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MICROENCAPSULATION OF PROTEOLYTIC ENZYMES FOR INDUSTRIAL APPLICATION

Abstract. The aim of the present study was to develop the technology of microencapsulation of proteolytic enzymes in the pseudo-boiling layer which based on the diffusion of maltodextrin into an enzyme, which allows maintaining high enzyme activity for a long time in meat products. It was found that maltodextrin provides high strength capsule walls their integrity during long-term storage. It was shown that the maximum activity of the proteolytic enzyme pepsin immobilized in a maltodextrin matrix shifts to the alkaline side with increasing pH of the reaction medium, which is not typical for a free enzyme. The results of determining the cutoff voltage of ham from pork ham confirmed that the immobilized enzyme remained active for 6 months of storage. While pure pepsin after 3 months of storage noticeably lost its proteolytic effect. Considering the results obtained microencapsulation of the proteolytic enzyme pepsin using maltodextrin with a coating thickness of 4 to 6 μm can be recommended which will expand the possibilities of using enzymes in the production of meat products.

Key words: microencapsulation, ham products, pepsin, activity, immobilized enzyme, maltodextrin.

Introduction. The modern food industry aims to increase the biological value of food products. In relation to ham products the scientific and technological problem is to accelerate the ripening and bating of meat which can be solved by the use of proteolytic enzyme preparations.

There are various methods of treating meat with enzyme preparations. The most common are aerosol. It means immersion of portioned meat pieces in an enzyme solution or injection of the drug by syringing. Due to the modern food science it possible to use microencapsulation of enzymes. Microcapsules are made with enzymes that have a directed effect on muscle and connective tissue proteins.

Given these circumstances, researchers have been searching for effective methods of capsulation [1,2,3,4,5]. Often, an extrusion method is used for the production of microcapsules which involves the external gelation of hydrocolloids using various gelling agents (calcium chloride solution for alginate, potassium chloride for carrageenan and tripolyphosphate for chitosan, transglutaminase for caseinate). The suspension of the biologically active substance and the hydrocolloid solution is extruded in separate drops. which are collected in a container for thermal gelation. Then using pressing it can be possible to produce capsules of various diameters [6]. Extrusion technology is recommended for encapsulation of living cells – probiotics [6,7,8].

Encapsulating food substances is proposed using hydrolyzed and modified starches [9]. At the same time, it is noted in the work that hydrolyzed starch does not always ensure the stability of taste. The use of starch modified with octenyl succinate increases the stability of the emulsion.

Studies often come down to finding a protective substance for encapsulation which should have high rheological properties and be easily processed during encapsulation. Also, the protective substance must have emulsion and dispersion properties with high stability, they must be inert with respect to the

encapsulated substance during use and during storage of capsules; have good solubility; be affordable and affordable. It should also be taken into account that the use of the protective substances used in the encapsulation of food ingredients must be approved by state authorities.

The protective substances used for encapsulation cannot always combine all these characteristics, in some cases several substances are used for this, for example, modified cellulose, which has high emulsifying and mechanical properties [10].

A promising method of microencapsulation of enzymes is the application of a protective coating on them in a pseudo-boiling layer of a dispersion of maltodextrin. This treatment allows you to evenly distribute the film-forming substance over the entire surface of the enzyme.

Lipin et al. [11] propose a number of designs of technological devices for gushing action, which make it possible to ensure a uniform carrier gas velocity for stable gushing. The data obtained can be used in the development of industrial plants.

Modern research often boils down to the use of micro-capsules to add value to foods, primarily dietary supplements. In relation to meat products, studies are aimed at increasing their shelf life [12,13,14, 15]. While the use of microcapsules for the tendering of meat products is not well understood. This actualizes the problem of the possibility of using microcapsules for softening ham products.

In this regard, the **aim** of this study is to develop a technology for microencapsulation of proteolytic enzymes in a pseudo-boiling layer and to evaluate their tendering effect in the production of ham products.

To achieve this goal, the following **tasks** were set:

- to develop own technology and device for microencapsulation of pepsin in maltodextrin;
- to evaluate the effect of microcapsules of different diameters and thicknesses of the coating on the activity of pepsin;
- to determine the tendering effect of microencapsulated pepsin on samples of ham products.

Organization and research methods. Encapsulation was carried out in a specially designed glass apparatus by applying a pseudo-boiling layer (PBL) to the surface of the enzyme (figure 1).

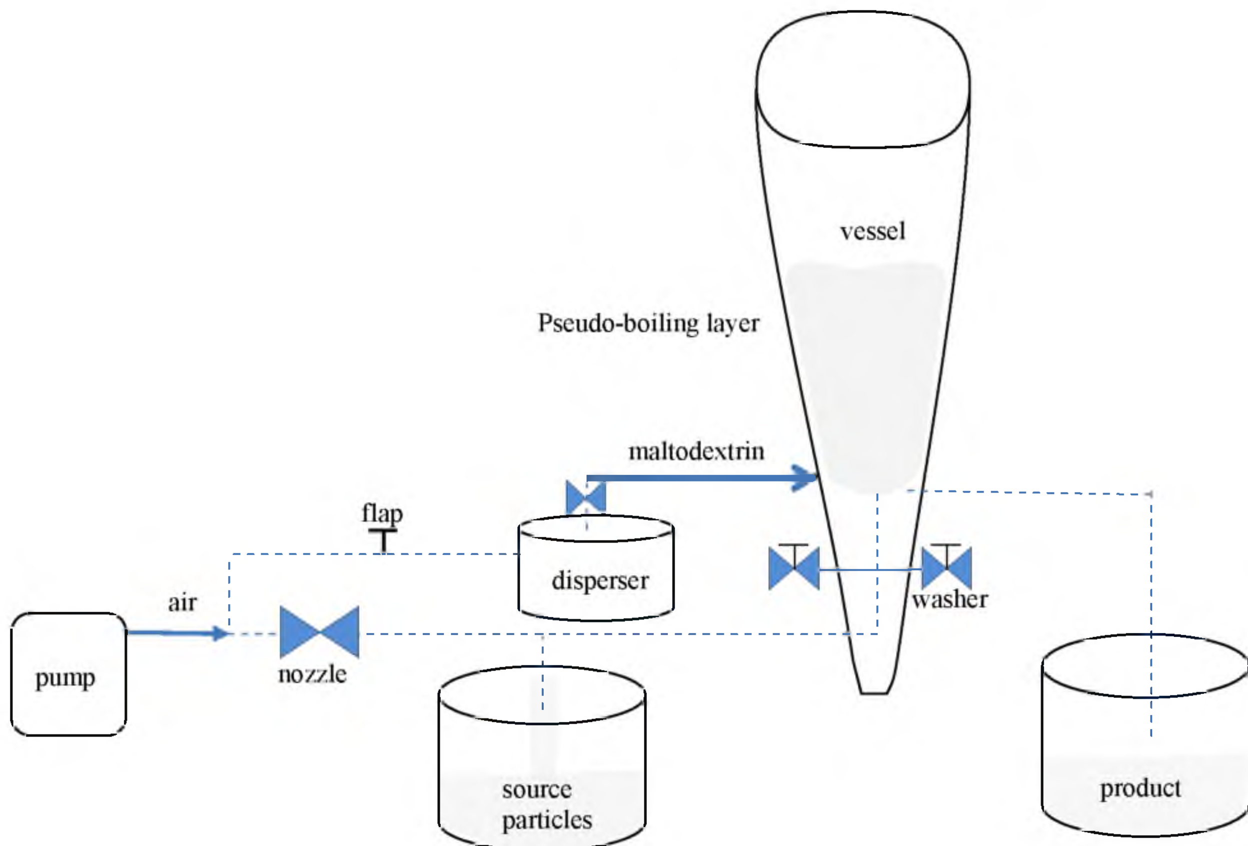

Figure 1 – Scheme of the device for microencapsulation of enzymes

Microencapsulation on the apparatus included the modified of known technology. A container with an enzyme was hermetically connected to the pipe, air was supplied from the pump, which, passing through interchangeable nozzles of various diameters, takes the initial (source) particles. Then, the particles in the turbulent flow are dried and enter the casing of the apparatus with pseudo-boiling layer in the form of gushing flows in the vessel. The correct selection of the velocities of these flows, depending on the properties of the enzyme particles, is ensured by washers of the required hole diameter and the size of the conical body of vessel (height and diameter). After preliminary drying of the particles in the nozzle and in the gushing flows of the apparatus, a washer was opened, and the air entered the dispersant for liquid components, previously filled with a portion of them. The dispersant is equipped with a replaceable nozzle. The choice of nozzle diameter provides dispersion of droplets of the required size, which are introduced through the nozzle into the gushing flows of the bottom of the vessel.

The size of the drops should create the overall surface of liquid components, on a par or even bigger than the consummate surface of the hard particles in the spouted layer of the apparatus. Covering the enzyme with drops should be intensive and fleeting, it comes in hand with the beginning of the desiccation of the glued drops by the spouted layer, which flush the particles from all sides. After all the required components are received, the nozzle is closed. After the desiccation has been made, the bottom throat section is occluded by the batch gate with flapper and the potion of the encapsulated enzyme falls freely in the storage vessel. Drying time was lasted from 5 to 8 minutes. Then the flapper is opened, and the cycle with hard and liquid particles is rehearsed in the same manner.

For the present experiment pepsin was used; as a protective layer, a 10% aqueous solution of maltodextrin obtained by the acid or enzymatic method from corn starch as a result of its partial hydrolysis and equivalent dextrose weight. When heated to 100 °C and a pH of 4.0–5.0, corn starch breaks down, resulting in maltodextrin and corn syrup.

The ratio of solid to liquid (S/L) was kept within 10/1 ÷ 11.5 / 1. The fluidizing agent including in the drying mode was room temperature air pumped through the apparatus.

For the experiment, five polished samples of encapsulated enzymes were prepared with a maltodextrin layer thickness of 2 μm, 4 μm and 6 μm. Pure unencapsulated pepsin was taken as a control.

Pepsin activity was determined in the range of pH between 1.5 to 5.0 by the amount of tyrosine as a result of the hydrolysis of casein. Casein was used as a substrate with Gamersten method according to Anson's work [16-19]. The amount of tyrosine was determined by the spectrophotometric method at a wavelength of 280 nm on an SF-46 spectrophotometer.

The object of the study was the rear ham of lean, boneless pork from chilled half-carcasses with 6.1 pH. The gammon was injected with brine in an amount of 15% by weight of the raw material with a density of 1077.7 kg / m^2, containing salt, sodium nitrite, the enzyme pepsin in an amount of 0.15% and granulated sugar. Raw meat filled with brine, was kept in salting for 5 hours. Then the salted samples were molded, poured and heated in accordance with the technology for the ham production. After cooling the shear stress was determined on an Instron 1022 testing machine.

Five groups of ham were developed. Each experimental study was performed in 5 times replicate. Statistical data processing was performed using Statistica 9 software package. The confidence level was 0.95 ($p \leq 0.05$).

Results and discussion. The duration of the processing of maltodextrin in the apparatus for micro-encapsulation determined the thickness of the protective layer of the capsules. It is defined that the thickness of the defensive coating from maltodextrin linear depends ($p \leq 0.05$) on the duration of its processing in the apparatus for microencapsulation (figure 2).

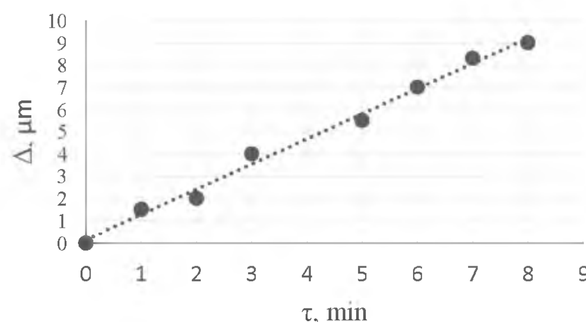

Figure 2 – Layer thickness maltodextrin and its duration on pepsin

After 2 min of processing by the maltodextrin mixture (figure 2) there was more than ¼of thickness of the surface layer from its average size on the pepsin grain at the end of the experiment, and after 6 min of depositing was 70%. And the rating speed of the air flow with the maltodextrin mixture in the bottle neck of the cone of the working chamber was on a par with the marginal terminal velocity of the large pepsin particles and was about 0,17 m/sec. Due to the equation of the flow continuity and in unison with the terminal velocity and pepsin particle entrainment there was a theoretical size of the belly of the apparatus cone during the depositing of the maltodextrin mixture made:

$$d_w = 4{,}4 \cdot d_n, \quad (1)$$

where d_w – the diameter of the broad cone part of the working chamber, d_n – the diameter of the narrow cone part of the working chamber.

The analysis of the pepsin activity and the coating thickness of the maltodextrin enzyme showed that the thicker the maltodextrin coating was, the longer the initial pepsin activity lasted (figure 3).

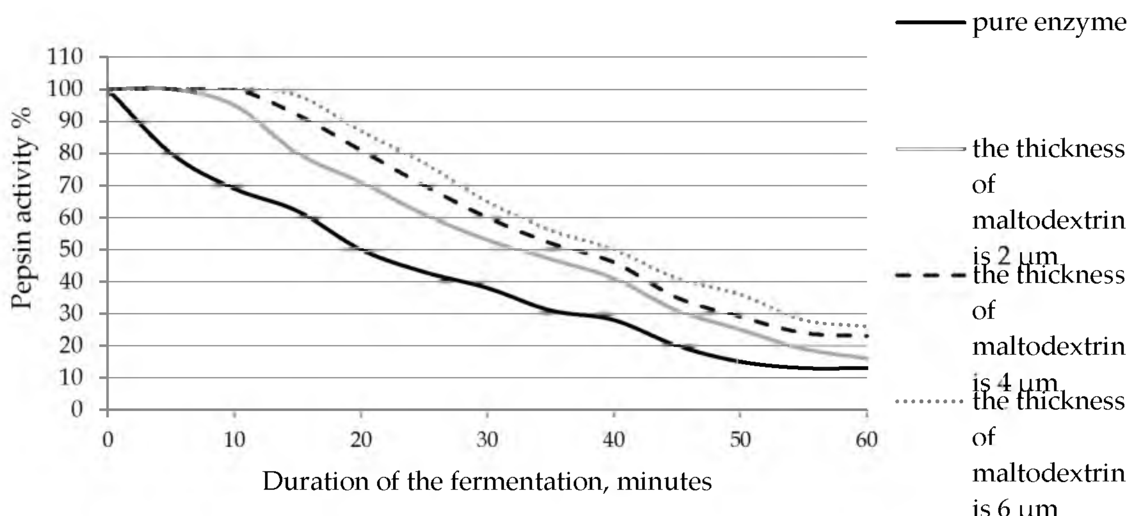

Figure 3 – The impact of the thickness of the maltodextrin on pepsin activity

The coating thickness of maltodextrin affects the activity of pepsin. The pepsin activity was the most stable when the thickness of the maltodextrin coating was 6 µm. As the thickness of the defensive coating decreases, the enzyme quickly loses its initial activity.

It was established that the activity of pepsin in microcapsules with a layer thickness of more than 6 µm was not investigated.

As can be seen in figure 4, maximum activity of the pepsin immobilized in the maltodextrin mixture moved roughly for 2 units pH in the acid direction compared with the free enzyme. It apparently can happen due to the limitation of the initial substance diffusion, when there is a lack of proton dispensation and limitations in diffusion as well.

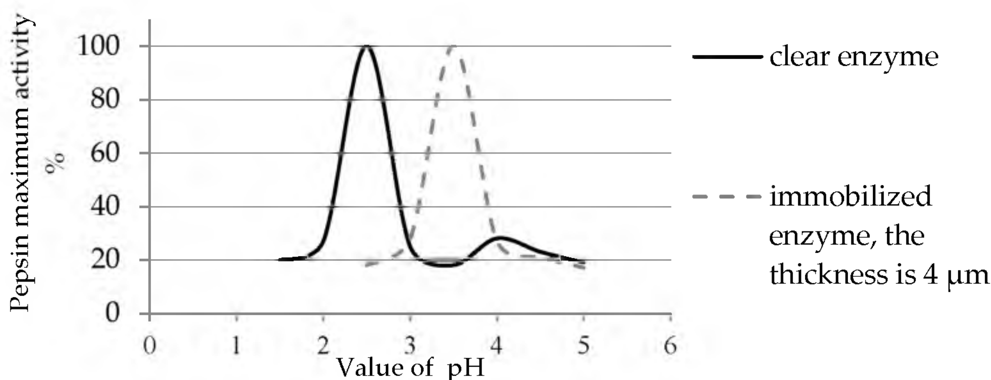

Figure 4 – Dependence of the pepsin activity from pH for the maltodextrin, thickness 4 µm

The results indicate that pepsin activity with the thickness maltodextrin of 4 μm is slightly lower than microcapsules of 6 μm. In this connection, it is advisable to use pepsin with maltodexrin coating of 4 μm. Therefore, in further experiments, an enzyme with the thickness of 4 μm was used.

The experiment of the pepsin proteolytic activity depended on the length of the conservation period under the temperature 0-2 °C revealed (figure 5) that immobilization of the enzyme with the maltodextrin made its activity much more stable practically for 6 months, whereas clear enzyme already started to lose its activity after 3 months.

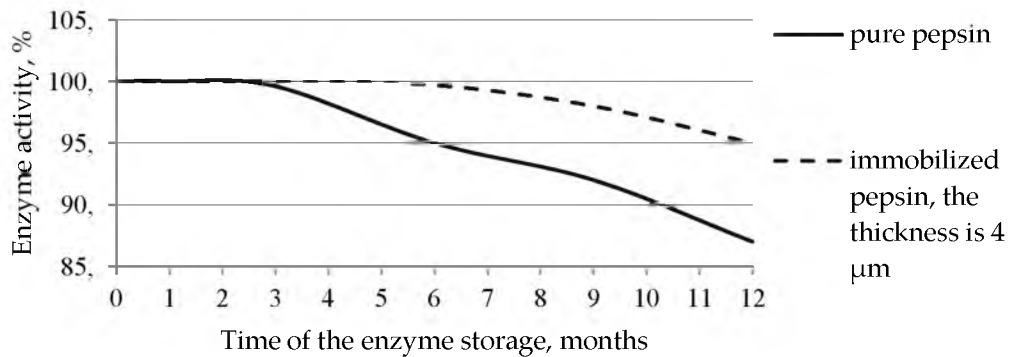

Figure 5 – Dependence of the proteolytic activity of the clear pepsin (Row 1) and the immobilised one, the thickness of the maltodextrin coating 4 μm (Row 2) on the length of conservation

During the experiment there were researches conducted on the connection between the length of the pure and encapsulated pepsin conservation and the structure and physical qualities of the ham. Enzymes microcapsules were stored in a dry, dark place at a temperature not exceeding 2 °C and a relative humidity of not more than 75% in compliance with the technical conditions. The shelf life of pepsin did not exceed 10 months. The immobilized enzyme for 6 months showed a great proteolytic activity (figure 6).

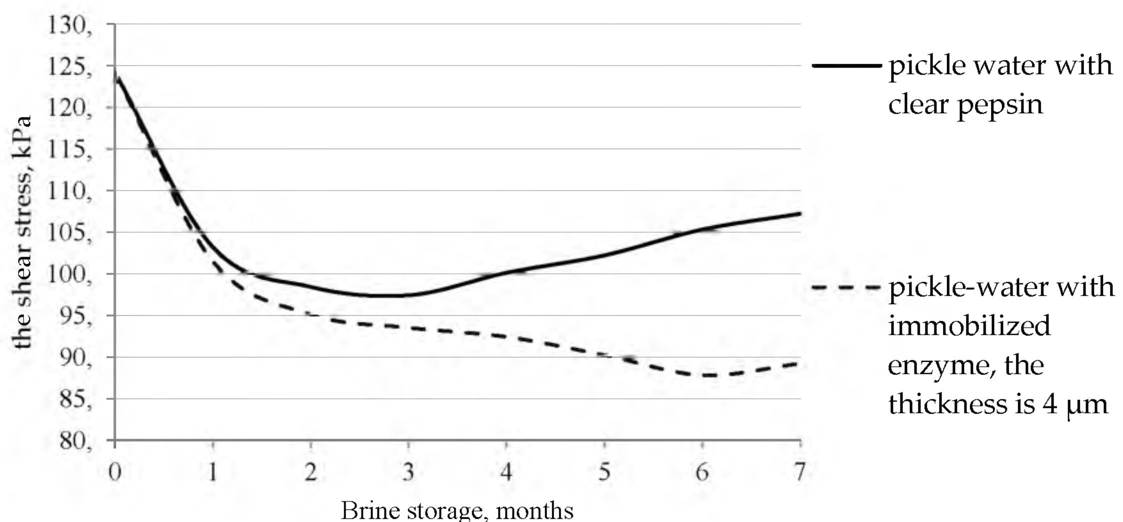

Figure 6 – The impact of the pepsin length of the storage on the structural-physical qualities of the ham pepsin (Row 1- pickle water with clear pepsin, Row 2- pickle-water with immobilized enzyme the thickness of the maltodextrin coating 4 μm)

It should be noted that within 1 month of storage, the proteolytic effect of pure and immobilized enzymes remained almost at the same level, as can be seen from the results of determining the stress value of the slice of ham. At the same time, pure pepsin noticeably lost its activity after 3 months and its proteolytic effect on the product was significantly reduced. This is evident by comparing the stress values of a slice of ham treated with an enzyme stored for up to 3 months and pure pepsin. At the same time, the immobilized enzyme did not lose its activity during 6 months of storage, which is evident from the

decrease in the voltage value of the ham cut. These results indicate the protective action of maltodextrin, preventing the inactivation of pepsin.

In accordance with the experiments upshots there was a way of making pepsin enzyme immobilization based on the annexation of the maltodextrin to the inert matrix. The impact of the thickness of the maltodextrin coating on the enzyme activity was proven; also, it is shown that immobilization of the pepsin by maltodextrin pushed the maximum activity roughly for 2 units in the alkali direction.

It was also proven that the conservation of the immobilized enzyme under the temperature 0-2 °C saved its proteolytic activity 2 times better compared with the clear enzyme.

Conclusion. As a result of the present research the technology for microencapsulation of proteolytic enzymes in a pseudo-boiling layer was developed and it was established:

- the efficiency of the developed microencapsulation technology is ensured by the diffusion of maltodextrin into the enzyme which allows to maintain high long-term activity of the enzyme in meat products;
- maltodextrin provides high hardness of the walls of the capsule during long-term storage the integrity is not broken;
- the maximum activity of pepsin immobilized in a maltodextrin solution, with an increase in the pH of the reaction medium, shifts to the alkaline side, which is not typical for a free enzyme;
- the results of determining the cutoff voltage of the ham confirm that the immobilized enzyme retains its activity for 6 months of storage. While pure pepsin after 3 months of storage had a less proteolytic effect, as evidenced by the magnitude of the shear stress of the finished product;
- Encapsulating the pepsin enzyme can be an effective way to ensure the quality of ham.

Bearing in mind the received outcomes, it is recommended the microencapsulation of the proteolytic pepsin enzyme with the use of maltodextrin with a coating thickness of 4 to 6 µm, which enables to broaden the usage of the immobilized enzymes during the production of meat products.

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ПРОТЕОЛИТТІК ФЕРМЕНТТЕРДІ ӨНЕРКӘСІПТЕ ҚОЛДАНУ ҮШІН МИКРОКАПСУЛДАУ

Аңдатпа. Мақала ферментке мальтодекстриннің диффузиясына негізделген жалған қайнататын қабатта протеолиттік ферменттерді микрокапсулдау технологиясын әзірлеу бойынша зерттеумен таныстырады, бұл ферменттің жоғары белсенділігін ет шикізатын ұзақ уақыт бойы сақтауға мүмкіндік береді. Микрокапсулдау келесі түрде жүргізілді: патрубкаға ферментпен сыйымдылық ғерметикалық қосылады және компрессордан ауа беріледі, ол түрлі диаметрлі ауысымды шүмектер арқылы өтіп, бастапқы бөлшектерді алады. Одан әрі турбуленттік ағындағы бөлшектер кептіріледі және фонтандау ағындары түрінде қайнаған қабаты бар аппарат корпусына түседі. Фермент бөлшектерінің диаметріне байланысты осы ағындардың жылдамдығын дұрыс таңдау тесіктердің қажетті диаметрі мен конустық корпустың өлшемі (биіктігі мен диаметрі) бар шайбалармен қамтамасыз етіледі. Бөліктерді патрубкада және аппараттың фонтандау ағынында алдын ала кептіргеннен кейін вентиль ашылады және ауа сұйық компоненттерге арналған ауыспалы шүмегі бар дисперғаторға түседі. Шүмектің диаметрі корпустың түптік бөлігінің фонтандаушы ағындарына патрубок арқылы енгізілетін қажетті көлемдегі тамшылардың диспергирленуін қамтамасыз етеді. Тамшылар көлемі аппараттың фонтандау ағынындағы қатты бөлшектер порциясының жиынтық бетіне қарағанда тең немесе бірнеше үлкен сұйық компоненттер порциясының жиынтық бетін қамтуы тиіс. Ферментті тамшылап жабу үдерісі қарқынды және жылдам ағады. Ол фонтандау ағындары, ферменттің бөлшектерін шаятындықтан жабысқан тамшыларды кептірумен бірге жүреді. Сұйық компоненттердің барлық порциясы берілгеннен кейін вентиль жабылады. Кептіру ұзақтығы 5-8 минутты құрайды.

Эксперимент үшін пепсин ферменті таңдалды, қорғаныс қабаты ретінде 10% – мальтодекстриннің сулы ерітіндісі қолданылды. Қатты заттың сұйықтыққа (Қ/С) арақатынасы 10/1 ÷ 11,5/1 шегінде шыдады. Күйік түсіруші ағент, оның ішінде кептіру режимінде бөлме температурасында аппарат арқылы сорылатын ауа болды.

Эксперимент үшін 2 мкм, 4 мкм және 6 мкм мальтодекстрин қабатының қалыңдығы бар капсулаланған ферменттердің бес эксперименталды өңделген үлгісі дайындалды. Бақылау ретінде таза капсулданбаған пепсин алынды. Пепсиннің белсенділігі казеин ғидролизінің нәтижесінде пайда болатын тирозин саны бойынша 1,5-тен 5,0 бірлікке дейінгі pH диапазонында анықталды. Субстрат ретінде Гаммерстен бойынша казеин қолданылды. Тирозин мөлшері СФ-46 спектрофотометрінде толқын ұзындығы 280 нм болғанда спектрофотометриялық әдіспен анықталған. Әзірленген технолоғия капсулалардың қорғаныш қабатының қалыңдығы түрлі микрокапсулаларды жасауға мүмкіндік береді. Бұл ретте, жағу ұзақтығына байланысты пепсин түйіршігіне мальтодекстрин ерітіндісін жағудың орташа қалыңдығының сызықтық тәуелділігі ($p \leq 0{,}05$) тәжірибелік жолмен анықталды. Мальтодекстрин капсула қабырғаларының жоғары беріктігін, ұзақ сақтау кезінде олардың тұтастығын қамтамасыз етеді. Мальтодекстрин қабатының қалыңдығы 4 мкм болатын пепсиннің белсенділігі мальтодекстрин қалыңдығы 6 мкм болатын пепсинге қарағанда шамалы төмен екені анықталды. Осыған байланысты 4 мкм-де мальтодекстриннің қорғаныс қабаты бар пепсинді қолданған жөн. Мальтодекстрин ерітіндісінде иммобилденген пепсиннің протеолитикалық ферментінің ең жоғары белсенділігі реакциялық ортаның pH жоғарылауы кезінде сілтілік жағына жылжиды, бұл еркін ферментке тән емес. Сонымен қатар, иммобилизацияланған фермент 6 ай сақтау кезінде өзінің белсенділігін жоғалтпады, бұл ветчина кесіндісінің кернеу мөлшерінің азаюынан көрінеді. Бұл деректер пепсиннің инактивациясына кедергі келтіретін мальтодекстриннің қорғаныш әсері туралы куәландырады және таза және иммобилизацияланған ферменттің белсенділігін анықтау нәтижелерін растайды. Иммобилизацияланған ферментті 0 – 2^{0}С температурада сақтау өңделмеген ферментпен салыстырғанда оның протеолит белсенділігін 2 есе сақтауға мүмкіндік беретіні анықталды.

Алынған нәтижелерді ескере отырып, 4-тен 6 мкм-ге дейінгі жабын қалыңдығы кезінде мальтодекстринді пайдалана отырып пепсиннің протеолитикалық ферментін микрокапсулдауды ұсынуға болады, бұл ет өнімдерін өндіру кезінде ферменттерді пайдалану мүмкіндігін кеңейтуге мүмкіндік береді.

Түйін сөздер: микрокапсулдау, пепсин, белсенділік, жұмсарту, сан еті, ветчина өнімдері.

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МИКРОКАПСУЛИРОВАНИЕ ПРОТЕОЛИТИЧЕСКИХ ФЕРМЕНТОВ ДЛЯ ПРОМЫШЛЕННОГО ПРИМЕНЕНИЯ

Аннотация. Статья знакомит с исследованием по разработке технологии микрокапсулирования протеолитических ферментов в псевдокипящем слое, которая основана на диффузии мальтодекстрина в фермент, что позволяет сохранить высокую активность фермента в течение длительного времени в мясном сырье. Микрокапсулирование проводилось следующим образом: к патрубку герметично подсоединяется емкость с ферментом и подается воздух из компрессора, который проходя через сменные сопла различных диаметров, забирает исходные частицы. Далее частицы в турбулентном потоке подсушиваются и поступают в корпус аппарата с псевдоожиженным слоем в виде фонтанирующих потоков. Правильный подбор скоростей этих потоков в зависимости от диаметра частиц фермента обеспечивается шайбами с необходимым диаметром отверстий и размером конического корпуса (высота и диаметр). После предварительной подсушки частиц в патрубке и в фонтанирующих потоках аппарата открывается вентиль, и воздух поступает в диспергатор со сменным соплом для жидких компонентов. Диаметр сопла обеспечивает диспергирование капель необходимых размеров, которые через патрубок вводятся в фонтанирующие потоки донной части корпуса. Размер капель должен создать суммарную поверхность порции жидких компонентов, равную или несколько большую, чем суммарная поверхность порции твердых частиц в фонтанирующих потоках аппарата. Процесс покрытия фермента каплями интенсивен и скоротечен. Он сопровождается началом сушки прилипших капель за счет фонтанирующих потоков, омывающих частицы фермента со всех

сторон. После выдачи всей порции жидких компонентов вентиль закрывают. Продолжительность сушки составляет 5-8 минут.

Для эксперимента был выбран фермент пепсин, в качестве защитного слоя использовали 10% - водный раствор мальтодекстрина. Соотношение твердого вещества к жидкому (Т/Ж) выдерживали в пределах 10/1 ÷ 11,5/1. Ожижающим агентом, в том числе и в режиме сушки, был воздух с комнатной температурой, прокачиваемый через аппарат.

Для эксперимента приготавливали пять экспериментальных отшлифованных образцов капсулированных ферментов с толщиной слоя мальтодекстрина 2 мкм, 4 мкм и 6 мкм. В качестве контроля был взят чистый некапсулированный пепсин. Активность пепсина определяли в диапазоне рН от 1,5 до 5,0 ед. по количеству тирозина, образующегося в результате гидролиза казеина. В качестве субстрата использовали казеин по Гаммерстену. Количество тирозина определяли спектрофотометрическим методом при длине волны 280 нм на спектрофотометре СФ-46.

Разработанная технология позволяет изготавливать микрокапсулы с различной толщиной защитного слоя капсул. При этом, опытным путем выявлена линейная зависимость ($p \leq 0,05$) средней толщины нанесения раствора мальтодекстрина на гранулу пепсина в зависимости от продолжительности нанесения.

Установлено, что мальтодекстрин обеспечивает высокую прочность стенок капсулы, их целостность при длительном хранении. Было выявлено, что активность пепсина с толщиной слоя мальтодекстрина 4 мкм незначительно ниже, чем у пепсина с толщиной мальтодекстрина 6 мкм. В связи с этим целесообразно использовать пепсин с защитным слоем мальтодекстрина в 4 мкм. Показано, что максимальная активность протеолитического фермента пепсина, иммобилизованного в растворе мальтодекстрина, при повышении рН реакционной среды сдвигается в щелочную сторону, что нехарактерно для свободного фермента. В то же время иммобилизованный фермент не терял своей активности в течение 6 месяцев хранения, что видно по уменьшению величины напряжения среза ветчины. Эти данные свидетельствуют о защитном действии мальтодекстрина, препятствующем инактивации пепсина и подтверждают результаты определения активности чистого и иммобилизованного фермента. Установлено, что хранение иммобилизованного фермента при температуре 0 - 2 ^{o}С позволяет сохранять его протеолитическую активность по сравнению с необработанным ферментом практически в 2 раза.

Учитывая полученные результаты, можно рекомендовать микрокапсулирование протеолитического фермента пепсина с использованием мальтодекстрина при толщине покрытия от 4 до 6 мкм, что позволит расширить возможности использования ферментов при производстве мясных продуктов.

Ключевые слова: микрокапсулирование, пепсин, активность, мягчение, окорок, ветчинные продукты.

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