiency

The effect of material used on the efficiency of capsaicin encapsulation was not statistically significant and the results for alginate sodium capsules with capsaicin was 89,33% and for capsules of alginate oligomers with capsaicin was 87,97%. Belščak et al. observed that alginate capsules containing different plant extracts obtained using the same method also differ in process efficiency. The polyphenol extract of olive leaves had the highest efficiency (89.39%), while the nettle extract had the lowest efficiency (80.88%) [15]. The microencapsulation efficiency of fish oil with alginate was found to be greater than 80% [16]. The efficiency of the encapsulation process using the ion gelation method varies greatly, as the efficiency of the process depends on many factors, such as the concentration and type of alginate, the nature and type of the immobilized substance, the concentration and type of cross-linking agents [17].

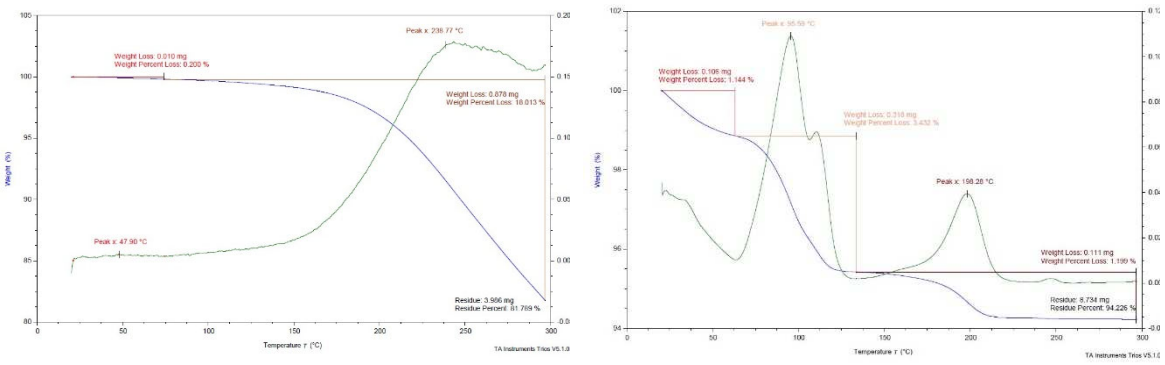
2.2.2. Thermo-Gravimetric Analysis (TGA)

The thermal decomposition of the AC (sodium alginate-capsaicin), AOC (sodium alginate oligomers-capsaicin) capsules, the paprika oleoresin with capsaicin (C), and the calcium chloride (CC) are presented in Figure 1. Thermal degradation of AC, AOC, CC is visible as a three-stage process with residue 49.373%, 69,028%, 94,226%, respectively, while the decomposition of capsaicin is in one stage with residue 81,789%. It was observed that the TGA of alginate capsules with oleoresin has a great loss at 20 – 200 °C corresponded to moisture evaporation. The next weight loss between 200 and 255 °C was due to the complexity of the process considered with sample degradation. The third weight loss was observed above 255 °C. Sodium alginate is decomposed by dehydration and degradation to sodium carbonate. Then at 550 - 750 °C, the material is carbonized and decomposed in nitrogen, which was observed by other authors [18, 19, 20]. The greatest weight loss of AOC was found in the third stage of thermal degradation between 220 - 300 °C. The percentages of residues of AC and AOC are about 49% and 69% respectively. When comparing total weight loss during thermal treatment, the AOC is more stable in a wide temperature range than AC. A great weight loss was found between 20 and 130 °C for the calcium chloride and corresponds to endothermic water removal. The second reduction was observed between 150 and 220 °C, which continues the endothermic dehydration of the material. When analyzing paprika oleoresin, thermal decomposition starts around 150 °C and is related to volatile compounds. These results are in agreement with Pereda, Poncelet and Renard [18].



(**a**) (**b**)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 November 2023 doi:10.20944/preprints202311.0090.v1



(**c**) (**d**)

Figure 1. TGA decomposition curves of (a) AC (b) AOC (c) paprika oleoresin with 3.3% pure capsaicin (C) (d) calcium chloride (CC).

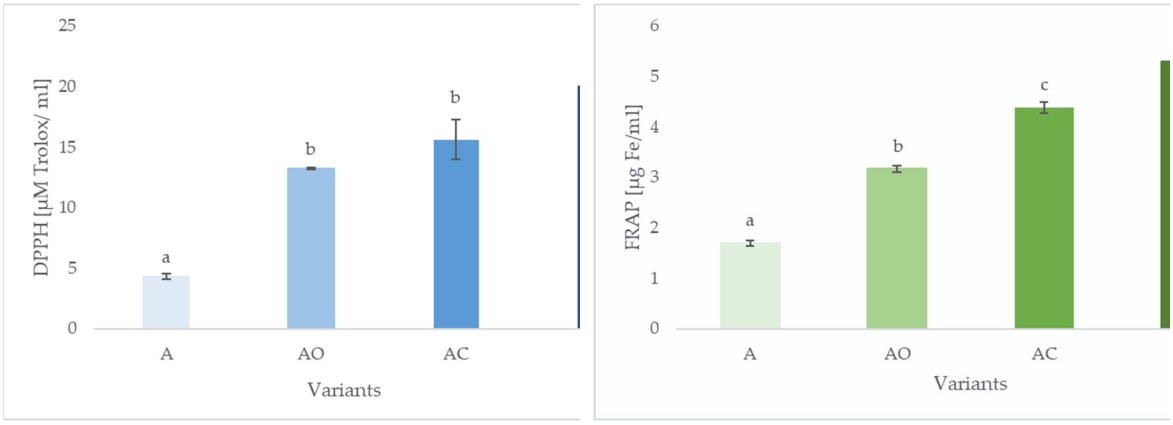
2.2.3. Antioxidant Properties

The results of the statistical analysis illustrating the impact of the materials used for encapsulation on their antioxidant capacity as free radical scavenging activity and ferric reducing antioxidant power are presented in Figure 2a and 2b, respectively. The type of core and shell substances used in the encapsulation processes affects the antioxidant properties of the final product.

The lowest ability to scavenge DPPH free radicals has a sodium alginate solution in an amount of 4.319 μM Trolox/ml. Statistical analysis did not show significant differences in the ability to remove free radicals for capsules with sodium alginate oligomers and double core capsules with alginate and capsaicin. The degradation process allowed the improvement of the antioxidant properties of the analyzed capsules solutions. As a result, alginate oligomers solution has the ability to neutralize free radicals at the level of 13.229 μM Trolox/ml. The process of creating double core capsules using capsaicin as the core material and sodium alginate as the shell material allows an increase in antioxidant activity to 15.618 μM Trolox/ml. The highest ability to scavenge free radicals at the level of 19.979 μM Trolox/ml, has capsules with sodium alginate oligomers in the capsaicin coating process. Capsaicin, thanks to its antioxidant properties, increases the ability to scavenge free radicals in capsules with sodium alginate and its oligomers. Amna et al. examined the antioxidant properties of 3% capsaicin solution and polyurethane capsules with capsaicin. The determination of DPPH shows that a 3% capsaicin solution has a free radical scavenging capacity of 40%, while encapsulated capsaicin has a capacity of 42% [21].

The ferric reduction capacity is significantly variable and depends on the material and core used for the production of capsules (Figure 2b). The use of appropriate encapsulation materials affects the final ability of the capsules to reduce iron ions. The lowest FRAP value was obtained for the sodium alginate solution and was 1.695 μg Fe/ml. After hydrolysis of sodium alginate using hydrogen peroxide, an increase in the ability to reduce ferric ions was observed, amounted to 3.166 μg Fe/ml. Coated capsaicin with sodium alginate increased the FRAP value to 4.382 μg Fe/ml compared to previous variants. However, the highest ferric reducing antioxidant power (5.294 μg Fe/ml) has AOC capsules. Furthermore, in our previous study, hydrolysis was observed to significantly improve the ferric reducing antioxidant power, comparing native sodium alginate and sodium alginate oligomers [7].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 November 2023 doi:10.20944/preprints202311.0090.v1



(a) **(b)**

Figure 2. Antioxidant potential of (a) DPPH and (b) FRAP of AO – alginate oligomers capsules, A – alginate capsules, AC – alginate with capsaicin capsules, AOC – alginate oligomers with capsaicin capsules.

2.2.4. Stability of Capsules under Gastric Conditions

No statistically significant changes were observed in the capsules during the process of exposing them to a solution that simulates gastric conditions. The capsules did not dissolve after 4 days of incubation in SGF solution at 37 °C. The type of encapsulation material did not affect the stability of the capsules under gastric conditions. Gioumouxouzis et al. also found no visible changes in the appearance of alginate capsules after incubation in an acidic SGF solution. The carboxyl groups of alginate in the chain remain protonated, creating a tight polymeric network that hinders water exchange and circulation [22]. Therefore, the insolubility of alginate, alginate oligomers, and their variation with capsaicin capsules in SGF is related to the formation of an acid gel, which is enhanced by increasing the concentration of H+ [23].

2.2.5. Stability of Capsules in Conditions of the Small Intestine

While maintaining the analyzed capsule variants in the PBS solution, statistically significant changes in the dissolution rate were observed and are presented in Table 4.

After 95 minutes, the alginate capsules without core dissolved in the PBS solution. Capsules made of sodium alginate oligomers obtained in the oxidative degradation process, as the shell material, were destabilized in the PBS solution after 1080 minutes, i.e. 18 hours. It has been shown that the type of encapsulation materials used affects the dissolution time of the capsules under conditions of the small intestine. The use of sodium alginate oligomers as a capsule material significantly increases the time needed for their decomposition in the PBS environment.

The assay examining the stability of capsules in the conditions of the small intestine is carried out to achieve a controlled release of the active substance from within the capsules. Gioumouxouzis et al. controlled the stability of the capsules in the conditions of the small intestine. After incubation in simulated intestinal fluid (SIF), it was observed that the alginate capsules dissolved after 90 minutes, while materials coated with sodium alginate destabilized after 150 minutes [22].

Table 4. Effect of the type of capsule material and the core used (AO – alginate oligomers capsules, A – alginate capsules, AC – alginate with capsaicin capsules, AOC – alginate oligomers with capsaicin capsules) on stability under the conditions of the small intestine.

Variants	SIF [min]
AO	1080.00 ^{b} ±2.00
A	95.00 ^{a} ±1.00
AC	92.00 ^{a} ±5.00

 doi:10.20944/preprints202311.0090.v1

AOC	1075.00 ^{b} ±5.00

$^{a–b}$ values with different letters within the same column differ significantly ($p < 0.05$).

3. Materials and Methods

3.1. Materials

Sodium alginate with M:G ratio = 1.4 extracted from *Laminaria digitata* was supplied by Danisco, Grindsted, Denmark (particle size max. 5% > 400 μm). Paprika oleoresin with 3.3% pure capsaicin (C) was purchased from Essence, Konstancin-Jeziorna, Poland. It is a solution obtained by extracting the fruit of red chilli paprika include oleoresin with derivatives of fatty acids and refined sunflower oil. Hydrogen peroxide (30%) was obtained from Pol-Aura, Morąg, Poland. Calcium chloride (CC) and ethanol (96%) were supplied by P.P.H. 'STANLAB' Sp. J., Lublin, Poland. Tween®80 was obtained from Sigma Aldrich, Poznań, Poland.

3.2. Sample Preparation

3.2.1. Preparation of alginate oligosaccharides by oxidation with hydrogen peroxide (H_2O_2)

Alginate oligosaccharides were prepared following the procedure described in Zimoch-Korzycka et al. [7].

3.2.2. Preparation of an Oleoresin Solution

An emulsion of paprika oleoresin with a capsaicin content of 0.245% was created. For this purpose, a solution of deionised water and ethyl alcohol (96%) was mixed in a 1:1 ratio and oleoresin and 0.682 g of Tween 80 were added. The solution was homogenized for 3 minutes at 3000 rpm using an IKA® T18 ULTRA-TURRAX® homogenizer (Staufen im Breisgau, Germany). The sample was left for 24 hours to check the stability of the emulsion.

3.2.3. Encapsulation of Capsaicin in a Solution of Sodium Alginate Oligomers

The encapsulation process was carried out using the 'BÜCHI' B-390 encapsulator (Flawil, Switzerland). In the study, single core capsules were produced using a 1.5% solution of sodium alginate oligomers and a 1.5% solution of sodium alginate. Furthermore, dual core capsules of core-shell type in the capsaicin-alginate and capsaicin-sodium alginate oligomers were obtained. The carrier substance of the capsules was solutions with a concentration of 1.5%. For the encapsulation process, a head with a diameter of 300 μm was used for single-core capsules and a shell material for dual-core capsules. A 150 μm head was used to dispense capsaicin. The gelation process was carried out in a cross-linking bath using a 0.5M solution of calcium chloride (CC). The capsules were washed with CC twice and then separated from the cross-linking agent using a 'BÜCHI' V-700 vacuum pump (Flawil, Switzerland). Table 5 shows the experimental setup of the study, which illustrates the combinations of capsules produced.

Table 5. Experimental design.

Coding	Alginate [A] concentration [%]	Alginate oligomers [AO] concentration [%]	Capsaicin [C] content [%]
AO	-	1.5	-
A	1.5	-	-
AC	1.5	-	0.245
AOC	-	1.5	0.245

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 November 2023 doi:10.20944/preprints202311.0090.v1

3.3. Methods

3.3.1. Contact Angle Measurement

The measurement of the contact angle was carried out using the sessile drop method using the DSA25 drop shape analyzer (KRÜSS, DSA 100 Hamburg, Germany). The tests were made for a 1% A solution (0 h of hydrolysis), 1% AO solution taken after 2, 3, 4 and 24 h of depolymerization. The drops were deposited on the Teflon material while maintaining a constant temperature of 20 °C. A 3.3 LS borosilicate glass 3.3 LS by MICROSYRINGES and a NE44 27749 needle with a diameter of 0.5 mm were used to dose the drops on the table surface. The determination was made in three repetitions for each tested sample.

3.3.2. Surface Tension Using the Pendant Drop Method

The surface tension of a 1% A solution (0h of hydrolysis), AO after hydrolysis times of 2, 3, 4 and 24 h was determined using the pendant drop method. The drop shape analyzer DSA25 (KRÜSS, DSA 100 Hamburg, Germany)was used for the determination. The drop was dosed using a 3.3 LS MICROSYRINGES borosilicate glass syringe and a NE44 27749 needle with a diameter of 0.5 mm. The surface tension was measured in three repetitions for each sample.

3.3.3. Determination of Encapsulation Efficiency

Encapsulation efficiency (EE) was determined according the procedure by Hudita et al. [24]. The efficiency was calculated according the following formula:

$$EE = \frac{A_{loaded} - A_{filtrated}}{A_{filtrated}} \cdot 100\%$$

where EE = encapsulation efficiency [%]; A_{loaded} - amount of encapsulated capsaicin; $A_{filtrated}$ - amount of capsaicin in the ultrafiltrate.

3.3.4. Thermal Gravimetric Analysis (TGA)

The thermal stability of AC and AOC, C, and CC was measured by thermal gravimetric analysis using a TGA 5500 thermogravimetric analyzer from TA Instruments Company (Tokyo, Japan). The analysis temperature was performed from room temperature to 300 °C in an inert nitrogen atmosphere with a flow rate of 25 ml / min and a warming rate of 10 K/min according to Kulig [25].

3.3.5. Antioxidant Properties

3.3.5.1. Free Radical Scavenging Activity (DPPH)

The free radical scavenging activity of DPPH was determined spectrophotometrically (UviLine 9400 SI Analytics, Mainz, Germany) at a wavelength of 517 nm according to Chen et al. [26]. The study was carried out for an A, AO after 24 h of hydrolysis and a homogenized solution of alginate-capsaicin and oligomer-capsaicin capsules. 1 ml of a 0.1% solution of the test sample and 1 ml of 96% ethyl alcohol were placed in the test tubes. The solutions were mixed using a Vortex V-1 plus and then 0.5 ml of 0.3 mM ethanolic DPPH radical solution was added to the samples. The samples were incubated for 30 minutes. The assay was performed in triplicate. The reagent sample was a mixture of 1 ml of H_2O, 1 ml of ethanol and 0.5 ml of 0.3 mM ethanol solution of DPPH radicals. The results were calculated based on the standard curve expressed in units of μg of Trolox needed to neutralize a 0.3 mM solution of DPPH radicals.

3.3.5.2. Ferric Reducing Antioxidant Power (FRAP)

The determination is carried out according to Benzie and Strain [27]. The study was carried out for 0.1% sodium alginate solution and 0.1% sodium alginate oligomers and a homogenized 0.1% solution of alginate-capsaicin and oligomer-capsaicin dual core capsules. For this purpose, 3 ml of

 doi:10.20944/preprints202311.0090.v1

the reagent was added to 1 ml of the test sample and the samples were incubated for 10 minutes. The absorbance was performed on a UviLine 9400 (SI Analytics, Mainz, Germany)spectrophotometer using a wavelength of 593 nm. The reagent test was a mixture of 1 ml of deionized water with 3 ml of working reagent. The assay was carried out in three repetitions. The content of Fe^{2+} ions in the sample was calculated on the graph of the standard curve graph.

3.3.6. Stability of Capsules under Gastric Conditions

The resistance of the capsules to the conditions of the stomach was tested using the SGF (Simulated Gastric Fluid) solution. The solution was prepared by adding 2 g of sodium chloride and 7 g of hydrochloric acid to 1000 ml of deionized water. By treatment with 0.1 M hydrochloric acid, the solution was adjusted to pH 1.2 [28, 29]. The determination was carried out for single-core capsules made of sodium alginate and sodium alginate oligomers and for dual-core capsules: sodium alginate - capsaicin, oligomers - capsaicin. For this purpose, 1 g of capsules was introduced into 20 ml of SGF solution. The samples were placed in a water bath with a shaking function, with a temperature of 37 °C and a speed of 95 rpm. The degree of dissolution of the capsules was visually controlled for 4 good. Analysis was performed in triplicate for each of the samples.

3.3.7. Stability of Capsules in Small Intestine

The resistance of the capsules to the conditions of the small intestine was tested using a PBS (Phosphate Buffered Saline) solution. The solution was prepared by adding 8 g of sodium chloride, 0.2 g of potassium chloride, 1.44 g of sodium hydrogen phosphate, 0.24 g of potassium dihydrogen phosphate to 800 ml of deionized water. By dosing a 0.1 M HCl solution, the pH of the solution was adjusted to 7.4. The solution was supplemented with water to a volume of 1000 ml [30]. The determination was carried out for single-core capsules made of sodium alginate and sodium alginate oligomers and for dual-core capsules: sodium alginate - capsaicin, oligomers - capsaicin. For this purpose, 1 g of capsules was introduced into 20 ml of PBS solution. The samples were placed in a water bath with a shaking function, with a temperature of 37 °C and a speed of 95 rpm. The stability of the capsules was visually checked. Analysis was performed in triplicate for each of the samples.

3.3.8. Statistical Analysis

The results obtained were statistically analyzed in the Statistica 13.3 program (StatSoft, Krakow, Poland). For this purpose, a one-way analysis of variance was performed using the Duncan test at the significance level $\alpha \leq 0.05$.

4. Conclusions

The chemical degradation process affects the physical and chemical properties of the sodium alginate solution. Sodium alginate oligomers formed in the chemical deg-radation can be used as a shell material in capsaicin encapsulation processes with sim-ilar efficiency. Double core capsules made from a solution of alginate oligomers and capsaicin have the highest ability to scavenge DPPH free radicals. The use of a solution of alginate oligomers as a coating material allows for an increase in the ferric reducing antioxidant power. The capsules obtained, regardless of the used encapsulation materials, are stable in gastric conditions. The use of sodium alginate oligomers increases the resistance of capsules to the conditions of the small intestine, expanding the possibilities of their use for the controlled release of drugs or bioactive substances. The thermal stability of the formed capsules allows them to be used in food production where thermal processes are required.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Conceptualization, A. Z.-K. and D.K.; methodology, D.K.; software, Ł.B.; validation, D.K., A. Z.-K. and A.J.; investigation, D.K.; data curation, A.S.; writing—original draft preparation, A.Z.-K. and D.K.; visualization, Z.K. – K. All authors have read and agreed to the published version of the manuscript."

Funding: This research received no external funding

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bede P. M., Prado da Silva M. H., Ben-Hur da Silva Figueredo A. Finotelli P. V., Nanostructured magnetic alginate composites for biomedical applications. Polímeros, 2017, 27(4), 267-272.
2. Bilal M., Iqbal H. M. N. Naturally-derived biopolymers: Potential platforms for enzyme immobilization. Int. J. Biol. Macromol., 2019, 130, 462-482.
3. Lopes da Silva T., Vidart J. M. M., Carlos da Silva M. G., Gimenes M. L., Vieira M. G. A. Alginate and Sericin: Environmental and Pharmaceutical Applications. In Biological Activities and Application of Marine Polysaccharides 1st edition, Shalaby E.A. Ed., Publisher IntechOpen, Internet, 2017. Crossref, doi:10.5772/62752.
4. Abd El-Mohdy H. L. Radiation-induced degradation of sodium alginate and its plant growth promotion effect. Arab. J. Chem.,2019, 10, 431-438.
5. Piacentini E. Encapsulation Efficiency. In Encyclopedia of Membranes, 1st edition, Drioli, E., Giorno, L. Eds, Publisher, Springer, Berlin, Heidelberg. 2016, 706-707.
6. Labre F., Mathieu S., Chaud P., Morvan P. Y., Vallée R., Helbert W., Fort S. DMTMM-mediated amidation of alginate oligosaccharides aimed at modulating their interaction with proteins. Carbohydr. Polym., 2018, 184, 427-434.
7. Zimoch-Korzycka, A.; Kulig, D.; Król-Kilińska, Ż.; Żarowska, B.; Bobak, Ł.; Jarmoluk, A. Biophysico-Chemical Properties of Alginate Oligomers Obtained by Acid and Oxidation Depolymerization. Polymers, 2021, 13, 2258. https://doi.org/10.3390/polym13142258
8. Chuyen H. V., Roach P. D., Golding J. B., Parks S. E., Nguyen M. H. Encapsulation of carotenoid-rich oil from Gac peel: Optimisation of the encapsulating process using a spray drier and the storage stability of encapsulated powder. Powder Technol., 2019, 344, 373-379.
9. Leong J. Y., Lam W. H., Ho K. W., Voo W. P., Leea M. F. X., Lim H. P., Lim S. L., TeyB. T., Poncelet D., Chan E. S. Advances in fabricating spherical alginate hydrogels with controlled particle designs by ionotropic gelation as encapsulation systems. Particuology, 2016, 24, 44-60.
10. Simó G., Fernández-Fernández E., Vila-Crespo J., Ruipérez V., Rodríguez-Nogales J. M. Research progress in coating techniques of alginate gel polymer for cell encapsulation. Carbohydr. Polym. 2017, 170, 1-14.
11. Echalier C., Valot L., Martinez J., Mehdi A., Subra G. Chemical cross-linking methods for cell encapsulation in hydrogels. Mater. Today Commun., 2019, 20, 100536
12. Segura-Campos R. M., Ruiz-Ruiz J. C., Chel-Guerrero L. A., Betancur-Ancona D. A. Capsicum chinense: Composition and Functional Properties. In Functional Properties of Traditional Foods, 1st edition, Kristbergsson, K., Ötles, S. Eds. Piblisher: Springer US. 2018, Chapter 20, 289-292.
13. Chen, L.; Bonaccurso, E. Effects of surface wettability and liquid viscosity on the dynamic wetting of individual drops. Phys. Rev.E 2014, 90, 022401.
14. Deng, W.; Zheng, H.; Zhu, Z.; Deng, Y.; Shi, Y.; Wang, D.; Zhong, Y. Effect of Surfactant Formula on the Film Forming Capacity, Wettability, and Preservation Properties of Electrically Sprayed Sodium Alginate Coats. Foods 2023, 12, 2197. https://doi.org/10.3390/foods12112197
15. Belščak-Cvitanović A., Komes D., Karlović S., Djaković S., Špoljarič I., Mršić G., Ježek D. Improving the controlled delivery formulations of caffeine in alginate hydrogel beads combined with pectin, carrageenan, chitosan and psyllium. Food Chem.:2011, 167, 378-386.
16. Bannikova, A., Evteev, A., Pankin, K., Evdokimov, I., & Kasapis, S. Microencapsulation of fish oil with alginate: In-vitro evaluation and controlled release. LWT, 2018, 90, 310–315. doi:10.1016/j.lwt.2017.12.045.
17. Łętocha, A.; Miastkowska, M.; Sikora, E. Preparation and Characteristics of Alginate Microparticles for Food, Pharmaceutical and Cosmetic Applications. *Polymers* **2022**, *14*, 3834. https://doi.org/10.3390/polym14183834
18. Pereda, M., Poncelet, D. & Renard, D. Characterization of Core-Shell Alginate Capsules. Food Biophys., 2019, 14, 467–478 https://doi.org/10.1007/s11483-019-09595-x.
19. Zhao, Y., Huang, Z., Zhang, J., Wu, W., Wang, M., & Fan, L. Thermal Degradation of Sodium Alginate-Incorporated Soy Protein Isolate/Glycerol Composite Membranes, 17th IAPRI World Conference on Packaging.2010, 402–405.
20. Soares, J.P., Santos, J.E., Chierice, G.O., Cavalheiro, E.T.G. Thermal behavior of alginic acid and its sodium salt. Eclet. Quim.2004, 29(2), 57–64.

21. Amna T., Gharsan F. N., Shang K., Hassan M. S., Khil M. S., Hwang I. Electrospun Twin Fibers Encumbered with Intrinsic Antioxidant Activity as Prospective Bandage. Macromol. Res. 2019, 27, 663–669.
22. Gioumouxouzis C. I., Chatzitaki A. T., Karavasili C., Katsamenis O. L., Tzetzis D., Mystiridou E., Bouropoulos N., Fatouros D. G. Controlled Release of 5-Fluorouracil from Alginate Beads Encapsulated in 3D Printed pH-Responsive Solid Dosage Forms. AAPS Pharm. Sci. Tech. 2018, 19 (8), 3362–3375.
23. Albadran, H.A., Chatzifragkou, A., Khutoryanskiy, V.V., Charalampopoulos, D. Development of surfactant-coated alginate capsules containing Lactobacillus plantarum. Food Hydrocoll. 2018. 82, 490-499.
24. Hudita A, Galateanu B, Costache M, Negrei C, Ion RM, Iancu L, Ginghina O. In Vitro Cytotoxic Protective Effect of Alginate-Encapsulated Capsaicin Might Improve Skin Side Effects Associated with the Topical Application of Capsaicin. Molecules. 2021 Mar 7;26(5):1455. doi: 10.3390/molecules26051455. PMID: 33800110; PMCID: PMC7962180.
25. Kulig, D.; Król-Kilińska, Ż.; Bobak, Ł.; Żarowska, B.; Jarmoluk, A.; Zimoch-Korzycka, A. Functional Properties of Chitosan Oligomers Obtained by Enzymatic Hydrolysis. Polymers, 2023, 15, 3801. https://doi.org/10.3390/polym15183801.
26. Chen, J.C.; Yeh, J.Y.; Chen, P.C.; Hsu, C.K. Phenolic content and DPPH radical scavening activity of yam-containing surimi gels influenced by salt and heating. Asian J. Health Inf. Sci. 2007, 2, 1–11.
27. Benzie, I.F.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": The FRAP assay. Analytic. Biochem. 1996, 293, 70–76.
28. Arenales-Sierra I. M., Lobato-Calleros C., Vernon-Carter E. J., Rodríguez-Hernández L., Alvarez-Ramírez J. Calcium alginate beads loaded with $Mg(OH)_2$ improve L. casei viability under simulated gastric condition. LWT, 2019, 112, 1–3.
29. Sorasitthiyanukarn F. N., Na Bhuket P. R., Muangnoi C., Rojsitthisak P., Rojsitthisak P. Chitosan/alginate nanoparticles as a promising carrier of novel curcumin diethyl diglutarate. Int. J. Biol. Macromol., 2019, 131, 1125-1136.
30. Gorbunova N., Bannikova A., Evteev A., Evdokimov I., Kasapis S. Alginate-based encapsulation of extracts from beta Vulgaris cv. beet greens: Stability and controlled release under simulated gastrointestinal conditions. LWT, 2018, 93, 442-449.