NOVEL OPERATIONS — AND MATERIALS — IN FOOD PROCESSING

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Wiesław Kopeć
and Małgorzata Korzeniowska

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PREFACE

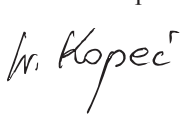
Combining new operations gives the chance to create any process. New opportunities are still searched for food preservation. One of the recent resolutions is an active packaging in which modification of the bioactive substances together with modified atmosphere is applied. It integrates food processing with the field of a novel material technologies. One of the good example is to use the chemical modification of novel packaging materials, like starch and chitozan, which can be used as a surface coatings with an antimicrobial activity.

A modern combined preservation techniques, such as an osmotic drying and a vacuum-microwaving, need to be still developed and described, especially in the case of the processing kinetics, in order to have a low-impact on the biologically active substance present in raw materials. Moreover, such techniques should be an alternative to classical, destructive for nutritional components methods of food preservation.

One of the most important requirements to meet by a method of food conservation is low-energy consumption and being an environmentally friendly. In this context irradiation as up to now remaining still a controversial technology for food purposes from the consumer concerns, should be consider as an energy-saving technology. High pressure is an emerging technology, which does not carry any consumers awareness due to the fact that it is not connected with chemically or biologically, including genetically modified organisms application, method of food preservation. New application of physical methods presented in this book showed that those techniques can be very innovative.

Another emerging challenge of food processing is to generate as less wastes as possible. Only operations created small amount of utilizable by-products are accepted. It is especially important in meat industry, which produces a very high biological loads for an environment, especially due to high content of proteins. Those proteins, which are still biologically valuable, and should be recycled like in the production of gelatin from bones or mechanically deboned meat. Moreover, plant origin by-products have to be source for manufacturing of new materials for example created in extrusion processes. Development of the new materials focusing on by-products utilization has to take into account all aspects assuring high quality and safety of the final products meeting high expectations of educated consumers. Special concerns should be put to inhibition of oxidation processes which easily lead to the spoilage of food materials.

Wiesław Kopeć



Małgorzata Korzeniowska

1

HIGH PRESSURE PROCESSED FOOD PRODUCTS

Introduction

Consumer's expectation for minimally processed, microbiologically safe, additives free and stable food products with "fresh like" characteristics has stimulated the interest of alternative food preservation technologies [Cardello et al. 2007, Deliza et al. 2003, Deliza et al. 2005].

Development of new technologies and modification of conventional preservation methods is associated with restriction to the thermal processes causing significant changes in the structure and ingredients of raw food product. Modern, alternative technologies of food preservation are mainly based on the concept of minimal processing with retention the high nutritional value and sensory attributes of natural food while maintaining the product quality and ensuring consumers health safety. Nonthermal processing of foods has essentially meant unprecedented opportunities for the industrial sector in providing better health and wellness for the consumers and unforeseen new food products of excellent quality without compromising safety. The challenges surrounding these emerging technologies are immense, but the long list of interested groups in support of their development is growing in an exponential fashion. Alternative, nonthermal processing technologies are being advanced and making a significant, positive impact in the food sector. Emerging processing facilitates the development of new products never envisioned before as a series of niche markets that will potentially receive wide attention in nearest time. The opportunities for such new products are countless and most of them will have superb quality and very attractive prices [Barbosa-Canovas et al. 2005, Farkas et al. 2000, Hendrickx et al. 2002].

Nonthermal technologies can be used for decontamination, pasteruization and in some aspects for sterilization but in all this cases of use the most important attribute of the processed product is excellent quality and fresh characteristics of most products. The short processing time is characteristic of most explored novel nonthermal technologies as well in both process energy and economic savings. The producing optimum quality and safe-processed products has become a top priority for food science and technology. During exploration and application of novel technologies should be taken into consideration the following relevant factors: food safety maximization, the kinds of inactivated microorganisms and achieved number of log cycles, lethal doses required for microorganisms inactivation, effect on enzyme activity related to food quality factors, searching the most attractive process combina-

tions to maximize processing parameters synergy, alterated food quality attributes, scale up laboratory and pilot plants achieved results to industrial applications, reliability of emerging technology, adoption costs including engineering the process, initial investment, operation of the process, maintenance and depreciation, energy savings, environmental impact, consumer perception and wellness of applied technology and processed food products for possible successfull implementation in industrail scale and food products convenience [Barbosa-Canovas et al. 2005, Devahastin 2011, Farid 2010, Singh and Heldman 2008].

These requirements are complied by the high pressure processing technology (HPP) which as one of the emerging technologies in food processing and preservation offers the opportunity of producing food of high quality, greater safety and increased shelf-life. High pressure processing has potential application for food preservation with prospects to inactivate undesirable microorganisms and enzymes with minimal heat treatment, resulting in the almost complete retention of sensory and nutritional characteristics of fresh food and increased shelf-life. Other advantages of HPP over traditional thermal processing include reduced process time, minimal thermal penetration of product, minimal undesirable functionality alterations, retention of sensory properties as freshness, flavour, texture and colour. Essential nutrients and vitamins retain stable or undergo minimal changes. The usefulness of high pressure treatment of food products is the settlement of such compression parameters which do not lower the nutrient value and sensory features of the product and affect directly on his persistence by the elimination or significant reduction unprofitable microbiological and enzymatic processes [Barbosa-Canovas et al. 2005, IFT Report 2000, Matser et al. 2004, Torres and Velazquez 2005]. The inactivation degree depends of applied high pressure processing parameters and the chemical composition of processed food products. The most effective method of inactivating microorganisms inactivation is considered to simultaneous effects of pressure and temperature. Effectiveness of high pressures on microbial morphology depends of the type of microorganisms and environmental conditions of their development (pH, temperature, water activity) [Barbosa-Canovas et al. 2005, Farkas and Hoover 2000, Hayashi 2002, Hendrickx and Knorr 2002, Houska et al. 2006, Indrawati 2010].

The commercial application of high pressure processing for food preservation generally concerns the highly industrialized countries which communities with relatively high incomes are interested in the acquisition and consumption minimally processed food products. Food products preserved by high pressure processing in the industrial scale are offered on the consumer market in Japan, USA and some European countries (France, Spain).

Description of food preservation by High Pressure Processing (HPP)

High Pressure Processing is nonthermal method of food preservation and processing under pressure in the range of 100÷1000 MPa. The published terminology appearing in the literature concerning this concept as high pressure technique, pressurization, pascalization, High Pressure Technology, Ultra High Pressure (UHP) has the same meaning. The first projects indicating the possibility of using this technology in the food industry were realized over 100 years ago. The processing parameters of certain food products preserved under high-pressure are presented in Table 1.

High pressure technology is currently considered in the world as the method for providing great opportunities in food preservation, characterized by prolonged durability, new

functional features, minimally processed, chemical preservatives free. It is believed that it could fully replace or support traditional, conventional thermal methods of food preservation and among new physical preservation methods will find the widest application. The inactivation of microorganisms and enzymes combined with the lack of negative impacts on the low molecular weight components (taste, smell, colour and vitamins) causes that high pressure processed food products are of high quality and durability with retained sensorial characteristics typical for fresh product. The influence of high pressure on food products should be considered with monitoring the quantity and activity of microorganisms, delaying or accelerating enzymatic reactions and changes in the food ingredients properties [Barbosa-Canovas et al. 2005, Farkas and Hoover 2000, Hayashi 2002, Hendrickx and Knorr 2002, IFT Report 2000, Patterson 2005, Sanchez-Moreno 2003a, 2003b, Torres and Velasquez 2005].

Table 1

High pressure processing parameters of selected food products

Food products	Pressure [MPa]
Fruit products	
Orange juice	100–800
Apple juice	150–621
Apricot juice	600
Jams and jellies	100–400
Apples (cubes)	400
Strawberry smoothie	200–500
Banana puree	500–700
Vegetable products	
Fresh vegetables (lettuce, asparagus, cauliflower, broccoli, green peas)	200–400
Carrot juice, Broccoli juice	600
Tomato juice	335–600
Salsas (guacamole)	545
Tofu	400
Sprout seeds	250–400
Vegetable oils	700
Eggs	100–400
Milk and dairy products	
Milk	100–600
Yoghurts	200–800
Ripened cheeses	50–1000
Meat products	
Beef	50–1000
Pork	200–827

Table 1. continuous

Lamb	200
Rabbit meat	200
Chicken meat	350–500
Turkey meat	200–400
Minced meat	600
Poultry gourmet pies	550
Pork ham	300
Sausages	400–550
Frankfurter sausages	300–700
Fish products, seafood	
Fishes	200–700
Minced fish meat	200–375
Salmon meat	150
Surimi	100–600
Oysters	207–345
Shrimps, octopus	400
Squids, calamares	150–400
Beer, sake, wine	300

Sources: [www.fresherizedfoods.com; www.avure.com; www.clearwater.ca; www.espuna.es; www.flowcorp.com; www.foodengineering.com; www.fresherunderpressure.com; www.gardenfreshsalsa.com; www.goosepoint.com; www.hormelfoods.com; www.lovittcider.com; www.minutemade.com; www.motivatit.com; www.nchyperbaric.com; www.ortogel.com, www.perdue.com, www.pressurefresh.com; www.sysco.com; www.theperfectoyster.com; www.tyson.com]

High pressure processing is based on the application of three physical parameters: pressure, time and temperature, among which the most important role is played by the pressure, imparting energy to the system. During pressurization exerting pressure has isostatic nature what means that it propagates in the medium at the same time in all directions with the same pressure value and thus the shape, size and type of product undergoing the compression are not affecting for the progress and effectiveness of the process. As compared to thermal methods the technique of high pressures is characterized by low energy consumption, generally, the temperature as one of the compression process parameters not exceed the range of 40°C. In pressure processing of food products on an industrial scale used pressure volumes are from the range of 300÷800 MPa. The upper limit of the applied pressures is determined by the structural strength of pressure chambers while the compression time of 20 minutes shall be considered as the maximum in respect to process economics. High pressure volumes are obtained in a very short time (eg, 1000 MPa - 90 seconds) and the compression energy is relatively low, for example, compression energy the water volume of 1 liter with exerted pressure of 400 MPa is 19.2 kJ when compared to energy of 20.9 kJ necessary to heat the water volume of 1 liter from temperature of 20°C to 25°C [Barbosa-Canovas et al. 2005, Devahastin 2011, Farid 2010, Hendrickx and Knorr 2002, Singh and Heldman 2008].

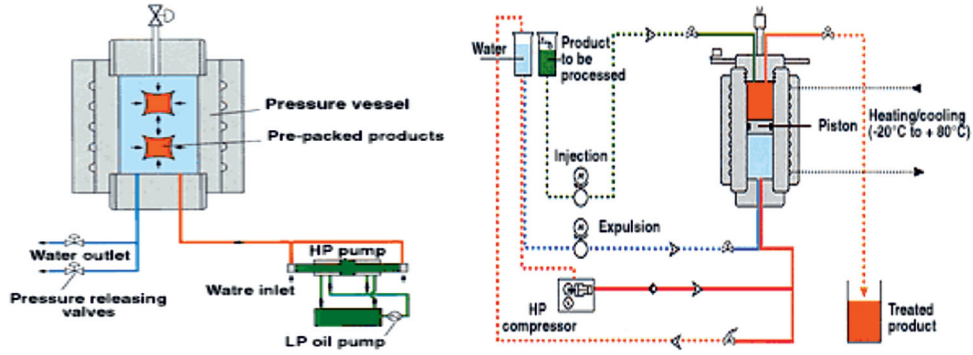
The food sterilization by HPP method involves applying a high temperature in range of 60÷90°C combined with simultaneous compression, where both pressure and temperature contribute to the products preservation, characterized by persistence and in many cases with higher quality than those preserved by conventional methods. The combined effect of temperature and pressure leads to adiabatic heating, the uniform distribution of temperature gradient and the relatively short period of time processing. Homogeneous product heating is an important advantage of the pressure sterilization by reducing the process time and maximum temperature inside the product even up to 10ºC which is beneficial in the case of food ingredients sensitive to the effects of high temperatures. By proper selection of compression parameters (pressure, temperature, processing time) and opportunity of adiabatic rise of temperature inside processed products is possible to achieve the effect of sterilization [Barbosa-Canovas et al. 2005, Caroll et al. 2003, Hartmann et al. 2003, Hendrickx and Knorr 2002, singh and Heldman 2008].

Depending of technical solutions high pressure processing of food is carried out with application of the direct method ("batch" system) or the indirect method ("semi-continuous" system) (Fig. 1).

The food products subjected to compression impact with the direct method ("batch" system) are placed prepackaged in the pressure chamber to avoid contact with the medium transferring generated pressure (Fig. 1a). High pressure processing of solid foods starts with removing as much air as possible from the flexible, high-barrier packaging containing food products. Air removal is essential to ensure that a maximum number of packagings/containers can fill the pressure vessel during each compression cycle and that compression work will not be wasted on air in the system. The packagings/containers are loaded into a carrier basket or placed directly into the pressure vessel. Commercial batch vessel volumes range from 30 to 600 liters. The typical process cycle consists of loading the vessel with the prepackaged food product and filling the remaining vessel voide space with water or glycol-water mix which acts as the pressure-transmitting fluid. The vessel is closed and the desired pressure process is achieved through addition of water delivered by an intensifer. After holding the product for the desired time at the target pressure, the vessel is decompressed by releasing the water.

The Spanish company Esteban Espuña S.A. offers meat products pressure processed in the "batch" pressure system. High pressure processing technological line designed for preservation prepackaged sliced boiled ham has process capacity of approximately 600 kg product per hour. The basic cycle of compression process at 400 MPa is performed in the time of 7 minutes with additional time of 8 minutes for handling operations (loading etc.) [www.espuna.es; www.nchyperbaric.com].

Liquid foods can be processed in batch or semi-continuous mode. In the batch mode the liquid product is prepackaged and pressure-treated as for packaged foods. The indirect method ("semi-continuous" system) is generally used for preservation of food products in liquid form (Fig. 1b) [www.avure.com; www.fresherunderpressure.com; www.hpp.vt.edu; www.iit.edu]. The liquid or concentrated food products processed by this method are used as pressure transfer medium and subjected to high pressure processing in the aseptic systems. After the compression process the preserved product is transported through sterile unloading port directly into sterile tanks or is aseptically packaged. Currently realized advanced projects are concentrated on developing the HPP production lines in the operation sequence with continuous processing method ("continuous" system) designed for preservation juices, sauces, purees and yoghurts, which can be pumped [www.flowcorp.com].



a) "Batch" system b) "Semi-continuous" system

Fig. 1. Patterns of high pressure processing devices designed for food preservation [28,30,37]

Flow International Corporation (USA), one of the leading HPP equipment manufacturers, offers in technical solutions the device so-called "Isolator" with inside placed the divider which separates processed product from the medium transferring the energy. After the compression cycle the processed product is pumped and packaged into sterile packagings. The continuity of high pressure processing in this system provides several sequentially placed pressure chambers. The "Isolator" system is equipped with an automatic monitoring of critical control points to ensure the health safety of the preserved food [www.avure.com; www.flowcorp.com].

The high pressure devices in the "semi-continuous" system and the "continuous" system are also suitable for the operation during preservation process in the pulse form consisting of several consecutive programmed compression and decompression cycles.

The development and improvement of high-pressure equipment units designed for commercially pressure processed food products have been based on specific requirements and needs of the food industry. Number of completely high pressure processing equipments operated in industrial scale around the world is still growing and in year 2009 was estimated in amount of 132 units. The use of high pressure in range of 300÷700 MPa for commercial applications around the world in HPP vessels ranging in capacity of 35÷420 L was an annual production rate higher than 150 000 tons in the year 2009.

An important problem related to HPP technology is packaging of food products. The type and nature of the packaging in a large extent depends of the characteristics and properties of the preserved food and created mechanical loads (especially welded closures) during compression process. The volume of most food products during the compression process is reduced which may lead to deformation of the package. To eliminate this occurrence and to ensure integrity of the package closure should be used the flexible and semirigid materials. Shape changes, mechanical properties, permeability and the migration level of packaging components determines the suitability of packaging materials designed for high pressure processing. The packaging designed for pressurized products must be complied with several requirements as sufficient flexibility, return to its original shape after the termination of high-pressure impact, low free volume not filled by product, no negative impact of packaging components on sensory characteristics of preserved food, low permeability to water vapor, the shape and dimensions enabling the optimal use of the pressure chamber capacity. The

most appropriate materials intended for packaging the pressurized food are polymers [Caner at al. 2003, 2004a, 2004b, Dobias et al. 2004, Lambert 2000, Lopez-Rubio et. al. 2005, Ozen and Floros 2001, Singh and Heldman 2008].

The packaging requirement for the high pressure processing varies depending on the type of HPP equipment ("batch", "continuous" or "semi-continuous"). The flexible or semirigid packaging with at least one flexible interface is the best suited for "batch processing" and variety od existing flexible packaging structures may be used. Because high-moisture foods is compressed by 15÷20% in the pressure range of 600 MPa at ambient temperature, HPP packagings materials must be able to accomadate these reductions in volume and then return to their original volume without loss of seal integrity or barrier properties. In the "batch" system most commonly used packaging is vacuum "skin pack" enabling to preserve the natural product features for a longer time period. The "semi-continuous" and "continuous" systems are used in the case of pumpable liquid products which are aseptically packaged after pressure treatment which is technically and economically preferable [www.avure.com; www.flowcorp.com].

Commercial applications of high pressure processing for preservation food products offered on the consumer market in the world

High pressure processing creates many new opportunities in food production (Tab. 2). Application of HPP technology to extend the durability and creation new functional features of fruit and vegetable juices, desserts, fruits, milk and dairy products, meat and meat products, poultry and poultry products, fishes and fish products with maintaining the natural nutrition values and appealing sensory characteristics of the raw product meets the expectations of the modern consumer.

The first commercial high pressure processed food products were produced by Meidi-Ya Company and marketed in Japan in the early 1990 years. The range of offered products included fruit jams: strawberry, apple and kiwi, packaged in plastic cups, pressure processed at 400 MPa for 20 minutes, packaged in plastic cups and fruit juices: grapefruit, lemon, orange, apple and tangerine, which were characterized by natural colour, taste and smell of raw materials. This innovation was the result of the research and development program initiated by Japanese scientist R. Hayashi from Kyoto University who created the "Association of High Pressure Application" composed of food manufacturers, HPP equipment suppliers and scientists, supported by Japanese government funding in the years 1989–1993. In subsequent years an offer was extended to other fruit products, among them the wide range of fruit juices, milk and dairy products as desserts, yogurts, dressings, meat products, fishes, seafood, surimi, rice cakes, ready-to-eat rice meals, beer and sake. From this time more than 100 pressure processed food products were introduced on the market in the Japan which gained consumer acceptance [Barbosa-Canovas et al. 2005, Sasagawa and Yamazaki 2002, Suzuki 2002].

The Japanese company Echigo Seika [www.echigoseika.co.jp] since the year 1994 started production of pressurized rice and cereal products processed at 200÷400 MPa with moderate heat treatments at temperature of 50^{o}C. Company produces four types of high pressure processed products, among them ready-to-eat brown rice and ready-to-eat white rice, packed

in single serve trays, designed for Japanese market. The products need only to be heated for 3 minutes in a microwave owen before consumption. Single portion of pressurized ready-to-eat cereals mix is composed of several kinds of grains including brown rice, black beans, soybean oats, barley and red rice. Hypoallergenic rice is prepared by high pressure treatment of partially hydrated brown rice to enhance rice cell wall porosity. The increased cell difusion facilitates salt-extraction of allergenic proteins. The product is dried after extracion [www.echigoseika.co.jp].

The high pressure treatment is used by the Japanese company Mitsunori specializing in seafood processing. The pressurization process at 300 MPa for 1 minute with sea-water as the compression medium is used for opening clams shells. The clams meat is removed manually, rinsed in sea-water and packaged in flexible pouches or trays also filled with sea-water. The refrigerated shelf life of processed product with preserved freshness is from 3 to 6 days depending on the type of seafood what is very important for Japanese consumers who are used to eating rawa seafood [www.mitsunori.co.jp].

High pressure treatment in the range of 200÷300 MPa provides a simple and efficient method for the removal of edible meat from shell and carapace of shellfish and crustceans. Industrial applications use high pressure to extract crustacean meat from crabs and lobsters, open oysters shells and other bivalves (shucking process). This activity is generally run by small seafood companies located in the United States and Canada which have invested in low-volume pressure units to meet developing market needs.

The French company UltiFruit, noting the development of HPP technology in Japan, was the the first producer in Europe which introduced on the market the food products preserved by high pressure processing. In the year 1994, UltiFruit company has began the production of pressure-pasteurized citrus juices (orange and grapefruit juices) to a local market. The citrus juices marketed as "freshly squeezed" packaged in polyethylene bottles were processed at 400 MPa and characterized with shelf life up to 16 days and high sensory quality. The UltiFruit company has progressively expanded high pressure processing production and actually its pressurized orange and grapefruit juices and line of smoothies, launched in year 2008, are marketed across France.

Current offer of HPP products on the European market include fruit juices and poultry gourmet pies (France), orange juice (Italy), apple juice (Portugal), fish steaks, meat products (ham), prepared meat dishes and vegetable dishes (ready-to-eat meal kits) from Spain [www.abraham.de; www. espuna.es; www.nchyperbaric.com; www.ortogel.com].

The Spanish company Esteban Espuña S.A. was the first European producer of pressure pasteurized meat products. The sliced cooked ham with the label "High Pressure Pasteurized Product Remain Fresh Until Eaten" was launched on the market in October of the year 1998 and even now is being sold and distributed in several supermarket chains in Spain. The pressurized ham slices are vacuum skin-packed with plastic film interleaves to facilitate the separation of slices by the consumers. The product is pressure processed at 400 MPa for 10 minutes and has refrigarated shelf life (in temperature 0÷5°C) of 60 days storage. In the year 2003 Spanish producer extended the range of offered pressurized meat products with various types of ready-to-microwave meat snacks consisting of small sausages (chorizos, pinchitos and morcilla), spicy diced chicken, turkey products, bacon and cheese rolls which have been successful on the market in Spain, Great Britain and France. Easy to prepare the meat snacks "Minute Snacks" are vacuum packed in plastic containers suitable for restitution in the mi-

crowave oven in time of 1 minute. The snacks have refrigarated shelf life (in temperature 0÷5°C) of 60 days. The offer includes wide range of hot meat snacks: Mini Chorizos and Mini Marinated Brochettes, Mini White Catalan Butifarra Sausage, Mini White Sausage, Mini Chicken Brochettes, Mini Turkey Brochettes and Mini Spanish Black Pudding. In the year 2005, the company offered on the consumer market the first sliced cured ham (Iberian, Serrano) pressure processed at 600 MPa and shelf-stable for 40 days at nonrefrigerated temperatures [www.espuna.es].

Abraham Schinken GmbH & Co. KG produces dry cured ham for the domestic German market and for export to the United States. Since the year 2005 vacuum-packed sliced dry cured ham is pressure processed at 600 MPa in time of 3 minutes [www.abraham.de].

Table 2

The offer of high pressure processed food products on the consumer market

High pressure processed food products	Producers
Citrus juices (orange, grapefruit) Smoothies	UltiFruit, Pernod Ricard Co. (France) Pampryl (France)
Ruby Red Orange juice	Ortogel SRL (Italy)
Apple juice	Frubaca Cooperativa (Portugal)
Sliced boiled ham Sliced dry-cured ham (Iberian, Serrano) Meat snacks „tapas" (Minute Snacks)	Esteban Espuña S.A. (Spain)
Fish steaks (cod, salmon, tuna)	CampoFrio Alimentacion (Spain)
Ready-to-eat vegetable meal kits	Grupo Alimentario IAN (Spain)
Guacamole and salsas Fruit juices, vegetable juices Fruit nectars and smoothies Ready-to-eat meal kits	Fresherized Foods (formelly Avomex, Inc. Co.) (USA)
Hummus	Hannah International Foods (USA)
Fruit juices	Lovitt Farms (USA) The Minute Maid Company (USA)
Fruit juices Fruit nectars, fruit drinks Vegetable juices	Fresh Samanta, Inc. (USA) Odwalla, Inc. (USA)
Fruit jams, jellies and desserts Fruits (cubes) Vegetable products, meat products	Tewari Fresh Foods (USA)
Tropical fruit juices and nectars	Maui Pineapple Company Ltd. (USA) Grupo Jumex (Mexico)
Ready-to-eat meat meal kits Prosciutto ham Citrus fruit juices	Hormel Foods Corp. (USA)
Ready-to-eat poultry meal kits	Kraft Foods, Inc. (USA) Perdue Farms, Inc. (USA) Tyson Foods , Inc. (USA)

Table 2. continuous

Oysters	Nisbet Oyster Company (USA) Joey Oysters (USA) OYSA (Australia)
Oysters, clams, shrimps, crabs, octopus, squid, lobsters, crabs meat	Motivatit Seafoods, Inc. (USA) Clearwater Seafoods (USA) Winsom's (USA) Calavo (USA) Leahy Orchards (USA)
Fruit jams, jellies and dressings	Meidi-Ya (Japan)
Citrus fruit juices Ready-to-eat rice meal kits	KSUN Corp. (Japan) Pokka Corporation (Japan), Echigo Seika (Japan)

Sources: [Barbosa-Canovas 2005, Sasagawa and Yamazaki 2002, Suzuki 2002, www.fresherizedfoods.com; www.avure.com; www.clearwater.ca; www.espuna.es; www.flowcorp.com; www.foodengineering.com; www.fresherunderpressure.com; www.gardenfreshsalsa.com; www.goosepoint.com; www.hormelfoods.com; www.lovittcider.com; www.minutemade.com; www.motivatit.com; www.nchyperbaric.com; www.ortogel.com, www.perdue.com, www.pressurefresh.com; www.sysco.com; www.theperfectoyster.com; www.tysonfoods.com]

The sandwich fillings contained cheese or mayonnaise mixed with a wide range of ingredients including ham, cooked vegetables, shrimps, smoked salmon and nuts, produced for the chain of sandwich shops owned by Spanish company Rodilla, since the year 2005 are pressure processed at 500 MPa for several minutes in 1 or 2 kg flexible pouches for shipment to Rodilla company shops all over Spain. High pressure processing prolonged refrigerated shelf life of sandwich fillings up to 21 days without changing the texture and flavor of the fillings [www.rodilla.com].

In the year 2001, the European Commission issued the positive decision for the French Danone Company with permission to place on the market fruit products preserved by high pressure processing as a kind of new foods (Novel Foods) according to the requirements of Regulation (EC) No. 258/97 of the European Parliament and the Council. Danone company did not commercialize the pressure preservation process [Commission Recommendation (EC) 1997, Commission Decision (EC) 2001, Regulation (EC) 1997].

The Australian company Donny Boy is processing and marketing high pressure preserved fruit-based products including purees, sauces and juices which are used in yoghurts, ice cream, food service and as beverages. The fruit products are pressure processed at 600 MPa for few minutes at room temperature. The company started the marketing its first pressurized products, apricot, peach and apple dice for use in yoghurts, in the year 2007 under the trademark "Preshafruit". In the year 2008 the range of pressure processed fruit preparations has been extended and include strawberry, cherry and mango. Donny Boy Company has also launched the line of pressurized exotic fruit purees packed into flexible transparent pouches and lines of high pressure presereved fruit juices and smoothies [www.preshafruit.com.au].

In Poland, High Pressure Research Center of Polish Academy of Science – UNIPRESS in Warsaw, in the year 2001 received the positive decision of National Health Institute allowed to apply high pressure processing on an industrial scale for the preservation of fruit products (fruit jams and jellies) in the range of 200÷600 MPa and for the extension durability

and reducing microbiological contamination of vacuum-packed meat products in the range of 350÷600 MPa.

The wide range of food products preserved by high pressure processing is available in the United States. Application of high pressure is used for extended durability of fresh fruits, fruit juices and fruit drinks, vegetable juices, salsas, sauces, yoghurts, fruit desserts, seafood (oysters, crabs, lobsters, shrimp, clams), meat products, fishes and a number of ready-to-eat dishes (ready-to-eat meal kits) [Barbosa-Canovas 2005, Guerrero-Beltran et al. 2004, Hayman et al., 2004, He et al. 2002, Suzuki 2002] (Tab. 2).

Guacamole, the popular avocado dip, pressurized by American company Fresherized Foods (formelly Avomex Inc. Co.) in the year 1997 was the first high pressure processed food product on the North American market. The company began the first industrail production of pressure-pasteurized guacamole at the plant in Mexico. Products were exported to the United States for food service use. The main reason of application this preservation method was necessity to extend the shelf life of guacamole for several weeks, allowing distribution the product over long distances without costs associated with importation of fresh avocado fruits from Mexico to the United States. Avocado pulp, the main ingredient of guacamole (pH 6.8÷7.0) is susceptible to colour change (enzymatic browning) and loss of natural sensory features caused by poliphenoloxidase activity (PPO). Previous attempts to prolong guacamole durability by rapid guacamole freezing or guacamole packaging in modified atmosphere did not given the expected results. Application of HPP method enabled to keep the sensory properties of fresh avocado fruits, inactivation of microorganisms, substantial reduction of poliphenoloxidase activity and to extend product shelf-life. The application of high pressure revolutionized the market for ready-to-eat avocado products and especially the guacamole market offering for consumers much higher quality products than those preserved by heat or freezing. High pressure-pasteurized avocado products have a refrigerated shelf life of over four weeks. The strong sales of pressurized avocado products by Fresherized Foods have led other avocado producers to invest in high pressure equipment for processing guacamole, avocado paste and salsas. Fresherized Foods is the leading American producer in the high-pressure treatment of foods based on number of high-pressure machines (vessels capacity 215÷350 L) and volume of production facilities in the United States, Mexico, Peru and Chile. Currently available offer of HPP products of this company on the markets besides guacamole and various kinds of salsas includes also fruit juices, vegetable juices, garlic puree, tropical fruits and vegetables [www.fresherizedfoods.com].

In the year 1999, the American company Motivatit Seafoods Inc. offered on the market pressurized raw oysters which quickly gained consumer acceptance [www.motivatit.com].

Hormel Foods Corp. and Perdue Farms Inc. successfully introduced on the consumer market ready-to-eat meat dishes (ready-to-eat meat kits, ready-to-eat poultry kits) preserved by high pressure processing [www.formelfoods.com; www.perdue.com; www.tyson.com].

AmeriQual as as major supplier of military food rations for US Army was interested in HPP technology to improve sensorial qualities of some of its shelf-stable products and found that high pressure combined with heat sterilization could greatly reduce process hold times. As contract manufacturer for Kraft Foods Inc. and Tyson Foods Inc., AmeriQual in the year 2007 started pressure-pasterurization oven-roasted chicken products like breasts, halves, thighs and bone-in whole birds. These products were the first bone-in packaged products industrially processed under pressure. The pressurization process at 600 MPa of several mi-

nutes duration at room temperature extended the refrigerated shelf life of vacuum-packed, preservatives-free, poultry products from 14 to 45 days [www.ameriqual.com]

Foster Farms started pressurization of ready-to-eat sliced turkey and chicken strips, preservatives-free, in the year 2007. The pressure treatment at 600 MPa for several minutes at room temperature extended refrigerated shelf-life of products to 55–60 days of storage period [www.fosterfarms.com].

SimplyFresco produces high-quality tomato sauce and a wide range of refrigerated premium pasta and salsa sauces preserved by high pressure for markets in Texas and southeastern states. The high pressure processing at 600 MPa in few minutes at room temperature yields the refrigerated shelf life of 100 days. The pressurized premium tomato sauce and salsa sauces, preservatives-free, have a unique sensorial quality [www.simplyfresco.com].

The application of high-pressure technology for food preservation in the United States has become one of the most popular alternative methods in relation to thermal processes. The list of pressurized food products offered on the U.S. market is increasing steadily and several of American companies operates with modern high pressure processing technological lines designed for food preservation. One of the largest manufacturers of completely technological lines designed for high pressure processing – Flow International Corp. (USA) – promoting the application of high-pressure for food preservation as "Fresher Under Pressure®" appeals to the imagination and awareness of American food producers and potential consumers [www.flowcorp.com; www.fresherunderpressure.com].

The food products preserved by high pressure processing are also an object of interest of U.S. Department of Defence and National Aeronautics and Space Administration (NASA). The carried out intensive researches and applications related to military combat rations for the U.S. Army in cooperation with research and industrial centers in the United States are coordinated by U.S. Army Natick Soldier Research, Development & Engineering Center (RDECOM), Combat Feeding Innovative Science Team in Natick, Massachusetts.

The performed tests included different types of pressure processed food products as spaghetti with meat sauce, rice dishes, fruit salads and fruit yoghurts packaged into polymer material "omni bowls" type in capacity of 8 oz. Tested pressurized food products were stored at the temperature of 2°C and 48°C for 120 days and were subjected to sensory and microbiological analysis in monthly intervals during storage period (Tab. 3) [www.natic.army.mil].

Table 3

High pressure processed food products tested by US Army

Food products	HPP parameters		Type of packaging
	Pressure [MPa]	Time [min]	
Spanish Rice	340	30	Saran coated nylon
Spaghetti with Meat Sauce	340	30	Saran coated nylon
Yoghurt with Peaches	340	30	Saran coated nylon
Citrus Fruits Salads	340	30	Saran coated nylon
Spanish Rice	580	15	Omni bowls
Lemon Pudding	580	15	Omni bowls
Yoghurt Drink	580	15	Omni bowls
Oriental Chicken (with rice)	580	15	Omni bowls

Table 3. continuous

Seafood Creole (with rice)	580	15	Omni bowls
Vegetarian Pasta	580	15	Omni bowls
Salsa	540	3	Scholle bags*
Apple Juice	540	3	Scholle bags*

* aseptic packaging

Source: [www.natic.army.mil]

Another big challenge in food science and technology is the processing of safe and nutritious foods for storage during long space missions where the required long storage of these foods is not the only requirement. On the request of NASA several lots of high pressure processed fruit yoghurts with extended shelf life were produced and tested by American company Avure Technologies, Inc. in cooperation with the science research center of Oregon State University [www.ohioline.osu.edu].

The precisely datas of high pressure processing of food products in the industrial scale are not widely available for public information. The detailed data of commercial applications may be considered confidential during the initial period of processing and establishing a market. In most countries improvements in food processing technologies may be not directly translate into stronger marketing position. According to the rarely published informations, in the year 2008 about 125 industrial high pressure processing machines were operating for worldwide food processing. Almost 85% of these HPP units were installed after the year 2000. The slow initial application of high pressure technology for food treatment can be attributed to the novelty of the process and a lack of knowledge of the marketing benefits of high pressure processing. Another problem were the limited capabilities of HPP units offered by equipment suppliers before the year 2000 and very high investment costs of high pressure processing units and equipment.

About 60% of total world's HPP industrial units are located in the United States, Mexico, Canada together with several units located in Peru and Chile. The operated high pressure processing vessels in Europe are estimated as 22% of total installed units in the worls and are located in Spain, Italy, Portugal, France, United Kingdom, Czech Republic, Germany, Belgium and The Netherlands. The remaining 18% of the HPP operating units are located in Asia, especially in Japan and recently in China and South Korea. The few units are located in Australia and New Zealand. The high pressure processing appears to be advancing with response for consumers expectations of premium convenient ready-to-eat food products, safety and with extended shelf-life.

Actually in the world about 60 food companies are marketing more than 250 different pressurized food products. The distribution of high pressure processing industrial machines is related to the type of processed food products: 36%–vegetable products such as ready-to-eat vegetables, primarily avocado products; 30%–meat and poultry products such as sliced or diced cooked pork (ham), chicken, turkey; 14%–juices and beverages such as smoothies; 14%–seafood and fish products; 6%–other products such as dairy products or for coprocessing or in tolling applications.

The development of high pressure processing for food preservation is faster in North America than in Europe due to the need of products characterized by extended safety and refrigerated shelf-life and legislation of such preserved foods more favorable to innovation in food production. The industrial manufacturers of pressure-assisted thermal processing must

demonstrate the successful application of these process to food safety authorities as the US FDA or European Food Safety Agency (EFSA). The safety of process must be demonstrated with commercial size process vessels as for any new thermal processing equipment. In the United States high pressure processing technology was approved by US FDA for its efficiency in food microbial pathogens inactivation with minimal negative effect on sensorial quality attributes. The European Union considered high pressure processing as novel technology and requires from the food manufacturers to comply with the requirements of Regulation (EC) No. 258/97 concernig Novel Foods. European food producers intending to use high pressure as the preservation method need to prove the safety of the process through a scientific study concerning not only the microbial safety of the pressurized product but also toxicological and allergenic safety. Scientific study should additionally demonstrate no detrimental effects of high pressure process to nutritional quality and the processed product should not mislead the consumers in perceived value [www.food.gov.uk; www.foodprocessing.com].

Cost calculations related to high pressure processing of food

Despite the wide range and applicability of high pressure processing for food preservation the participation of pressurized products on the food market is still insignificant. The lack of broad information campaign to prevalence knowledge about the novel food products and alternative technologies such as high pressure processing and relatively high price of pressurized products are not conducive to widespread and the demand for these food items. The commercial application of HPP takes place primarily in highly industrialized countries where the community with relatively high incomes is interested in the acquisition and consumption of minimally processed food. The relatively high price of pressure processed food products offered on the market is directly related to still very high investment cost of technological production lines and equipment designed for high pressure processing. Another factors are high depreciation costs of installed production equipment, spare parts and production periodicity depended of the degree of consumers demand for the assortment of offered pressurized food products.

An example of costs calculation associated with capital investment costs of installation line designed for high pressure processing of food products and final unit production cost is presented in Tab. 4. The calculations of investment costs and production profitability were carried out independently by the financial experts teams of American companies Unilever and Basic American Foods. With adopted in the calculations assumption of the possibility high pressure processing technological line for preservation the various types of food products, offer prices analysis of the main equipment manufacturers, the current technical capabilities and the capacity of HPP equipment these expert teams have received similar results as calculation the production cost in amount of 0,05 USD per pound. It was also found that there is a real further possibility of cost reduction to amount of 0,03 USD per pound by modifying and improving the currently offered high pressure processing equipment in terms of productivity and reducing processing cycle time [www.flowcorp.com].

The capital costs of high pressure processing equipment for food preservation in the industrial scale are relatively high compared to the relatively low production costs during the operation processes. Depending of the equipment size (pressure chamber capacity) and the

degree of automation the price of high pressure processing device designed for food preservation in commercial scale is at the level of approximately 0,5÷2,5 million of USD.

The effective cost of food production by high pressure processing is calculated within 0,05÷0,20 USD depending of the product nature. The calculation has been carried out assuming the compression process with the pressure of 700 MPa at the temperature of 20÷50°C, the optimal operation cycle time for 2 minutes and the operation of HPP equipment for 20 hours daily in 360 days. The efficiency of the pressure chamber was defined in the scale of continuous operation in the period of seven years and with carried out about one million production cycles. The calculations of some food producers provided the unit cost production of food preserved by high pressure processing in amount of 0,05 USD per pound for meat products and in amount of 0,07 USD per liter for juices [www.avure.com; www.cfsan.fda.gov; www.flowcorp.com; www.hormelfoods.com; www.lovittcider.com; www.minutemaid.com; www.perdue.com; www.sysco.com].

Table 4

Costs calculation of high pressure food processing

HPP technological line equipment	Specification: pressure chambers: capacity of 215 ltrs. maximum pressure: 690 MPa, max. temperature: 100ºC, min. compression time: 90 s min. decompression time: 30 s compression pumps, monitoring equipment Manufacturer: Flow International Corp., USA Total costs:	USD 3 500 000
Preservation time	6÷8 minutes	
HPP technological line productivity	Technological line: 5 pressure chambers with capacity of 215 ltrs. productivity: 330 days in year daily production: 392 325 lbs per day annual production: 135 000 000 lbs per year	
Depreciation period	10 years	
Capital costs	HPP technological line equipment: Others (infrastructure, additional equipment): Total costs:	USD 17 500 000 USD 10 500 000 USD 28 000 000
Unit costs	Production costs: Repair costs: Depreciation costs: Total costs:	USD 0.0090/lb USD 0.0180/lb USD 0.0185/lb USD 0.0455/lb

Source: [www.flowcorp.com]

The fruit jams produced in Japan from fresh fruits, sugar and pectins additive are pressure processed at 400÷600 MPa in time of 10÷30 minutes. As the result of pressurization is achieved accelerated sugar penetration and gelation together with the effect of pasteurization. The product retains with fresh appearance and flavour during storage period of 2 months at the temperature 4ºC (after opening – 1 week). The retail price of such a jam is more than twice compared to the same product produced by conventional method. Grapefruit juice is pressuri-

zed at 200 MPa with process temperature of 15ºC for 10÷15 minutes to inactivate the enzymes responsible for the bitter taste of juice and can be stored for 3 months at the temperature of 20ºC. The retail price of high pressure processed grapefruit juice is twice higher than the price of juice produced in the standard way [Sasagawa and Yamazaki, 2002; Suzuki, 2002].

According to the information of Spanish company Esteban Espuna S.A. the production cost of high pressure processed sliced cooked ham and various types of meat snacks (morcilla and chorizos) is around 0.80 Euro per kilogram. The last retail price of pressurized sliced cooked ham (Jamón Cocido Extrafino, 150 g package) offered in Spanish supermarket chain El Corte Ingles was calculated in amount of 2.00 Euro for one package [www.espuna.es].

The suggested selling price of high pressure processed sets of prepared meals (ready-to-eat meal kits) as chicken fajitas, beef fajitas, chicken chipotle, chicken enchiladas and chicken quesadillas offered on the consumer market in the United States by American company Hormel Foods Inc. was calculated in amount of 7.99 USD. The retail price of the guacamole set containing two packages with capacity of 8 oz. each one was calculated in amount of 4.0÷5.0 USD [www.fresherizedfoods.com; www.hormelfoods.com].

The cost of carrying out the one series of high-pressure tests at the High Pressure Research Center of Polish Academy of Science – UNIPRESS in Warsaw, Poland is about 1500 PLN. For comparison, the cost of similar services for high pressure processing the samples of tested food products at the High Pressure Processing Laboratory, Department of Food Science and Technology, Virginia Tech. in the United States is calculated in amount of 650 USD for two working hours [www.hpp.vt.edu].

The high pressure processing has been successfully used in the commercial preservation of foods and the number of installed high pressure systems is constantly growing in the world. The increasing application of high pressure processing by food producers reflects industry needs for safety, refrigerated, convenient packaged foods characterized by freshness and reasonable shelf life period.

Conclusions

High pressure processing has potential application for food preservation with prospects of almost complete retention of sensorial and nutritional characteristics of fresh food and increased shelf-life.

The participation of pressure processed food products on the consumer market in the world is insignificant and generally is concerned with selected foods produced in the highly industrialized countries.

The price of pressurized food products offered on the market is directly related to still very high investment costs of HPP equipment.

The unit cost of high pressure processing in the industrial scale is relatively low.

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2

THE EFFECT OF A VACUUM-MICROWAVE DRYING AFTER OSMOTIC PRE-TREATMENT IN SODIUM CHLORIDE SOLUTIONS ON THE QUALITY OF PUMPKIN SLICES

Introduction

There are many different methods of fruits or vegetables drying such as hot air drying, freeze-drying, vacuum drying or microwave assisted drying. Osmotic dehydration can be considered as a potential pre-treatment, which improves the quality of the finish product and reduces the energy consumption [Le Maguer 1988]. The osmotic dehydration usually is performed in sucrose or sodium chloride (NaCl) solutions as well as in solution containing these both ingredients [Ade-Omowaye et al. 2003, Ravindra and Chattopadhyay 2000]. During that process, three types of mass transfer occur at different intensity [Raoult-Wack 1994]. The first type is water flux from the raw material to the osmotic solution. The second type is the solids transfer from the solution to the raw material, while the third type consists of natural solutes migration from the raw material to the solution. The intensities of particular types of mass transfer are depended on the temperature, concentration and kind of the osmotic solution. The optimal concentration of NaCl solution assures high osmotic potential and improves the taste of the dried product.

Osmotic dehydration in NaCl solution was applied to many raw materials such as mushroom [Torringa et al. 2001], tomato pomace [Al-Harahsheh et al. 2009], potato or carrot [Chua et al. 2004]. Another raw material worth consideration is pumpkin (*Cucurbita pepo*) Pumpkin is a valuable source of vitamins (C, E, B_6) tannins, and riboflavin, as well as minerals, e.g. potassium, phosphorus, magnesium, iron and selenium [Terazowa and Ito 2001, USDA National Nutrient Database 2004]. Pumpkin flesh is a delicious and fully appreciated additive in diverse products for children and adults. Pumpkin fruits are being processed to obtain juice, pomace, pickles and dried products.

However, osmotically pre-dried plant material requires finish drying in order to reduce the moisture content until the safe level and to ensure the attractive texture of the finish product. Among several methods used for this purpose, vacuum-microwave (VM) is worth consideration. During VM drying the energy of microwaves is absorbed by water located in the whole volume of the material being dried. This creates a large vapour pressure in the centre of the material, allowing rapid transfer of moisture to the surrounding vacuum and preventing structural collapse [Lin et al. 1998]. As a consequence, the rate of drying is considerably

higher than in traditional methods of dehydration [Sharma, and Parasad, 2004]. The puffing phenomenon, that accompanies the rapid process of dehydration, creates a porous texture of the food and facilitates obtaining a crispy and delicate texture [Sham et al., 2001], and in this way it reduces the product's density as well as shrinkage.

The VM technique has already been satisfactory applied to reduce the moisture content of many plant materials, such as carrots [Cui et al. 2004], cranberries [Sunjka et al. 2004], strawberries [Krulis et al. 2005], peanuts [Delwiche et al. 1986], bananas [Mousa and Farid 2002], apples [Sham et al. 2001], pumpkin [Nawirska et al. 2009] and garlic [Cui et al. 2003]. However, at the beginning of VM dehydration the intensive water evaporation from the material being dried may exceed the vacuum pump capacity. This would require a reduction in the raw material subjected to drying or application of a large vacuum installation. This problem can be overcome by pre-drying of the material using osmotic dehydration in the NaCl solution. As a result of pre-drying the mass loads of a VM equipment can be radically decreased [Hu et al. 2006]. Pre-drying of the material by convective method before VM finish drying reduced the total cost of dehydration and improved the quality of dried tomatoes [Durance and Wang 2002], nutritional value of strawberries [Böhm et al. 2006] and improved the quality of beetroot cubes [Figiel 2010]. However, applying of the osmotic pre-drying in NaCl additionally influence the dielectric properties of the material finish dried with microwaves [Torringa et al. 2001].

No scientific work has yet been reported on the combined drying of pumpkin consisted of osmotic pre-drying in NaCl solution and VM finish drying. This method of drying could make a significant contribution to the vegetable processing industry. However, it is not obvious what concentration of NaCl solution should be applied to ensure the best quality of dried product. Therefore the aim of this work was to determine the effect of sodium chloride concentration on the drying kinetics of pumpkin slices dehydrated by the osmotic pre-treatment and VM finish drying as well as quality of the finish product in terms of shrinkage, colour, texture and sensory attributes.

Materials and methods

Sample preparation: Pumpkin of "Hokkaido" variety was purchased at a local marked. Slices of the raw material (5 mm thick and 18 mm in diameter) were prepared with the aid of a cutter (Gastrotech, Kraków, Poland) and a steel-made blanking tool, which was cylindrical in shape and pointed on one of the sides. The slices were mixed in a plastic container and then were dried by the combination of osmotic dehydration and vacuum-microwave drying.

Drying: Three osmotic solutions of sodium chloride (NaCl) 5, 10 and 15% were prepared in separate containers. The solutions were distributed into 70 ml beakers immersed in water bath of temperature 40°C. The ratio of osmotic solution to pumpkin slices was maintained at 3:1. The mass of the samples was measured after 0.5, 1, 2, 4 and 6 hours of the osmotic dehydration. The samples were taken out from the solution by using a tea strainer and the surplus moisture was gently eliminated from their surfaces with a tissue paper just before measuring of their mass.

VM finish drying was carried out in an SM-200 drier (Plazmatronika, Wrocław, Poland). Pre-dried in osmotic solutions samples of a mass corresponded to the initial mass of

60 g were placed in a cylinder rotating at a speed of 6 rev·min^{-1}. The pressure in the cylinder varied from 4 to 6 kPa. Microwave power amounted to 360 W.

The VM drying kinetics was determined on the basis of mass losses of pumpkin samples. The moisture ratio *MR* was determined from the equation:

$$MR = \frac{M(t) - M_e}{M_0 - M_e} \tag{1}$$

The moisture content of dehydrated samples was determined in vacuum dryer (SPT-200, ZEAMiL Horyzont, Krakow, Poland) for 24 hours at temperature 60°C.

Temperature measurement: During VM finish drying the vacuum-drum was rotating in order to avoid the local overheating of pumpkin samples. Nevertheless, the temperature of individual slices differed despite of the drum rotation. The temperature of pumpkin slices was measured with an infrared camera Flir i50 immediately after taking them out of the VM dryer. The external temperature of most heated slices was recorded. It was supposed that the temperature measured with this method reflected the course of mean temperature during drying. A direct internal temperature measurement of the slices in the drying chamber under vacuum is practically not possible because the measuring elements inserted into the dried material are heated by the microwave emission.

Shrinkage: Shrinkage *S* of the dried product were determined from the equation (2):

$$S = \frac{V_0 - V}{V_0} \tag{2}$$

The volume of pumpkin slices before drying V_0 and after drying V was determined with the use of a gas picnometer HumiPyc-M2 (InstruQuest Inc., USA).

Colour: Colour of dried samples was evaluated by a Minolta Chroma Meter CR-400 (Minolta Co. Ltd., Osaka, Japan). Instrumental colour data were expressed as CIE L^*, a^*, b^* coordinates, which define the colour in a three-dimensional space: L^* (dark – light), a^* (green – red) and b^* (blue – yellow). Samples before measurement were ground using an electric mill.

Texture Profile Analysis (TPA): The TPA (Texture Profile Analysis) of pumpkin slices was determined with an Instron 5566 strength-testing machine (Instron, High Wycombe, UK) equipped with the strain gauge of 1 kN range. In this test the sample was placed between flat plate and the cylindrical probe with diameter 5 mm fixed to the measuring head. While the test the head was moving at a speed of 60 mm·min^{-1}. The sample was subjected to double compression cycles imitating the double bite of the human jaws. Shifting of the head amounted to 50% of the initial sample height. The maximum force was achieved at first compression. Upward shift of the head caused decreasing of the compressive force and created a gap between the deformed sample and the surface of the probe. The subsequent compression took place at lower deformation of the slightly recovered sample. The test was completed at the initial position of the head. On the basis of a TPA curve (Fig. 1), three basic parameters were determined: hardness, cohesiveness and springiness. Hardness was defined as the first force peak on the TPA curve. Cohesiveness was the ratio of the force area during the second compression to that during the first compression. Springiness was understood as the recovered sample deformation in the second compression.

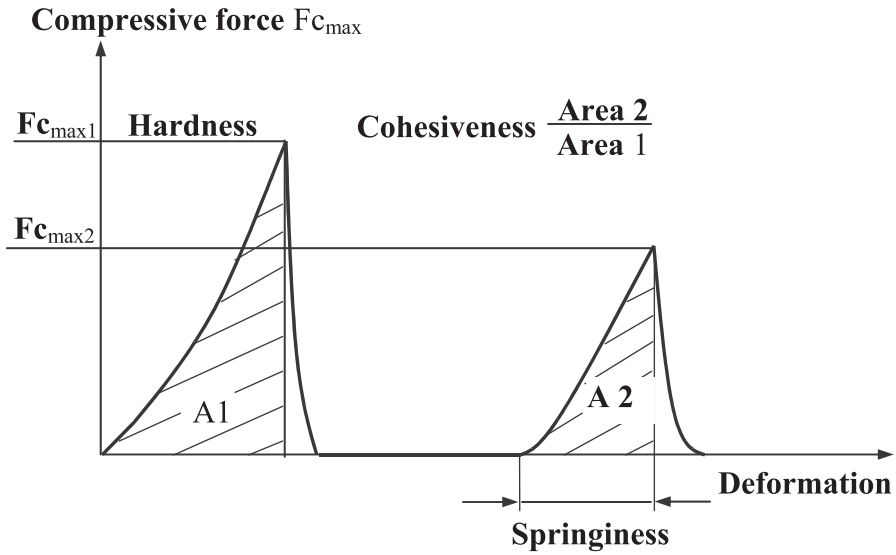

Fig. 1. TPA curve. Fc_{max1} – maximum compressive force at first compression, Fc_{max2} – maximum compressive force at second compression, A1 – force area during the first compression, A2 – force area during the second compression

Sensory evaluation: Sensory evaluation with trained panel was used to discriminate the intensities of the main characteristics of dried product in terms of colour, flavour, taste and texture. The samples were tested by a panel of 8 panellists, ages 25 to 33 years (7 female and 1 male, all members of the Wroclaw University of Environmental and Life Sciences), with sensory evaluation experience and trained in descriptive evaluation of fruits and vegetables.

Measurements were performed in individual booths according with ISO-PN 8586-1:1996 and ISO-PN 8589:1998 standards. The individual samples were scored for the intensity of evaluated attributes on a scale of 0 to 10, where:

- 0 = Non perceptible intensity,
- 10 = extremely high intensity.

The dried samples were presented in 100 mL plastic containers, which stood at room temperature for 30 min prior to analyses.

Results and discussion

Drying kinetics

The changes of pumpkin slices mass during osmotic dehydration in NaCl solution was shown in Fig. 1. It was found that the mass of samples during osmotic pre-treatment was rapidly decreasing at the beginning of the process and then was slightly increasing. This was because at the beginning of the osmotic pre-treatment the intensive water flux from the raw material to the osmotic solution was much higher than the NaCl solids transfer from the solution to the raw material. Afterwards, water loss was getting smaller than the NaCl gain. The experimental points show that the increase in NaCl concentration speeded up the

osmotic process, but has any effect on the final level of mass changing, which was similar amounting to 1.4 g. However, the final moisture content of all samples was different and amounted to 83.3, 80.4 and 77.2% wet basis for osmotic solution concentrations 5, 10 and 15% respectively.

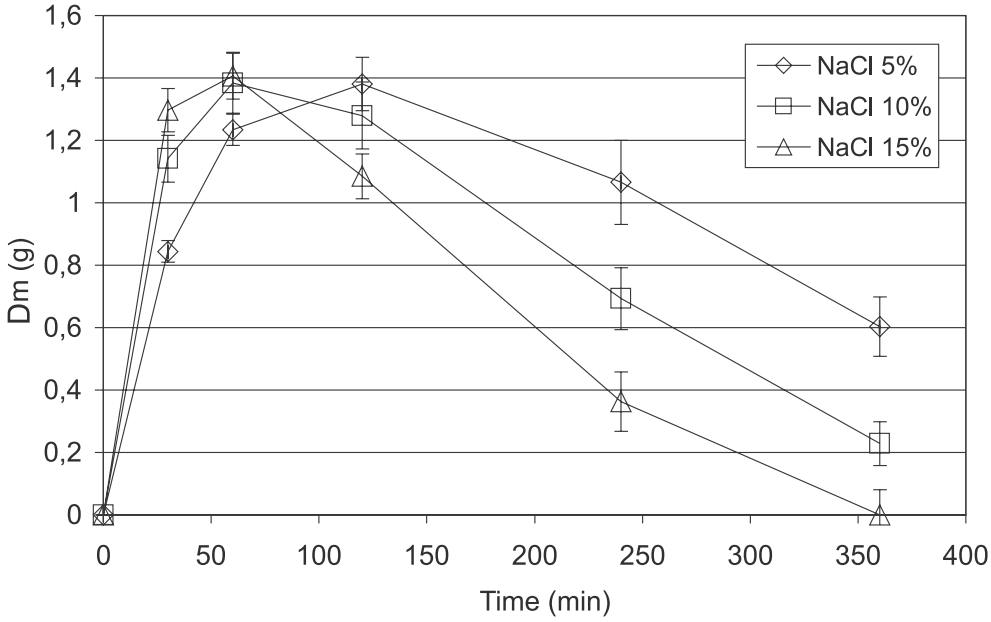

Fig. 2. Changes of pumpkin slices mass during osmotic dehydration in NaCl solutions

The vacuum-microwave (VM) drying kinetics of pumpkin slices without pre-treatment as well as pre-dried in NaCl solution of concentrations 5, 10 and 15% was shown in Fig 3. It was found that the decrease in moisture content of the pumpkin slices during VM finish drying could be described with an exponential equation (3) at very high determination coefficient R^2 (Table 1).

$$MR = a \cdot e^{-k \cdot \tau} - b \tag{3}$$

The drying time of the samples without pre-treatment was 33 min. This drying time decreased until 20 min after pre-treatment in NaCl solution despite of the level of concentration.

Table 1

Coefficients of the equation describing the drying kinetics of pumpkin slices

NaCl concentration (%)	$MR = a \cdot e^{-k \cdot \tau} - b$			
	a	b	k	R^2
0	1.048	0.0276	0.115	0.9978
5	0.9	0.0749	0.135	0.9963
10	0.77	0.0833	0.122	0.9963
15	0.676	0.107	0.102	0.9888

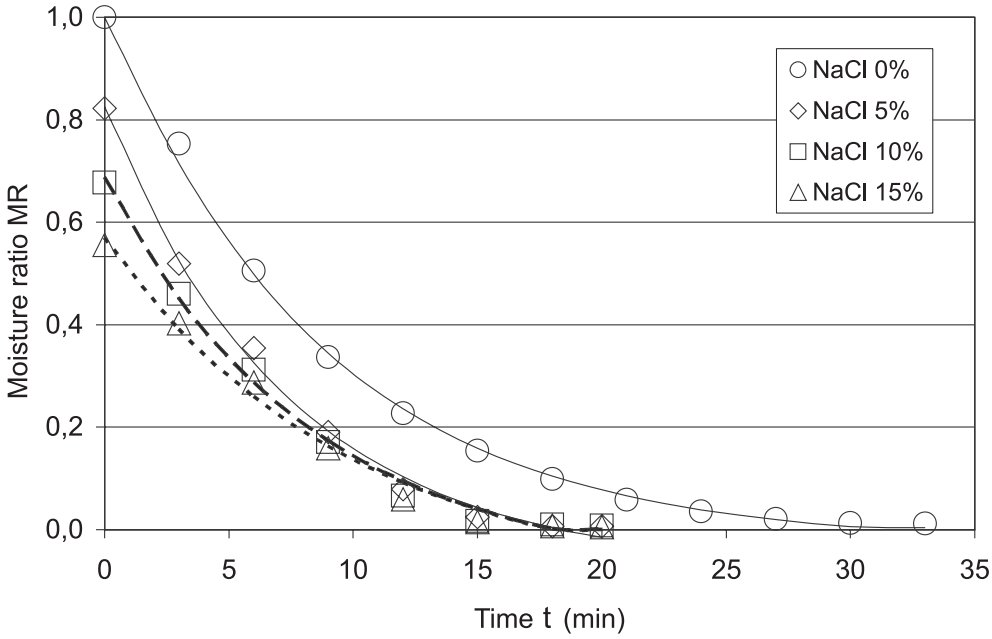

Fig. 3. VM drying kinetics of pumpkin slices pre-dried in different concentrations of NaCl solution

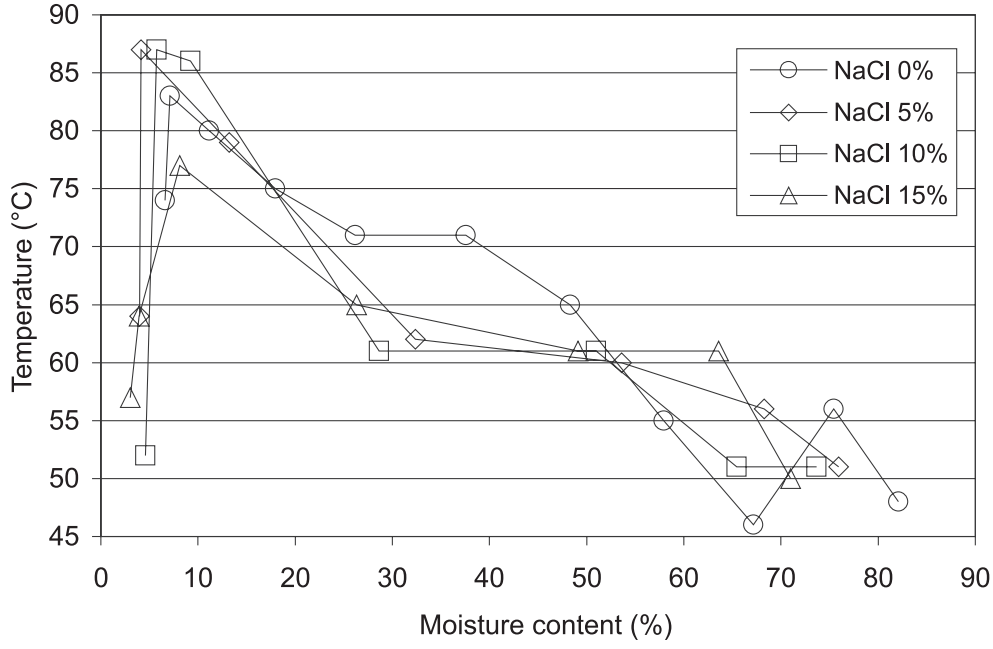

Fig. 4. Temperature profile for pumpkin slices during VM finish drying after pre-drying in different concentrations of NaCl solution

It was stated that while VM finish drying the temperature of samples was increasing until the certain moisture content and then was decreasing (Fig. 4). The peak temperatures 83, 87, 87 and 77°C were found for the critical moisture contents 11.1, 4.1, 5.7 and 8.1% wet basis for the sample without pre-treatment as well as samples pre-treated at NaCl concentrations 5, 10 and 15% respectively. One can presume, that the course of temperature versus moisture content depends on two phenomena [Figiel 2010]. The first is the generation of heat energy by water dipoles in microwave field [Tang 2005] while the other one is the absorbing of that energy by water evaporating from the surface of the material. The increase in the material temperature until critical moisture content results from the excess of the energy generated over the energy necessary for water evaporation. Naturally, the amounts of water generating the energy and water evaporating are decreasing with decreasing moisture content. Beyond the critical moisture content the energy generated by water dipoles is lower than the sum of the energy necessary for water evaporation and that transferred from the material to the ambient of lower temperature. In this study the increase in NaCl concentration generally decreased the critical moisture content and increased the peak temperature. The relatively low peak temperature determined for the sample pre-dried at the highest NaCl concentration might results from the lowest amount of water at the very beginning of VM finish drying.

Shrinkage

The increase in NaCl concentration of the osmotic solutions decreased shrinkage of VM finish dried pumpkin slices (Fig. 5).

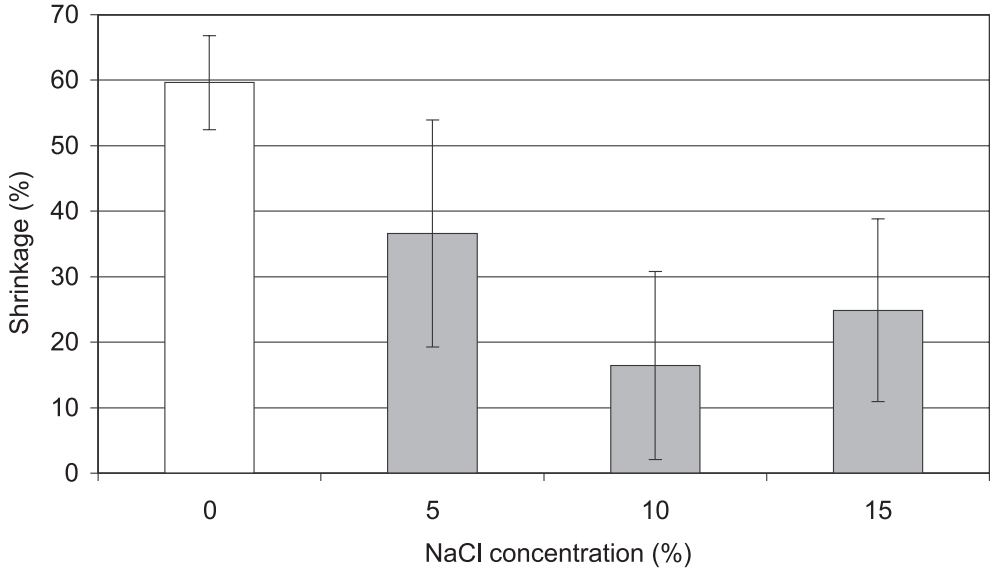

Fig. 5. Effect of NaCl concentration on shrinkage of VM finish dried pumpkin slices

The highest value of shrinkage (59.6%) was found for the sample without pre-treatment and the lowest (16.4%) for the sample pre-dried in the NaCl solution of concentration 10%. Torringa et al. [2001] also reported that the increase in NaCl concentration of the osmotic solution decreased the shrinkage of the mushroom samples finish dried by combined microwa-

ve-hot-air drying method. The VM method usually ensures lower shrinkage than traditional methods of drying due to the puffing phenomenon [Lin et al. 1998]. This study revealed that optimal addition of salt enhances the puffing effect.

Colour

The increase in NaCl concentration of the osmotic solutions slightly increased *L**, and decreased *a** and *b** colour parameters of VM finish dried pumpkin slices (Fig 6). This means that the colour of the slices was getting lighter and was shifting towards greenness and blueness. The values of *L**, *a** and *b** determined for the sample without pre-treatment were 68.9, 15.6 and 57.5 respectively while the values determined for the sample pre-dried at the NaCl concentration 15% were 77.8, 6.8 and 53.7 respectively. Higher brightness usually makes the colour of the product more attractive for the potential consumers.

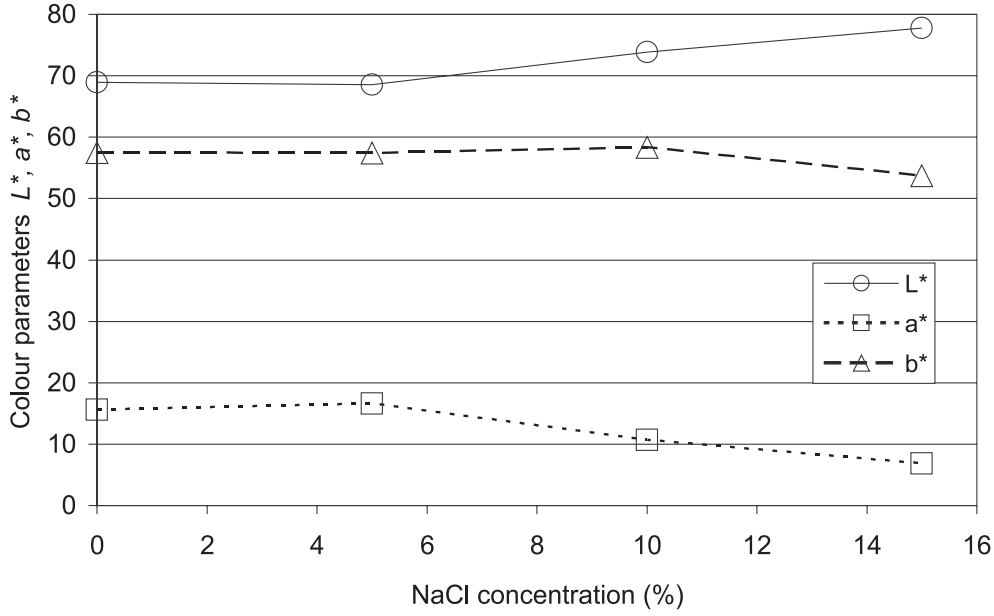

Fig. 6. Effect of NaCl concentration on colour parameters of VM finish dried pumpkin slices

TPA parameters

The results of TPA test reviled that the increase in NaCl concentration of the osmotic solution from 5 to 15% increases the hardness of VM finish dried pumpkin slices from 17.6 to 42.5 N (Fig. 7). However, the highest value of hardness (49.7 N) was found for the control sample, which was not pre-treated in the NaCl solution.

On the other hand, the increase in NaCl concentration of the osmotic solution from 5 to 15% decreased the cohesiveness of the VM finish dried product from 0.47 to 0.16 J/J at the highest value 0.51 J/J obtained for the control sample (Fig. 8). The springiness was also decreasing from 1.92 to 1.07 mm for pre-treated samples (Fig. 9). However, the value of this parameter for control sample was 1.57 mm. The increased hardness associated with decreased cohesiveness and springiness consequently may invoke the impression of increased

crispiness. This is a positive effect, which enhances the attractiveness of the product for the potential consumers [Szcześniak 1971].

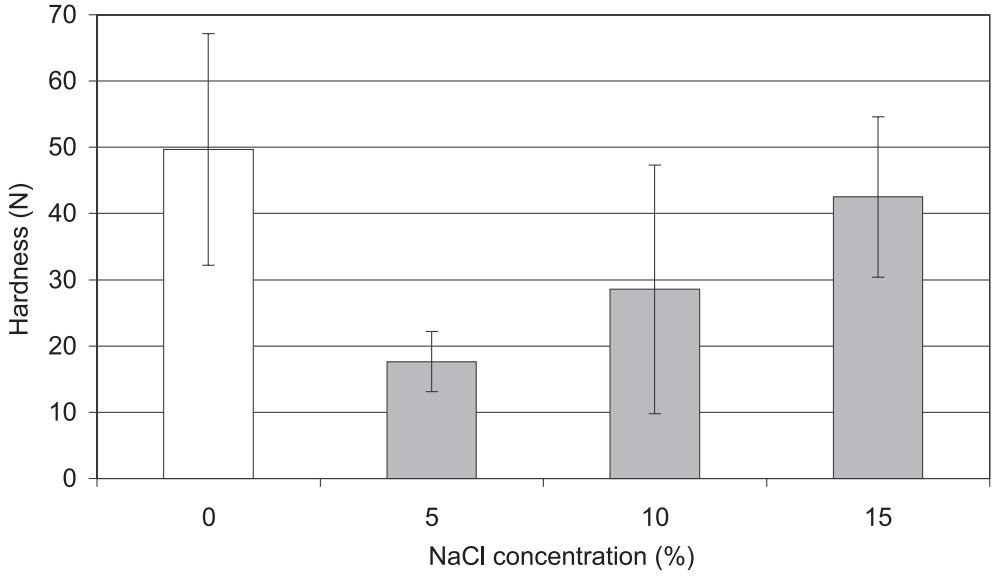

Fig. 7. Effect of NaCl concentration on hardness of VM finish dried pumpkin slices

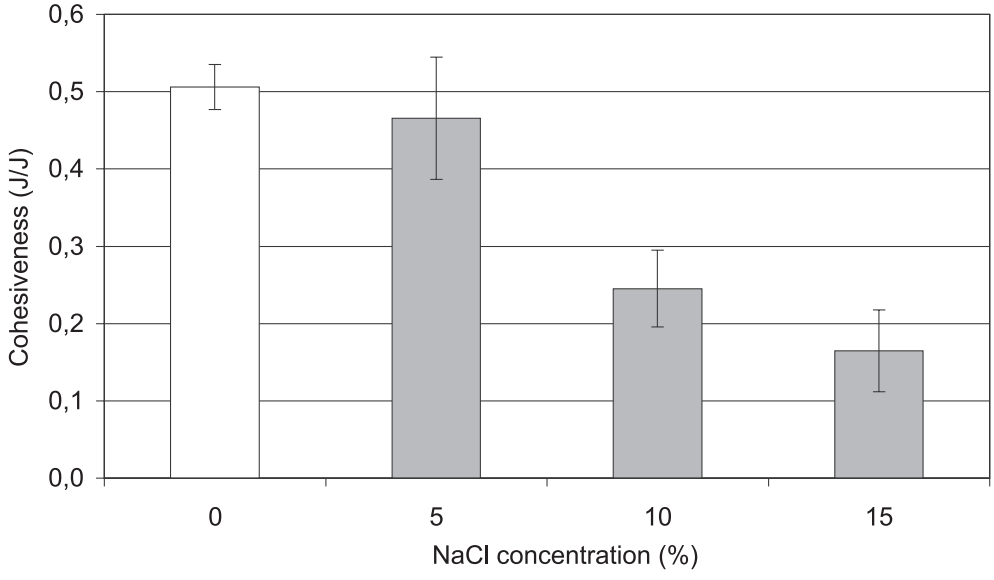

Fig. 8. Effect of NaCl concentration on cohesiveness of VM finish dried pumpkin slices

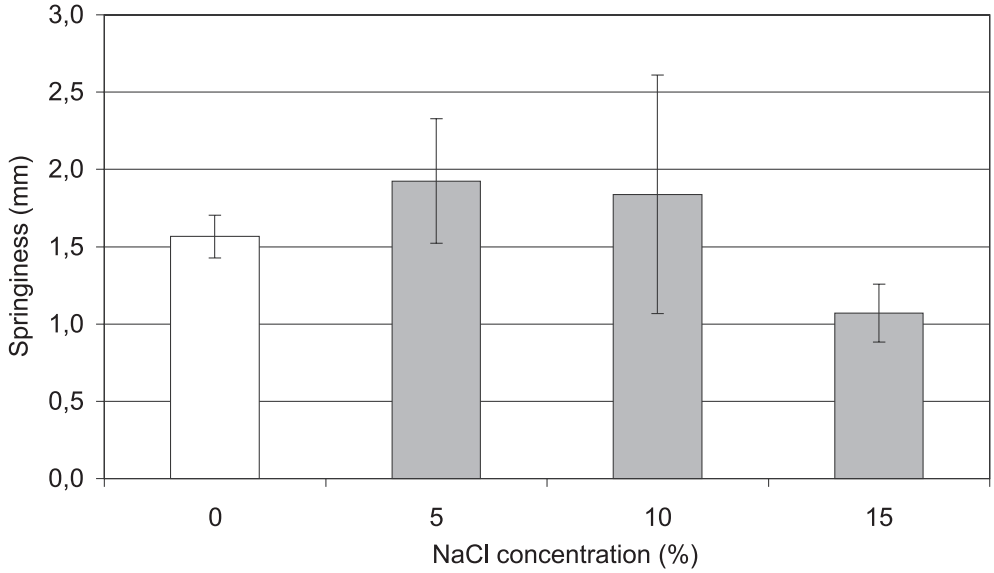

Fig. 9. Effect of NaCl concentration on springiness of VM finish dried pumpkin slices

Sensory evaluation

The results of the sensory assessment of dried pumpkin samples osmotically pre-dried at different NaCl concentrations were compiled in Tables 2 and 3.

Table 2

Sensory assessment of appearance, flavour and taste for pumpkin samples osmotically pre-dried at different NaCl concentrations

NaCl concentration (%)	Appearance		Flavour	Taste
	colour	colour uniformity	typical	typical
0	6.25 ±0.65a	3.94 ±0.90a	6.86 ±0.78a	6.31 ±0.65a
5	7.19 ±0.91a	5.94 ±0.94a	6.57 ±0.84a	3.88 ±0.55bc
10	5.63 ±0.69a	5.94 ±0.72a	7.21 ±0.51a	4.50 ±0.85ab
15	2.43 ±0.50b	4.79 ±1.07a	5.00 ±0.67a	1.79 ±0.62c

Different letters at mean values indicate significant differences (Duncan test, $p<0.05$)

Table 3

Sensory assessment of texture for pumpkin samples osmotically pre-dried at different NaCl concentrations

NaCl concentration (%)	Texture				
	hardness	crispiness	gumminess	fibrousity	tooth packing
0	7.31 ±0.61a	2.75 ±0.92a	5.00 ±1.13a	5.13 ±1.27a	4.50 ±1.11a
5	4.38 ±0.50b	2.56 ±0.68a	6.00 ±0.98a	4.75 ±1.18a	4.81 ±1.04a
10	4.69 ±0.65b	4.00 ±0.98a	5.88 ±0.86a	4.31 ±1.22a	4.50 ±1.05a
15	5.14 ±0.93b	4.43 ±1.03a	4.71 ±0.94a	4.07 ±1.18a	3.43 ±0.94a

Different letters at mean values indicate significant differences (Duncan test, $p<0.05$)

In most cases the differences between mean values were not significant. The significant differences were found only for colour, taste and hardness. However, the test reviled that the pumpkin samples without pre-treatment were characterised by the most typical taste and positive flavour, the highest hardness and fibrousity, the lowest crispiness and colour uniformity, while the samples pre-dried at the NaCl concentration 15% exhibited the highest crispiness and the lowest gumminess, fibrousity, tooth packing, but in the same time the worst scores regarding appearance, flavour and taste. High crispiness is a positive attribute of the food texture [Szcześniak 1971]. It can be stated that the best product in terms of taste and flavour does not require the pre-treatment in NaCl solution but in terms of texture involves pre-drying at NaCl concentration amounted to 15%. This increased crispiness evaluated in sensory assessment is associated with decreased cohesiveness (Fig. 8) and springiness (Fig. 9) determined in TPA test. Taking into account the all sensory attributes it can be stated that the optimal quality of the VM finish dried pumpkin slices can be obtained by pre-drying at NaCl concentration of 10%. It is also worth mentioning a relationship between hardness determined in sensory evaluation and TPA test (Fig. 10).

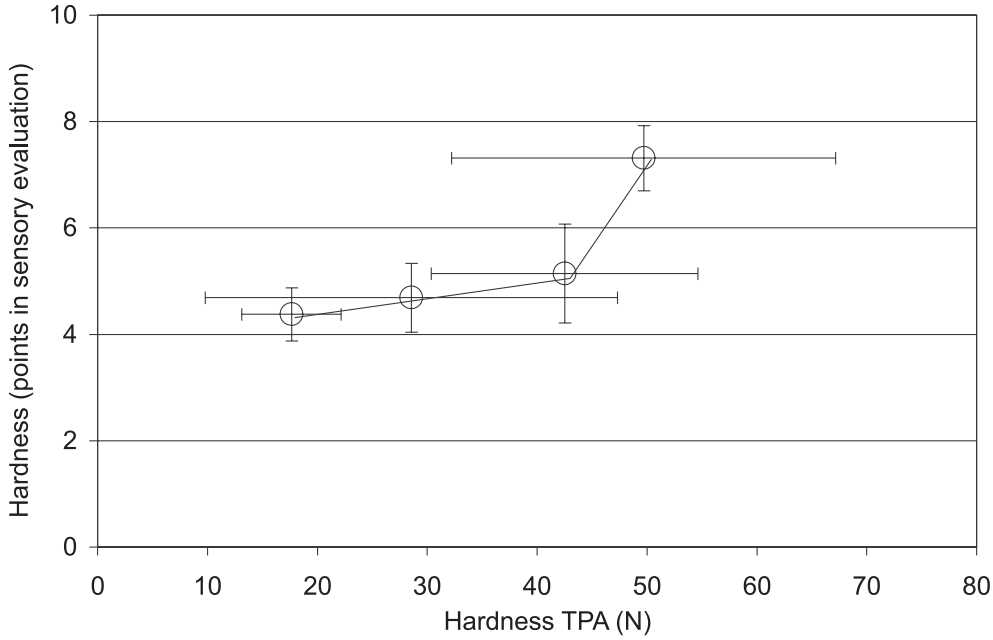

Fig. 10. Relationship between hardness determined in sensory evaluation and TPA test

Conclusions

The mass of pumpkin samples during osmotic pre-treatment was rapidly decreasing at the beginning of the process and then was slightly increasing as the result of water flux from the raw material to the osmotic solution and solids transfer from the solution to the raw material.

The increase in NaCl concentration decreased the final moisture content of the pre-treated samples.

The decrease in moisture content of the pumpkin slices during vacuum-microwave (VM) finish drying could be described with an exponential equation.

During VM finish drying the temperature of the samples was increasing until the critical moisture content and then was decreasing as the result of the balance of energy generated within the dried material by dipoles of water and the energy necessary for water evaporation.

The increase in NaCl concentration decreased shrinkage, cohesiveness springiness as well as colour parameters *a** and *b** but in the same time increased hardness and the brightness of the finish-dried product.

The best product in terms of taste and flavour does not require the pre-treatment in NaCl solution but in terms of texture involves pre-drying at NaCl concentration amounted to 15%. The optimal quality of the VM finish dried pumpkin slices can be obtained by the osmotic pre-drying in NaCl solution with concentration of 10%.

The hardness determined in sensory evaluation was confirmed by the result obtained in TPA test.

Nomenclature

a*	Redness
b*	Yellowness
a, b	Equation coefficients
k	Drying constant (s^{-1})
L*	Lightness
MR	Moisture ratio
M_e	Equilibrium moisture content (kg/kg db)
M_0	Initial moisture content (kg/kg db)
R^2	Coefficient of determination
S	Shrinkage (%)
t	Time (min)
TPA	Texture Profile Analysis
V	Volume (m^3)
V_0	Initial volume (m^3)
VM	Vacuum-microwave
Dm	Change of mass (g)

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3

APPLICATION OF SODIUM CHLORIDE OSMOTIC SOLUTIONS AND VACUUM-MICROWAVES AS DRYING METHODS FOR BEETROOT SLICES

Introduction

Osmotic dehydration of fruits or vegetables is considered as a potential pre-treatment to other methods of drying such as hot air drying, freeze drying, vacuum drying or microwave assisted drying for improving the quality of the finish product and reducing the energy consumption [Le Maguer 1988]. The osmotic dehydration usually is performed in sucrose or sodium chloride (NaCl) solutions as well as in solution containing these both ingredients [Ade-Omowaye et al. 2003, Ravindra and Chattopadhyay 2000]. During that process, three types of mass transfer occur at different intensity [Raoult-Wack 1994]. The first type is water flux from the raw material to the osmotic solution. The second type is the solids transfer from the solution to the raw material, while the third type consists of natural solutes migration from the raw material to the solution. The intensities of particular types of mass transfer are depended on the temperature, concentration and kind of the osmotic solution. The optimal concentration of NaCl solution assures high osmotic potential and improves the taste of the dried product.

Osmotic dehydration in NaCl solution was applied to many raw materials such as mushroom [Torringa et al. 2001], tomato pomace [Al-Harahsheh et al. 2009], potato or carrot [Chua et al. 2004]. Another raw material worth consideration is Beetroot (*Beta vulgaris)* This vegetable is rich in valuable, active compounds such as carotenoids [Dias et al. 2009], glycine betaine, [de Zwart et al. 2003], saponins [Atamanova et al. 2005], betacyanines [Patkai et al. 1997], folates [Jastrebova et al. 2003], betanin, polyphenols and flavonoids [Váli et al. 2007]. Therefore, beetroot ingestion can be considered a factor in cancer prevention [Kapadia et al. 1996].

However, osmotically pre-dried plant material requires finish drying in order to reduce the moisture content until the safe level and to ensure the attractive texture of the finish product. Among several methods used for this purpose, vacuum-microwave (VM) drying seems to be appropriate. During VM drying the energy of microwaves is absorbed by water located in the whole volume of the material being dried. This creates a large vapour pressure in the centre

of the material, allowing rapid transfer of moisture to the surrounding vacuum and preventing structural collapse [Lin et al. 1998]. As a consequence, the rate of drying is considerably higher than in traditional methods of dehydration [Sharma and Parasad 2004]. The puffing phenomenon, that accompanies the rapid process of dehydration, creates a porous texture of the food and facilitates obtaining a crispy and delicate texture [Sham et al. 2001], and in this way it reduces the product's density as well as shrinkage.

The VM technique has already been satisfactory applied to reduce the moisture content of many plant materials, such as carrots [Cui et al. 2004], cranberries [Sunjka et al. 2004], strawberries [Krulis et al. 2005], peanuts [Delwiche et al. 1986], bananas [Mousa and Farid 2002], apples [Sham et al. 2001], pumpkin [Nawirska et al. 2009] and garlic [Cui et al. 2003]. However, at the beginning of VM dehydration the intensive water evaporation from the material being dried may exceed the vacuum pump capacity. This would require a reduction in the raw material subjected to drying or application of a large vacuum installation. This problem can be overcome by pre-drying of the material using osmotic dehydration in the NaCl solution. As a result of pre-drying the mass loads of a VM equipment can be radically decreased [Hu et al. 2006]. Pre-drying of the material by convective method before VM finish drying reduced the total cost of dehydration and improved the quality of dried tomatoes [Durance and Wang 2002], nutritional value of strawberries [Böhm et al. 2006] and improved the quality of beetroot cubes [Figiel 2010]. However, applying of the osmotic pre-drying in NaCl additionally influence the dielectric properties of the material finish dried with microwaves [Torringa et al. 2001].

No scientific work has yet been reported on the combined drying of beetroots consisted of osmotic pre-drying in NaCl solution and VM finish drying. This method of drying could make a significant contribution to the vegetable processing industry. However, it is not obvious what concentration of NaCl solution should be applied to ensure the best quality of dried product. Therefore the aim of this work was to determine the effect of sodium chloride concentration on the drying kinetics of beetroot slices dehydrated by the osmotic pre-treatment and VM finish drying as well as quality of the finish product in terms of shrinkage, colour, texture and sensory attributes.

Materials and methods

Sample preparation: Beetroots of "Alto F1" variety were cultivated in a field situated close to Wroclaw (Poland). Slices of the raw material (5 mm thick and 18 mm in diameter) were prepared with the aid of a cutter (Gastrotech, Kraków, Poland) and a steel-made blanking tool, which was cylindrical in shape and pointed on one of the sides. The slices were mixed in a plastic container and then were dried by the combination of osmotic dehydration and vacuum-microwave drying.

Drying: Three osmotic solutions of sodium chloride (NaCl) 5, 10 and 15 % were prepared in separate containers. The solutions were distributed into 70 ml beakers immersed in water bath of temperature 40°C. The ratio of osmotic solution to beetroot was maintained at 3:1. The mass of the samples was measured after 0.5, 1, 2, 4 and 6 hours of the osmotic dehydration. The samples were taken out from the solution by using a tea strainer and the surplus moisture was gently eliminated from their surfaces with a tissue paper just before measuring of their mass.

VM finish drying was carried out in an SM-200 drier (Plazmatronika, Wrocław, Poland). Pre-dried in osmotic solutions samples of a mass corresponded to the initial mass of 60 g were placed in a cylinder rotating at a speed of 6 rev·min^{-1}. The pressure in the cylinder varied from 4 to 6 kPa. Microwave power amounted to 360 W.

The VM drying kinetics was determined on the basis of mass losses of beetroot samples. The moisture ratio *MR* was determined from the equation:

$$MR = \frac{M(t) - M_e}{M_0 - M_e} \tag{1}$$

The moisture content of dehydrated samples was determined in vacuum dryer (SPT-200, ZEAMiL Horyzont, Krakow, Poland) for 24 hours at temperature 60°C.

Temperature measurement: During VM finish drying the vacuum-drum was rotating in order to avoid the local overheating of beetroot samples. Nevertheless, the temperature of individual slices differed despite of the drum rotation. The temperature of beetroot slices was measured with an infrared camera Flir i50 immediately after taking them out of the VM dryer. The external temperature of most heated slices was recorded. It was supposed that the temperature measured with this method reflected the course of mean temperature during drying. A direct internal temperature measurement of the slices in the drying chamber under vacuum is practically not possible because the measuring elements inserted into the dried material are heated by the microwave emission.

Density and shrinkage: Density ρ and shrinkage S of the dried product were determined from the equation (2) and (3) respectively.

$$\rho = \frac{m}{V} \tag{2}$$

$$S = \frac{V_0 - V}{V_0} \tag{3}$$

The mass of the samples m was measured with the use of balance of accuracy 0.001 g, while their volume before drying V_0 and after drying V was determined with the use of a gas picnometer HumiPyc-M2 (InstruQuest Inc., USA).

Colour: Colour of dried samples was evaluated by a Minolta Chroma Meter CR-400 (Minolta Co. Ltd., Osaka, Japan). Instrumental colour data were expressed as CIE L^*, a^*, b^* coordinates, which define the colour in a three-dimensional space: L^* (dark – light), a^* (green – red) and b^* (blue – yellow). Samples before measurement were ground using an electric mill.

Texture Profile Analysis (TPA): The TPA (Texture Profile Analysis) of beetroot slices was determined with an Instron 5566 strength-testing machine (Instron, High Wycombe, UK) equipped with the strain gauge of 1 kN range. In this test the sample was placed between flat plate and the cylindrical probe with diameter 5 mm fixed to the measuring head. While the test the head was moving at a speed of 60 mm·min^{-1}. The sample was subjected to double compression cycles imitating the double bite of the human jaws. Shifting of the head amounted to 50% of the initial sample height. The maximum force was achieved at first compression. Upward shift of the head caused decreasing of the compressive force and created a gap between the deformed sample and the surface of the probe. The subsequent compression

took place at lower deformation of the slightly recovered sample. The test was completed at the initial position of the head. On the basis of a TPA curve, three basic parameters were determined: hardness, cohesiveness and springiness. Hardness was defined as the first force peak on the TPA curve. Cohesiveness was the ratio of the force area during the second compression to that during the first compression. Springiness was understood as the recovered sample deformation in the second compression.

Sensory evaluation: Sensory evaluation with trained panel was used to discriminate the intensities of the main characteristics of dried product in terms of colour, flavour, taste and texture. The samples were tested by a panel of 8 panellists, ages 25 to 33 years (7 female and 1 male, all members of the Wroclaw University of Environmental and Life Sciences), with sensory evaluation experience and trained in descriptive evaluation of fruits and vegetables.

Measurements were performed in individual booths according with ISO-PN 8586-1:1996 and ISO-PN 8589:1998 standards. The individual samples were scored for the intensity of evaluated attributes on a scale of 0 to 10, where:

- 0 = Non perceptible intensity,
- 10 = extremely high intensity.

The dried samples were presented in 100 mL plastic containers, which stood at room temperature for 30 min prior to analyses.

Results and discussion

Drying kinetics

The changes of beetroot slices weight during osmotic dehydration in NaCl solution were shown in Fig. 1. It was found that the mass of samples during osmotic pre-treatment was rapidly decreasing in the first hour of the process and then was slightly increasing. This was because at the beginning of the osmotic pre-treatment the intensive water flux from the raw material to the osmotic solution was much higher than the NaCl solids transfer from the solution to the raw material. Afterwards, water loss was getting smaller than the NaCl gain. The experimental points show that the increase in NaCl concentration increased the mass loss of the pre-treated beetroot samples within the first three hours of the pre-treatment process. Then the difference in mass loss for samples pre-treated in NaCl solutions of concentrations 5 and 10% was getting smaller. However, the final moisture content of all samples was different and amounted to 83.2, 79.0 and 76.3% wet basis for osmotic solution concentrations 5, 10 and 15% respectively.

The vacuum-microwave (VM) drying kinetics of beetroot slices without pre-treatment as well as pre-dried in NaCl solution of concentrations 5, 10 and 15% were shown in Figures 2–5. It was found that the decrease in moisture content of the beetroot slices during VM finish drying could be described with an exponential equation at very high determination coefficient R^2. The drying time of the samples without pre-treatment was 36 min. This drying time decreased until 21 and 18 min after pre-treatment in NaCl solution of concentrations 5, and 10 or 15% respectively.

It was stated that while VM finish drying the temperature of samples was increasing until the certain moisture content and then was decreasing. The peak temperatures were found for the critical moisture contents amounted to 14% wet basis for the sample without

pre-treatment and around 5% wet basis for the pre-treated samples. One can presume, that the course of temperature versus moisture content depends on two phenomena [Figiel 2010]. The first is the generation of heat energy by water dipoles in microwave field [Tang 2005] while the other one is the absorbing of that energy by water evaporating from the surface of the material. The increase in the material temperature until critical moisture content results from the excess of the energy generated over the energy necessary for water evaporation. Naturally, the amounts of water generating the energy and water evaporating are decreasing with decreasing moisture content. Beyond the critical moisture content the energy generated by water dipoles is lower than the sum of the energy necessary for water evaporation and that transferred from the material to the ambient of lower temperature.

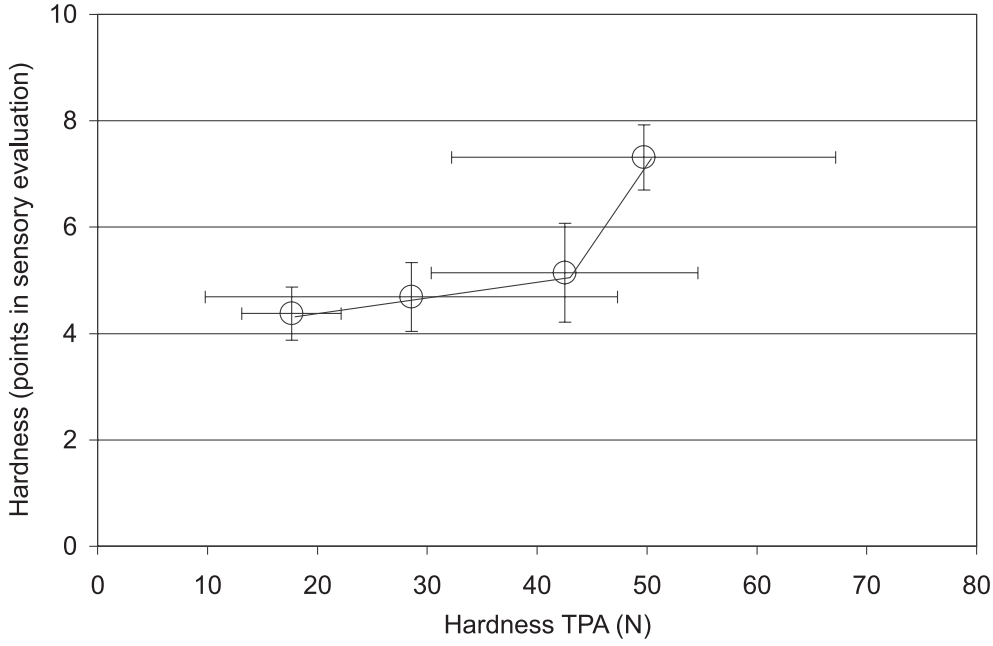

Fig. 1. Changes of weight of beetroot slices during osmotic dehydration in NaCl solution

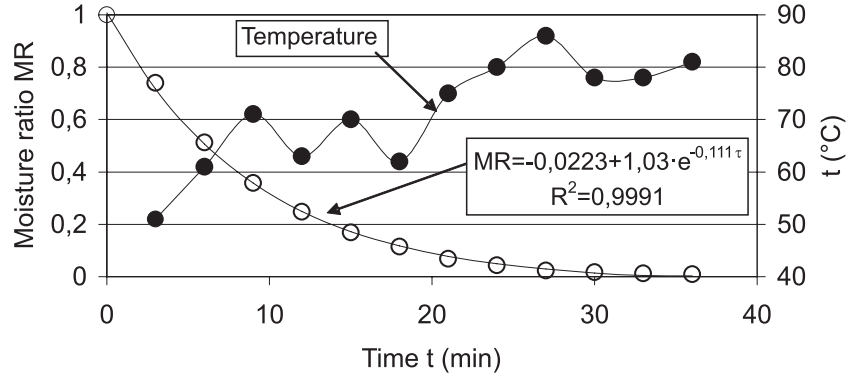

Fig. 2. VM drying kinetics of beetroot slices without pre-drying in NaCl solution

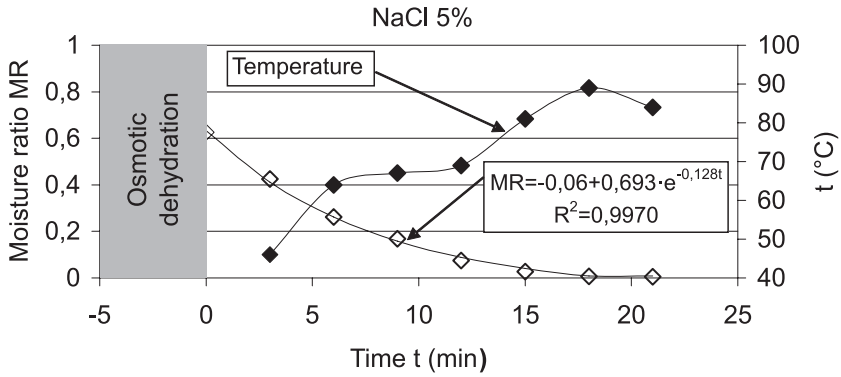

Fig. 3. VM drying kinetics of beetroot slices pre-dried in NaCl solution of concentration 5%

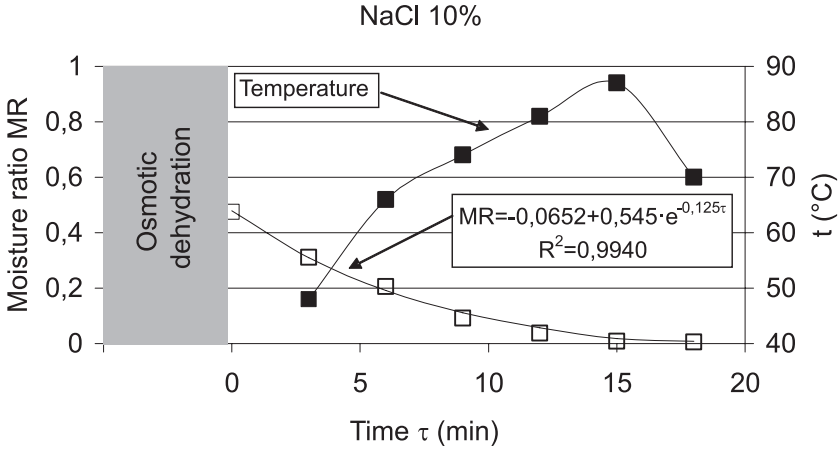

Fig. 4. VM drying kinetics of beetroot slices pre-dried in NaCl solution of concentration 10%

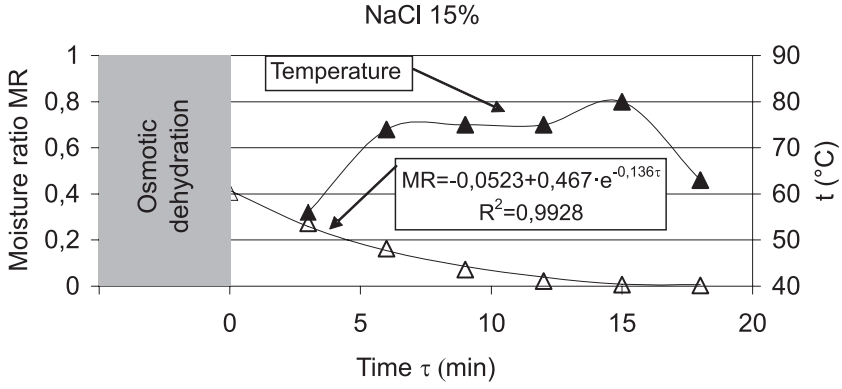

Fig. 5. VM drying kinetics of beetroot slices pre-dried in NaCl solution of concentration 15%

Density and shrinkage

The increase in NaCl concentration of the osmotic solutions decreased density and shrinkage of VM finish dried beetroot slices (Figs 6–7). The highest values of density (1.13 g/cm^3) and shrinkage (66.25%) were found for the sample without pre-treatment and the lowest (0.92 g/cm^3 and 31.33% respectively) for the sample pre-dried in the NaCl solution of concentration 10%. Torringa et al. [2001] also reported that the increase in NaCl concentration of the osmotic solution decreased the shrinkage of the mushroom samples finish dried by combined microwave-hot-air drying method. The VM method usually ensures lower shrinkage than traditional methods of drying due to the puffing phenomenon [Lin et al. 1998]. This study revealed that optimal addition of salt enhances the effect of puffing.

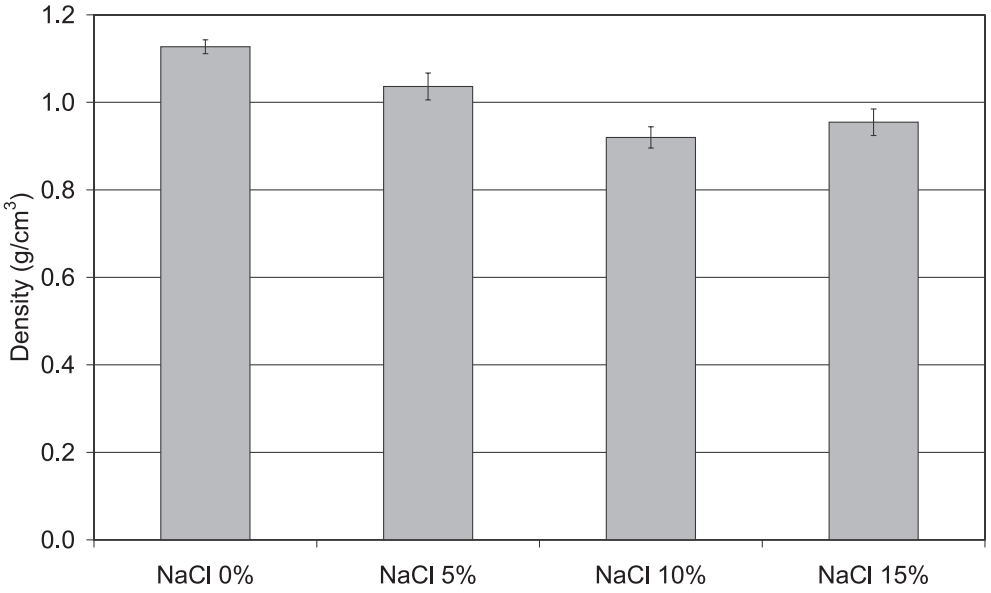

Fig. 6. Effect of NaCl concentration on density of VM finish dried beetroot slices

Colour

The increase in NaCl concentration of the osmotic solutions increased colour parameters *L**, *a** and *b** of VM finish dried beetroot slices (Figs 6–7). This means that the colour of the slices was getting lighter shifting towards redness and yellowness. The lowest values of brightness *L**, redness *a** and yellowness *b** determined for the sample without pre-treatment were 34.1, 15.1 and 3.3 respectively while the highest values determined for the sample pre-dried at the NaCl concentration 15% were 41.4, 27.4 and 6.0 respectively. Higher brightness usually makes the colour of the product more attractive for the potential consumers, while red colour is typical for beetroot and thus the increasing of *a** parameter can be considered as the positive alteration.

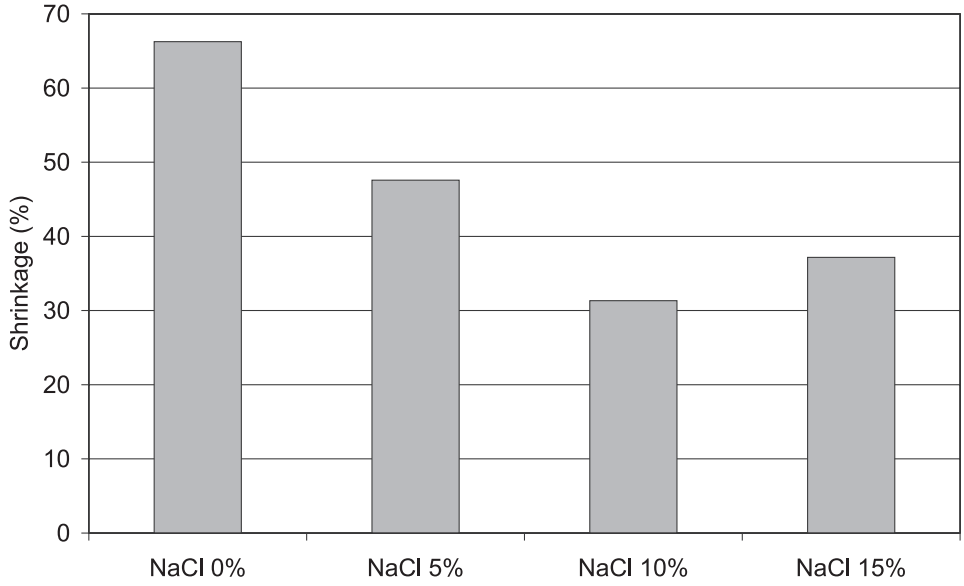

Fig. 7. Effect of NaCl concentration on shrinkage of VM finish dried beetroot slices

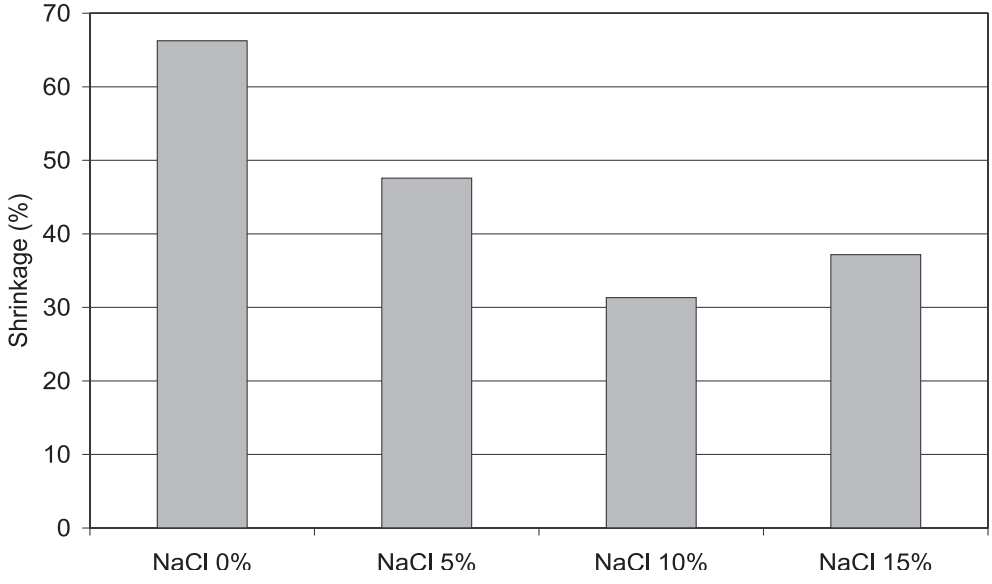

Fig. 8. Effect of NaCl concentration on colour parameters of VM finish dried beetroot slices

TPA parameters

Generally, it can be stated that the increase in NaCl concentration of the osmotic solution increases the hardness of VM finish dried beetroot slices (Fig. 9). However, the highest value of hardness (57.11 N) was found for the sample pre-dried at 5% of NaCl concentration and the following increase in NaCl concentration did not result in increasing of this parameter.

On the other hand, the increase in NaCl concentration of the osmotic solution from 0 to 15% decreased the cohesiveness of the VM finish dried product from 0.46 to 0.16 J/J (Fig. 10). The springiness was also decreasing with increasing of NaCl concentration, but this decrease from 1.56 to 1.20 mm was not much significant. The increased hardness associated with decreased cohesiveness and springiness consequently may invoke the impression of increased crispiness. This is a positive effect increasing the attractiveness for the potential consumers [Szcześniak 1971].

Sensory evaluation

The results of the sensory assessment of appearance, flavour and taste for VM dried beetroot samples osmotically pre-dried at different NaCl concentrations were compiled in Table 1, while the results of the sensory assessment of texture for these samples were shown in Table 2. In most cases the differences between mean values are not significant. However, the test reviled that the beetroot samples without pre-treatment were characterised by the most typical taste and flavour, the highest fibrousity and tooth packing, the lowest hardness, crispiness and colour uniformity, while the samples pre-dried at the NaCl concentration 5% exhibited the high crispiness, the most intensive colour, intensive taste as well the lowest hardness and tooth packing. The lowest intensive taste, colour attribute, hardness, crispiness, gumminess and fibrousity were found for samples pre-dried at the NaCl concentration 15%. High crispiness is a positive attribute of the food texture [Szcześniak 1971]. It can be stated that the best product in terms of taste and flavour does not require the pre-treatment in NaCl solution but in terms of texture involves pre-drying at NaCl concentration amounted to 15%. This increased crispiness evaluated in sensory assessment is associated with decreased cohesiveness and springiness determined in TPA test. The optimal quality of the VM finish dried beetroot slices can be obtained by pre-drying at NaCl concentration of 5%.

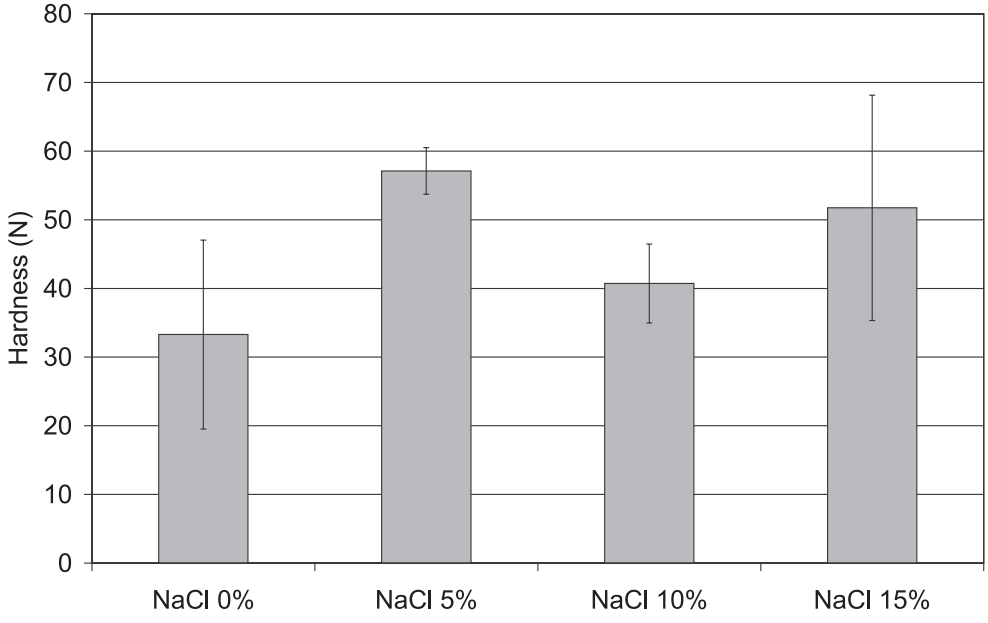

Fig. 9. Effect of NaCl concentration on hardness of VM finish dried beetroot slices

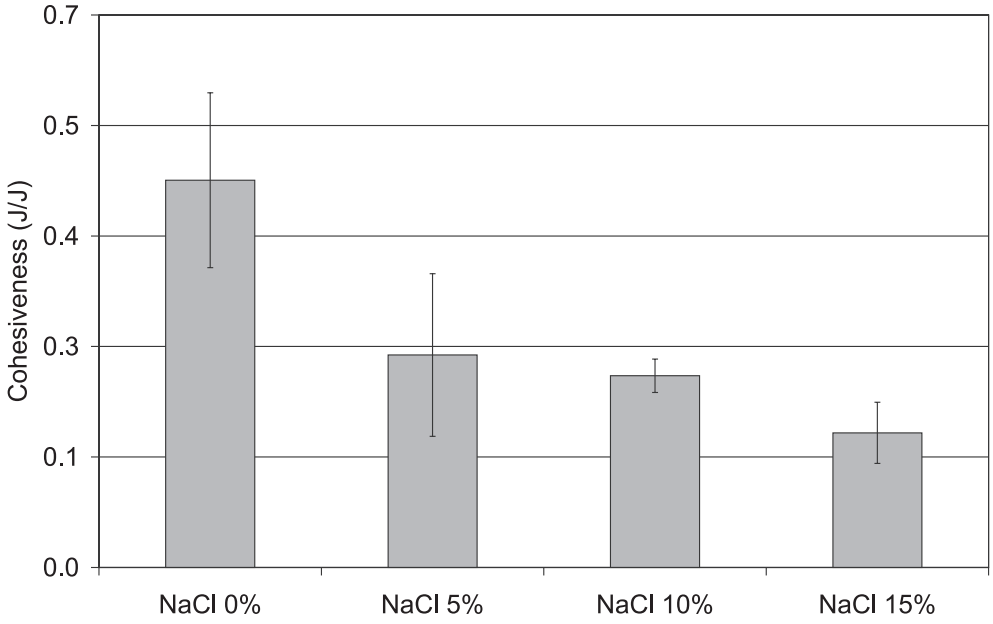

Fig. 10. Effect of NaCl concentration on cohesiveness of VM finish dried beetroot slices

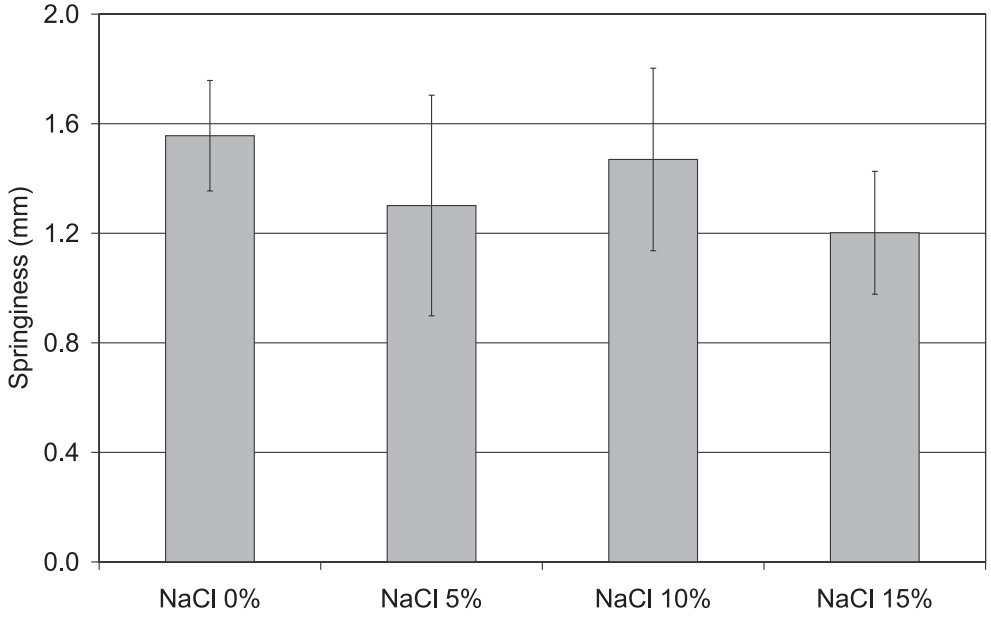

Fig. 11. Effect of NaCl concentration on springiness of VM finish dried beetroot slices

Table 1

Sensory assessment of appearance, flavour and taste for beetroot samples osmotically pre-dried at different NaCl concentrations

NaCl concentration (%)	Appearance		Flavour	Taste
	colour	colour uniformity	typical	typical
0	6.56±0.79ab	5.13±1.10a	7.00±1.28a	7.56±0.63a
5	7.75±0.43b	6.00±1.05a	6.14±0.85a	7.31±0.82a
10	5.50±0.84ab	6.06±0.80a	3.79±0.95a	3.69±0.96b
15	5.13±0.70a	5.88±1.15a	4.93±0.80a	3.25±0.89b

Different letters at mean values indicate significant differences (Duncan test, $p<0.05$)

Table 2

Sensory assessment of texture for beetroot samples osmotically pre-dried at different NaCl concentrations

NaCl concentration (%)	Texture				
	hardness	crispiness	gumminess	fibrousity	tooth packing
0	4.94±0.77a	3.06±0.93a	5.63±0.80a	5.06±1.11a	4.56±1.15a
5	5.13±0.70a	7.00±0.96b	4.50±0.85a	4.63±1.27a	3.13±0.70a
10	5.44±0.71a	5.00±1.00ab	6.13±0.79a	4.63±1.15a	4.69±1.15a
15	5.13±0.55a	7.13±1.01b	4.13±0.93a	3.75±1.21a	3.25±0.83a

Different letters at mean values indicate significant differences (Duncan test, $p<0.05$)

Conclusions

The mass of samples during osmotic pre-treatment was rapidly decreasing in the first hour of the process and then was slightly increasing as the result of water flux from the raw material to the osmotic solution and solids transfer from the solution to the raw material.

The increase in NaCl concentration decreased the final moisture content of the pre-treated samples.

The decrease in moisture content of the beetroot slices during vacuum-microwave (VM) finish drying could be described with an exponential equation.

While VM finish drying the temperature of samples was increasing until the certain moisture content and then was decreasing as the result of the balance of energy generated within the dried material by dipoles of water and the energy necessary for water evaporation.

The increase in NaCl concentration decreased density, shrinkage, cohesiveness and springiness but in the same time increased hardness and the colour parameters of the finish-dried product.

The best product in terms of taste and flavour does not require the pre-treatment in NaCl solution but in terms of texture involves pre-drying at NaCl concentration amounted to 15%. The optimal quality of the VM finish dried beetroot slices can be obtained by the osmotic pre-drying in NaCl solution with concentration of 5%.

Nomenclature

a^*	Redness
b^*	Yellowness
L*	Lightness
m	Mass (kg)
MR	Moisture ratio
M_e	Equilibrium moisture content (kg/kg db)
M_0	Initial moisture content (kg/kg db)
R^2	Coefficient of determination
S	Shrinkage (%)
t	Time (min)
TPA	Texture Profile Analysis
V	Volume (m^3)
V_0	Initial volume (m^3)
VM	Vacuum-microwave
Dm	Change of mass (g)
ρ	Density (g/cm^3)

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4

IRRADIATION OF FOOD PRODUCTS

Introduction

Among the emerging technologies irradiation has gained the widest application for food preservation. Food irradiation is one of a set of processing technologies that can be used to increase the microbiological safety and shelf-life of a wide range of foods and currently is applied for preservation of more than sixty types of food products in about 40 countries in the world. Food irradiation is the physical processing of food products by ionizing radiation for the purposes of destruction pathogens, reduction of microbial load, insect infestation and extension of shelf-life. Current applications of radiation processing are concentrated in the following areas: medical diagnosis and therapy, sterilization of medical equipment and disposables, sterilization of pharmaceuticals, structural modification and improvement of polymers, effluent treatment, sterilization of animal feed, sterilization of food packaging materials, sterilization of hospital diets, sterilization of prepared meals for specific requirements (for military and space flights purposes) and food pasteurization [Arvanitoyoannis 2010, Loaharanu 2003, Molins 2001, Singh & Heldman 2008, Smith 2004, Zhang et al. 2011].

Ionizing radiation is sufficiently high in energy to remove an electron from water which is the main component of foods and living organisms and to create highly reactive compounds including free radicals as hydroxy radical and hydrogen peroxide. The prodominant useful of irradiation rely on reaction of this high reactivity radicals with the DNA of microorganisms causing inactivation [Arvanitoyoannis 2010, Barbosa-Canovas et al. 2005, Loaharanu 2003, Molins 2001].

Food irradiation consists of subjecting the foodstuffs with ionizing radiation which depending of the applied dose is aimed primarily at: reducing the number of undesirable microorganisms and/or insects in food, the elimination of pathogenic organisms in food, the inhibition of plants germination, delayed ripening of fruits and vegetables, shelf-life extension of perishable products (meat, poultry and seafood). Overall target of food irradiation is to prevent adverse developments in food products, increasing stability and improving the sanitary quality of food as a result of the destruction pests, parasites and microorganisms [Arvanitoyoannis 2010, Loaharanu 2003, Molins 2001].

Irradiation of food products is allowed when there is a reasonable need for the use of ionizing radiation and irradiated foods do not pose a threat to human health and irradiation process will be carried out under appropriate conditions, but does not replace the hygienic

practices and proper health care or good manufacturing and agricultural practice. Irradiation of foodstuffs is actually permitted however it is compulsory to indicate on the label that foods have been irradiated even when the irradiated ingredients constitute less than 1% of the final product. Food irradiation carried out under conditions of normal manufacturing or agricultural practice is considered as the safe method of food preservation, but also causes much controversy. Food irradiation as no other method of food preservation has been covered by the wide range of researches on its impact on human health. To determine and establish the rules for the safe application of ionizing radiation for food preservation were performed a series of works and investigations both in theory and practical tests, covering topics from various fields of the science [Anonymous 1999a, Anonymous 1999b, Anonymous 2003, Arvanitoyoannis 2010, Codex Alimentarius Commission 2003, Codex General Standard for Irradiated Foods Standard 2003, Recommended International Code of Practice for the Operation of Irradiated Facilities Used for Treatment of Foods 2003, Zhang H.Q. et al. 2011]

Irradiation techniques

In commercial radiation of processing products such as foods and medical or pharmaceutical devices are used three types of ionizing radiation: radiation coming from high energy gamma rays, X-rays and accelerated electrons. In accordance with the Codex General Standard for Irradiated Foods [Codex Alimentarius Commission 2003, www.codexalimentarius.net] only these three sources of ionizing rays are approved and authorized to be used in food irradiation applications. Their energy is high enough to dislodge electrons from atoms and moleculesand convert them to electrically charged ions.

In operating facilities designed for irradiation ionizing radiation may come from different sources:

- Gamma ray generated by radioactive substances (radioisotopes). The officially approved and allowed sources of gamma rays designed for food irradiation are the radionuclides: the most common cobalt-60 (^{60}Co) (with energy level of 1,17÷1,33 MeV) and cesium-137 (^{137}Cs) (with energy level of 0,66 MeV).
- Electron beams generated by accelerators such a linear accelarator or a Van de Graaff generator at nearly the speed of light. Energy level not exceed the level of 10 MeV.
- X-ray generated by accelerators. Energy level not exceed the level of 5 MeV.

Gamma ray, electron beams and X-ray sources are used for a variety of industrial processes.

Irradiatian facilities using as the radiation source radionuclides are generally designed for the food treatment and for nonfood applications as sterilization of medical supplies, pharmaceuticals, cosmetics and veterinary products. Electron accelerators are used in the process of producing packagings materials and improvement its properties. Commercial irradiatian facilities may be operated in batch or continuous mode with the wide range of applied radiation doses. The maximum energies permitted for each of radiation sources is determined by the need to avoid induced radiactivity in rialable isotopes in food (Tab. 1) [Anonymous 1999b, Arvanitoyoannis 2010, Barbosa-Canovas et al. 2005, Cleland 2006, Codex Alimentarius Commission 2003, Codex General Standard for Irradiated Foods Standard 2003, Molins

2001, Recommended International Code of Practice for the Operation of Irradiated Facilities Used for Treatment of Foods 2003].

Table 1

Characteristics of radiation sources

Radiation source	Characteristics
Radionuclides – cobalt-60 (^{60}Co) (Gamma rays)	high penetration power, permanent radioactive source, high efficiency, radiation source replenishment needed, low througput
Electron beam	low penetration power, switch on – switch off capability, high efficiency, high througput, power supply and cooling requirements, technically complex
X-rays	high penetration power, switch on – switch off capability, low efficiency, high througput, power supply and cooling requirements technically complex

There are two main sources of radiation that have practical application in the food industry and whose usage is economically viable: the radioisotopes sources and the machine sources (electron accelerators). Radionuclide irridiators are characterized with good penetration power of gamma rays which can can penetrate deeply and can be used for food treatment in large packaging units, low dose rate of radiation, high reliability but it is need to replenish radionuclide source. Food treatment with ionizing electrons is often more easily accepted because radioactive substances are not present in the preservation process. Electron beams generated by electron accelarators have relatively limited penetration power (the depth of penetration is shallow - to a depth up to 5 cm) so the food to be treated must be no thicker than this for it to be treated all way through. This is a disatvantage of electron radiation compared with gamma rays produced by radionuclides. Accelarators designed for irradiation are characterized by high electric power consumption and requirements of cooling but the machines can be switched off immediately with stopped radiation process. Small units of equipment could be integrated into a production line of processed products. X-rays are capable for irradiation of thicker items but the process is extremely expensive resulting by intensive energy consumption. In this case large quantities of products would have to be irradiated to make the radiation process affordable. Although several irradiation techniques are avaiable for food preservation the most widely used is radionuclides radiation and the majority of food is still irradiated with gamma rays (^{60}Co) [Arvanitoyoannis 2010, Barbosa-Canovas et al. 2005, Cleland 2006, Codex General Standard for Irradiated Foods Standard 2003, Loaharanu 2003, Molins 2001, Recommended International Code of Practice for the Operation of Irradiated Facilities Used for Treatment of Foods 2003].

Irradiation applications for food preservation

In order to accurately determine the energy absorbed by matter as a result of irradiation was necessary to introduce the relevant units as measurement of ionizing radiation. A measure of absorbed dose is the amount of energy absorbed by matter subjected to ionizing

radiation. Radiation energy transferred to a weight of the substance unit determines the average absorbed dose by the substance. The universally applicable unit of absorbed dose of energy according to SI standards is Gray (Gy) where 1 Gy equals 1 J of radiation energy transferred to the substance of 1 kg weight (1 Gy=1 J/1 kg). The measure unit used in practice is 1 kGy.

Process of irradiation is carried out in specially contained areas where the food is exposed to a defined dose of radiation in a continuous or batch processes. The level of exposure is designed to take into account interdependent parameters such as the type of operation (batch or continuous), the optimum energy required to successfull and sufficient food safety and ionizing radiation source. The irradiation process in continuous mode by electron beams is sufficient for treatment of most prepackaged food items while the irradiation of large bulk food quantities in batch mode are preferable by more effective ionizing radiation sources generating gamma rays or X-rays.

The doses of ionizing radiation depend of the purpose and type food products tratment. In selection of ionizing radiation dose is to be considered what minimum dose is necessary to achieve intended purpose of radiation and what may be the maximum dose allowed due to possible adverse changes in the irradiated product. Additionally, the amount of dose depends of the irradiation conditions (temperature, presence of oxygen, vacuum).

The doses of 25 kGy and higher are used for polymer treatment and medical sterilization purposes whereas for food irradiation are usually used maximum doses of 10 kGy [Anonymous 1999b, Anonymous 2003, Arvanitoyoannis 2010, Cleland 2006, Loaharanu 2003, Molins 2001, Recommended International Code of Practice for the Operation of Irradiated Facilities Used for Treatment of Foods 2003, Singh & Heldman 2008, Zhang 2011].

Depending on the intended purpose of food irradiation in industrial practice and the dose of ionizing radiation are applied the following processes: radiation pasteurization and radiation sterilization (Tab. 2).

Table 2

The application of ionizing radiation for food preservation

Purpose of irradiation	Food products
Microbial decontamination	spices, fresh and frozen poultry, dried fruits, vegetables, mushrooms, herbs, tea, egg powder, fresh fishes and fish products, shrimps
Sprouting inhibition	potatoes, onion, garlic
Disinfestation	dried fruits and vegetables, grains, cereals, rice, cocoa beans, nuts, dried fishes
Shelf-life extension	fresh strawberries and other fruits (mango, papaya, avocado), fresh mushrooms, poultry, meats, fishes
Sterilization	special diets for hospital patients, meals for specific requirements (for military and space flights purposes)
Improvement of the technological properties	grapes and dates (increasing juice extracting efficiency), dehydrated vegetables (shorter cooking time)

The small doses of radiation are sufficient for destroying parasites while in the case of bacterial inactivation bacterial doses must be higher.

Based on performed surveys it was found that:

- bacterial vegetative cells are destroyed at doses of about 1,0÷3,5 kGy
- yeasts and moulds are inactivated at doses higher than 5 kGy
- spore forms of microorganisms are inactivated at doses higher than 10 kGy.

The low radiation doses, up to 1 kGy, are used to increase the sustainability of certain agricultural products, the prevention parasitic disease of and food poisoning. The low radiation doses, less than 1 kGy are inhibited sprouting process in food products such as potatoes, onion and garlic and prevented insect infestation in grains and citrus fruits (through sterilization of the insects and interruption of the breeding cycle). The low irradiation doses can also delay the ripening of certain fruits. This application has particular importance in improving the quality of imported tropical fruits by irradiating at peak maturity rather than harvesting and shipping immature produce however this effect is not applicable to all fruits and can also vary with the cultivar type and growing conditions.

The medium radiation doses act destructively on microorganisms causing reduction of its total number by several logarithmic cycles, thus seriously reducing and sometimes eliminating the risk of food poisoning. The irradiation process reduces also microorganisms resistance to thermal and chemical damage what can be used in the associated methods of food preservation. The doses of 1÷10 kGy are used primarily to extend the storage period of meat, fish, fruits, vegetables and other food products. The applied radiation doses of 1÷3 kGy reduce the numbers of spoilage microorganisms present in foods what influence on extension shelf-life of products such as soft fruits, meat and fish. It has particular commercial importance for the distribution of perishable soft fruits such as strawberries. Food poisoning microorganisms such as *Salmonella*, *Campylobacter* and *Listeria* are slightly more resistant but its reductions in counts in practical value can be achieved within this radiation dose range. Bacterial spore forms such are much more radiation-resistant and are unlikely to be affected at practical applied food irradiation doses. Viruses are highly resistant to irradiation and are unaffected at the radiation dose of 10 kGy which is officially permitted and approved in the irradiation process [Anonymous 1999b, Arvanitoyoannis 2010, Barbosa-Canovas et al 2005, Codex General Standard for Irradiated Foods Standard 2003, Loaharanu 2003, Molins 2001, Smith J. 2004, Zhang et al. 2011].

The typical doses used for irradiation fresh vegetables and fruits, grains, raw beef, pork, poultry and seafood are 0,75 kGy÷2,5 kGy. Irradiation process increases the durability of food due to a significant reduction of the total number of microorganisms, slowing their rapid proliferation and death of living cells without affecting the spore forms, therefore the food products require further preservation by traditional conventional methods. In the radiation process aimed to inactivation the most vegetative pathogens (except viruses) is obtained the effect equivalent to process of pasteurization. The typical radiation doses applied in this case to raw fish, poultry, beef, cocoa beans and some spices are 2,5 kGy÷10 kGy. Practically according to the officialy approved legislation requirements the ionizing radiation can be used for food, but in doses no higher than 10 kGy.

Based on further researches it was found that higher radiation doses are also safe for food. Currently, it is acceptable to use doses higher than 10 kGy, but just in case if it is necessary to ensure proper preservation process.

The high radiation doses, about 30 kGy and even up to 70 kGy are authorized for use only in special cases.The process of radiation sterilization is equivalent to heat sterilization resulting in the destruction and inactivation of present microflora in approximately 98% (eli-

mination of vegetative bacteria forms, viruses and reduction of spores) while the enzymes that are highly resistant to ionizing radiation are not subject to total inactivation. Additionally, high doses of ionizing radiation used in the process of radiation sterilization may cause adverse effects such a chemical and organoleptic changes in preserved food products causing loss of product quality. The radiation doses applied in the radiation sterilization of spices, deep-frozen foods, canned meat and dietary foods are higher than 30,0 kGy. The use of high doses of radiation, in the range of 10÷60 kGy did not cause the formation of toxic substances in food for humans but there are present marked changes in the organoleptic and chemical properties, reducing the consumer and nutritional properties of such preserved foods [Arvanitoyoannis 2010, Loaharanu 2003, Molins 2001] (Tab. 3).

Table 3

The radiation doses used in food irradiation processes

Radiation dose level	Radiation target	Application for food products
Low [up to 1,0 kGy]	slowdown in the processes of sprouting and maturation of plants, pest elimination, cereals, fruits and vegetables decontamination, elimination of some parasites	potatoes, onion, garlic, ginger, bananas, mangoes, grains and legumes, dried vegetables, dried fish and dried meat, pork
Medium [1,0÷10,0 kGy]	effect of pasteurization – a significant reduction or elimination of certain pathogens (excluding viruses) and food spoilage microorganisms	strawberries, grapes, dried vegetables, fresh fish, fresh and frozen seafood, poultry and meats
High [10,0÷ 50,0 kGy]	fffect of sterilization – the elimination of microbial vegetative forms and significant reduction spore forms of almost pathogens	meat, poultry, seafood, spices, prepared sterile hospital diets, food additives of natural origin

The exact radiation doses required for food preservation application should be established taking into consideration the contamination level, the hazard involved, the efficacy of the radiation treatment and the fate of preserved product during manufacturing, stoarage, distribution and culinary preparation of foods. Taking into consideration the maintaing organoleptic and nutritional quality of food products designed for irradiation it is desirable to use the lowest possible radiation doses necessary to achieve desired levels of microbiological and parasite control on a commercial scale for distributed foods. The important factors are established effiacy of the radiation treatment and threshold doses depending of food quality changes [Arvanitoyoannis 2010, Molins 2001].

Among the food preservation technologies irradiation is probably the most versatile method. The irradiation process is carried by any one of officially permitted and acceptable radiation sources:

- insect disinfestations such as fruit flies on papayas and mango seed weevils
- shelf-life extension by delaying the ripening and senescence of some fruits
- inhibiting the sprouting of tubers such as potatoes, onion and garlic
- decontamination of poultry, meats, seafood, herbs and spices by inactivation harmful and food spoilage microorganisms (in case of poultry, meats and seafood refrigeration should follow irradiation process)

- sterilization by completely inactivation all microorganisms in meat products and prepared meals (designed for military purposes, space travel and hospital patients)
- product properties improvement such as minimizing oligosaccharides content in beans and increasing fruit juice extraction (Tab. 4)

Insect disinfestation as a quarantine treatment of plant foods (called also as phytosanitary measure) is one of the most useful applications of irradiation. In the tropical or subtropical regions many species of insects invade fruits, vegetables, nuts, dried fruits and grains. In the case of transport and handling fruits and vegetables from an infested area to a nonifested consumer market the products first must be treated with an acceptable quarantine treatment procedure. Chemical fumigation of this food products is no longer allowed or used in many countries. The postharvest shelf-life and quality of cherries, blueberries, cranberriers and perishable strawberries can be extended with low dose of irradiation. Grains and cereals are treated with low doses of irradiation to eliminate fungi since some of this organisms can produce mycotoxins. Irradiation doses in the range of 0,20÷1,00 kGy are effective in controlling insect infestation in cereals and increasing the radiation dose to 5 kGy totally inactivates the spores of many fungi which can survive lower doses. The radiation doses are applied to prolong the shelf-life of fruit and vegetables, grains, legumes, nuts and dried foods.

The irradiation process of potatoes, onion, garlic and other tubers at a very low dose (0,02÷0,15 kGy) is a very feasible and practical way of extending their shelf-life by retarding their sprouting for 6–9 months. It is good alternative to ensure the supply of this products while modulating market demand with seasonal fluctuation [Arvanitoyoannis 2010, Loaharanu 2003, Molins 2001, Zhang et al. 2011].

The ripening of several tropical fruits such as banana, mango and papaya can be delayed with low doses of irradiation (0,12÷0,75 kGy) increasing their marketable life. Only the limited number of climacteric fruits benefit from such radiation treatment which mainly involves the slowing of changes in protopectins into water soluble pectins by irradiation.

The applied medium radiation doses (1,00÷10,0 kGy) are used for inactivation of food-poisoning bacteria in the process decontamination of poultry, meat and seafood. The irradiation is effective in preventing or delaying the microbial spoilage of fresh meat and poultry. The radiation doses used in practice are following: for poultry 3,00÷4,50 kGy (refrigerated/frozen), for red meats 4,5÷7,00 kGy. In the year 1997 the U.S. Food and Drug Administration officially approved the use of ionizing radiation to inactivate pathogenic microorganisms present in red meat. The Food Safety and Inspection Service of the United States endorses the use of ionizing radiaton as technique for processing meat and poultry products to provide consumers with a safe, wholesome and nutritious food supply.

Dried herbs, spices and vegetable seasonings are often contaminated with bacteria and insects. The radiation doses allowed and used in practice for their decontamination varies from 10 kGy to 30 kGy.

The precooked meat, prepared meals for special purposes and sterile hospital diets required are sterilized in irradiation process by radiation doses in the range of 50 kGy.

Another several potential applications of irradiation is improvement of the technological properties of some food products such as the decrese the oligosaccharides contnent in freshly germinating soy beans (dose of 7,50 kGy), increase juice extraction from grapes and dates (doses up to 16,00 kGy) and decrease cooking time by softening the vegetables [Arvanitoyoannis 2010, Chmielewski & Migdał 2005, Molins 2001].

Table 4

Functional effects of food irradiation

Functional effects	Radiation dose [kGy]	Food examples
Low doses [up to 1,0 kGy]		
Sprouting inhibition	0,02÷0,15	potatoes, onion, garlic
Insect disinfestation and parasite disinfection	0,15÷0,50	cereals, dried fruits, pork
Delayed ripening	0,12÷0,75	fresh fruits and vegetables
Medium doses [1,0 ÷10,0 kGy]		
Reduction of spoilage microorganisms	1,0÷3,0	fish, strawberries
Reduction of pathogenic microorganisms (non sporing)	2,0÷7,0	poultry, shellfish
Microbial load reduction in dry products	7,0÷10,0	herbs, spices
High doses [higher than 10,0 kGy] Sterilization	25,0÷50,0	sterile hospital diets, precooked meat, prepared meals

Effects of irradiation on microorganisms

The ionizing radiation affects microorganisms such as bacteria, yeasts and moulds by causing lesions in genetic material of cells, effectively preventing it from carrying out the biological processes for its continued existence. The principial targets of irradiation are nucleic acids and membrane lipids. Alteration in microorganisms membrane lipids leads to perturbation of membranes and deleterious effects on various membrane functions such as permeability. The activity of membrane enzymes may be affected as a secondary effect of lipid degradation. Ionization radiations act through changes induced in the DNA structure of irradiated microbial cells which result in prevention of replication or other functions. The applied energy levels are sufficient to disrupt certain bonds in the molecules of DNA making impossible the cell reproduction. Nucleic acids are the main targets of free radicals generated by irradiation. Because the bacteria genetic material is intrinsically very sensitive, the cell destruction and consequently the lethal damage occurs as a result of exposure to irridiation.

The main factors which affect on the susceptibility of microorganisms and effectiveness of irradiation are: radiation dose level, temperature, atmosphere (presence or absence of oxygen), medium and type of microorganism (size, cell wall characteristics, Gram positive or Gram negative type in nature, cell's age). Bacterial spores are more resistant for ionizing radiation than theirs corresponding vegetative bacteria cells. The sensivity of microorganisms to irradiation depends on both microbial species and strain and also on environment influence such as food type and pH. The microorganisms resistance increases at reduced temperatures so in this case the reduction of microbial load present in frozen food products requires doses of up to 7 kGy. The decontamination of dry herbs and spices requires the highest approved radiation dose of 10 kGy. Although the microbial reduction increases with increasing radiation dose, in practice the maximum deliverable dose is limited by minor changes in food components that can affect the sensory quality of food [Arvanitoyoannis 2010, Barbosa-Canovas et al. 2005, Chmielewski & Migdał 2005, Loaharanu 2003, Molins 2001, Zhang et al. 2011].

Effects of irradiation on basic food components

The major advantage of irradiation is that the changes of food components caused by recommended radiation doses are minor. The changes in nutritional value caused by ionizing radiation are not higher than in the case of other methods of food processing and preservation. The observed changes in the organoleptic characteristics can be reduced by properly chosen irradiation conditions of foods subjected to ionizing radiation. The occurrence of chemical changes in irradiated food is one of the arguments of opponents to application of ionizing radiation in food industry.

The ionizing radiation doses of about 10 kGy causes minor changes in composition and physicochemical properties of food. The probability of changes in the low-molecular compounds is small while in the case of macromolecular compounds undiserable changes increases proportionally to the molecular weight. These changes increases with applied higher radiation dose which contributes with changes in sensory properties that normally limit the permissible radiation dose. After crossing the ionizing radiation dose of 10 kGy in certain food products (milk, eggs, raw meat) there are adverse changes in sensory attributes, referred as the radiation taste which is not is sensory acceptable.

The changes in sensory properties are the results of the several chemical reactions. Irradiation initiates the normal process of autoxidation which gives rise to rancid off-flavours. Highly unsaturated fats are more readily oxidized than less unsaturated fats although this process can be slowed by eliminating oxygen or food irradiation by vacumm or modified atmosphere. In lipids, particulary unsaturated fatty acids radiolytic decomposition is connected with the break at the level of the carbonyl function of the double bond what induces the formation of peroxides and some volatile compounds responsible for off-flavours. This leads to the formation of free radicals as principial intermediatesand and ultimately to particular end products such as CO_2, CO, H_2 and hydrocarbons, mainly alkanes and aldehydes. Unsaturated fatty acids also form dimers and polymers of which the amounts are increased by the presence of oxygene. Irradiated fats in the presence of oxygen are primarily subjected to oxidative processes with formation peroxides, hydroxides and carbonyl compounds. The mechanism of free radicals formation induced by ionizing radiation is similar to the formation of free radicals in the process of fats rancidity. The free radicals in the presence of oxygene, either during irradiation process or later, cause autoxidation of the lipids through the formation of hydroperoxides, aldehydes, ketones etc.In order to minimize negative oxidation processes of irradiated fats radiation treatment should be carried out without of presence oxygen, light and as well at low temperatures or with the addition of antioxidants [Arvanitoyoannis 2010, Barbosa-Canovas et al. 2005, Loaharanu 2003, Molins 2001, Zhang et al. 2011].

Irradiation of proteins may cause molecular uncoiling, coagulation, unfolding and even molecular cleavage and splitting of amino acids. Generally the peptide linkages are not affected and the main undesirable effects are concentrated around sulphur linkages and hydrogen bonds. The sequence of changes of proteins caused by ionizing radiation is concerned with following bonds: -S-CH_3, -SH, imidazole, indole, alpha amino, peptide and proline. At applied radiation dose of 10 kGy the overall increase in total free amino acids was observed mainly due to the rise in the levels of glycine, valine, methionine, lysine, isoleucine, leucine, tyrosine and phenylalanine. Amino acids are characterized by a fairly diverse resistance to radiolytic degradation. The most resistant are arginine, lysine, glutamic acid, and the most sensitive are sulphur amino acids, aromatic amino acids, valine and isovaline. The irradiation process influence on

unfloding of the protein molecule leading to the avaiablity of more reactions sities. In the effect of ionizing radiation on amino acids primarily occurs the break the bonds of the amino group $–NH_2$ (deamination) and carboxyl group -COOH (decarboxylation). The radiolytic changes of amino acid are the main cause of organoleptic changes in irradiated food product and the formation off-flavours compounds. Characteristic also is the release of hydrogen sulfide (H_2S). The effects of ionizing radiation on proteins is not only the sum of radiolytic transformations of separately amino acids, but also changes in enzymatic activity, ability to water and salt binding and decrease of the solubility and viscosity. The irradiation of proteins also generates radiolytic products, generally small molecules such fatty acids, mercaptans and other sulphur compounds which although minor presence become components of irradiated food products and influence on sensory characteristics of irradiated foods. The irradiation process also affects on the functional properties of proteins. The activity of enzymes is unaffected at normal radiation doses and the continuing enzymes activity limits the achievable shelf-life extension of fruits and vegetables. The sensitivity of enzymes on radiation ionizing varies within fairly wide limits and depends of many factors, including volume of absorbed dose, pH of the environment, the presence of oxygen, temperature, enzyme concentration and its chemical structure. The enzymes present in food chemical complex are more resistant to changes caused by irradiation because other food components act in relation to them the protective function [Arvanitoyoannis 2010, Barbosa-Canovas et al. 2005, Loaharanu 2003, Molins 2001].

The irradiation can break high-molecular-weight carbohydrates into smaller units leading to depolimerization. This process is responsible for the softening of fruits and vegetables through the breakdown of cell wall structure as for example pectin. The effect degree depends of number of factors such as type, variety and maturity of fruit. The effect of softening can be disatvantage but may be advantageous in increasing juice yield and in reducing the drying and cooking times of dehydrated food products. The sugars may be hydrolyzed or oxidazed when subjected to gamma irradiation with increased initial total reducing sugars content. The effect of radiation on carbohydrates is complex due to different radiolytic products produced under varying conditions and doses. The important factor in the radiolysis of carbohydrates is the hydroxyl radical. The radicals form can further react by disproportonation, dimerization and dehydratation. Applied high radiation doses may contribute to hydrolysis, oxidation, degradation and depolymerization of carbohydrates [Arvanitoyoannis 2010, Barbosa-Canovas et al. 2005, Loaharanu 2003, Molins 2001].

Sensitivity and loss of vitamins depends largely of the absorbed dose of ionizing radiation, temperature and oxygen. In the food irradiated by the dose of 1 kGy generally is observed the minor loss of vitamins. The most sensitive to ionizing radiation are vitamins: B_1, C, A and tocopherols (vitamin E). Irradiation of vitamins in their pure state and in solution destroys them in a greater extent than is the case in natural systems. Probably it is caused by the presence of protective substances such as proteins, natural antioxidants and the fact that many of the vitamins present in biological systems are bonded and therefore more permanent. The destruction extent of vitamin C, E and K depends of the applied radiation dosage. Among the B vitamins, thiamine (vitamin B_1) is the most radiation labile. Substantial loss of vitamin B_1 occurs in meat and meat products. Vitamin B_2 (riboflavin) is fairly resistant to irradiationas as well as choline, pantothenic acid, biotin and folacin when present in foods. Niacin, pyridoxine, vitamin B_6 and vitamin B_{12} are moderately affected by irradiation. Vitamin C in solution is quite labile to irradiation but in fruits and vegetables is quite stable at low radiation doses. Vitamins with antioxidant activity such as A, C, E, K are degraded when

irradiation process is carried out in the presence of oxygene. Process of irradiation usually causes little loss of vitamin D while vitamin E as an unsaturated lipid is easily affected by irradiation especially in the pressence of air.

Mineral compounds as micronutrients are not changed in any significant way by irradiation of food products [Arvanitoyoannis 2010, Loaharanu 2003, Molins 2001].

Undesired changes in sensory characteristics can be minimized by combining the irradiation process with other preservation treatments and such combination processes have the advantage of enhancing effects on present microorganisms. The typical processes that can be used in combination with irradiation are refrigeration, modified-atmosphere packaging and mild heat treatment. The successful combinations of this processes can permit reduction both in the applied radiation dose and in the level of associated process.

Economic cost of irradiated food

The conomics of food irradiation is dependent of several factors: applied radiation dose, which is related to purpose and application; packaging density, which influences on structural design and radiation physics; throughput, which determines operation time use of irradiation facility; radiation dose uniformity (related to tolerance dose depended of specific irradiated food), maximum dose (according approved government regulations) and minimum dose (for quarantine treatment purposes).

Depending of type od irradiation facility concerned with radiation source an economic feasibility study should be carried out on the planned irradiator. The study should take into consideration and involve the following steps: developing a irradiator model; defining product type and describing the process of irradiation; making necessary assumptions; deciding on the type of irradiator; defining the annual throughput; calculating total processing cost and unit processing cost. When the calculated unit processing cost is competitive with other processing costs and the irradiated food product has a share of the desired market then the use of irradiation can be considered ecomically feasible.

The processing cost is a combination of capital and operating costs or fixed and variable costs. The capital cost for free standing irradiation facility includes the cost of hardware (irradiator, processing equipment – carriers, conveyors, control systems and ancillary eqypment), radiation source (radionuclide or accelerator), land, shielding, warehouse and laboratories. The operating costs includes salaries, utilities, packaging, maintenance, taxes and insurance, interest of loans, supplies and chemicals, transport, source replacement and return to equity [Arvanitoyoannis 2010, Cleland 2006].

The unit processing cost of food irradiation is quite varied and radiation is determined by the degree of use of installations for radiation preservation of food, the degree of commercialization of food irradiation, as well as the amount of radiation dose, the range of the irradiation process and type of food subjected to irradiation. In the United States typical costs for disinfesting fruits can vary from 0,20 to 0,40 USD/kg. For decontaminating meat and poultry unit processing cost can vary from 0,40 to 0,70 USD/kg (approximate cost of fruit packer or meat packer in delivery fruits or meats to an irradiation plant for custom commercial irradiation). Average unit cost of food irradiation depends on the following factors: radiation dose; the size of food party; the utilization of radiation installations; the costs of food transport to the place of irradiation process. Major cost factors are concerned with initial investment (ca-

pital costs), throughput (the utilization of the irradiation process reduces unit processing cost) and the adsorbed radiation dose (the higher the dose – the higher the cost). Economy of scale is important in operating an irradiation facility as well as seasonality of treated products. If the irradiation facility can be used throughout the year, the processing cost will be much lower than if the facility is used only part of the year. Most commercial irradiation plants are for irradiating high volume of foods and high markup disposal medical products. Also both the gamma irradiator or the linear accelarator are expensive. Depending on the size stimulates throughput the gamma irradiator can cost 2÷6 million USD while the linear accelerator can cost 3÷10 million USD (approximate cost includes the radiation source, shielding, buildings and hardware) [Molins 2001, Zhang et al. 2011].

Actually irradiated food products in a particular country are only sold in that country. There has been no bilateral or international agreement or policy on the trade of irradiated food, until policies and implmented new regulations concerning properly irradiated food products as the quarantine treatment will be established, especially to export certain fruits and vegetables.

Legislation status of food irradiation

A joint FAO/IAEA/WHO Expert Committee of Food Irradiation concluded that irradiation of food up to an overall average radiation dose of 10 kGy causes no toxicological human health hazards and introduces nospecial nutritional or microbiological problems. Also other organisations such as the U.S. Food and Drug Administration, U.S. Department of Agriculture, Health Canada and European Commission's Scientific Committee on Food officially supported and approved this radiation dose limit. They endorsed food irradiation as a safe and effective method to increase food safety and reduce the incidence of foodborne illness [Anonymous 2003, Arvanitoyoannis 2010, Communication from the Commissions on foods and food ingredients authorised for treatment with ionising radiation in the Community 2001, Codex Alimentarius Commission 2003, Codex General Standard for Irradiated Foods Standard 2003].

The safety and effectiveness of irradiation process as the method of food processing and preservation have been recognized by the Codex Alimentarius Commission and regulated in Codex General Standard for Irradiated Foods. The irradiation of food and agricultural products is currently allowed in about 50 countries in the world. The most common irradiated food products for commercial use are species and dry vegetable seasonings. The safety issues of irradiated food products are grouped in respect to the following factors: residual radioactivty; free radicals and radiolytic products; carcinogenic and mutagenic properties; nutrient quality; polyploidy; toxicity; microbiological safety; operator safety during irradiation processing. The majority of performed researches, toxicological studies and feeding trials have shown no evidence for toxic effects.

The rules on food irradiation and its labelling are defined by the Codex Alimentarius Commission FAO / WHO. The International Committee on Food Labelling has approved a special Radura irradiation symbol which are marked irradiated food products. The labelling of such preserved foods should be strictly adhered to in each country. These guidelines are reflected in the legislation of the European Community and EC member states [Codex Alimentarius Commission 2003, Codex General Standard for Irradiated Foods Standard 2003].

In the European Union rules for production, trade and imports of food treated with ionizing radiation is regulated by Directive No. 1999/2/EC of the European Parliament and the Council of 22 February 1999 on the approximation of the laws of EU Member States concerning foods and food ingredients treated ionizing radiation, known as the Framework Directive. According to the contained record the exposing food to ionizing radiation can be done only in follwing cases: after obtaining a special permission; if there are reasonable technological reasons; when there is no health risk to human health; when the irradiation process is beneficial in point of the consumer view; when it can not be a substitute for good practices (GAP, GHP, GMP); the process is to be carried out in accordance with established conditions.

The European Community member states may apply irradiation of foods only to: reduce the number of food poisonings by destroying pathogens present in food; reduce food spoilage by inhibiting or preventing degradation processes, as well as the destruction of organisms responsible for these processes; reduce loss of foodstuffs resulting from premature ripening, germination or sprouting; elimination from food harmful organisms affecting on plants or plant products. In terms of food irradiation Directive No. 1999/2/EC is complemented by Directive No. 1999/3/EC of the European Parliament and the Council of 22 February 1999 on the establishment of a Community list of food and food ingredients treated with ionizing radiation.

Poland is one of about 50 countries, where regulations allow the use of ionizing radiation for the treatment of foods. General principles of food irradiation in Poland are contained in Chapter 5 of the Act of Food Safety and Nutrition of 25 August 2006, OJ No. 171, item. 1225. The records contained in the Act are adapted to European Community law. The Act of Food Safety and Nutrition, in accordance with the guidelines of the Directive No. 1999/2/EC sets out the conditions for the use of ionizing radiation for food irradiation and the general rules for obtaining the authorization by a decision of Chief Sanitary Inspector in Poland for entities applying food irradiation. The Act authorizes the use of ionizing radiation in food processing, unless it constitutes no threat to human life and health, and is technologically justified. Food irradiation can not substitute for compliance with the hygienic conditions of food production. The provision of food irradiation is the Regulation of Ministry of Health of 20 June 2007 on food irradiation by ionizing radiation. OJ 2007 No. 121, item. 841. The regulation, in force since July 2007, contains a list of foods that can be treated with ionizing radiation, the maximum allowable doses, and also allowed the source of ionizing radiation (in accordance with Directive No. 1999/2/EC) [Act of Food Safety and Nutrition of 25th of August 2006; Regulation of Ministry of Health of 20th of June 2007] (Tab. 5).

Table 5

Maximum permissible doses of ionizing radiation in Poland

Food product	Radiation dose [kGy]	Radiation target
Potatoes	0,025÷0,10	Inhibition of sprouting
Onion	up to 0,06	Inhibition of sprouting
Garlic	0,03÷0,15	Inhibition of sprouting
Champignons	1,0	Inhibition of growth and aging of the fungi
Dry spices, including dried herbs, spices and vegetable seasonings	10,0	Dry spices, including dried herbs, spices and vegetable seasonings

Table 5. continuous

Dried champignons	1,0	Dry spices, including dried herbs, spices and vegetable seasonings
Dried vegetables	1,0	Dry spices, including dried herbs, spices and vegetable seasonings

The Regulation also sets out detailed conditions of food irradiation by ionizing radiation and the requirements for packaging and labeling of irradiated foods, as well as the conditions for imports from third countries of foods irradiated by ionizing radiation. Based on the Codex Alimentarius Commission and Directive No. 1999/2/EC in the Regulation of Ministry of Health of 20 June 2007 on food irradiation by ionizing radiation was established that the average total dose of ionizing radiation absorbed by the foodstuff treated with irradiation must not exceed 10 kGy. Irradiation of foodstuffs in Poland, despite existing regulations and approved service providers in the field of food irradiation is limited and relates mainly spices [Chmielewski & Migdał 2005].

Consumer attitudes toward irradiated food

The performed marketing studies concerning irradiated food indicated that many consumers are receptive toward irradiated food products and they will purchase such preserved food in preference to a non-irradiated equivalent when they perceive the benefits, especially in freshness and food safety. The majority of the public knows very little about the process of food irradiation. Many consumers are not adequately educated about safety of irradiated foods. In many countries although a number of food products have been officially approved by government health authorities for commercial irradiation, the level of public knowledge about food irradiation is very low. This creates a need for consumer education which should include the informations concerning benefits of the food irradiation process, health safety and quality of irradiated food products, processing and environmental safety in the irradiation facilities and endorsements of food irradiation by credible reputable international organisations such as FAO, WHO and IAEA. Government authorities, the food industry and scientists should together provide more consumer education about the purposes, benefits and safety of food irradiation for acceptation this preservation technology [Anonymous 1999a, Anonymous 2003, Arvanitoyoannis 2010]

Conclusions

Irradiation has become one of the successful emerging techniques to preserve food with minimum change to the functional, nutritional and sensory properties of food products.

Food irradiation as the process of exposing food to a controlled source of ionizing radiation influence on reduction the microbial load, destruction of pathogens, desinfestation and extension of food products shell-life and has been proven to be an effective food safety measure based on continuous international researches of this area.

The radiation treatment of various foods is now legally accepted in more than 40 countries in the world but is still prohibited in others. Food irradiation has received offi-

cial legislation approval for use in several food catergories by international and domestic authorites.

Informations about the increasing use of ionizing radiation are mainly due to the widespread use of this method of food preservation in the United States. In the Europe, food irradiation is aining acceptance very slowly and there is limited use of this technology of food preservation.

Food irradiation still continues to generate controversy inhibiting widespread acceptance and application in the world.

The adopting irradiation technology is still slow because of the high investement cost of commercial facilities and industry tends to seeks other less expensive ways of maintaining plant sanitation and minimizing contamination of food products.

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5

INFLUENCE OF PACKAGING MATERIALS ON PH, WATER BINDING CAPACITY, DRIP LOSS AND COOKING LOSS OF TURKEY THIGH MUSCLES PACKED AND STORED UNDER MODIFIED ATMOSPHERE

Introduction

It is generally known that the functional and sensory attributes of meat are influenced by the following factors: pH, kind and biochemical state of muscle, microorganisms, chemicals and environmental parameters, especially temperature and atmosphere composition. For the customers, appearance is the major factor for purchase selection and initial evaluation of meat quality. Other quality attributes, such as pH, drip loss, cooking loss and water holding capacity are important for the consumers after purchasing the product, as well as for the processors when producing meat products [Allen et al. 1998]. It is known, that poultry meat with low pH has been associated with low water holding capacity, which results in increased cooking loss and drip loss. Meat with low pH has also been reported to increase shelf life and decrease tenderness. Poultry meat with high pH creates an ideal enviroment for rapid microbiological spoilage in some packaging conditions.

To extend the shelf life of fresh chilled meat and provide its atractive appearance one can use packaging and storage in modified atmosphere (MA). The principal gases used in MA packaging for preservation of meat and poultry are carbon dioxide, oxygen and nitrogen [Krala 1996, Gimenez et al. 2002, Sőrheim et al. 2004, Patsias et al. 2006, Veberg et al. 2006, Acton et al. 2007, McMillin 2008]. The use of CO_2 enriched atmosphere extends the shelf life of raw poultry by inhibiting the bacterial growth [Saucier et al. 2000, Fraqueza et al. 2004, Acton et al. 2006]. The CO_2 concentration as high as 60% was recommended for packaging the poultry meat under MA [Baker et al. 1985, Sawaya et al. 1995, Krala 1999]. A major function of oxygen in MA is to maintain myoglobin in its oxygenated form, oxymyoglobin [Zhao et al. 1994]. The MA containing low oxygen concentrations are suitable for poultry [Krala 1999, Saucier et al. 2000, Jeremiah 2001]. Stiles [1991] reported, that to provide extended storage, low gas and moisture vapour transmission rates are essential. Smith [1993] mentions barrier properties (permeability to various gases and water vapour transmission rate and anti-fog properties (good product visibility) among of the factors taken into account when choosing a packaging film. For fresh meat in vacuum and in modified

atmosphere packages low oxygen transmission rate from 10 to 100 $cm^3/m^2 \cdot 24h \cdot 0.1MPa$ is desired [Brody 1989, Zhao et al. 1994, Jeremiah 2001]. Commonly used for meat and meat products pakaging in MA are usually plastic laminates produced from polyethylene (PE) and polyamide (PA) [Kotzekidou and Bloukas 1996, Krala et al. 1997, Ahn et al. 1998, Saucier et al. 2000, Smiddy et al. 2002, Pettersen et al. 2004a, John et al. 2005]. Polyamide has a good O_2- barrier properties, whereas polyethylene has a good humidity barrier and sealing properties [Zhao et al. 1994, Pettersen et al. 2004].

Not many studies investigated the effect of the packaging film oxygen transmission rate (OTR) on the quality attributes of the fresh chilled meat.

There isn`t any information on the moisture vapour transmission rate (MVTR) of the packaging material on the quality of meat packed in MA. The effect of barrier properties of packaging material on the quality of the turkey thigh muscles stored in MA and the use of the packaging material with an antifog layer is not known.

Taking into account the importance of the problem, the following investigation was conducted. The objective of the work was to determine the effect of barrier properties of three types of packaging films (with different gas and moisture vapour transmission rates) on pH, water holding capacity, drip loss and cooking loss of the turkey thigh muscles stored in a modified atmosphere consisting of 75% CO_2, 20% N_2, 5% O_2.

Material and Methods

Three packaging materials with different gas and moisture vapour transmission rates and four periods of storage (4, 8, 12, 15 days) were studied. The experimental material covered the thigh muscles portions (without skin and bones, average mass ± 0.5 kg) cut out 24 h after slaughter from the 18-week-old industrially slaughtered turkeys. The samples were wrapped in polyethylene bags and transported in a cool box containing ice cubes to the laboratory of the Department of Animal Food Technology, University of Economics. The experiments were repeated five times for each types of polyamide – polyethylene film pouches (PA/PE, PA/PE+AF, PA/ARE/PE – the characteristics of packaging films are given in Table 1). Each pouch was 350 mm wide x 260 mm long. Every time 25 muscles were investigated. Twenty thigh muscles were placed in the polypropylene (PP) container, which measured 227 x 178 x 80 mm (l x w x high) with absorption pad (120/160 mm – with 2,51 l/m^2 absorbing capacity) and next were used to put in one of three types of film pouches (20 samples were packed in the same kind of film, five samples were examined on each of these days (on the 4^{th}, 8^{th}, 12^{th}, 15^{th} day of storage). The remaining five muscles were used for fresh sample analysis carried out 24 h after slaughter and were used as initial values for all three films. The thigh portions were packed using the Tepro packaging machine type PP5 (Tepro SA, Koszalin, Poland) in a modified atmosphere (MA) with compositions of 75% CO_2, 20% N_2, 5% O_2. The samples were stored in a refrigerator at $+1^oC$ and were examined in 24 h after slaughter (unpacked muscles) and after the 4^{th}, 8^{th}, 12^{th}, 15^{th} day of storage (muscles packed in MA). The determinations were performed for each of the muscle.

The pH was measured with the digital pH-meter Meratronic – 517, directly in the fillet with a Double Pore Slim electrode (Hamilton).

The water holding capacity (WHC) was determined according to procedure described by Wierbicki et al. [1962]. It was expressed as a percentage of water absorbed by meat.

Table 1

Mechanical and barrier properties of films* used to packaging turkey thigh muscles in modified atmosphere

The type of the film	PA/PE	PA/PE+AF	PA/ARE/PE
Total thickness [mm]	60	80	60
Thickness of individual layers [mm]	10/50	20/60	15/5/40
Permeability of:			
Oxygen [$cm^3/m^2 \cdot 24h \cdot 0.1MPa$]	70.0	50.0	14.0
Carbon Dioxide [$cm^3/m^2 \cdot 24h \cdot 0.1MPa$]	287	175	47.0
Nitrogen [$cm^3/m^2 \cdot 24h \cdot 0.1MPa$]	-	18.0	54.0
Moisture Vapour [$g/m^2 \cdot 24h$]	10.30	3.00	4.30

PA/PE – polyamide – polyethylene film
PA/PE+AF – polyamide – polyethylene film with anti-fog properties
PA/ARE/PE – polyamide – polyethylene film with amorphous polyamide layer
*The films` characteristics were obtained from the manufacturer

The drip loss was calculated as the difference in muscle weight before and after storage. It was expressed as a percentage of relation to the initial muscle weight.

The cooking loss was determined according to a procedure described by Kotter et al. [1968]. It was expressed as a percentage with respect to the initial muscle weight.

The obtained data were submitted to analysis of variance using Statistica software program, version 8.0 (Statsoft, INC., 2008). Two-way analysis of variance (Anova) and two-way interactions main effects (the three packaging materials and the four periods of storage) were applied to the data. Tukey`s multiple range test was used to separate means for significant ($p<0.05$) affects.

Results and discussion

The pH of the turkey muscles packed in all three films increased gradually up to the 12th day of storage, and then decreased on the 15th day (Tab. 2). At the end of the storage, muscles packed in PA/PE and PA/PE+AF foils had similar pH to the unpacked samples. However, samples packed in PA/ARE/PE film were characterized by a higher pH values than unpacked muscles.

A significant increase of pH in muscles packed in each type of films was noticed on the 8th day of storage, in relation to the values noticed in unpacked samples (Table 2). The increase of pH in muscles from the 8th day could be associated with increase of the total number of bacteria and the number of *Enterobacteriaceae* (data in press). Many types of bacteria, including *Enterobacteriaceae*, produce alkaline compounds, such as ammonia [Gram et al. 2002, Skandamis et al. 2005, Nychas and Skandamis 2005], which leads to the progressive alkalining of stored muscle, and thus to the increase of pH. The decrease of pH in muscles on the 15th day could result from the accumulation of lactic acid in samples produced by the bacteria of the genus *Lactobacillus* and/or the accumulation of lipid oxidation products. According to Gill [1996] and Seydim et al. [2006] meat pH can be affected by many factors; however the growth of lactic acid bacteria, resulting in the lactic acid production, is the major factor of pH decrease in packaged meat.

Table 2

Mean values (± standard deviation) of pH, water holding capacity (WHC), drip loss and cooking loss in the turkey thigh muscles packed in different films under modified atmosphere and stored at +1°C for up to 15 days

	Packaging films	Storage time	(days)*			
		0	4	8	12	15
pH						
	PA/PE	5.95^{A} ± 0.07	5.96^{Aa} ± 0.06	6.08^{Ba} ± 0.06	6.14^{Ca} ± 0.08	5.94^{Aa} ± 0.07
	PA/PE+ AF	5.95^{A} ± 0.07	5.97^{Aa} ± 0.08	6.10^{Ba} ± 0.04	6.15^{Ca} ± 0.06	5.97^{Aa} ± 0.08
	PA/ARE/PE	5.95^{A} ± 0.07	6.01^{Aa} ± 0.06	6.15^{Bb} ± 0.06	6.21^{Cb} ± 0.09	6.06^{Db} ± 0.06
WHC (%)						
	PA/PE	85.59^{A} ± 6.11	84.87^{Aa} ± 5.62	99.37^{Ba} ± 6.47	107.83^{Ca} ± 6.64	$74,26^{Da}$ ± 5.17
	PA/PE+ AF	85.59^{A} ± 6.11	85.69^{Aa} ± 6.26	102.69^{Ba} 7.25	109.62^{Ca} ± 5.20	77.11^{Da} ± 5.09
	PA/ARE/PE	85.59^{A} ± 6.11	88.57^{Aa} ± 5.31	108.74^{Bb} ± 5.76	118.34^{Cb} ± 6.41	83.90^{Db} ± 3.11
Drip loss (%)						
	PA/PE	-	1.60^{Aa} ± 0.10	2.38^{Ba} ± 0.27	3.16^{Ca} ± 0.19	3.77^{Da} ± 0.20
	PA/PE+ AF	-	1.48^{Aa} ± 0.11	2.12^{Ba} ± 0.13	2.92^{Ca} ± 0.26	3.45^{Da} ± 0.26
	PA/ARE/PE	-	1.35^{Aa} ± 0.13	1.67^{Bb} ± 0.19	2.04^{Cb} ± 0.18	2.52^{Db} ± 0.23
Cooking loss (%)						
	PA/PE	28.67^{A} ± 0.63	27.93^{Aa} ± 1.86	26.03^{Ba} ± 1.15	23.58^{Ca} ± 1.31	24.66^{Da} ± 1.44
	PA/PE+ AF	28.67^{A} ± 0.63	27.91^{Aa} ± 1.11	25.91^{Ba} ± 1.76	23.45^{Ca} ± 1.56	24.38^{Da} ± 1.43
	PA/ARE/PE	28.67^{A} ± 0.63	27.88^{Aa} ± 1.43	24.61^{Bb} ± 1.84	22.42^{Cb} ± 0.83	23.29^{Db} ± 1.48

*The data are average values of 75 tests for storage time 0; 25 tests for storage time 4, 8, 12, 15
A, B, C, D – values with different letter in the same row, differ at $p \leq 0.05$ in view of time of storage
a, b, c – values with different letter in the same column, differ at $p \leq 0.05$ in view of packaging films
The characteristics of packaging films are given in Table 1

Daniels et al. [1985] and McMullen and Stiles [1991] reported that a storage of meat in the modified atmosphere rich in CO_2 gave rise to a lowering of pH. This effect has been related to the absorption of CO_2 by meat, which results in the production of carbonic acid.

However, Krala [1999] observed that CO_2 dissolved in water contained in muscle tissue reduced pH of chicken meat only by 0.2 units. Urbaniak and Niewiarowicz [1977] showed that the lipid oxidation products could cause the decrease of pH in the muscle tissue.

Also, from the 8th day of storage, samples packed in PA/ARE/PE film had the highest pH, in comparison to the samples packed in PA/PE and PA/PE+AF bags. Concurrently, significant differences in pH muscles packed in PA/PE and PA/PE+AF bags, were not observed during cold storage (Tab. 2).

The highest pH value in the muscles stored in PA/ARE/PE film, could result from the lowest number of *Lactobacillus*, compared to the other two packaging materials (data in press). Differences in the pH of samples packaged in films with different barrier could also be due to a higher stage of oxidative changes in lipids. In the muscles stored in PA/PE and PA/PE+AF films, compared to samples packaged in PA/ARE/PE film, a higher content of malonic aldehyde was found (unpublished data). Therefore, a lower pH of muscles packed in foil PA/PE and PA/PE+AF foils could be caused by the accumulation of higher amount of acidic products in these samples, which resulted from lipolysis, lipid oxidation and accumulation of bacterial metabolites.

Tsigarida and Nychas [2001] studied the change of pH in beef fillets inoculated with *Pseudomonas sp.* and *Lactobacillus sp.*, packed in modified atmosphere (100% CO_2) in two films with different values of oxygen permeability (low OTR – 28 $cm^3/m^2 \cdot 24h. \cdot 0.1$ Mpa and high OTR – 2600 $cm^3/m^2 \cdot 24h. \cdot 0.1$ Mpa). The researchers observed that the OTR of the packaging films had significant effect on the pH of the meat samples inoculated with *Pseudomonas sp.* The pH of meat packaged in low permeable film remained constant throughout the storage period. However, the pH of samples stored in film with a higher oxygen transmission rate, increased throughout the storage period by 0.55 units, in relation to the pH noticed at the beginning of the storage and it was 6.0. The same authors stated that the OTR of the packaging films did not have an influence on the pH of meat inoculated with *Lactobacillus sp*; the pH of samples dropped slightly and was in the range of 5.35 to 5.55 throughout the storage period. The lack of the influence of OTR on the pH of meat can be explained by the lack of significant differences in the number of *Lactobacillus sp.* in meat stored in films with both low and high OTR.

Probably, the differences in results of our research and data obtained by Tsigarida and Nychas [2001] concerning the effect of packaging material on the pH of meat, could result from different: atmosphere, and/or range of OTR of packaging material, various kinds of meat (turkey, beef) and its initial microbiological level (the type and number of bacteria).

Changes of water holding capacity in the turkey thigh muscles packaged in materials with different barrier properties, proceeded in a similar way as changes of pH. A significant increase of WHC in muscles packed in each type of films was found on the 8th day of storage, in comparison to the values noticed in unpacked samples. The WHC increased gradually up to the 12th day, and then decreased on the 15th day in samples packed in all three films (Tab. 2).

Wołoszyn et al. [1999] and Przysiężna and Wołoszyn [2003] also found the increase, and then the decrease of WHC in duck legs and turkey breast muscles stored under vacuum. These mentioned authors explained the changes in WHC by the increase of the number of microorganisms in the examined muscles.

The barrier properties of the investigated films had a significant effect on the WHC in turkey thigh muscles. From the 8th day of storage, samples packed in PA/ARE/PE film

had the highest WHC, in comparison to the samples packed in PA/PE and PA/PE+AF bags. Although significant differences in WHC muscles packed in PA/PE and PA/PE+AF bags were not observed during the storage, however the samples packed in PA/PE film – with the highest OTR and the highest transmission of moisture vapour, had the lowest WHC (Tab. 2).

The storage time had a significant effect on the cooking and drip loss in the turkey thigh muscles. The changes of cooking loss in muscles packed in all types of investigated films depended on the pH and the WHC. From the 8th to the 12th day, with the increase of pH and WHC in muscles, the decrease of cooking loss was observed in the samples, regardless of the type of packaging material. With the fall of pH and WHC on the 15th day the increase of cooking loss, in all packaging films, was discovered (Tab. 2).

A significant effect of packaging material on the amount of cooking loss was noticed also on the 8th day of storage in the investigated muscles. From the 8th until the 15th day the samples packed in PA/ARE/PE film had significantly lower cooking loss in comparison to the samples packed in the other two films. Although significant differences in the amount of cooking loss muscles packed in PA/PE and PA/PE+AF bags, were not observed during the storage, the samples packed in PA/PE film, with the lowest barrier properties, had the highest cooking loss (Table 2).

Thus, the higher barrier properties of packaging material, the higher WHC, the lower cooking loss of stored muscle, the higher productivity and the more juicy product. As pointed by Sörheim et al. [2004], consumers prefer juicy meat.

The drip loss of the turkey thigh muscles packed in all three films increased gradually up to the 15th day of storage. On the 15th day the drip loss rose by 186,67%, 233,11%, 235,63% for samples packed in PA/PE, PA/PE+AF, PA/ARE/PE films, respectively in comparison to the amount of drip loss noticed on the 4th day (Table 2).

According to Krala [1985] the increase of weight loss in geese breast muscles stored in controlled atmosphere (40 to 100% CO_2, 20 to 60% N_2) for 21 days was due to the decomposition of nitrogen compounds contained in the muscle tissue and due to the loss of water.

The increase of drip loss in muscles during storage was also found by: Rammouz et al. [2004] in turkey thigh muscles (1.9%); and by Krala [1985, 1996a, 1998] in:

- geese breast muscles stored in controlled atmosphere (1.5–1.8%),
- chicken breast muscles stored in modified atmosphere (1.9–2.5%),
- chicken and turkey breast muscles stored in vacuum containers and in polyethylene bags closed without venting (2.7% and 3.4% – turkey muscles; 3.5% and 4.5% – chicken muscles).

The results of our research, concerning the drip loss of the muscles stored in MA (75% CO_2, 20% N_2, 5% O_2) are higher than the data obtained by the mentioned authors. According to Krala [1996a] differences in the amount of drip loss inside the packaging with meat stored in modified atmosphere, depend on their properties and methods of cooling after slaughter of birds carcasses. The concentration of CO_2 included in MA and final partial pressure in the plastic bags have significant impact on the drip loss inside the packaging with cuts of poultry meat. CO_2 is more quickly absorbed by packed meat when the ratio of the package volume to packaged meat mass is too low. In this case, the packaging tightly clinges to the surface of the meat, causing an increase of leakage. A similar effect can occur when carbon dioxide is transmitted quite rapidly through plastic packaging materials (packaging film has too high permeability of carbon dioxide).

Similarly to the cooking loss, on the 8th day of storage a significant effect of packaging material on the amount of drip loss was found. From the 8th until the 15th day the samples packed in PA/ARE/PE film had significantly lower drip loss in comparison to the samples packed in the other two films. Although considerable differences in the amount of drip loss in the muscles packed in PA/PE and PA/PE+AF bags, were not observed during the storage, the samples packed in PA/PE+AF film, with the lower oxygen and moisture vapour transmission rate in comparison to PA/PE film, had lower drip loss (Table 2).

The obtained data indicate that higher barrier properties of films reduce the amount of drip loss in stored muscle, which results in a reduction of their weight loss.

The outcomes of our own study showed that the greater drip loss in muscles was associated with the higher microbiological contamination. From the 8th day of storage the samples packed in PA/PE film with the lowest barrier properties (the highest drip loss), had a higher number of bacteria than those in films with higher barrier properties (data in press).

Conclusions

Barierr properties of packaging films determined the functional properties of turkey muscles such as: pH, WHC, drip loss and cooking loss. It is important from the culinary and technological viewpoint. The thigh muscles packed in PA/ARE/PE bags had the lowest concentration of drip loss, cooking loss and the highest pH values and water binding capacity in comparison with the other two films. Significant differences in drip and cooking loss, water binding capacity and the pH values between samples packed in PA/PE+AF and PA/PE films were not observed (films with the greatest differences in water vapour permeability: 7.3 g/m^2·24h). Significant differences in the pH values, water binding capacity, drip and cooking loss between samples packed in PA/ARE/PE film and PA/PE and PA/PE+AF bags can be explained by a considerable influence of the oxygen transmission rate on these parameters.

The turkey thigh muscles packed in PA/ARE/PE film proved to be the best suited for consumption and processing as compared to those packed in PA/PE+AF and PA/PE films.

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6

THE INFLUENCE OF ACTIVE PACKAGING ON THE HARDNESS CHANGES OF THE SOFT *KLEO* CHEESE DURING STORAGE TIME

Introduction

Texture is an important characteristic used to differentiate many cheese varieties [Antoniou et al. 2000, Wendin et al. 2000] and is considered by the consumer as a determinant of overall quality and preference [Lee et al. 1978, Adda et al. 1982, McEwan et al. 1989, Guinard and Mazzucchelli 1996], therefore its evaluation is an important step in product development and quality control [Meullenet and Gandhapuneni 2006].

Soft fresh cheeses are those cheeses that are unripe. White cheese is usually made from raw milk without of starter culture [Abdalla et al. 1997], souring the milk, either with lemon juice or vinegar. Creamy curds were formed then strained to produce a simple cheese. These cheeses have high moisture content, are usually mild and have a very soft texture. These cheeses are typically the most perishable. Cheeses in the fresh category include Italian Style Mascarpone, and Ricotta, Chevre, Feta, Cream cheese, Quark and Cottage cheese.

Optimal packaging solutions could prevent or minimize quality changes, resulting in increased shelf life as well as quality maintenance. Different types of cheeses have to be packed in different packaging concepts. [Mortensen et al. 2005]. Most fresh cheeses are packed in air atmosphere due to the short shelf life required. Some experiments proved that chemical composition and sensory characteristics, colour and body of white cheese made from pasteurized cow milk during the storage period 45 days in vacuum packaging did not significantly change [Osman et al. 2009]. It was found that packaging and cold storage of Sudanese white cheese in metal containers is better than in plastic containers as low total bacterial, coli forms, *E.coli* and yeast counts were obtained in cheese packed in metal containers. [Ahmed and Alhassan 2010].

The potential use of Modified atmosphere packaging (MAP) and active packaging for extending the shelf life of dairy products, including cheese, has been demonstrated [Floros et. al, 2000]. Limited work has been conducted to date on the use of different gas composition MAP for shelf life extension of soft, creamed-style, and whey cheeses [Pintado et al. 2001, Papaioannou et al. 2007, Dermiki et al. 2008].

In order to optimize product and packaging compatibility, materials with improved barrier properties should be used. Optimization may include new areas such as active packaging concepts, and nanocomposite technology. [Mortensen et al. 2005]. A study conducted by the

Department of Food Science at Cornell University concluded that FreshPax™ is effective in extending the shelf-life of oxygen-sensitive foods, and its use decreases mould and mould spoilage of commercial cheese. (FreshPax™ Oxygen Absorbing Packets]. Different antimicrobial packaging systems were used as active agents to increase the shelf life of Mozzarella cheeses. [Conte et al. 2007].

The objective of this study was to investigate the effect of packaging materials and the technology on textural hardness in connection with moisture content and water activity during storage time.

Materials and methods

Kleo cheese characteristics

Experiments were carried out at the Department of Food Technology, Latvia University of Agriculture in 2011. The object of the research was *Kleo* cheese – a regional white soft fresh cheese manufactured in a local cheese making factory from pasteurized (78–82°C), and normalized cow milk – for experiments was bought on the local supermarket in Latvia. The albumen from the milk had been set down by addition of acid cheese whey, after that it wasfilled in a self compressing vat for moulding for 16 to 18 hours. The pressed unripe cheese pieces have a cylindrical shape with rounded off side edges, before packaging they are rubbed with salt (NaCl), salt content – from 0.3 to 0.8%. The consistency of cheese is mild, and has a homogenous slightly grainy texture, with irregular breaks, the surface slightly wet, the colour from white to slightly yellowish. The moisture content of the cheese should not be more than 64%, fat content – 35±2%.

Packaging and storage of samples

Kleo cheese in currently is sold in the market in a polymer PA/PE pouch vacuum packaging (VP) weight of 0.3–0.8 kg in each and its shelf-life is not more than 15 days at a temperature 0 to +6°C. Four different polymer films were used: PA/PE, PE/OPA, Multibarrier 60 and OPP. The structure of performed experiments is shown in Figure 1. Packaging materials for experiments were selected with different water vapour transmutation rate and various thicknesses. The manufactured *Kleo* cheese cylindrical pieces were cut into four parts each, packed by 100±10 g beforehand from roll stocks made from polymer pouches, size 110 × 120 mm. For shelf life extension the use of both regular MAP conditions as well as with oxygen scavenger commitment in the pouch was investigated. Modified atmosphere consisting of carbon dioxide CO_2 (E 290) 30% and nitrogen N_2 (E 941) 70% was used, while vacuum packaging (VP) was selected as the control. For reduced oxygen packaging (ROP) creation (O_2 – 0%) in pouches an iron based oxygen scavenger sachets of 50 cc obtained from Packaging Solutions OÜ were used. The samples were hermetically sealed by MULTIVAC C300 vacuum chamber machine and stored in a Commercial Freezer/Cooler "Elcold" at a temperature of +4.0±0.5°C, controlled by MINILog Gresinger electronic. At the storage time of up to 32 days the physical and chemical properties: pH, moisture content, water activity and hardness of the cheese were evaluated. At each time of measurement, two identical packages for each packaging material were randomly selected on sampling days (day 0) and after 5th,

11th, 15th, 18th, 22nd, 25th, 29th and 32nd day of storage; six measurement repetitions of each sample were performed.

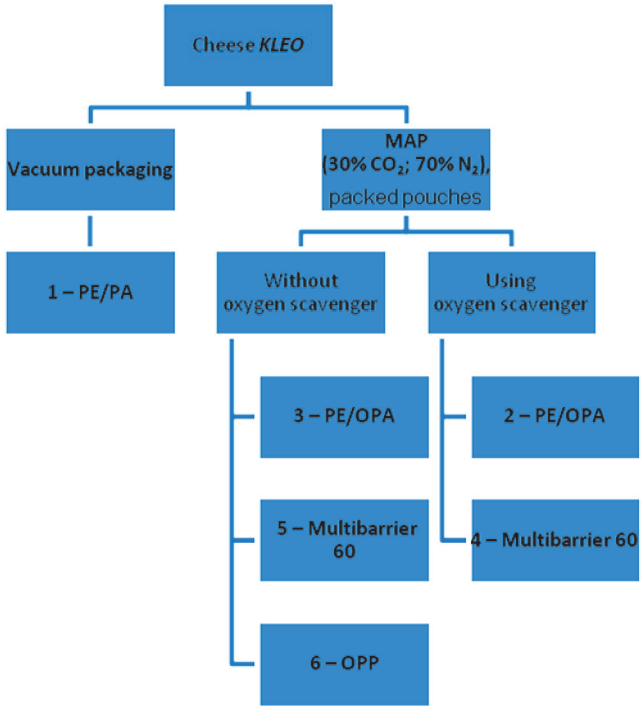

Fig. 1. The structure of performed experiments

Physical- chemical analysis

Texture analyses were conducted on the Texture Analyzer, TA-XT2i Texture Analyzer; Stable Micro Systems, NY. A spherical probe (P/1S – Ball Stainless) was used measuring the hardness of cheese samples. Test speed, distance and trigger force were 2 mm s^{-1}, 5 mm and 0.0493 N, respectively. Moisture content was determined by ISO 6496:1999, water activity by ISO 21807: 2004 AquaLab LITE device and pH levels by LVS 1132:2001 – Jenway 3510.

Statistical analysis

The results were processed by mathematical and statistical methods. Statistics on completely randomized design were determined using the General Linear Model (GLM) procedure SPSS, version 16.00 and MS Excel programmes.

Results and discussion

Hardness of *Kleo* cheese, during the storage time, had been decreasing slightly in all cheese samples packaged in different packaging materials used in the research. At the beginning – before the cheese was packed, the hardness of the cheese was 20 N and by the influence of LAB had gradually decreased to 15±3 N (Fig. 2).

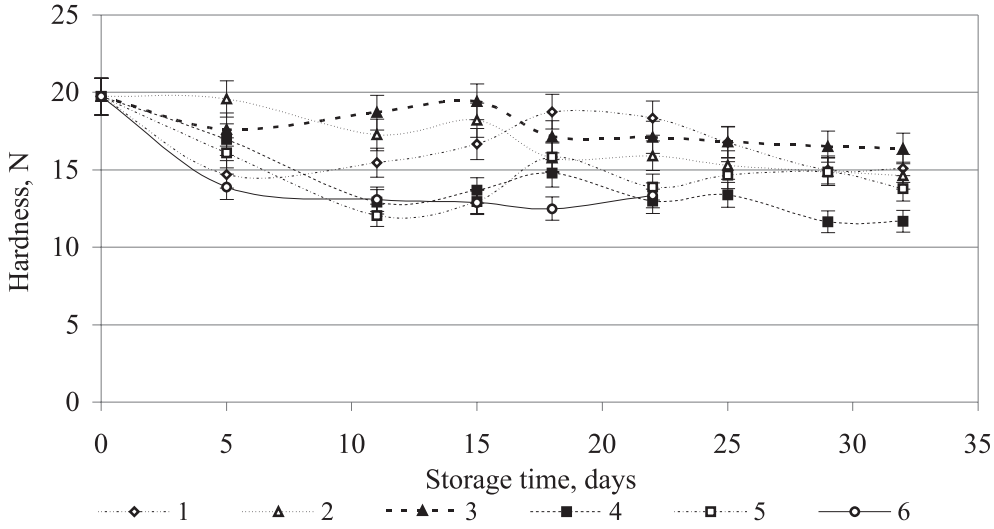

Fig. 2. Dynamics of the hardness in *Kleo* cheese during the storage time, N
1 – vacuum, PA/PE; **2** – MAP, PE/OPA; **3** – MAP, PE/OPA, oxygen scavenger*;* **4** – MAP, Multibarrier 60, oxygen scavenger; **5** – MAP, Multibarrier 60; **6** – MAP, OPP

The highest decrease to 12 N in hardness was noticed in cheese samples packed in Multibarrier 60 film without an oxygen scavenger. The most considerable changes in the hardness of the cheese were found in the cheese samples packed and stored in PE/OPA with oxygen scavengers – from 20 to 13 N. The presence of an oxygen scavenger in the packaging materials made from PE/OPA and Multibarrier 60 (APA/TIE/PA/EVOH/PA/TIE/PE/PE) comparing to the simple packaging materials with MAP, the hardness of the cheese had increased by 13%.

Water loss during the storage period is one of the factors that might influence the hardness of the cheese samples. Dosebry and Hardy [2000] noted that a 2.5–5.0% weight loss of cheese due to insufficient barrier properties is normal. They also found that dehydration of fresh cheese should be avoided, because a dehydrated surface is a major quality defect in those products. Moisture loss was the main factor of weight loss from the cheese during the storage time caused by different water vapour barrier properties of packaging materials. The highest decrease in moisture content was observed in those cheese samples which were packed and stored PE/OPA with an oxygen scavenger (Fig. 3). The decrease in moisture content reached the peak point on the 5th day and afterwards increased.

Relationship between changes of hardness and moisture content during the storage was found in those cheese samples which were packed in the PE/OPA and OPP (r=0.700).

Water activity a_w of *Kleo* cheese at the beginning of the experiment was 0.98 and during the storage time it slightly increased to 0.99 (Fig. 4). The increase of the water activity can be related to the water vapour barrier properties.

As shown in Figure 5, initial pH value of *Kleo* cheese was 5.8 decreasing within 32 storage days up to 5.0 of the control sample (VP) and 4.8 in samples packed under MAP in PE/OPA without oxygen scavengers. Using the oxygen scavengers, pH values decreased till 4.9 in PE/OPA and till 5.0 in Multibarrier 60 film packaging. In the correlation analysis it

was found that strong correlation between pH and hardness existsted in cheese samples which were packed in the PE/OPA without an oxygen scavenger (r=0.91) and Multibarrier 60 with an oxygen scavenger (r=80) and good correlation – PE/OPA with an oxygen scavenger (0.64).

The lowest decrease in pH values was shown in a sample packed in Multibarrier 60 film without an oxygen scavenger. The higher decrease of cheese pH value was observed in cheese packed in MAP, OPP – already after 22 storage days it was 4.8, therefore all samples in this packaging become misty, wherewith this packaging material doesn't ensure the cheese quality during storage.

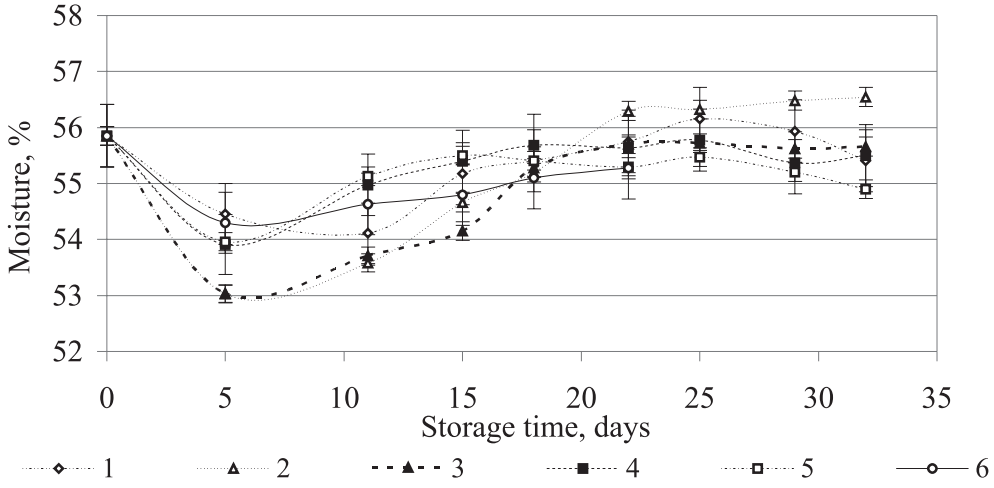

Fig. 3. The dynamics of moisture content in *Kleo* Cheese during the storage time, %
1 – vacuum, PA/PE; **2** – MAP, PE/OPA; **3** – MAP, PE/OPA, oxygen scavenger*;* **4** – MAP, Multibarrier 60, oxygen scavenger; **5** – MAP, Multibarrier 60; **6** – MAP, OPP

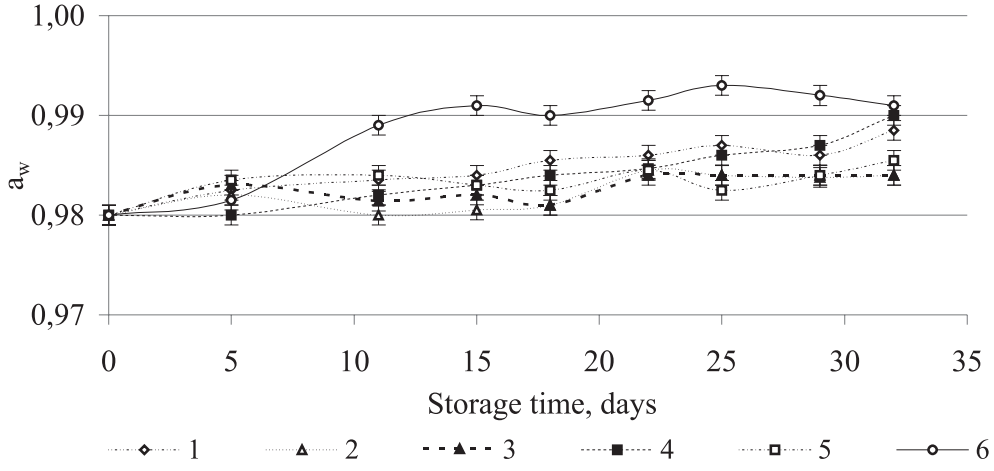

Fig. 4. The dynamics of water activity (a_w)
1 – vacuum, PA/PE; **2** – MAP, PE/OPA; **3** – MAP, PE/OPA, oxygen scavenger*;* **4** – MAP, Multibarrier 60, oxygen scavenger; **5** – MAP, Multibarrier 60; **6** – MAP, OPP

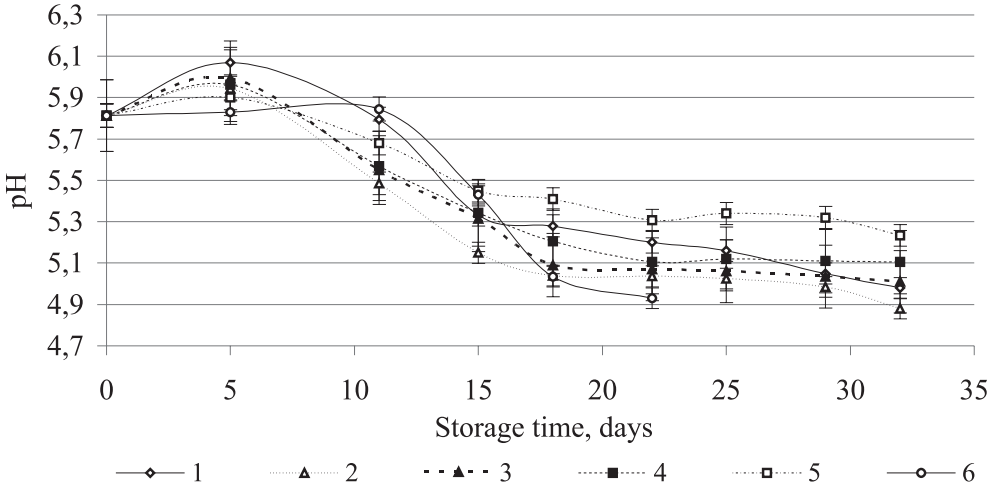

Fig. 5. pH dynamic in vacuum and in MAP packed *Kleo* Cheese during the storage time
1 – vacuum, PA/PE; **2** – MAP, PE/OPA; **3** – MAP, PE/OPA, oxygen scavenger*;* **4** – MAP, Multibarrier 60, oxygen scavenger; **5** – MAP, Multibarrier 60; **6** – MAP, OPP

Conclusions

During the 32 days' storage time of Kleo cheese considerable changes in its quality by using oxygen scavengers in the packaging material were not noticed. The hardness of the cheese had decreased slightly from 20 to 13 N. In the correlation analysis it was found that strong correlation between pH and hardness was noticed in the cheese samples which were packed in the PE/OPA without an oxygen scavenger (r=0.91) and Multibarrier 60 with an oxygen scavenger (r=80) and good correlation – PE/OPA with an oxygen scavenger (0.64) and between hardness and moisture content – in cheese samples packed in the PE/OPA with an oxygen scavenger (r=0.7) and OPP (r=0.7).

Acknowledgement

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7

THE EFFECT OF SELECTED PLASTICIZERS ON STRENGTH PROPERTIES OF EDIBLE FILMS

Introduction

The use of edible coatings was found large attention in last few years, mainly because of the possibility for extending the shelf life of foods. An edible coating or film has been defined as a thin, continuous layer of edible material placed on foods or between food components. The aim of this study is to produce the natural biopolymeric coating which may be eaten together with the food and present specific properties,. Their function is to provide a barrier to mass transfer (water, gas and lipids), to serve as a carrier of food ingredients and additives (pigments, flavours, antioxidants and antibiotics), or to provide mechanical protection. Edible coatings for suitably designed mechanical properties can replace synthetic packaging [Krochta et al. 1997, Tendej 2001].

Protective films are manufactured mostly from natural polymers, animal and plant origin, such as proteins, gums, lipids, which are completely biodegradable and safe for the environment [Cao 2007, Debeaufort et al. 1998, Guilbert et al. 1996]. Additive substances, which may present following properties or activity: antimicrobial, antioxidant, plasticizing (glycerol, sorbitol, mannitol, polyethylene glycol), colouring and light-absorbing substances are used in production of edible coatings. Films created solely from polymers are brittle, while the additive of plasticizer increases their flexibility [Fernandez 2007].

A plasticizer is defined as a substance or material incorporated in a material (usually a plastic or an elastomer) to increase its flexibility or workability.

Functionality hydrocolloid coatings depends on their chemical composition, structure and properties of a polysaccharide and a plasticizer used [Yang L. et al. 2000].

Mechanical properties, relevant to the application of edible coatings on the product, determine the sustainability and strength of coatings and stability during the storage time [Fernandez et al. 2207, Jones 2002, Kaya 1997].

The aim of this study was to determine the effect of selected plasticizers (glycerol, polyethylene glycol, sorbitol) on the mechanical properties of protective polysaccharide films.

Materials and methods

In production of protective film were used: hydroxypropylmethylcellulose (HMPC 100 PA 2208 FG) from Wolff Cellulosics and carrageenan (WG 2000) AMCO and following

plasticizers, such as: D – sorbitol (POCH. SA), polyethylene glycol PEG 400 (POCH. SA), glycerol (ZCHG "Pollena–Stum").

Experiment was executed according to bifactor model, by assumption: three different concentrations hydroxypropylmethylcellulose 100 (HPMC 100), carrageen (0, 1, 2%) and plasticizer (1, 1,4, 2%).

The first step in obtaining experimental coatings was to obtain an aqueous solution of hydrocolloids, which was placed in a water bath for 20 minutes at 75°C. The cooled sol was stirred using a stirrer speed of R50D CAT 250 r / min, gradually adding the plasticizer (Table 1). The resulting mixture was poured to forms coated with Teflon (80x200mm) and dried for about 24 hours at 25ºC with a humidity of 60%.

The dried films were used to measure the tensile strength and puncture and elongation at break. Designation of selected mechanical parameters was performed using a Materials Testing Machines Zwick / Roell Z010. Measurement of tensile strength and elongation tensile was made using a test – a simple extension of the appointment F_{maxw} penetration test was performed by using a compression stem F_{maxp} setting. The requirements for this test were as follow: the sample dimensions 15x7x0.046 mm, drive speed of head for the tensile force was 1 mm/s, for the puncture force was 0.5 mm/s and 6.0 mm shank diameter (ASTM 1996).

Statistical analysis was performed using the Software STATISTICA (Version 7.0, Statsoft, Inc.). Response surface methodology (RSM) was used to investigate the simultaneous effect of two experimental factors. This test allows for the calculation of the coefficient estimates of quadratic equation. Polynomials were fitted to the experimental data as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_{11} + \beta_{22} X_{22} + \beta_{12} X_{12}$$

Where Y is the estimated response, β_0, β_1, β_{11}, β_{22}, β_{12} are constant and regression coefficients of the model, and X_1, X_2 are levels of independent variables, i.e., the concentrations of carrageen and plasticizer, respectively. The results of measurements of tensile strength film, puncture and tensile elongation were evaluated by analysis of variance requesting uniform groups based on Duncan's test at $\alpha<0.05$, and response surface method, verifying the importance of factors based on the so-called volatility assess the effects.

Table 1

Experimental design

Code	HPMC 100 [%]	Carrageen [%]	Glicerol [%]	Code	HPMC 100 [%]	Carrageen [%]	Sorbitol [%]	Code	HPMC 100 [%]	Carrageen [%]	PEG 400 [%]
H0G1	0	2	1	H0S1	0	2	1	H0P1	0	2	1
H0G1.4	0	2	1.4	H0S1.4	0	2	1.4	H0P1.4	0	2	1.4
H0G2	0	2	2	H0S2	0	2	2	H0P2	0	2	2
H1G1	1	1	1	H1S1	1	1	1	H1P1	1	1	1
H1G1.4	1	1	1.4	H1S1.4	1	1	1.4	H1P1.4	1	1	1.4
H1G2	1	1	2	H1S2	1	1	2	H1P2	1	1	2
H2G1	2	0	1	H2S1	2	0	1	H2P1	2	0	1
H2G1.4	2	0	1.4	H2S1.4	2	0	1.4	H2P1.4	2	0	1.4
H2G2	2	0	2	H2S2	2	0	2	H2P2	2	0	2

Results and discussion

Puncture strength

Significant effect of HPMC and glycerol on the variability of F_{maxp} obtained from puncture test was found. It was observed that increasing of glycerol content and HPMC content coatings decreases strength to puncture. Similar conclusions are reached by Vanina et al., showing a negative effect of glycerol on the strength of films puncture (F_{maxp} = 8.9 N for 0.6% added glycerol, and F_{maxp} = 18.28 N at 0.2% added glycerol).

The lowest F_{maxp} value (22.72 N) was obtained for variant H2G2 (Fig. 1). The highest value of (46.74 N) was determined for coatings containing 1.4% glycerol.

Analyzing the puncture strength of experimental coatings with the addition of polyethylene glycol (PEG 400) and sorbitol had a significant effect of plasticizers on the variability of parameter F_{maxp}. It was observed that increasing levels of sorbitol (1%–1.4%) caused the increases of F_{maxp} to 9.22%. Further increase of tested plasticizer concentration (1.4%–2%) reduces the value of this parameter (Fig. 2).

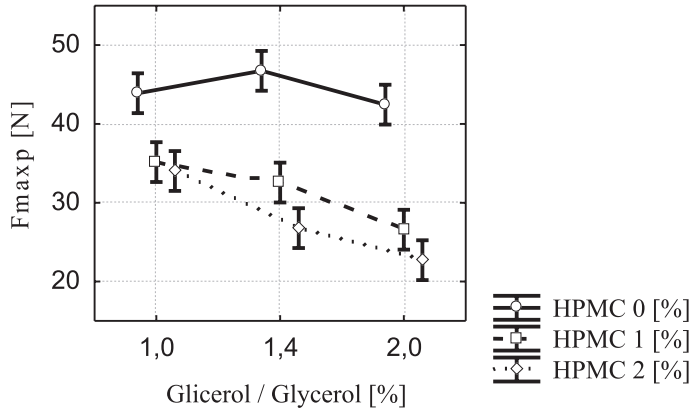

Fig. 1. Influence of HPMC 100 and plastificators on F_{maxp} Glicerol / Glycerol

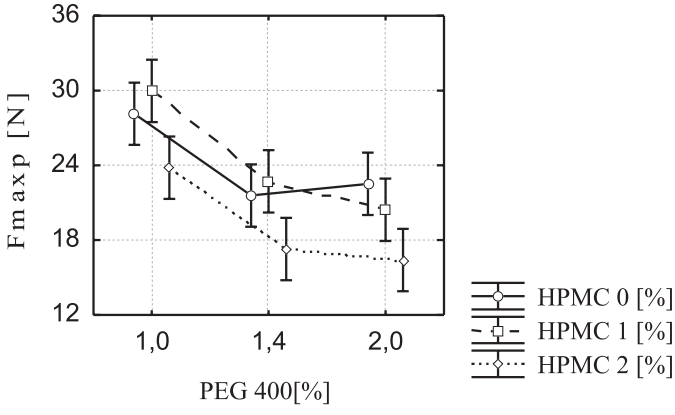

Fig. 2. Influence of HPMC 100 and plastificators on F_{maxp} PEG 400

The maximum of penetration force of coatings with addition PEG 400, showed a linear downward trend (Fig. 3).

The highest F_{maxp} value among all tested coatings was obtained for variant H0S1. 4 (51.75 N) while the lowest (16.40 N) for the H2P2.

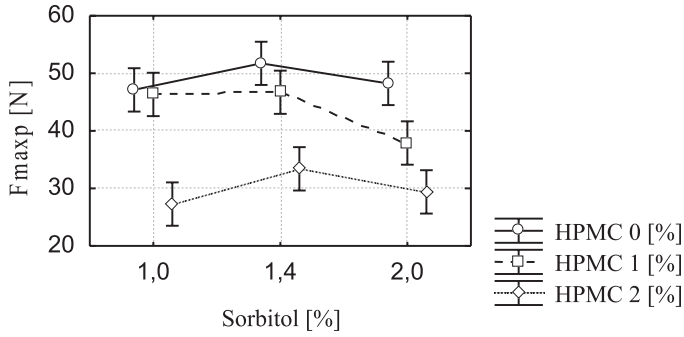

Fig. 3. Influence of HPMC 100 and plastificators on F_{maxp} Sorbitol

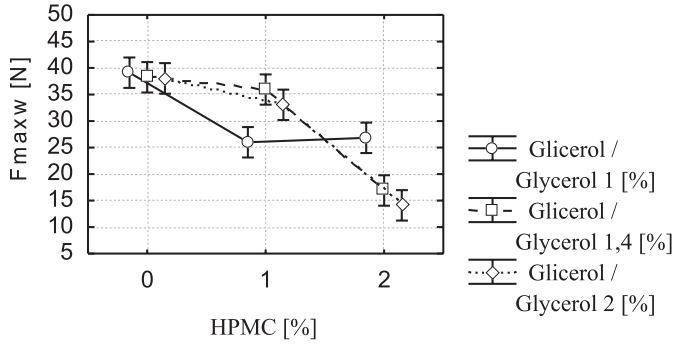

Fig. 4. Influence of HPMC 100 and plastificators on F_{maxw} Glicerol / Glycerol

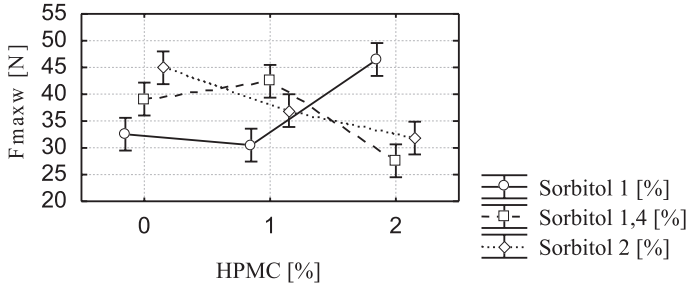

Fig. 5. Influence of HPMC 100 and plastificators on F_{maxw} Sorbitol

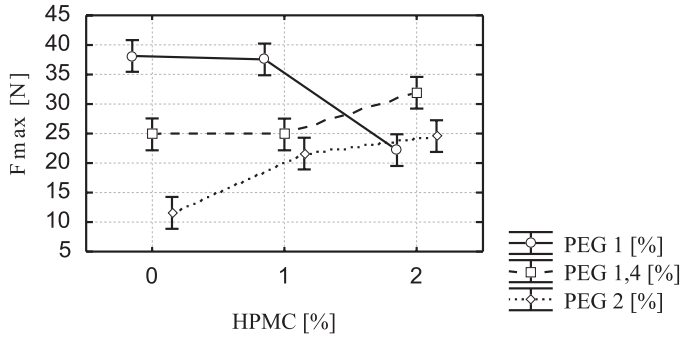

Fig. 6. Influence of HPMC 100 and plastificators on F_{maxw} PEG 400

Tensile strength

The coatings produced with the addition of glycerol at 1.4 and 2% showed no significant differences in the assessment of tensile strength. Increase of HPMC level in the experimental films reduces the value of F_{maxw}. The highest F_{maxw} value in protective films was characterized by a content obtained without hydroxypropylmethylcellulose (Fig. 4–6). In a study conducted by Tang et al. and Cho et al. found a negative effect of the addition of glycerol on the value of the maximum elongation force (Cho, Rhee 2002, Tang et al. 2008).

It was observed that in addition to experimental coatings PEG 400 at 1.4 and 2% and HPMC at 2%, increases F_{maxw}. Comparing hydrocolloid effects on the strength of the experimental coatings was found that regardless of the type of plasticizer, comparable values were obtained for films F_{maxw} protection from participation HPMC at 1%. The results obtained in the tensile test showed that the highest value F_{maxw} (53.03 N) was characterized by a variant of H2S1, and the lowest (11.56 N) for variant H0P2 (Fig. 6). However, in a study conducted by Sothornvit et al., where the base of β-lactoglobulin was found that the highest resistance to elongation were characterized by a the coating with the addition of glycerol, and the lowest of PEG 400 [Sothornvit et al. 2001] comparing the mechanical properties of experimental films made with glycerol or sorbitol, showed that the glycerol-containing the coating have higher coefficient F_{maxw} [Mali et al. 2005].

Elongation at break (εz)

It was noted that value of elongation at break for experimental coatings produced with carrageenan 2%, increased with the increasing concentration of plasticizer. Vavin and Tang were proved that the addition of glycerol increases the flexibility of the protective film [Tang et al. 2008, Vanin et al. 2005]. However Martelli was found that the coatings prepared with sorbitol were characterized by low values of the coefficient εz [Martelli et al. 2006].

Films obtained with PEG 400 were presented the highest values of elongation – the maximum value of 16.32% for εz H2P1.4. Sothornvita and others were obtained low strength elongation of coatings containing PEG 400, what is in opposite with our results [Sothornvit Krochta 2001]. Analysing the influence of plasticizer concentration on the variability of the elongation at break, coatings produced with glycerol and sorbitol were characterized by similar values of these parameter (Fig. 8 and 9).

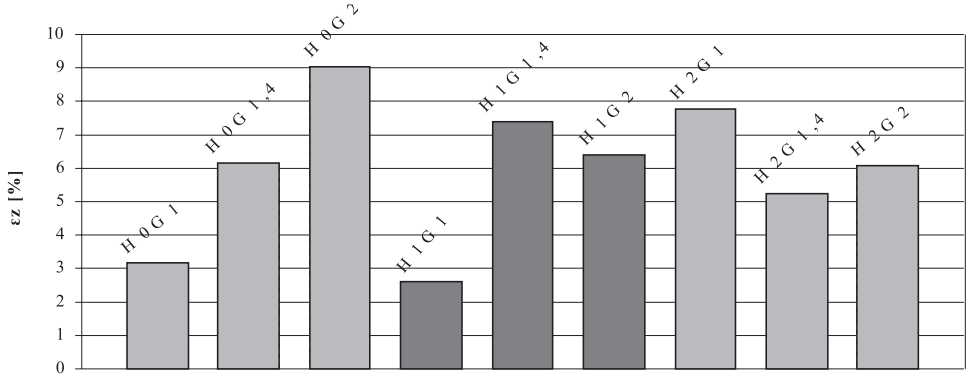

Fig. 7. Influence of HPMC 100 and glycerol on ε_z

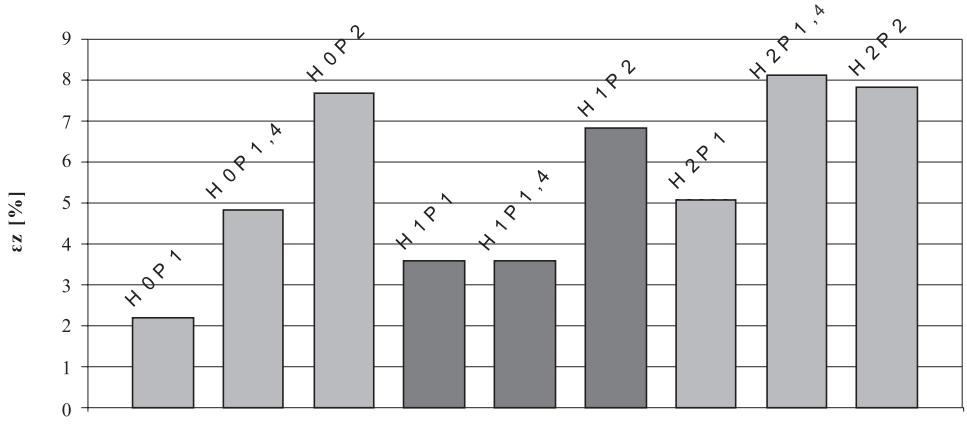

Fig. 8. Influence of HPMC 100 and sorbitol on ε_z

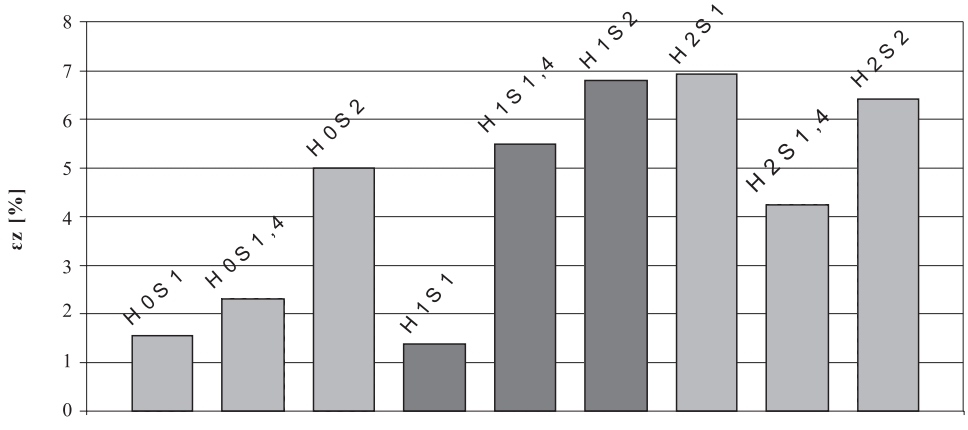

Fig. 9. Influence of HPMC 100 and PEG 400 on ε_z

Conclusions

Results of the experiment showed that increasing the concentration of hydroxypropylmethylcellulose in films reduces both the maximum value of puncture force, and the maximum force of elongation.

The lowest strength puncture and the highest capacity for elongation at break were observed for coatings containing PEG 400 as plasticizer.

Protective films prepared with 1% of glycerol, without addition of HPMC 100 showed the highest elongation and puncture strength.

The coatings obtained with the addition of sorbitol characterized the lowest values of strength parameters which were evaluated.

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8

ATTACHING OLEIC ACID INTO ACETYLATED STARCH BY ENZYMATIC TRANSESTERIFICATION

Introduction

Hydrophillic character of starch is main cause of their limited industry use [Singh et al 2004]. Possible solution of this problem is modification of native starch [Yan et al. 2010], which imbeds it with desired physico-chemical and functional properties [Fortuna et al. 2002]. Esters of saccharides and fatty acids are relatively new group of modificates [Ward et al. 1997]. They are classified as natural non-ionic biosurfactants [Kołakowski et al. 2005], which find use both in food, chemical, cosmetic, as well as pharmaceutical or biomedical industry [Degn et al. 2001]. Saccharides, as polyhydroxy compounds, offer possibility of esterification of free hydroxyl groups present in their molecule. It allows to obtain complexes with dramatically different physico-chemical properties [Lortie 1997, Kołakowski et al. 2005]. Esters of fatty acids and saccharides are compounds characterized by stabilizing and emulsifying properties [Ye et al. 2010]. Exploit of fatty acid with saccharide esters is also a point of interest for cosmetic industry. They can be found in body creams, toothpastes, lipsticks, shampoos, hair conditioners as well as dishwashing, or laundry detergents. They are characterized by softening properties, eliminate problem of hard water, and show antibacterial properties [Ye et al. 2010]. Use of saccharide esters also increase in pharmaceutical and biomedical industry. They facilitate dissolvment of vitamins and antibiotics in fats, without mucosa irritation during their reception. Additionally they may be used as fillers in bone cavities, or even as their complete replacements [Rajan et al. 2006, 2008].

The main method of saccharides with fatty acids esters obtainment is a method with chemical agents usage. Synthesis of esters is a reaction requiring large amounts of energy, while toxicity and poor selectivity of reagents used in process, commonly generates undesired side-products [Tsitsimpikou et al. 1997, Degn et al. 2001, Chen et al. 2003]. It seems that, synthesis may be alternatively processed by enzymes- particulary lipases. Main reaction is conducted in lower temperature (30–60°C) and obtained product has specified chemical structure, is devoid of color impurities, and equally important, it is natural [Debreucq et al. 2000]. Currently, mechanisms of saccharides esterification with fatty acids are known, but there are no reports concerning transesterification of saccharide and fatty acid esters. This raises need to investigate possible method of those esters obtainment with the above-mentioned reaction. Taking into account great usability of saccharide esters in virtually all industry

branches, it is appropriate to conduct research for new methods of their synthesis with maintained environmental care.

The purpose of this study was obtainment of high acetylated potato starch esterified with oleic acid by enzymatic transesterification, conducted by utilization of diversified fractions of immobilized lipase of microbial origin, as well as comparison of obtained products properties.

Materials and methods

The research material consisted of: high acetylated starch, produced in the Department of Food Storage and Technology of Wrocław University of Environmental and Life Sciences, molecular sieve 4A Sigma-Aldrich, oleic acid from Fluka Chemist company, tert-Butanol from Riedel-de Haën company, preparations of extracellular lipases, derived from Candida antarctica yeasts, immobilized on acrylic resin trade named Nowozym 435 and fractions A and B of this enzyme, from Sigma-Aldrich company.

Obtainment of high acetylated starch

Natural dry potato starch, in an amount of 200 g, was placed in a reaction vessel and 620 ml of acetic anhydride was added. The mixture, continuously agitated, was heated to 60°C and sequentially, to initiate the reaction, 16 ml of 50% NaOH was added. The resulting slurry was cooled to 30°C and adjusted to pH 7 with 25% NaOH. The resulting product was centrifuged and dried at 40°C for 48 hours. Obtained starch modificate was placed in a reaction vessel and then subjected to acetylation in the manner described above.

Enzymatic transesterification

Reaction mixtures of the following composition were prepared: acetylated starch, oleic acid, tert-butanol, molecular sieve, and enzyme (lipase A and B, as well as Novozyme 435). Simultaneously control samples were created – without enzyme addition. Composition of reaction mixtures: for 1 mol of glucose equivalent 1 mol of oleic acid was weighed, tert-butanol was double the mass equivalent of oleic acid and esters of saccharides, preparations of immobilized lipase (Novozyme 435 and its fractions A and B) – 1g for ten day reaction and 3 g for three day reaction, molecular sieve (absorption of water formed during the reaction) – 0,2 times mass equivalent of saccharide esters and oleic acid. Mixtures were subject to reaction, which was carried with continuous mixing (350 rev / min) for 3 or 10 days at 60°C, after which excess fatty acids were extracted with 150 ml of acetone at 35°C for 1 hour. The resulting precipitate was filtered to separate the molecular sieve and washed with acetone to remove residuals of unreacted oleic acid. Preparations were washed, and than dried at room temperature (20–25 degrees C).

Analysis of the chemical bonds present in the preparations – the method of magnetic resonance ^{1}H NMR.

Samples containing 10 mg of tested preparations and 1 ml of deuterated dimethyl sulfoxide (DMSO) were prepared. The mixture was heated in a water bath at 70°C for 7 days. Then 0,6 ml of sample was collected and transferred to NMR tubes. ^{1}H NMR analysis was carried out at 70°C using a spectrometer AVANCE AMX 300 from Bruker. Basing on the obtained spectra presence of the following signals was confirmed: CH_3 end groups of oleic

acid at 0,79–0,87 ppm, protons of glucose ring in range 5,0–5,5 ppm, C=C bonds derived from oleic acid at 4,2 ppm, CH_3 groups of acetic acid at 2,01–2,10 ppm.

Determination of degree of substitution (DS) of starch with acetic and oleic acids – **the method of magnetic resonance ^{1}H NMR.**

The degree of substitution with oleic acid was calculated from the formula:

where:

$$DS_{acetic} = \frac{3 \cdot a}{a + b + c} \qquad DS_{oleic} = \frac{3 \cdot b}{a + b + c}$$

a – total area of the peaks of acetic acid residues at 2.01–2.10 ppm,

b – peak area of the terminal methyl group of oleic acid at 0.85 ppm,

c – total area of the peaks of the OH group in the glucose ring at 5.0–5.5 ppm.

Analysis of the functional groups occurring in preparations by absorption spectroscopy FTIR.

0.2g of tested samples were weighted, and compressed into a pill made of alkali metal halides (KBr). To determine the functional group analysis on spectrometer Mattson FTIR--300 was performed.

Determination of the thermal characteristics of the substance using a differential scanning calorimeter DSC – Mettler-Toledo Model 822e.

To aluminum dish, of 100 µl capacity, 5mg of tested substance calculated dry mass was weighted. Sample was conditioned for 30 minutes at 20–25°C and then placed in a chamber of differential scanning calorimeter. The sample was heated from temperature 25 to 250ºC at rate of 10°C·min^{-1}. Obtained results allowed to determine specific heat of phase transitions, initial and final temperatures of phase transitions and extrapolated centers of peaks.

The manner of results preparation

Obtained results were subject of statistical interpretation using two-factor one-way analysis of variance. Homogeneous groups were determined with Duncan's test with significance level α=0.05 in the program Statistica 9.1.

Results and discussion

Table 1 presents a degree of high acetylated starch substitution with oleic and acetic acids, as well as its derivatives obtained during ten days reaction. In this case, the highest activity of transesterification was presented by fraction B of used enzyme. The value of degree of substitution with oleic acid was DS=0.63 and with acetic acid – DS=2.07. The lowest efficiency of transesterification was showed by N fraction of the enzyme, allowing a degree of substitution DS=0.21 – for oleic acid and DS=2.38 – for acetic acid.

Application of enzyme fraction A resulted with simultaneous connection of oleic acid to the starch chain in the amount of DS=0.58 and a complete disconnection, present before reaction, groups derived from acetic acid (deacetylation). The degree of substitution of pure high acetylated starch with acetic acid is DS=2.52

By analyzing the data in Table 2, it can be concluded, that in the case of 3-day transesterification of high acetylated starch with oleic acid, similarly to the reaction carried out for

Table 1

Degrees of substitution (DS) of high acetylated starch and its derivatives, obtained in the reaction carried out for 10 days.

Sn.	Preparation	Degree of substitution (DS)	
		acetic acid	oleic acid
1.	High acetylated starch	2.52	-
2.	Complex of high acetylated starch and oleic acid	2.38	0.17
3.	Product of high acetylated starch transesterification with oleic acid obtained with enzyme A	0*	0.58
4.	Product of high acetylated starch transesterification with oleic acid obtained with enzyme B	2.07	0.63
5.	Product of high acetylated starch transesterification with oleic acid obtained with enzyme N	2.38	0.21

*IR and ^{1}HNMR spectra did not show bonds from acetic acid

Table 2

Degrees of substitution (DS) of high acetylated starch and its derivatives, obtained in the reaction carried out for 3 days

Sn.	Preparation	Degree of substitution (DS)	
		acetic acid	oleic acid
1.	High acetylated starch	2.52	-
2.	Complex of high acetylated starch and oleic acid	2.38	0.17
3.	Product of high acetylated starch transesterification with oleic acid obtained with enzyme A	1.67	1.10
4.	Product of high acetylated starch transesterification with oleic acid obtained with enzyme B	1.54	1.24
5.	Product of high acetylated starch transesterification with oleic acid obtained with enzyme N	1.62	1.19

ten days, fraction B of enzyme used proved to be the most effective (DS=1.24 – for oleic acid and DS=1.54 – for acetic acid). The lowest degree of substitution was obtained in a reaction catalyzed by the enzyme of A fraction (DS=1.10 – for oleic acid and 1.67 – for acetic acid).

The HNMR spectra, for reactions lasting ten days, as well as three days, all show signals from protons of CH_3 group of fatty acid in range 0.79–0.87 ppm, the signals of the CH_3 group of acetic acid in the range of 2.01 to 2.10 ppm, and peaks derived from the glucose ring in 5.0–5.50 ppm.

Analysis of the functional groups present in investigated preparations was performed using FTIR spectroscopy. High acetylated starch, complex of high acetylated starch and oleic

acid, as well as products of high acetylated starch transesterification with oleic acid using enzymes showed stretching vibrations originating from the residues of acetic acid, and oleic.

Table 3 shows the initial phase transition temperatures of high acetylated starch and its derivatives, obtained during heating of the material to a temperature of 250ºC in an anhydrous medium, as well as the effect of synthesis length on the products initial temperature of phase-change. The ten-day samples were characterized by higher initial phase transition temperature (190.24ºC) in contrast to the material obtained from a three-day reaction (152.65ºC). Thus they form two homogeneous groups. Considering the differences between products it can be noted that the highest initial temperature was characterizing high acetylated starch (225.49ºC), whereas the lowest was noted for complex of high acetylated starch with oleic acid (135.23ºC). Each of the products was a separate homogeneous group.

Table 4 shows the values of end temperatures for high acetylated starch and its derivatives, determined during heating of samples to temperature of 250ºC in an anhydrous medium, as well as relations between length of synthesis and the end temperatures of phase transition. The highest end temperature was reached for products of ten days reaction (212.35ºC) and the lowest for products of three-days reaction (186.42ºC). They were described as two homogeneous groups. The product characterized with the highest end temperature was high acetylated starch (237.82ºC), and the product that showed the lowest values of this parameter – high acetylated starch esterified with fatty acid using the enzyme N. Products were set to four homogeneous groups.

Table 5 shows the extrapolated temperature of phase transition peak center for high acetylated starch and its derivatives, determined during heating of samples to a temperature of 250ºC in an anhydrous medium, as well as influence of enzymatic synthesis length on the value of this parameter. Compounds obtained in ten day reaction achieved higher values of temperature – 204.3ºC. The products of three day reaction were characterized by lowest temperature – 178.4ºC. Among the products, the highest temperature was recorded during heating of high acetylated starch (227.2ºC) and lowest (166.6ºC) for the complex of high acetylated starch and oleic acid. The products differ among themselves significantly, falling into five homogeneous groups.

Table 6 shows the specific heat capacity of phase transition for high acetylated starch and its derivatives, determined during heating to a temperature of 250ºC in an anhydrous medium, as well as the relationship between the values of specific heat capacity and the length of synthesis. High acetylated starch is characterized by the lowest specific heat capacity value (–10.34 $J \cdot g^{-1}$), whereas ester of high acetylated starch obtained with enzyme A in 3 day reaction with highest – 75.50 $J \cdot g^{-1}$. In statistical analysis, accounting the reaction length, samples from ten day reactions, were characterized with the lowest average values of specific heat capacity (16.78 $J \cdot g^{-1}$), while the three day synthesis products reached an average value of 43.06 $J \cdot g^{-1}$

Starch is an irreplaceable raw material in almost all sectors of the economy. Versatility of this material is the main cause of continued growth in demand for this raw material. The main reason for under-utilization of starch in the industry is caused by its hydrophilic nature [Singh et al. 2004, Kapuśniak et al. 2007]. In order to obtain suitable properties such as solubility, texture, consistency or a high resistance to various external factors, it is subjected to modifications [Fortuna et al. 2002]. Starch as a polysaccharide compound has three free hydroxyl groups, wherein substitution may undergo on one, two or all of these groups. This creates favorable conditions for esterification of saccharides with for example fatty acids.

Table 3

Initial temperature (T_p) of phase transition for high acetylated starch and its derivatives, determined during heating to 250^{0}C in anhydrous medium

Sn.	Product	Initial temperature of phase transition [°C]						
		3 day reaction			10 day reaction			
		Repeats		Average	Repeats		Average	
		I	II		I	II		
1.	High acetylated starch	222.53	228.46	**225.48**	222.53	228.46	**225.48**	LSD=3.63
2.	Complex of high acetylated starch and oleic acid	135.84	134.62	**135.23**	135.84	134.62	**135.23**	
3.	Product of high acetylated starch transesterification with oleic acid obtained with enzyme A	121.36	121.09	**121.22**	239.58	242.87	**241.22**	
4.	Product of high acetylated starch transesterification with oleic acid obtained with enzyme B	127.88	131.90	**129.89**	219.63	218.15	**218.89**	
5.	Product of high acetylated starch transesterification with oleic acid obtained with enzyme N	152.35	150.50	**151.42**	130.51	130.20	**130.35**	
		LSD=2.30						

Table 4

End temperature (T_k) of phase transition for high acetylated starch and its derivates, determined during heating to 250^{0}C in anhydrous mediumw środowisku bezwodnym

Sn.	Product	End temperature of phase transition [°C]						
		3 days reaction			10 days reaction			
		Repeats		Average	Repeats		Average	
		I	II		I	II		
1.	High acetylated starch	237.77	237.88	**237.82**	237.77	237.88	**237.82**	LSD=1.92
2.	Complex of high acetylated starch and oleic acid	177.76	179.54	**178.65**	177.76	179.54	**178.65**	
3.	Product of high acetylated starch transesterification with oleic acid obtained with enzyme A	163.83	163.85	**163.84**	250.01	249.91	**249.96**	

Table 5. continuous

4.	Product of high acetylated starch transesterification with oleic acid obtained with enzyme B	168.98	168.86	**168.87**	222.94	220.38	**221.66**	LSD=1.92
5.	Product of high acetylated starch transesterification with oleic acid obtained with enzyme N	182.84	182.86	**182.85**	175.69	171.61	**173.65**	
		LSD=1.21						

Table 5

Extrapolated temperature of peak center (T_e) of phase transition for high acetylated starch and its derivatives, determined during heating to 250^{0}C in anhydrous medium

Sn.	Product	Extrapolated temperature of peak center of phase transition [°C]						
		3 days reaction			10 days reaction			
		Repeats		Average	Repeats		Average	
		I	II		I	II		
1.	High acetylated starch	224.40	229.97	**227.18**	224.40	229.97	**227.18**	LSD=2.91
2.	Complex of high acetylated starch and oleic acid	166.37	166.77	**166.57**	166.37	166.77	**166.57**	
3.	Product of high acetylated starch transesterification with oleic acid obtained with enzyme A	156.51	156.44	**156.47**	246.11	245.43	**245.77**	
4.	Product of high acetylated starch transesterification with oleic acid obtained with enzyme B	164.14	164.76	**164.45**	220.62	218.71	**219.66**	LSD=2.91
5.	Product of high acetylated starch transesterification with oleic acid obtained with enzyme N	177.46	176.95	**177.20**	162.78	161.81	**162.29**	
		LSD=1.84						

Currently, the majority of saccharides esters are obtained by synthesis with chemical catalysts. This method was described in 1956 by Osipov et al. Its advantage comes from that when conducted under appropriate conditions it does not result in the destruction of starch granules, and the changes within the starch molecules are small. Unfortunately, very low so-

Table 6

Specific heat capacity (Q_w) of phase transition for high acetylated starch and its derivates, determined during heating to 250^{0}C in anhydrous medium

Sn.	Product	Specific heat capacity of phase transitiona [J*g^{-1}]						
		3 days reaction			10 days reaction			
		Repeats		Avera-ge	Repeats		Avera-ge	
		I	II		I	II		
1.	High acetylated starch	-10.46	-10.22	**-10.34**	-10.46	-10.22	**-10.34**	LSD=3,71
2.	Complex of high acetylated starch and oleic acid	49.43	54.67	**52.05**	49.43	54.67	**52.05**	
3.	Product of high acetylated starch transesterification with oleic acid obtained with enzyme A	73.44	77.57	**75.50**	-11.55	-11.99	**-11.77**	
4.	Product of high acetylated starch transesterification with oleic acid obtained with enzyme B	56.68	56.15	**56.41**	-8.93	-9.23	**-9.08**	
5.	Product of high acetylated starch transesterification with oleic acid obtained with enzyme N	41.32	41.98	**41.65**	59.96	66.09	**63.02**	
		LSD=2.34						

lubility of saccharides in hydrophobic solvents requires to conduct reaction in polar organic solvents, which are harmful to human health. Commonly the are dimethyl sulfoxide (DMSO), toluene, pyridine. In addition, the process has exothermic nature which results in formation of colored impurities [Kołakowski et al. 2005].

Esterification reactions can be successfully conducted by biotechnology means, using enzymes of microbial origin [Saxena et al. 2003, Adamczak et al. 2004]. Mainly lipases are used, which are obtained from the yeasts *Candida antarctica*, *Candida cylindracea*, *Candida rugosa*, molds *Rhizomucor miehei* and bacteria *Bacillus subtilis* [Kennedy et al. 2006]. They may be used in the esterification, transesterification and interesterification [Bornscheuer 1999] and their main task is to catalyze hydrolysis of fats into free fatty acids [Debreucq et al. 2000].

Above mentioned microbial lipases have been "tested" by Seino et al, in terms of usefulness for the synthesis of saccharides esters. A series of reactions was conducted in which: sucrose, glucose, fructose and sorbitol, as well as fatty acids: oleic, stearic and linoleic took part. The reactions were conducted in phosphate buffer medium. The highest activity of esters synthesis was shown by enzyme from *Candida cylindracea* [Seino et al. 1984].

Presented work, enzyme from *Candida antarctica* was used as a biocatalyst in esterification reaction of natural starch and transesterification reaction of high acetylated starch with

oleic acid, as well as A and B fractions of this enzyme. Ward et al, in their studies, have used eight different enzymes, as a catalysts of saccharide esters synthesis reactions with arachidonic acid, including those originating from Candida antarctica. As a result of experiments they concluded that the best results were obtained for lipase derived from *Candida antarctica* [Ward et al. 1996]. These observations were confirmed by Tsitsimpikou et al. [1997]. They used a lipase from *Candida antarctica*, as well as from *Candida rugosa* in the synthesis of saccharides esters. Acylation of arabinose, mannose, fructose and glucose with lauric acid in the environment of hexane led them to conclusion that *Candida antarctica* is characterized by higher activity of saccharides esters synthesis than *Candida rugosa* [Tsitsimpikou et al. 1997].

This paper describes the characteristics of: products obtained by transesterification and complexes formed from saccharide esters with oleic acid, as well as starting compounds. Total amount of five preparations were tested.

First qualitative analysis was performed using nuclear magnetic resonance. ^{1}HNMR spectra, additionally to confirmation that reaction of transesterification with oleic acid took place, were used to calculate the degree of substitution (DS). The degree of substitution of acetic acid was calculated as the ratio of the area of methyl protons from acid groups of ester – from 1.97 to 2.10 ppm to the surface of signals from protons of glucose ring in the starch chain in the range from 5.0 to 5.5 ppm. In case of substitutions with oleic acid, the signal from 0,80 to 0,87 ppm was sought, which belongs to the terminal methyl group of oleic acid (in some cases, this signal shifted towards higher values of – 1,09 ppm and contained within standard shift range) as well as signals of glucose proton ring in starch chain in the range from 5.0 to 5.5 ppm.

Degree of substitution for starch high acetylated with acetic acid was 2.52, while complex of high acetylated starch with oleic acid 2.38 (both for three and ten day reactions).

The values of the degree of substitution for products of high acetylated starch transesterification with oleic acid, obtained by ten day reaction synthesis were as follows: high acetylated starch complex with oleic acid (0.17 – oleic acid, 2.38 – acetic acid); products of high acetylated starch transesterification with oleic acid conducted by fractions A enzyme (0.58 – oleic acid, 0 – acetic acid); products of high acetylated starch transesterification with oleic acid conducted by fractions B enzyme (0.63 – oleic acid, 2.07 – acetic acid); and products of high acetylated starch transesterification with oleic acid conducted by fractions N enzyme (0.21 – oleic acid, 2.38 – acetic acid). Reaction carried out for three days resulted in significantly higher degrees of substitution: products of high acetylated starch transesterification with oleic acid conducted by fractions A enzyme (0.17 – oleic acid, 2.38 – acetic acid); products of high acetylated starch transesterification with oleic acid conducted by fractions B enzyme (1.24 – oleic acid, 1.54 – acetic acid); and products of high acetylated starch transesterification with oleic acid conducted by fractions N enzyme (1.19 – oleic acid, 1.62 – acetic acid). It is noteworthy, that the substitution degree, for both esterification and transesterification products, obtained in three day reaction is higher than the literature data. Kapuśniak et al, conducted thermal esterification reaction of potato starch with linoleic acid, and obtained the degree of substitution was ranging from 0.09 to 0.68 [Kapuśniak et al. 2007].

Rajan et al, in an article published in 2006, noted the degree of substitution for cassava and maize starch, obtained in various reaction conditions. The highest degree of substitution was obtained during the synthesis of starch with fatty acids derived from coconut oil with the participation of *Themomyces lanuginosa* lipase in a microwave environment (DS=1.55).

The lowest degree of substitution (DS=0.07) was achieved during 36 hour reaction of cassava starch with fatty acid. The publication of the 2008 reported, however, that it is possible to obtain esters of cassava starch with palmitic acid with lipase AYS and dimethylsulphoxide as the reaction medium. The resulting compound was characterized by a degree of substitution equal to 1.05 [Ryan et al. 2006, 2008].

Boruczkowska et al. [2008] conducting enzymatic esterification of starch and its derivatives with lauric acid, obtained the degree of substitution from 0.22 to 1.00. They pointed out that differences in the amount of attached fatty acid groups are linked to the type of output product and the solvent applied [Boruczkowska et al. 2008].

In this paper, ten day samples were different from three day, both in time and amount of enzyme used. Samples that were subject of prolonged enzymatic reaction proved to be "lower" substituted, than it was in the case of products obtained by the shorter reaction. As it is known, a natural byproduct of this reaction is water. Perhaps the molecular sieve, contained in the reaction mixture, was not able to absorb all of the formed water or it became to fragmentated. In consequence this phenomenon could shifted the balance toward deesterification which would explain lower degree of substitution of those samples. However, it can be conclude that in this case, the time was not a determining factor effecting the degree of substitution of obtained modificates. A significant impact was therefore the quantity of enzyme used in the course of the experiment. Samples with the greater enzyme addition carried out modification reaction in shorter time, giving an impressive degree of substitution of saccharides with oleic acid. The ability of rapid reaction is highly desired by industry, but it should be noted that the enzyme preparation for an industrial scale is still costly.

Many modifications made to the starch and its derivatives often cause changes in their physicochemical properties. Thermal analysis of natural starch, high acetylated starch and their derivatives, was performed with a differential scanning calorimeter (DSC). Although results of determinations carried out on DSC, published by different authors, not always coincide with each other, it was decided to determine: initial, end, extrapolated center of the peak and the peak phase change temperatures, as well as heat capacity of phase transitions of obtained preparations.

Heating of natural starch and its derivatives (ten and three day) to temperature of 250ºC in an anhydrous medium allowed to conclude that in each of the investigated temperatures show the same dependence. Determined values are lower than those achieved by the natural starch, and the three day samples are characterized by slightly higher temperatures than the derivatives synthesized in ten day process. When it comes to heating up to 250ºC high acetylated starch and its derivatives the situation look otherwise. Products of three and ten days reactions were characterized by lower temperatures than the high acetylated starch. Additionally, the esters obtained by shorter variant process, were characterized by lower temperatures than products of ten day synthesis. Specific heat capacity of phase transition for products of three day reaction (natural starch derivatives) was determined as positive values (endothermic reaction), while the specific heat capacity of natural starch derivatives from ten day process had negative values (exothermic reaction). This difference may be caused by degradation of previously formed ester bonds.

Specific heat capacity of phase transition for high acetylated starch (both ten and three day samples) are more difficult to interpret because of all modificates, only complex of high acetylated starch with oleic acid, as well as transesterification product of high acetylated starch with oleic acid obtainment by enzyme N proved to be endothermic reactions.

The literature reports, that in the case of rice starch, addition of oleic and linoleic acids did not significantly affect the gelatinization temperature, peak temperature of phase transition or the specific heat capacity of phase transition [Zhou et al. 2007]. More often, however, published reports are contradicting proposals of Zhou et al. [2007].

Rajan et al. [2006, 2008] conducted a series of experiments on preparation of the esterified cassava and maize starch. They claimed that the degree of substitution of starch with fatty acid has a significant influence on the temperature of phase transitions. Esterification with fatty acids, according to them, increases thermoplasticity of starch. Thermal decomposition of starch is related to the dehydration reaction in which the main product of decomposition is water. A higher degree of substitution means less free hydroxyl groups. Thus, this phenomenon is correlated with degree of substitution of starch. Subsequently change of mechanical and thermoplastical properties of starch occurs, so it becomes impossible to create bonds between its hydroxyl groups and water. This creates the possibility of starch utilization in products, where water absorption must be minimal [Ryan et al. 2006, 2008].

Methods of saccharides enzymatic esterification are subject of continuing research. Quite clearly new trends and directions of food ingredients modification are being promoted. Sooner or later, the biotechnology methods (focused on environmental protection) will supplant the use of chemical agents as catalysts. Basing on the results obtained in this study it was concluded that it is possible to obtain starch esters by synthesis with lipases utilization. The resulting products require more detailed studies, however, it can be already stated that they are characterized by completely different properties than starting materials

Conclussions

It is possible to conduct enzymatic transesterification of high acetylated starch with oleic acid by use of *Candida antarctica* derived lipase, as well as A and B fractions of this enzyme.

Enzymatic reaction of transesterification of high acetylated starch with oleic acid in presence of tert-butanol organic solvent, led to formation of three esters in case of both time variants.

Detention of high acetylated starch in mixture of oleic acid and solvent caused formation of complexes acetylated starch – fatty acid.

Synthesis conducted for three days with excess of enzyme led to formation of esters with higher degree of substitution, than in ten day reaction, carried out in the same conditions.

Temperatures of phase transitions of obtained starch esters were different from respective temperatures for input material, which proves obtainment of new products.

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9

THE EFFECT OF HYDROLYSED SILK PROTEIN ON SELECTED PHYSICAL PROPERTIES OF CHITOSAN FILMS

Introduction

White silk can be synthesized from the *Bombyx mori* and contains fibroin in 74.45%, sericine in 21.67%, minerals in 1.28% and colorful and waxy substances in 2.60%. Fibroin has a secondary structure of protein beta. The structure of the hydrogen bonds is formed between the peptide bonds of different polypeptide chains or different parts of the same polypeptide chain. Polypeptide chains neighboring in the beta structure affect the strength and stiffness of the structural proteins [Hames et al. 2006]. There has been a growing interest in soluble silk fibroin of biomedical industry recently. Silk may be successfully used as a biomaterial in: joint replacement, wound healing, bone plates and cement, dental implants, blood vessel prostheses, heart valves, skin repair devices, contact lenses [Nair et al. 2009]. Crystalline silk fibers are 4 times stronger than steel fibers, due to their regular structure induced by intermolecular hydrogen and hydrophobic bonds [Rabek 2008]. However, silk films may be very brittle, what can be improved by addition of other substances with good mechanical properties. This kind of polymer is chitosan

Four percent chitosan solution creates strong films with a thickness of 0.02 and 0.04 inches, having a tensile strength of 1.780 g and 2.970 g and puncture of 1.500 g and 2.035 g respectively. The use of chitosan coating on fresh mangoes extends their shelf life about 18 days, without changing the taste and the development of microorganisms [Srinivasa et al. 2002]. Biocomposites chitosan with pectin, characterized by reduced solubility compared to the individual polymers, whereas in combination with HPMC, chitosan forms a concise, flexible and resistant to stretching films [Fell et al. 2003]. Yin obtained transparent and brittle coating using chitosan/hydroxypropylmethylcellulose combination [Yin et al. 2006]. Chitosan coatings are characterized by moderate water permeability therefore they may be used to prevent drying of food, prolonging their freshness [Guilbert et al. 1996]. In addition, chitosan has the ability to reduce lipid oxidation and discoloration, while maintaining the sustainability of fresh foods with high water activity during storage at low temperatures. However, during frying breaded products, protective films prepared with chitosan may reduce the absorption of oil [Guilbert 2000].

The aim of this study was to determine the effect of hydrolyzed silk proteins on the variability of physical properties (puncture strength, elongation, elongation at destruction, water vapor permeability, WVP) of chitosan edible coatings.

Materials and methods

Hydrolysate of silk protein (Proteina), low molecular weight chitosan (Sigma-Aldrich), anhydrous glycerin 95.5% (P.P.H. Stanlab), acetic acid 80.0% (P.P.H. Stanlab), hydrochloric acid (P.P.H. Stanlab).

Production of chitosan films with hydrolysed silk protein

The experimental coatings were made by experimental design presented in Table 1. Low molecular weight chitosan in constant concentration of 1%, silk proteins in three different concentrations (0, 1.5, 3.0%) and glycerol at three levels (0, 15, 30%) were used for the coatings production. Chitosan was dissolved in 2% acetic acid at room temperature and with addition of plasticizer. The mixture was stirred for 12 h to complete dissolution of chitosan. The next step was pH reduction of silk proteins solution with 1 M HCl from pH 6.6 to pH=4.09. All components were mixed together as shown in table 1. Sols, before transferring on the glass plates, were degassed and then dried for 72 h in climatic chambers (Binder type KBF-LKC 240), with constant temperature 25°C, and relative humidity of 65%.

Table 1

Experimental design of chitosan films

Variants codes	Chitosan [%]	Silk protein [%]	Glycerol [%]
S0G0	1	0	0
S0G15			15
S0G30			30
S1,5G0		1.5	0
S1.5G15			15
S1.5G30			30
S3.0G0		3.0	0
S3.0G15			15
S3.0G30			30

Water vapor permeability (modified ASTM method, 2000)

Analyses were performed using glass measuring cups in the shape of a cylinder with dimensions of Ø=80 mm and L=60 mm. Tanks were filled with distilled water to 100 ml each, leaving a space of 40 mm between the water and upper cover. Then, the vessels were tightly closed with experimental films in the cover. Effective area of water vapor transfer was 2826 mm^2. The prepared samples were weighed and placed in a climatic chamber at a temperature of 25°C and a relative humidity of 60%. Weighing process was repeated every hour for 6 hours. Linear weight loss as a function of time was determined on the basis of the results, which was needed to calculate water vapor transmission rate – WVTR as follows:

Formula 1.

$$WVTR = \frac{(m_i - m_f)}{t \cdot A}$$

m_i – initial mass [g],
m_f – final mass [g],
t – time [s],
A – tested area [m^2].

On the basis of the above parameter, the water vapor permeability was calculated as follows:

Formula 2.

$$WVP = \left[\frac{(WVTR)}{s \cdot (R_1 - R_2)}\right]$$

S – saturation vapor pressure of water at the test temperature 25°C=3167.7394 Pa
R_1 – RH in glass cup [%],
R_2 – RH in climatic chamber [%],
d – film thickness [m].

Determination of mechanical properties of chitosan films

Determination of the tensile strength was performed using a Materials Testing Machines Zwick/Roell Z010. The speed of the head was set at 60 mm/min. Initial tensile strength was 0.07 N/mm. Rheological material was tested after 72 hours of storage in a climate chamber. Each sample film was cut in the shape of a rectangle measuring 6x2 cm (length x width) with 7 mm of constriction on both sides of the sample in its central part. Samples were made in 20 replications. The analysis of tensile strength of films was performed to obtain following rheological parameters: F_{max} [N/mm], F_{max} breaking [N/mm].

Determination of the resistance to puncture was performed using the same testing machine as in tensile strength measurement. The speed of the head was set at 30 mm/min. Mandrel diameter was 5 mm and was oval shaped, which pierced the film, spacing the support edges of the film 14 mm. Rheological material was tested after 72 h of drying in the chamber. Before performing measurements on each film, sample was cut in the shape of a rectangle 6x2 cm (length x width). Samples were also made in 20 replications. F_{max} [N] was fixed in puncture test of experimental films.

Statistical analysis

Statistical analysis was performed using Statistica 7.0. The analysis results are presented using the method of plane response. This method enables to describe the relationship between the analyzed factors which determining the coefficients based on a quadratic equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X^2_1 + \beta_{22} X^2_2 + \beta_{12} X_1 X_2$$

where:
Y – discriminant experiments; X_1, X_2 – factors levels [%]; β_0 β_1 β_2 β_{11} β_{22} β_{12} quadratic equation coefficients.

The rest of results were analyzed using Pareto test, where significance of difference was defined at $p \leq 0.05$.

Results and discussion

Statistical analysis showed a significant effect of glycerol, silk proteins and their interactive effects in linear correlation on the variability of the F_{max} of tensile strength (Tab. 2). Fitting model coefficient R^2 was 0.852 (Tab. 3). The addition of protein and glycerol presented negative effect on this parameter, but the interaction of both factors was positively significant (Tab. 2). It was showed that adding of glycerol from 0% to 15% results in reduction in the value of this ratio, while increasing the dose to 30% of plasticizer does not affect lowering tensile strength. The maximum value was observed for sample without any addition of glycerol with F_{max}=61.49 N and the minimum F_{max}=19.00 N was obtained for sample with 30% addition of plasticizer (Fig. 1). Casariego and others [2008] observed that an increase in the addition of a plasticizer in the production of chitosan coatings, causes an increase in elongation at break and reduces their strength. Elongation of coatings increases with the polarity of plasticizing substances and decreases with increasing molecular weight of plasticizer. Glycerol is a simple polyol compound with low molecular weight, 92.09 g/mol. It was found that with increasing participation of silk proteins, tensile strength significantly decreases. Experimental films produced on the basis of protein 3% characterized ten or twenty fold lower value of F_{max} compared to the coatings not containing this component. Minimum value of F_{max}=3.16 N for sample with maximum concentration of silk protein and glycerol was noted (Fig. 1). It was observed that the maximum value F_{max}=3.92 N for films with 3% of protein was achieved without glycerol dosage. Tensile strength of silk fibroin/chitosan films were tested by Kweon and others [2001]. Those researchers also obtained lower results of above mentioned analysis. Silk fibroin content was a reason of weaker strength in chitosan films, what may be caused by water presence in the sample and therefore its use in films production.

A significant effect of silk protein, glycerol and their interactive effects on the variability of breaking strength was proved by statistical analysis. Lack of glycerol influence in linear correlation on braking F_{max} was observed. Interaction of silk protein and glycerol and addition of glycerol in quadratic correlation affected breaking force negatively breaking force (Tab. 2). Fitting model coefficient R^2 was 0.778 (Tab. 3). It was observed that the parameter of damage F_{max} decreases significantly with increasing dose of glycerol, from 59.59 N/mm for 0% to 26.11 N/mm for 15% respectively (Fig. 2). Samples with 30% of glycerol were significantly different, with varying addition of silk protein from 17.91 N/mm to 1.87 N/mm. It was also found, that the films without plasticizer and proteins content reached the highest value of damage force. The lowest force was needed to destroy films with 3% of proteins, independent of glycerol concentration. Therefore it may be concluded that variable concentrations of silk proteins influence on breaking strength of films. It was found that F_{max} decreased significantly with increasing protein concentration in the experimental coatings. Similar effects of decreasing breaking strength of silk fibroin/chitosan films with increasing protein content were obtained earlier by other researcher [Basal et al. 2010].

Basing on statistical analysis a significant effect of silk protein, glycerol and their interactive effects on the changes of puncture F_{max} were found. Addition of silk protein to the

experimental films caused negative influence in linear and quadratic correlation on force of puncture (Tab. 2). Fitting model coefficient R^2 was 0.947 (Tab. 3). It was showed that resistance to puncture of experimental coatings decreases significantly with increasing concentration of silk proteins from 61.13 N to 4.76 for 0% and 3% of this substance respectively (Fig. 3). The biggest changes in value of puncture F_{max} were observed with rising concentrations of both factors. Minimum force amounting to 4.31 N for film contained 3% of protein and 30% of glycerol was noted. It was found that 1.5% of protein in the coatings results in more than five-fold reduction in the results of puncture test. It was noted that the maximum of force puncture was achieved for the experimental coatings with just chitosan (Fig. 3).

Table 2

Main effects and interaction of analyzed factors on mechanical parameters

Quadratic equation coefficients	Parameters		
	F_{max} of tensile strength [N/mm]	F_{max} of braking strength [N/mm]	F_{max} of puncture [N]
β_0 (constant/average)	55.798*	1.080	58.198*
β_1 (silk protein L)	-31.419*	5.501*	-40.928*
β_2 (glycerol L)	-1.569*	0.265*	-1.079*
β_{11} (silk protein Q)	4.350*	-0.040	7.661*
β_{22} (glycerol Q)	0.015*	-0.006*	0.016*
β_{12} (silk protein x glycerol)	0.460*	-0.040*	0.219*

* code identifying the significance of quadratic equation coefficient for $p \leq 0.05$

Table 3

Fitting model coefficients fixed for mechanical parameters

Fitting model coefficients	Parameters		
	F_{max} of tensile strength [N/mm]	F_{max} of breaking strength [N/mm]	F_{max} of puncture [N]
R^2	0.852	0.778	0.947
R	0.847	0.771	0.945

A significant effect of silk protein, glycerol and their interactive effects on the variability of WVP was proved by Pareto statistical analysis. Silk protein in quadrate correlation and interaction of both factors were negatively significant on water vapor permeability. It was noted that the maximum value of the WVP was obtained for film with 15% of glycerol and 3% of silk protein and amounted to 1.31E–11 g/m*Pa*s (Fig. 5). The lowest permeability of water was noted for chitosan film with any addition of components and was 8.49E–11 g/m*Pa*s (Fig. 5). It suggests that addition of silk protein and/or glycerol may improve a transfer water vapor, what is important in wound dressing production [Kweon et al. 2001]. It was also observed that the increase in the proportion of glycerol from 15% to 30% causes a slight decrease of permeability. Silk protein 3% addition to the experimental films significantly changed WVP, what was observed in lower barrier ability (Fig. 5). Increasing content of glycerol in chitosan coatings did not show a significant increase in value of vapor transfer, but impact of this parameter was found for samples with or without glycerol addition. It was

also proved by other scientists, that every plasticizer would help improve the elasticity of films, but at the same time would decrease their barrier properties to water vapor [Sothornvit et al. 2001, Sobral et al. 2005].

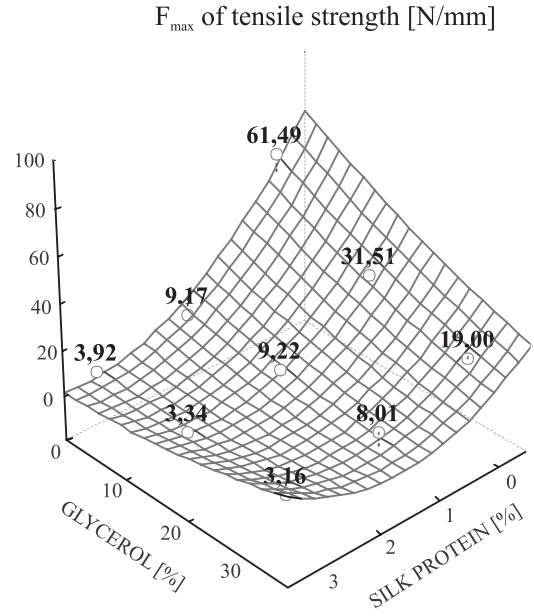

Fig. 1. Influence of silk protein and glycerol content on F_{max} of tensile strength

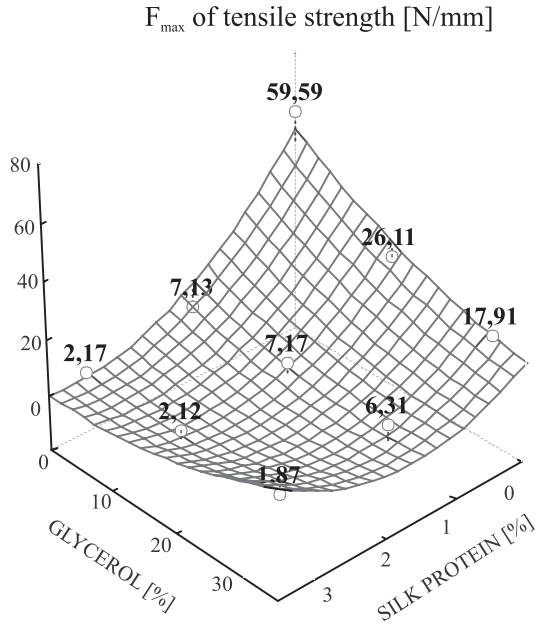

Fig. 2. Influence of silk protein and glycerol content on F_{max} of breaking strength

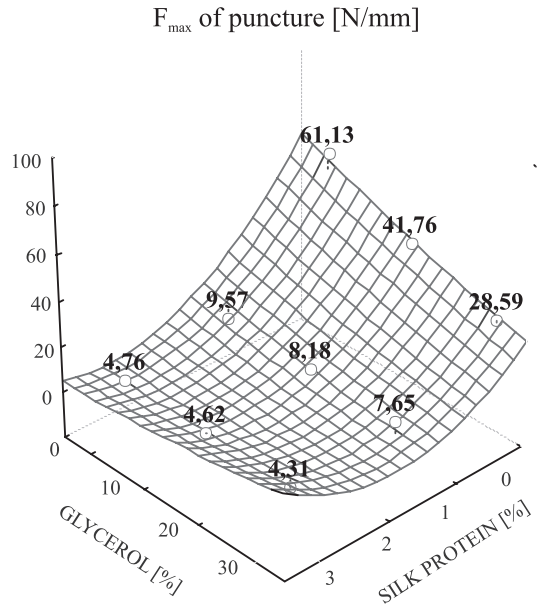

Fig. 3. Influence of silk protein and glycerol content on F_{max} of puncture

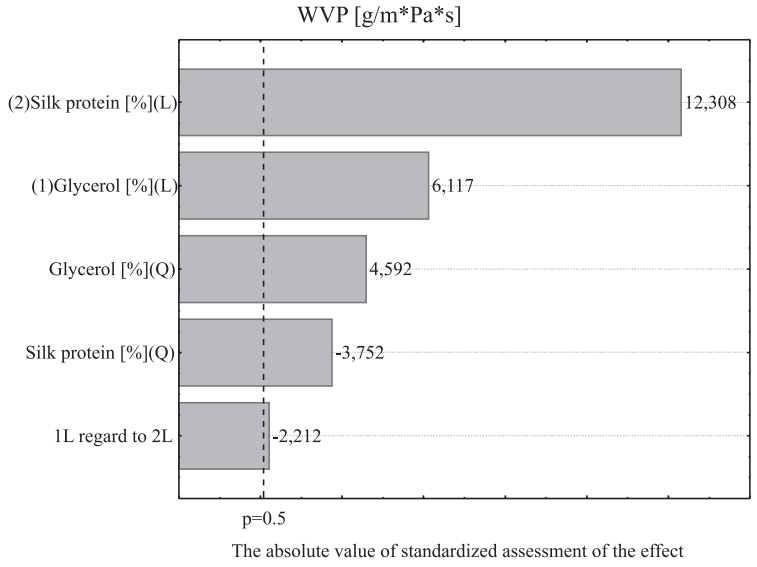

Fig. 4. Influence of silk protein and glycerol content on WVP

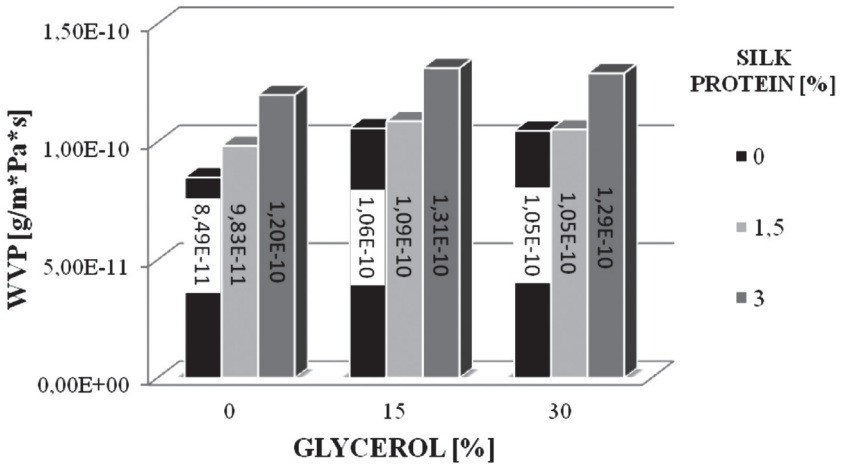

Fig. 5. WVP variations

Conclusions

It was showed that with increasing concentration of silk proteins in the composition of the experimental chitosan coatings their tensile strength and puncture are reduced.

It was observed that the increased addition of glycerol to the films composition caused in lowering their tensile strength and puncture.

The highest water vapor permeability of the experimental coatings for sample produced with a 3.0% of silk proteins and 15% of glycerol was obtained.

The biggest barrier to water vapor was achieved for chitosan films with no glycerol and silk proteins.

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10

THE EFFECT OF DEACETYLATION DEGREE ON THE PROPERTIES OF CHITOSAN

Introduction

Shells chitin of marine crustaceans such as crabs, lobsters, and shrimps are an example of maritime industrial waste which is hard to utilize. They are a rich source of protein and heteropolisaccharide – chitin, which is after cellulose the most abundant in nature biorenewable polymer. Chitin is a major structural component of the exoskeleton of invertebrates, insects, yeast and fungal cell walls [Tharanathan and Kittur 2003].

Chitosan is N-acetylated derivative of chitin, containing 2-amino-2-deoxy-β-D-glucose (GlcN) connected by bond β (1–4) with the molecules of N-acetylglucosamine (GlcNAc), which occasionally occur in the chain. The chemical structure of both chitin and chitosan molecule resembles cellulose, except the C-2, which have acetamide group and/or amino group respectively.

Chitosan is obtained by chemical or enzymatic deacetylation of chitin (Fig. 1). Strong acids and alkalis are used in chemical reactions of deacetylation. These substances are necessary to purify chitin shells of crustaceans from other ingredients.

Mineral salts such as $CaCO_3$ or $Ca_3(PO4)_2$ and the lipids are extracted with hot HCl solution, while the proteins are removed with hot NaOH solution. Discoloration process takes place in the presence of H_2O_2 or NaClO solution, followed by washing and drying. The process of NaOH washing is repeated in a suitable temperature with the aim to deesterify N-acetyl groups, and then rinsed and dried to obtain chitosan [Srinivasa and Tharanathan 2007]. The process of enzymatic deacetylation of chitin is used in order to obtain chitosan with lower molecular weight and water solubility. The reaction is often performed, because it alters the structure of the polymer and enables to control the process [Sakai et al. 1991]. The most frequent enzymes used for this purpose are found in fungi, bacteria or plants, namely chitinases and chitosanase.

The acetylation degree of chitosan has an impact on such physicochemical properties as molecular weight, viscosity and solubility. Many analytical techniques have been tested to determine this parameter. IR spectroscopy is considered to be the most appropriate and rapid method. Dependence of deacetylation degree (DD) with the acetylation degree (DA) of chitosan molecules is described by equation as follows:

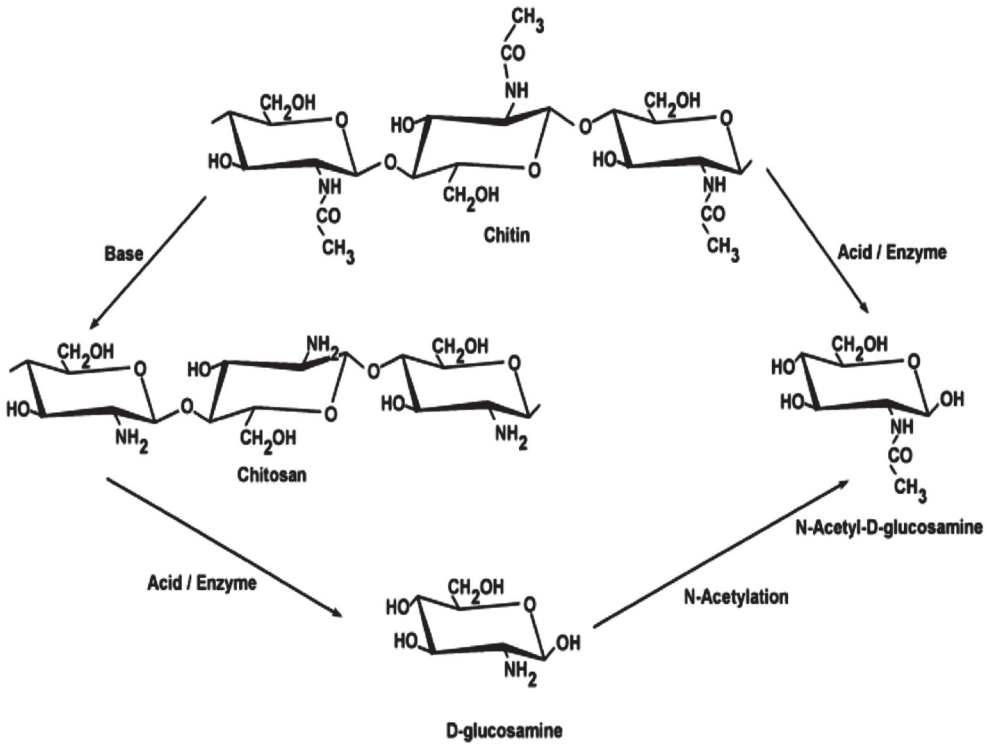

Fig. 1. Deacetylation of chitin to chitosan [Shahidi et al. 1999]

$$DD = 100 - DA$$

The value of the deacetylation degree assigns the boundary of qualitative transformation of chitin to chitosan. It is widely recognized that the chitin with 70% of deacetylation degree is chitosan [Li et al. 1997].

The aim of this study was to produce chitosan with different degrees of deacetylation and evaluate selected parameters of rheological and mechanical strength of properly prepared sols and protective films, assuming a constant level of plasticizer.

Materials and methods

Chitin (Sigma-Aldrich), sodium hydroxide (P.P.H. Stanlab), acetic acid 99.5% (Poch S.A.), lactic acid 85.0% (Sigma Aldrich), sodium acetate – granulate 95.5% (P.P.H. Stanlab), hydrochloric acid – 0.10 M (Hempur), anhydrous glycerin 95.5% (P.P.H. Stanlab).

Production process of chitosan with various deacetylation degrees

The experiment was carried out for two variants of time (1 and 11 hours) and three levels of repetition cycles of washing chitin with sodium hydroxide (1, 2, 3 – fold) (Tab. 1).

Table 1

The parameters of the chitin deacetylation process

Variants codes	Time [h]	Repetition cycles [multiplicity]	Temperature [°C]	Chitin weight [g]	NaOH concentration [%]
CH1x1	1	1	121	10	40
CH2x1		2			
CH3x1		3			
CH1x11	11	1			
CH2x11		2			
CH3x11		3			

Chitin flakes were treated with 40% NaOH in the ratio 1 to 30 and placed in an autoclave in 121°C for 1 or 11 hours in order to obtain deacetylated chitin. Cooling process of the samples followed directly in the autoclave and then experimental material was neutralized to pH≈7 using water. The cycles where repeated two or three times in autoclave with the same parameters. Neutralized chitosan was dissolved in 2% of acetic acid, the suspension was stirred with the speed of 300 rpm by 12 h. The next step was to precipitate chitosan by addition 0.1 M of NaOH to reach pH≈6.7. Magnetic stirrer (CAT M5) was used to distribute the sodium hydroxide solution in chitosan solution evenly. Precipitated chitosan was separated by centrifugation (Sigma-Aldrich, type 3K30) with the specified parameters: 14000 rpm of speed, 30 minutes duration and temperature 15°C. Then centrifuged chitosan was transferred to the glass Petri dishes and dried at 50°C for 72 h. Immediately after the drying process, the resulting product was weighed and the yield of the process was calculated.

Determining chitosan deacetylation degree

Experimentally produced chitosan samples were dissolved in 0.1 M HCl by magnetic stirrer (CAT M5) at speeds 300 rpm for 2 hours. To such prepared solution 10 ml of H_2O was added, and then titrated with 0.1 M NaOH, recording the pH every 5 ml. Titration curves with two inflexion points were based on the obtained data. The volume difference between these two points (Fig. 2) was used to calculate the deacetylation degree of chitosan from following formulas (1 and 2) [Tan et al. 1998]:

Formula 1.

$$\varphi = \frac{(N_A \cdot V_A) - (N_g \cdot V_g)}{1000}$$

where:
N_A – molarity of HCl,
V_A – volume of HCl,
N_B – molarity of NaOH,
V_B – volume of NaOH.

Formula 2.

$$DD = \frac{\varphi}{\left[\frac{W - 161\varphi}{204 + \varphi}\right]}$$

where:
W – weight of chitosan.

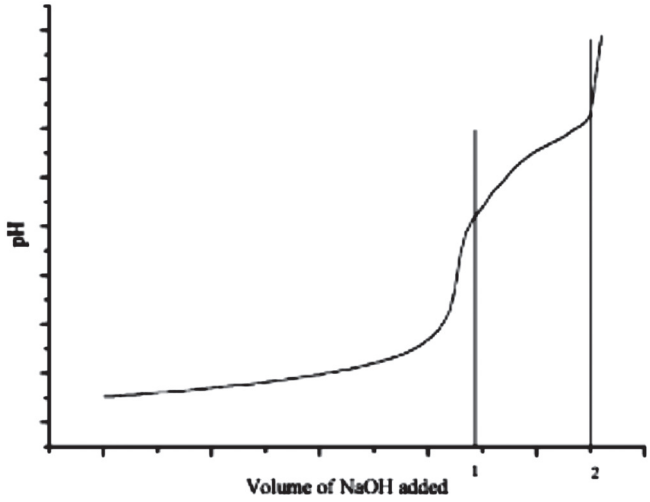

Fig. 2. Titration curve with two inflexion points [Jiang et al. 2003]

Determining chitosan molecular weight

To determine the molecular weight the viscosity method of dilute solutions was used. 0.2 g chitosan was dissolved in 100 ml of 0.3 M AcOH / 0.2 M AcONa buffer and stirred using a magnetic stirrer (CAT M5) at a speed of 300 rpm by 5h. Then the stock solution with a concentration of 0.2% was used for preparing working solutions with concentrations: 0.01%, 0.025%, 0.05%, 0.10%. The viscosity was measured for each sample, where the only reference was the buffer. Ubbelohde capillary viscometer with a diameter of 0.7 mm capillary and a constant K=0.063 ml /g was used to determine the viscosity. To the viscometer 20 cm^3 of the solution was poured, then liquid in the capillary was raised above the upper reservoir using a rubber pear. After that, the free movement time of substances between the upper and lower line of the capillary was measured. The analysis was performed in triplicate. Intrinsic (formula 3) and reduced (formula 4) viscosity was calculated for each solution and graph of the reduced viscosity (η_{sp}) versus polymer concentration (C) was drawn. The reduced viscosity was calculated by extrapolating the function to zero. Viscosity average molecular weight was calculated on the basis of the Mark-Houwink equation, where K=0.06521 mg/l, α=0.83 for chitosan-buffer system (formula 5).

Formula 3.

$$\eta_{ink} = \frac{[\ln (t_x - t_0)]}{C}$$

where:
η_{inh} – intrinsic viscosity,
t_x – time of outflow of stock solution,
t_0 – time of outflow of solvent,
C – concentration of solution.

Formula 4.

$$\frac{\eta_{sp}}{C} = \frac{t - t_0}{t_0 \cdot C}$$

where:
η_{sp} – reduced viscosity,
t_x – time of outflow of stock solution,
t_0 – time of outflow of solvent,
C – concentration of solution.

Formula 5.

$$\eta = K(M)^{\alpha}$$

where:
η – intrinsic viscosity,
K and α – are constant for solute-solvent system and temperature,
M – average molecular weight of polymer.

Measuring viscosity dynamic

Dynamic viscosity measurement was performed on rotational viscometer (Rheotest RN 4.2, Medingen GmbH). The sample containing 0.5% chitosan solution dissolved in 2% acetic acid was examined. Samples were subjected to constant shear rate test using coaxial cylinders. H1 internal cylinder, which is capable of measuring the viscosity in the range 10 to 105 mPa*s and shear rate from 0.20 to 2000 s-1, was used. Test parameters were as follows: time of 2 min. and speed of inner cylinder rotation 40 rpm.

Preparing experimental films

Films were prepared from six kinds of chitosans produced in the earlier stage of research. Each sample was dissolved in 2% acetic acid by stirring at the speed 300 rpm for 12 h. Then from thus prepared sample 25 ml was collected and 200 µl of glycerol was added. The final sample volume was 50 ml each time (after adding distilled water solution). The mixtures were made each time in triplicate. The obtained solution was poured on glass plates coated with Teflon (8x20 cm) and dried in a climatic chamber (Binder, type KBF-LKC 240). The conditions in the chamber where as follows: 25°C, humidity 60%, drying time of films was 24 h. The dried films were used to measure the tensile strength and puncture.

Tests for tensile strength and puncture

Measurements of resistance to puncture of experimental films and tensile strength were performed using Materials Testing Machines Zwick Roell Z010. The analysis of tensile

strength of films was performed to obtain following rheological parameters: F_{max} [N/mm], F_{max} breaking [N/mm]. F_{max} [N] was also fixed in puncture test of experimental films.

Statistical analysis

Statistical analysis was performed in program Statistica 6.0 using analysis of variance (ANOVA). Duncan's multiple-range test was used for the multiple means comparisons, where significance of difference was defined at $p \leq 0.05$.

Results and discussion

The highest yield of production process of chitosan was obtained after one hour of alkali treatment in one repetition and equaled 72.63%. Chitosan obtained after eleven hours of NaOH treatment presented 20% less yield than mentioned above (Fig. 3). Those results suggested that production of chitosan in shorter time gives higher yield of the process. Conversion of chitin to chitosan reduces the molecular weight, change of N-acetylation degree and charge [Rosca et al. 2005, No et al. 2006]. Average molecular weight of chitin is 1.03–2.5 x 10^6 Da, but after N-deacetylation process it is lowered to 1.0–5.0 x 10^5 Da [Ravikumar, 2000], what is in relation with our results. All chitosans, which were obtained in the experimental production process have the same viscosity – average molecular weight (M_v), and is 209.41 Da.

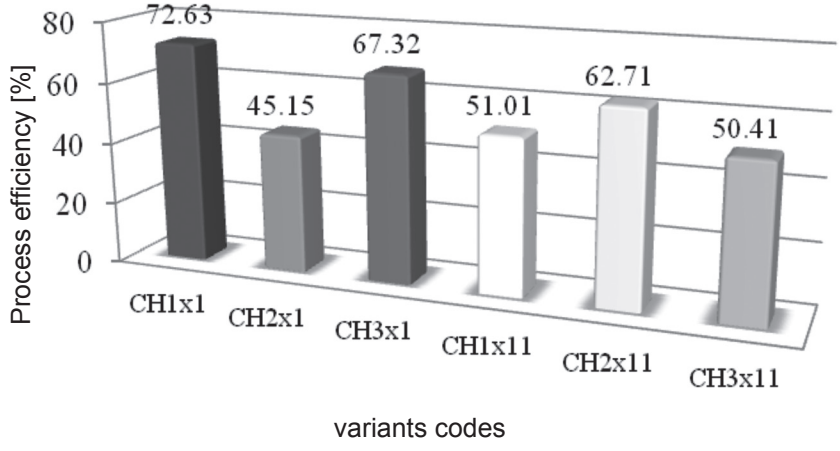

Fig. 3. Yield of production process of chitosan

Deacetylation degree was obtained on the basis of titration curve, presented on figure 4. Statistical analysis showed a significant effect of time and multiplicity of alkali treatment on the deacetylation degree variability of chitosans. DD value increased from 58.76% to 65.35% with increasing detention time of chitosan in sodium hydroxide (Tab. 2). It was also found that the effect of increasing the number of cycles on the DD value was changed from 67.02% to 53.70%. The highest DD was noted for chitosan treated with alkali just once (Tab. 2). Analyses of interactions of both factors on DD changes of chitosans, proved

the hypothesis that degree of deacetylation decreased with the increasing times of leaching cycles (Tab. 2). The highest DD of polymer was obtained after the single treatment of the sodium hydroxide solution for 1 h (72.59%) and the lowest after three times of washing for 1 h (41.72%). Aranaz and others [2009] confirmed that the maximum degree of deacetylation can be achieved by a single treatment of chitin. Efficiency of multistep deacetylation for 11 h remained at the same level as the one-step process, which DD values are in the range from 61.44 to 68.94%. The slowdown of the deacetylation process is related to the decrease of temperature in the autoclave, which after 11 h was 35°C. Kham and others [2002] proved that with decreasing temperature and the alkali concentration, degree of deacetylation of chitosan decreases.

Table 2

DD changes in experimental chitosans

Main effects				Interactions	
Time (T) [h]	DD [%]	Repetition cycles (RC) [multiplicity]	DD [%]	TxRC	DD [%]
1	58.76 [a]	1	67.02 [a]	1x1	72.59 [a]
				1x2	61.97 [b]
		2	65.45 [b]	1x3	41.72 [c]
11	65.35 [b]			11x1	61.44 [d]
		3	53.70 [c]	11x2	68.94 [e]
				11x3	65.69 [f]

a–f – means significantly different at $p \leq 0.05$ according to the ANOVA and Duncan's test

The dynamic viscosity of chitosans reduction in the value of this parameter along with repeatability of the process of alkali washing, and with long exposure of chitin in sodium hydroxide solution was investigated (Fig. 4). The highest viscosity (0.523 Pa*s) was obtained for chitosan with the highest degree of deacetylation. Wang and Xu [1994] showed that the dynamic viscosity increases with the increase in DD, which is associated with strong intermolecular interactions of chitosan. Significant reduction in the viscosity of investigated experimental systems was observed in sols obtained in three step process after 11 h of base treatment (0.091 Pa*s). Lower viscosity of chitosan solutions and higher degree of deacetylation are caused by time extension and temperature increases of chemical reaction of deacetylation [Mucha 2010].

A significant effect of time on tensile strength and the damage force of experimental films were observed. The longer the time of the deacetylation process of chitosan, the lower the values of above mentioned parameters. Decreases of tensile strength from 22 N/mm to 12.98 N/mm, and breaking force from 21.28 N/mm to 11.93 N/mm were noted (Tab. 3). No effect of increasing the cycle times of chitosan deacetylation on the maximum tensile strength and breaking films were observed. It was found that the value of the tensile strength of films obtained from chitosan (1 h) shows the highest value of the maximum tensile strength above 22 N/mm independent of cycle repetition (Tab. 3). This is confirmed by Nunthanid studies [2001), who received more brittle films with a lower DD chitosan due to the smaller amount of water absorbed by the polymer. The lowest value was recorded for samples CH1x11

(11.45 N/mm) (Tab. 3). Changes in maximum breaking force were caused by interactions of both analyzed factors, time of process and repetition cycles. Similar statistical effects of breaking force were obtained for chitosans produced after 1 and 11 h in three-step processes and amounted to 18.62 N and 14.39 N respectively. The other statistical groups contain samples CH1x1 and CH2x1 and the latter CH1x11 and CH2x11 (Tab. 3).

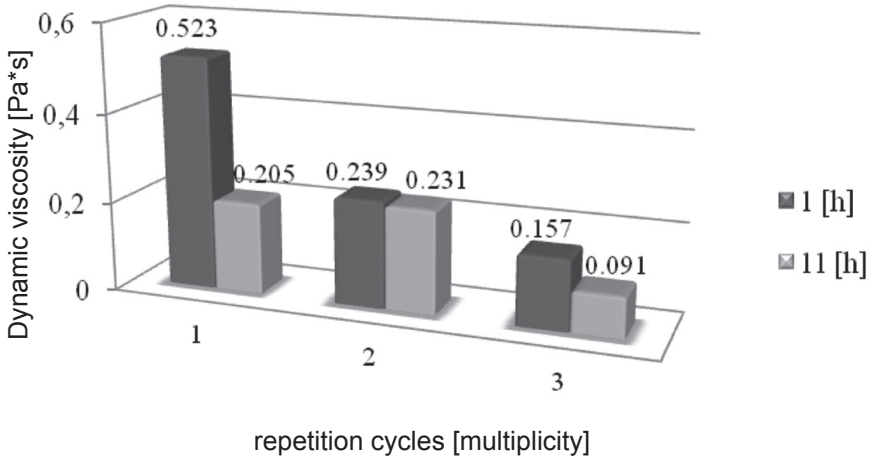

Figure 4. Dynamic viscosity of experimental chitosans

Lack of time influence on treatment was observed for puncture force of chitosan films (Tab. 4) together with statistical signification of repetition cycles in F_{max} values of puncture. The interaction values of F_{max} in puncture test for chitosan films samples vary from 6.69 N/mm to 13.38 N/mm. The highest value of maximum force of puncture was obtained for film with chitosan treated twice by alkali for 11 h, and the lowest in one-step process at the same time (Tab. 4).

Table 3

F_{max} of tensile strength and breaking changes in experimental chitosans

Main effects						Interactions		
Time (T) [h]	F_{max} of tensile strength [N/mm]	F_{max} of breaking [N/mm]	Repetition cycles (RC) [multiplicity]	F_{max} of tensile strength [N/mm]	F_{max} of breaking [N/mm]	TxRC	F_{max} of tensile strength [N/mm]	F_{max} of breaking [N/mm]
1	22.33 [a]	21.28 [a]	1	16.57 [a]	15.71 [a]	1x1	22.84 [a]	22.37 [a]
						1x2	23.66 [a]	23.09 [a]
			2	18.68 [a]	17.60 [a]	1x3	20.61 [a]	18.62 [a,b]
11	12.98 [b]	11.93 [b]				11x1	11.45 [b]	10.27 [c]
			3	17.87 [a]	16.66 [a]	11x2	13.14 [b]	11.50 [c]
						11x3	14.67 [b]	14.39 [b,c]

a–c – means significantly different at $p \leq 0.05$ according to the ANOVA and Duncan's test

Table 4

F_{max} of puncture test changes in experimental chitosans

Main effects				Interactions	
Time (T) [h]	F_{max} of puncture [N]	Repetition cycles (RC) [multiplicity]	F_{max} of puncture [N]	TxRC	F_{max} of puncture [N]
1	10.94 [a]	1	9.52 [a]	1x1	12.63 [a, d]
				1x2	9.54 [b]
		2	11.31 [b]	1x3	10.61 [b, d]
11	9.80 [a]			11x1	6.69 [c]
		3	10.39 [a, b]	11x2	13.38 [a]
				11x3	10.13 [b]

a–d – means significantly different at $p \leq 0.05$ according to the ANOVA and Duncan's test

Conclusions

The value of the deacetylation degree of chitosan decreases with increasing number of washing chitin with a sodium hydroxide solution.

The effectiveness of a multistage deacetylation of chitin for 11 hours remains at a similar level as the one-step process.

Viscosity value of experimental sols measured at a constant temperature and at constant shear rate decreases with a reduction in the deacetylation degree of chitosan molecules.

Mechanical strength of films on tensile and puncture decreases with increasing time and the multiplicity of the process of chitin deacetylation.

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11

THE EFFECTS OF THERMAL PROCESSING AND ADDITION OF ALGINATE ON FUNCTIONAL PROPERTIES OF RESTRUCTURED MEAT PRODUCTS

Introduction

Restructuring of meat products enables the use of less valuable meat components to produce palatable meat products at reduced cost. To achieve this goal, numerous nonmeat functional ingredients, mainly proteins and polysaccharides, have been applied as binders, fillers and extenders to improve their quality. These ingredients are primarily used for their water binding ability and/or texture-modification functionality [Unklesbay et al. 1998].

Conventional restructured meat production consists of meat processing wastes such as trimmings, cuts or small pieces of meat restructured to resemble fresh intact muscle cuts.

Meat to meat binding in restructured meat products may be achieved through the formation of gels that are set thermally (hot-set) or chemically (cold-set). Conventional restructured meat products depend on hot-set binding (thermal) of myofibrillar proteins that are extracted from meat with the combined effects of salt, phosphate and mechanical action. With this technology, the product must be sold either precooked or frozen because the product bind is not very high in the raw state [Boles and Shand 1998].

Cold-set binders would enable food processors to make more desirable and more acceptable products to be sold as raw chilled, and refrigerated using chemically induced gels instead of thermal set gels. One cold-set binder system widely used is an alginate system because of its capacity to form a gel at room temperature, from the reaction between alginate salt and a calcium source. The gel produced holds food pieces together and is thermally stable or thermo-irreversible; therefore, restructured products maintain their structural integrity through subsequent heating. With this system the quality of the gels, which in turn affects the binding strength, can be modified by controlling gel hydration, setting time and reaction rate by the use of acidulants and sequestrants [Suklim et al. 2004].

Alginate, a polysaccharide extracted from brown seaweed, can be used for binding comminuted or diced meat pieces. Sodium alginate is the form most often used in meat applications. Common ingredients in alginate binding systems are an alginate salt, a calcium source, an acidulant and a sequestrant. When calcium ions are introduced into an alginate solution, thermo-irreversible gels are formed. Acidulants and sequestrants can be used to modify the reaction rate, thus controlling the hydration rate and the gel setting time [Boles, Shand 1998].

The objective of this research was to assess the effect of thermal processing and addition alginate on the functional properties (dry matter, protein content, fat content, water holding capacity) and textural profiles (hardness, springiness, cohesiveness gumminess and chewiness) of meat products.

Materials and methods

The study was conducted on two types of meats: fresh meat (ham) and scalded meat (cooked ham). The pork meat was obtained from Meat Plant „Edward i Grzegorz Dworaccy" placed in Golejowo.

The ingredients used in the production of restructured meat product included: xanthan (0.5%, Amco Poland), guar gum (0.5%, Amco Poland), carboxymethylcellulose CRT 70 (0.25%, Wolff Cellulosics), microbial transglutaminase Active (0.3%) and calcium sulfate (1%), curing salt (1.6%, containing 99.5% sodium chloride and 0.5% sodium nitrite, ZHU Żuk-Pol, Wrocław), calcium sulfate (1%, POCH S.A.), polyphosphates Hamina-S (0.4%, ZHU Żuk-Pol, Wrocław), sodium isoascorbinate (0.15%, ZHU Żuk-Pol, Wrocław). Model meat products (Tab. 1) were prepared with addition of scalded meat at three levels (100, 50 and 0%) and alginate (Protanal RF 6650) also at tree levels (0.5, 0.75 and 1%). The ingredients were mixed for 30 s using BÜCHI „MIXER B-400" (9000 rev/min). Final products were stored in chilling conditions (4°C) for 24 hours – variant I. The variant II included the whole activities from the variant I, and next the samples were heated to a final internal temperature of 72°C and after that they were cooled to 14°C in an ice bath.

In restructured meat product the content of the following physicochemical properties were analyzed: dry matter (by thermal drying method in 105°C according to PN-ISO 662:2000), protein content according to Kjeldahl's method, using Kieltec^{TM} 2300; free fat content by Soxlet's method according to PN-ISO 1444:2000 Polish standard, water-holding capacity, textural profiles.

Table 1

Experimental design
Variant I – without thermal processing/Variant II – thermal processing

Variant I	raw meat/ scalded meat	Alginate [%]	Variant II	raw meat/ scalded meat	Alginate [%]
MS100A05	100/0	0.5	GMS100A05	100/0	0.5
MS100A075		0.75	GMS100A075		0.75
MS100A1		1.0	GMS100A1		1.0
MS50A05	50/50	0.5	GMS50A05	50/50	0.5
MS50A075		0.75	GMS50A075		0.75
MS50A1		1.0	GMS50A1		1.0
MS0A05	0/100	0.5	GMS0A05	0/100	0.5
MS0A075		0.75	GMS0A075		0.75
MS0A1		1.0	GMS0A1		1.0

The modified Grau-Hamm [1957] procedure was used to measure WHC (Water Holding Capacity) of the model meat products and was expressed as the ratio of moisture retained in the sample to the initial moisture content. Cooking loss was estimated by the equation: (weight before cooking minus weight after cooking /weight before cooking) x 100.

Textural characteristics of meat products were analysed according to the texture profile analysis (TPA) method using a Zwick/Roell type Z010 machine. The samples were compressed twice to 75% of their original height at a constant cross – head speed of 60 mm/min. The TPA parameters, namely hardness (peak force on first compression [N]), cohesiveness (ratio of the active work done under the second force – displacement curve to that done under the first compression curve [-]), springiness (distance the sample recovered after the first compression [mm]),gumminess (hardness x cohesiveness [N]), chewiness (hardness x cohesiveness x springiness [N x mm]) were computed [Pietrasik 2003].

The data were analyzed statistically, using STATISTICA v.6.0 software. Response surface methodology (RSM) was used to study the simultaneous effect of the experimental of thermal processing and addition alginate. Significant differences between the mean values were determined using Duncan's test (α=0,05).

Results and discussion

The results of alginate on physical properties of model meat products are presented in Table 2. The dry matter content of the meat products under investigation ranged from 17.20% to 21.53%. The highest content of dry matter and the lowest content of water, was observed in samples with addition of 100% scalded meat and 1% alginate (MS0A1). The measurements of water content in meat products showed that the highest content was observed in model meat products with addition of 100% raw meat (MS100A05). An application of alginate had influence on dry matter content.

The protein content depends on the scalded meat level. The meat products made with addition of scalded meat characterize with higher value of protein. Addition of alginate was unable improve their parameters.

The measurements of free fat content in meat products showed that the highest content was in samples with addition of 100% scalded meat. Addition of alginate to the meat products resulted in decrease in free fat level.

Table 2

Variant I. The effect of alginate level on physical properties of model meat products

Variable	Parameters			
	Dry matter [%]	Water [%]	Protein [%]	Free fat [%]
MS100A05	$17.20^{a}\pm0.16$	$82.80^{i}\pm0.16$	$12.04^{b}\pm0.02$	$1.44^{b}\pm0.02$
MS100A075	$17.67^{b}\pm0.51$	$82.33^{h}\pm0.51$	$12.39^{b,c}\pm0.35$	$1.19^{a}\pm0.14$
MS100A1	$18.20^{c}\pm0.04$	$81.80^{g}\pm0.04$	$12.87^{c,d}\pm0.76$	$0.97^{d}\pm0.18$
MS50A05	$18.74^{d}\pm0.06$	$81.26^{f}\pm0.06$	$13.50^{a,d}\pm0.01$	$1.61^{c}\pm0.34$
MS50A075	$18.98^{e}\pm0.05$	$81.02^{e}\pm0.05$	$13.72^{a}\pm0.23$	$1.47^{b,c}\pm0.04$
MS50A1	$19.44^{f}\pm0.12$	$80.56^{d}\pm0.12$	$13.94^{a}\pm0.01$	$1.23^{a}\pm0.10$

Table 2. continuous

MS0A05	19.98^{g}±0.32	80.02^{c}±0.32	14.58^{e}±0.06	2.66^{g}±0.09
MS0A075	21.11^{h}±0.35	78.89^{b}±0.35	14.86^{e}±0.00	2.29^{f}±0.09
MS0A1	21.53^{i}±0.04	78.47^{a}±0.04	15.03^{e}±0.08	2.12^{e}±0.12

a,b,c – mean values denoted by various letters and placed in the columns differ statistically significantly at $p \leq 0.05$

Addition of scalded meat and alginate caused were variables that influence the dry matter content (Tab. 3). The highest content of dry matter were found in samples without raw meat. The measurements of the protein content in restructured meat products showed that the highest content was in samples with addition of 100% scalded meat and 1% of alginate. Alginate level had no effect on these properties of the products.

The results obtained in the study showed that addition of scalded meat and alginate level was responsible for free fat content. The lower fat content in samples without scalded meat is probably related with releasing more amount of this component to meat juice during process of heat treatment.

The water holding capacity ranged from 53.57 to 59.05% (Tab. 4). The highest WHC (sample MS0A1) was observed in restructured meat products with addition of 100% scalded meat and with 1% alginate level and the lowest in sample MS50A05.

Water-holding capacity of meat products (ability to retain inherent water) is an important property of meat as it affects both the yield and the quality of the end product.

The water-binding capacity of meat products are mainly myofibrillar proteins, with the largest share of water-protein interaction is attributed to myosin and actomyosin. Loss of water through the model meat products is probably due to denaturation of the complex actomyosin that occurred already during the first heating. Denaturation is the change of protein structure during cooking which brings a decrease in diameter and thickness of the protein and so a less juicy and tougher cut [Joo et al. 1999, Westphalen et al. 2005]. In the variant II (Tab. 5), the analysis of the results showed that the highest water holding capacity was in restructured meat products without raw meat (GMS0A1). The lowest WHC was in model meat products with addition of 50% scalded meat and 0.5% alginate.

Table 3

Variant II. The effect of thermal processing and alginate level on physical properties of model meat products

Variable	Parameters			
	Dry matter [%]	Water [%]	Protein [%]	Free fat [%]
GMS100A05	17.51^{a}±0.22	82.49^{i}±0.22	12.72^{b}±0.33	1.71^{b,c}±0.29
GMS100A075	18.25^{b}±0.26	81.75^{h}±0.26	12.81^{b}±0.01	1.58^{a,b}±0.13
GMS100A1	18.42^{c}±0.28	81.58^{g}±0.28	12.81^{b}±0.01	1.54^{a}±0.29
GMS50A05	19.11^{d}±0.04	80.89^{f}±0.04	13.73^{a}±0.27	2.23^{d}±0.14
GMS50A075	19.74^{e}±0.03	80.26^{e}±0.03	13.89^{a}±0.07	1.96^{e} ±0.32
GMS50A1	20.46^{g}±0.01	79.54^{c}±0.01	14.00^{a}±0.01	1.74^{c} ±0.03
GMS0A05	20.33^{f}±0.15	79.67^{d}±0.15	14.69^{c}±0.00	2.75^{g} ±0.13

Table 3. continuous

GMS0A075	$20.89^{h}\pm0.05$	$79.11^{b}\pm0.05$	$14.92^{c}\pm0.01$	$2.49^{f}\pm0.08$
GMS0A1	$21.80^{i}\pm0.13$	$78.20^{a}\pm0.13$	$14.80^{c}\pm0.31$	$2.14^{d}\pm0.05$

a,b,c – mean values denoted by various letters and placed in the columns differ statistically significantly at $p\leq0.05$

In its ability to alginates to form gels with calcium ions it is possible to use alginates as an binder in the production of restructured meat products. Alginate gel/calcium provides relevant binding meat cuts, in both raw and cooked, and does not significantly affect the smell and taste the finished meat products.

Table 4

Variant I. The effect of alginate level on water holding capacity of model meat products

Variable	WHC [%]
MS100A05	$57.28^{a}\pm4.35$
MS100A075	$57.48^{a}\pm1.25$
MS100A1	$57.92^{g}\pm2.48$
MS50A05	$53.75^{c}\pm1.61$
MS50A075	$54.32^{d}\pm3.06$
MS50A1	$56.05^{f}\pm2.33$
MS0A05	$54.71^{e}\pm2.61$
MS0A075	$58.74^{b}\pm1.64$
MS0A1	$59.05^{b}\pm5.59$

a,b,c – mean values denoted by various letters and placed in the columns differ statistically significantly at $p\leq0.05$

Table 5

Variant II. The effect of thermal processing and alginate level on water holding capacity of model meat products

Variable	WHC [%]
GMS100A05	$44.76^{b}\pm0.25$
GMS100A075	$49.87^{e}\pm3.33$
GMS100A1	$51.11^{f}\pm2.07$
GMS50A05	$44.61^{a}\pm4.61$
GMS50A075	$46.68^{c}\pm3.45$
GMS50A1	$48.32^{d}\pm4.03$
GMS0A05	$51.47^{g}\pm2.14$
GMS0A075	$59.88^{h}\pm0.94$
GMS0A1	$69.49^{i}\pm2.08$

a,b,c – mean values denoted by various letters and placed in the columns differ statistically significantly at $p\leq0.05$

In the variant II (Tab. 4), the analysis of the results showed that the highest water holding capacity was in restructured meat products without raw meat (GMS0A1). The lowest WHC was in model meat products with addition of 50% scalded meat and 0.5% alginate.

In its ability to alginates to form gels with calcium ions it is possible to use alginates as an binder in the production of restructured meat products. Alginate gel/calcium provides relevant binding meat cuts, in both raw and cooked, and does not significantly affect the smell and taste the finished meat products.

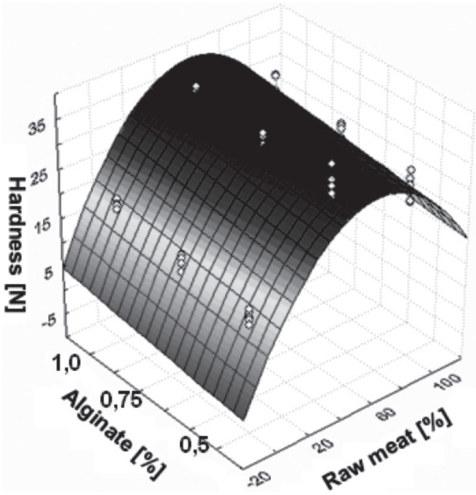

Fig. 1. Effect of addition alginate and raw meat on hardness meat products – variant I, R^2=0,939

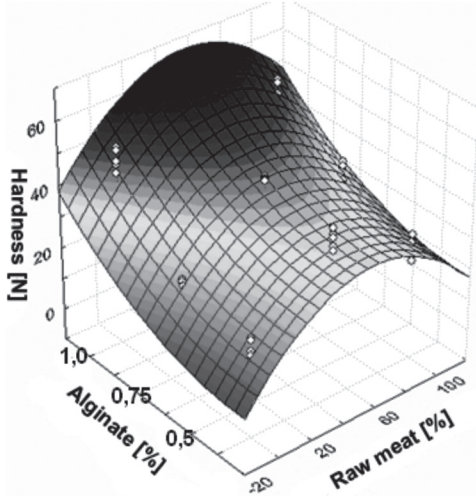

Fig. 2. Effect of addition alginate and raw meat on hardness meat products – variant II, R^2=0,851

Textural properties of model meat products depend on the type of meat (Fig. 1–10). Statistical analyze proved significant differences in value of textural properties for restructured meat products with addition of alginate. In variant I, the hardness values measured for model products were highest for samples with addition of 50% scalded meat. The addition alginate had not influence on hardness (Fig. 1). The temperature of thermal process, in variant II, had a strong impact on textural properties. In variant II, hardness values depend on the alginate level and on the thermal processing (Fig. 2). Addition of alginate to the restructured meat products resulted in a increasing in their hardness.

Higher temperatures cause changes in the constituents of meat, which affects its quality. One of these changes is the denaturation of proteins, in which proteins lose almost all functional properties [Ibanoglu 2005]. The products created with less involvement of proteins are characterized by a looser, less compact structure resulting in a lower hardness determined by testing of finished products.

In Figure 3 and 4 TPA test results for cohesiveness are shown. In variant I, the results showed that cohesiveness of the restructured meat products increased with an decreasing scalded meat level. The cohesiveness values were highest for sample with addition of 100% raw meat (Fig. 3). Impair of textural properties by the increased temperature was observed in variant II. The cohesiveness values were highest for sample with 1% alginate and 100% scalded meat (Fig. 4).

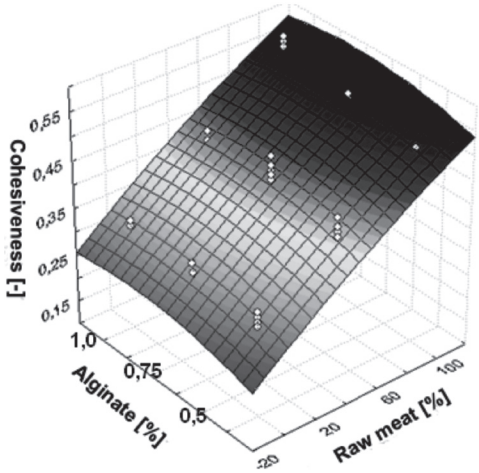

Fig. 3. Effect of addition alginate and raw meat on cohesiveness meat products – variant I, R^2=0,954

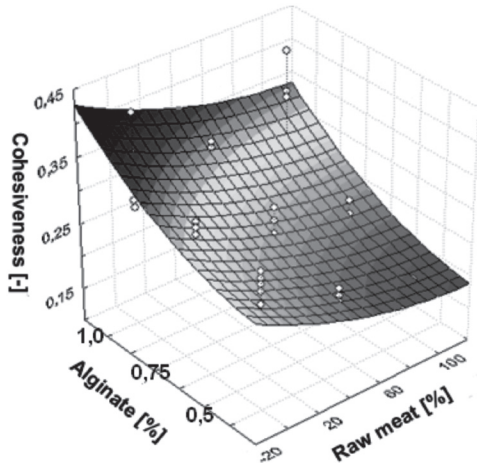

Fig. 4. Effect of addition alginate and raw meat on cohesiveness meat products – variant II, R^2=0,689

Figure 5 and 6 shows springiness parameters of TPA test of model meat products. The results obtained in the study show that the springiness depend on the thermal processing and alginate level. In variant I (Fig. 5), addition of scalded meat increases the springiness value. In variant II (Fig. 6), the temperature of thermal processing reduces the parameter examined.

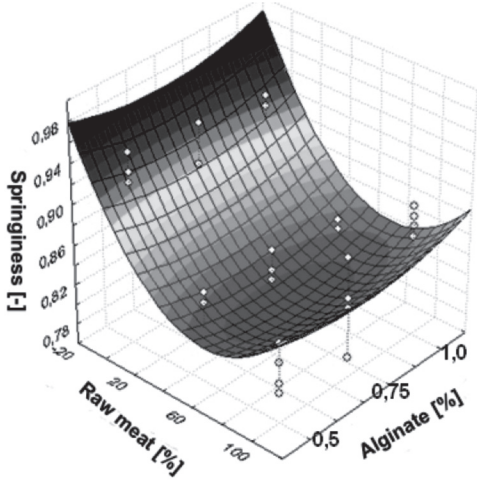

Fig. 5. Effect of addition alginate and raw meat on springiness meat products – variant I, R^2=0,657

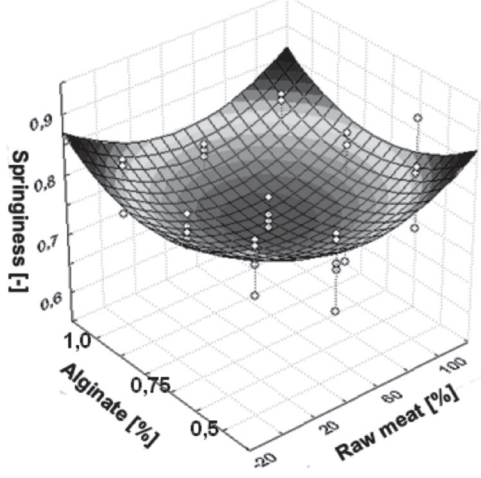

Fig. 6. Effect of addition alginate and raw meat on springiness meat products – variant II, R^2=0,216

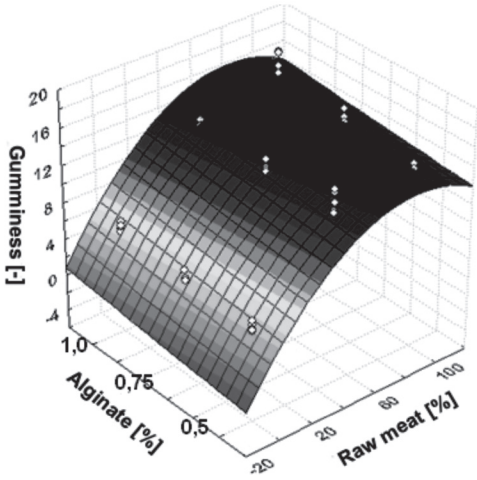

Fig. 7. Effect of addition alginate and raw meat on gumminess meat products – variant I, R^2=0,929

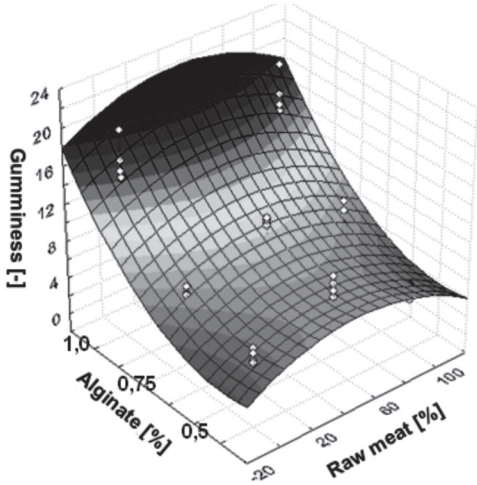

Fig. 8. Effect of addition alginate and raw meat on gumminess meat products – variant II, R^2=0,873

In variant I and II the behaviour of gumminess and chewiness was similar. In variant I, the gumminess (Fig. 7) values and chewiness (Fig. 9) values were highest for restructured meat products without scalded meat. The addition of alginate had not influence on parameters measured. In variant II, the addition of alginate increases the gumminess and chewiness values. Addition of scalded meat did not change the parameters of model meat products.

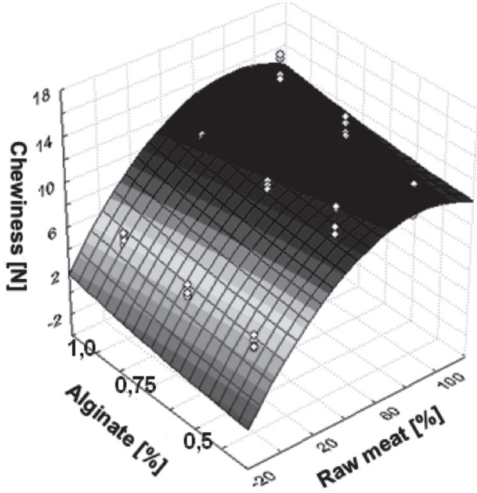

Fig. 9. Effect of addition alginate and raw meat on chewiness meat products – variant I, R^2=0,896

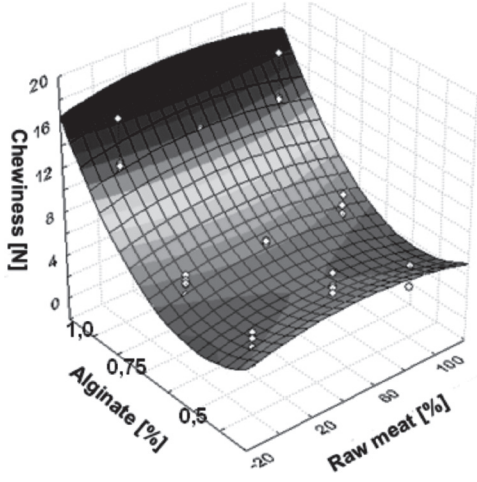

Fig. 10. Effect of addition alginate and raw meat on chewiness meat products–variant II, R^2=0,901

Conclusions

It was not observed significant difference between variant I and variant II for dry matter content, protein content and free fat content. On a dry matter content and fat had an effect both scalded meat and alginate. Additions of alginate had not influence on the protein content. In both variants additions of alginate and scalded meat had influence on water holding capacity in restructured meat products. The biggest difference was observed in profile texture measurements. In the variant I the addition of scalded meat had the biggest influence on gumminess and chewiness. In the variant II values of these parameters depended on the addition of the alginate.

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12

OXIDATION OF LIPIDS AND PIGMENTS, AND COLOUR MODIFICATIONS DURING REFRIGERATED STORAGE OF PORK LIVER PÂTÉS WITH DATE PALM BY-PRODUCTS

Introduction

Quality and acceptability of meat and meat products is mainly limited by lipid oxidation, what also affects free radicals production, flavour, texture, colour and nutritional deterioration. Lipid oxidation is related with complex reactions where polyunsaturated fatty acids are degraded via formation of free radicals [Gardner 1989].Thus, lipid oxidation is favoured during handling, processing and storage [Morrissey, Sheehy et al. 1998]. For instance, lipases are released when animal tissues are minced and macerated; thus, free fatty acids are also released, which accelerate the development of rancidity [Soyer and Ertas 2007]. Moreover, in meat products, the ingredients may act as antioxidants, but also as pro-oxidants, according to their chemical properties, the environmental conditions, and their interactions with the lipids. Also, oxidation initiators, as heat, light, high energy radiation, metal ion or metalloproteins, such as hemoproteins, and certain enzymes are very important in food systems [Coupland and McClements 1996, Greene and Price 1975]. In animal foodstuffs, among the different oxidation promoters, iron showed an important pro-oxidant activity [Love and Pearson, 1974]. Non-heme iron is considered the most important oxidation promoter in meat systems [Kanner et al. 1988], increasing when heme iron decrease, due to the breakdown of the heme molecule during cooking and storage [Miller et al. 1994]. These reactions are also associated with the destruction of the iron-porphyrin complex of myoglobin [Schricker and Miller 1983].

Colour changes in cooked products during refrigerated storage have been also related to oxidation processes, and they are influenced by several factors such as the characteristics and amount of fat, the packaging method and the presence of antioxidants [Jo et al. 1999]. Colour is very important for the meat industry, since consumers' decision when purchasing is highly influenced by colour acceptability. Additionally, meat and meat products appearance is highly affected by colour. Therefore, consumers use colour as an indicator of quality, composition and freshness [Pérez-Álvarez 1996]. Thereby, colour analysis should be considered in the development of new products to evaluate the effect of the addition of the novel ingredients or technological processes [Fernández-López et al. 2004; Martín-Sánchez A.M., Sayas-Barberá et al. 2009].

As a result, liver pâtés, with high amounts of fat and iron, are expected to suffer some oxidative deterioration, including heme pigments, which are oxidized, affecting also the product colour [Estévez and Cava 2004]. Furthermore, pâtés are usually marketed by bulk, in 1 kg containers, so this favours reactions between food components and atmospheric oxygen (oxidative rancidity), what leads to alterations, principally in fats and meat pigments, reducing the product quality [Perlo et al. 1995]. Thereby, the presence of antioxidants will influence on their susceptibility to oxidative processes during refrigerated storage, being able to retard lipid oxidation. Therefore, the addition of new ingredients should be accompanied by assessments about their effects on the product and the susceptibility of lipids to oxidation, developing methods to prevent or retard lipid oxidation in foods. Antioxidants can be used to retard oxidation, and according to the consumers' demand towards more natural and healthier ingredients, natural antioxidants are preferred. Thus, this work study the possibility of using a date palm by-product rich in antioxidant compounds [Biglari et al. 2008] as an intermediate food product (IFP) [Linden and Lorient 1999]. In addition, there is an increasing demand in developed countries towards sustainable food production [Weber and Matthews 2008]. Thus,]he incorporation of functional ingredients from by-products from agro-food industries could be a strategy to develop healthier meat products, but also to increase the eco-efficiency in the food industry [Martín-Sánchez et al. 2009].

Date palm (*Phoenix dactylifera* L.), is widely cultivated in some Mediterranean regions. In Europe, the main date palm cultivars are concentrated in the south east of Spain (Elche and Orihuela) due to the arid and semi-arid climatic conditions. In the last years, the average production in the main grove (Elche) is around 4.000 metric tons, of which less than 100 tons are commercialized as fresh date [Vilella-Esplá 2008]. Date harvesting is habitually accompanied by fruit losses during picking, selection, storage and conditioning processes [Besbes et al. 2009]. Moreover, fresh dates are harvested during a short period of time, and generally in different ripening states within the same cluster. For these main reasons, together with the high perishability of fresh dates due to its high moisture and sugar content, the amount of "second and low-grade dates" in Spain reach very high percentages of the production. Generally, many tons of these by-products are discarded or used for animal feeding [Yousif et al. 1996]. However, dates are rich in compounds potentially beneficial for human health, such as fibre, vitamins, minerals and antioxidants [Al-Farsi and Lee 2008].

Therefore, the aim of this work was to evaluate the oxidation of lipids and pigments, and colour modifications produced by the addition of several concentrations of a novel IFP from date palm by-products to a pork liver pâté during refrigerated storage.

Materials and Methods

Samples Preparation

Campagne type pork liver pâtés were elaborated according to the traditional formula: 75% dewlap, 25% pork liver; and the rest of ingredients related to meat: 15% water, 1.8% salt, 1.5% caseinate, 8% egg, 0.2% white pepper, 0.03% thyme, 0.03% garlic powder, 0.03% nutmeg, 0.05% sodium ascorbate and 125 $mg \cdot kg^{-1}$sodium nitrite. In addition, three more batches were prepared adding 5, 10 and 15% of a novel IFP: a scalded date palm by-products paste from the variety Confitera, khalal stage (inmature). The dewlap was scalded at 100°C

for 10 min in a hot water bath. The liver after soaking and the dewlap were minced in a grinder IPS (Mainca, Barcelona, Spain) through a 10 mm grinder plate. After comminution, the other ingredients and additives were added and mixed for 5 min. The mixture was packed in steel containers and cooked in a heat oven A23 (Alphatech, London, England) until 72°C were reached in the geometric centre of each container. After cooking samples were cooled until reach the room temperature and then stored at 1–4°C.

The packs were stored for 5 days in the steel containers covered with an aluminium lid, similar to the marketed by bulk. Samples from each batch were taken at day 0,2 and 4 to analyse pH, moisture, water activity, CIELAB parameters, lipid oxidation (TBA test), metmyoglobin, heme iron, total iron, non heme iron and sodium chloride content.

Physicochemical Analysis

The CIELAB colour space (L*: lightness; a*: redness/greenness; b*: yellowness/ blueness) was studied following the recommendations of the AMSA [Hunt M.C., Acton J.C. et al. 1991) by means of a Minolta CM-2600 (Minolta Camera Co., Osaka, Japan) spectrophotometer with illuminant D_{65} and 10°C observer. The pH was determined directly with a Crison 507 pH-meter (Crison Instruments S.A., Barcelona, Spain) equipped with a combined electrode for solid samples (Cat. No. 52, Crison Instruments S.A., Barcelona, Spain), inserting the electrode in five different parts of the pâtés. Moisture was determined by loss in weight after heating the pâtés to constant weight at 105ºC.Water activity (aw) was measured at 25°C in an electrolytic hygrometer (Novasina TH-500, Axair Ltd., Pfaeffikon, Switzeland). Sodium chloride was determined (g/100g sample) according to the standard ISO/DIS 1841.27 [1981].

Pigment and Lipid Oxidation

Lipid oxidation was assessed in triplicate by the 2-thiobarbituric acid (TBA) method of Botsoglou et al. [1994], and expressed as mg malonaldehyde (MA)·kg^{-1} sample. Total iron concentration was determined in the ash of the samples using the ferrozine assay [Stookey 1970]. Heme iron was analysed using the method of Hornsey [1956] with acid acetone extraction; and non heme iron was calculated as the difference between the total iron and the heme iron. The percentage of metmyoglobin (MMb) was obtained following the method of Ulu [2004].

Statistical Analyses

Statistical analyses were carried out using the statistical package SPSS 19.0 (IBM SPSS Statistics, Chicago. Data from the samples was analysed by Analysis of Variance (ANOVA) with two factors: time and treatments. The Tukey post hoc test ($P<0.05$) was used for comparison of means.

Results and Discussion

Physicochemical Analyses

All values are shown in Table 1. For the pH values, statistically were found significant differences (SD) ($P<0.05$) among the batches, decreasing according to the date paste con-

Table 1

Parameters analysed of the four sample batches with SD and of the date paste (mean values±standard error).
^{A,B,C} Values with different letters in the same column are significantly different (P<0.05).
^{a,b,c} Values with different letters in the same row are significant different (P<0.05). No letter means no significant differences

		Time (days)	CONTROL	5% DATE PASTE	10% DATE PASTE	**15% DATE PASTE**	**DATE PASTE**
Physico-chemical parameters	pH	0	^{A}6.34±0.00^{c}	^{A}6.32±0.00^{bc}	^{A}6.30±0.00^{ab}	^{A}6.28±0.00^{a}	5.93±0.07
		2	^{B}6.36±0.00^{c}	^{B}6.34±0.00^{c}	^{B}6.32±0.00^{b}	^{A}6.29±0.00^{a}	
		4	^{AB}6.35±0.00^{b}	^{A}6.31±0.00^{a}	^{B}6.32±0.00^{a}	^{A}6.31±0.00^{a}	
	aw	0	^{A}0.956±0.00^{b}	^{A}0.949±0.00^{a}	^{A}0.950±0.00^{a}	^{A}0.949±0.00^{a}	0.941±0.03
		2	^{A}0.953±0.00^{a}	^{A}0.950±0.00^{a}	^{A}0.947±0.00^{a}	^{A}0.948±0.00^{a}	
		4	^{A}0.952±0.00^{b}	^{B}0.952±0.00^{b}	^{A}0.949±0.00^{ab}	^{A}0.947±0.00^{a}	
	Moisture (%)	0	44.67±1.16^{a}	48.62±1.29^{a}	49.38±1.00^{a}	47.68±1.69^{a}	66.4±0.54
		2	47.83±0.73^{a}	48.92±0.21^{a}	49.80±0.26^{a}	49.29±0.32^{a}	
		4	47.99±0.72^{a}	49.66±0.11^{ab}	51.33±0.54^{b}	50.07±0.41^{ab}	
	NaCl (%)	0	0.90±0.00^{a}	0.87±0.00^{c}	0.84±0.00^{b}	0.80±0.00^{a}	
Colour parameters	L* Lightness	0	^{A}61.96±0.55^{b}	^{A}59.52±0.69^{a}	^{B}60.42±0.43^{ab}	^{A}59.42±0.41^{a}	65.75±0.33
		2	^{B}64.18±0.42^{c}	^{A}61.42±0.42^{b}	^{A}58.62±0.57^{a}	^{A}59.60±0.36^{a}	
		4	^{AB}62.34±0.58^{b}	^{A}61.62±0.70^{b}	^{A}57.83±0.45^{a}	^{A}58.10±0.64^{a}	
	a* Redness	0	^{A}2.93±0.13^{ab}	^{B}2.19±0.15^{a}	^{B}2.89±0.18^{ab}	^{B}3.24±0.32^{b}	3.51±0.03
		2	^{A}2.90±0.13^{b}	^{A}1.34±0.12^{a}	^{A}1.27±0.05^{a}	^{A}1.63±0.26^{a}	
		4	^{A}2.52±0.15^{b}	^{A}1.27±0.13^{a}	^{A}1.29±0.18^{a}	^{A}1.22±0.21^{a}	
	b* Yellowness	0	^{A}15.82±0.13^{b}	^{B}14.05±0.33^{a}	^{B}14.75±0.39^{ab}	^{C}14.29±0.53^{a}	28.64±0.23
		2	^{A}15.29±0.22^{b}	^{A}12.18±0.30^{a}	^{A}12.16±0.20^{a}	^{B}12.64±0.22^{a}	
		4	^{A}15.31±0.18^{c}	^{B}13.65±0.48^{b}	^{A}12.01±0.49^{ab}	^{A}10.69±0.46^{a}	
Pigment oxidation	MMb (%)	0	^{A}32.78±1.35^{a}	^{A}38.63±1.19^{b}	^{A}35.84±0.19^{ab}	^{A}38.61±1.21^{b}	
		2	^{A}37.10±0.82^{a}	^{A}39.26±0.10^{a}	^{B}39.95±0.80^{a}	^{A}39.41±0.73^{a}	
		4	^{B}42.70±1.33^{a}	^{A}42.56±1.21^{a}	^{C}42.88±0.10^{a}	^{A}41.63±0.62^{a}	
	Total iron (mg·kg^{-1})	0	26.2±0.54^{a}	25.74±0.52^{a}	25.18±0.34^{a}	24.41±0.36^{a}	
	Heme iron (mg·kg^{-1})	0	3.45±0.14^{b}	3.00±0.21^{ab}	2.85±0.00^{ab}	2.57±0.13^{a}	
		2	3.19±0.24^{a}	2.84±0.00^{a}	2.79±0.31^{a}	2.54±0.02^{a}	
		4	3.00±0.15^{b}	2.93±0.00^{ab}	2.89±0.00^{ab}	2.61±0.05^{a}	
	Non heme iron (mg·kg^{-1})	0	22.73±0.14^{b}	22.31±0.21^{ab}	22.22±0.00^{ab}	21.66±0.13^{a}	
		2	22.99±0.24^{b}	22.47±0.00^{ab}	22.28±0.31^{ab}	21.69±0.02^{a}	
		4	23.18±0.15^{c}	22.39±0.00^{b}	22.18±0.00^{b}	21.65±0.06^{a}	

Table 1. continuous

Pigment oxidation	Heme iron (%)	0	13.20±0.55	11.85±0.84	11.38±0.03	10.62±0.55	
		2	12.20±0.93	11.21±0.00	11.13±1.25	10.48±0.09	
		4	11.48±0.59	11.56±0.00	11.51±0.00	10.77±0.24	
	Non heme iron (%)	0	86.79±0.55	88.14±0.84	88.62± 0.03	89.37±0.55	
		2	87.79±0.93	88.78±0.00	88.86±1.25	89.51±0.09	
		4	88.51±0.59	88.43±0.00	88.48±0.00	89.22±0.24	
Lipid oxidation	TBARs (mg MA·kg^{-1})	0	^{A}0.59±0.11^{a}	^{A}0.58±0.11^{a}	^{A}0.47±0.00^{a}	^{A}0.47±0.00^{a}	
		2	^{B}1.86±0.00^{c}	^{B}1.45±0.17^{b}	^{A}0.47±0.00^{a}	^{AB}0.48±0.00^{a}	
		4	^{B}1.83±0.00^{c}	^{B}1.29±0.25^{b}	^{A}0.49±0.00^{a}	^{B}0.48±0.00^{a}	

centration, but the differences were very low. All values, in general, were close to pH values of 6.3, similar to those values of Estévez et. al. [2005]. No important differences occurred during storage time. Therefore, pH decrease was directly related with date paste content. This could be due to the pH of the date paste (5.92), lower than the pH of the control pâté (6.35). However, date addition did not compromise the pH of pâtés, since they were higher than 5.6–5.8, what does not affect water holding capacity neither the formation of curing colour [Feiner 2006].

In general, aw was higher in the control than in the pâtés with the paste, ($P<0.05$). Conversely, moisture was minor in the control, being significant different ($P<0.05$) in general. No SD ($P>0.05$) appeared through the storage time, but in all batches the tendency was to increase. These differences in moisture and water activity could be due to the fibre and sugar content of dates, since they show high water holding capacity [Sánchez-Zapata et al. 2011].

For colour parameters, nearly all of them were reduced by the date paste content, except a* with 15% date on day 0, though $P>0.05$; however, the colour parameters of the date paste were higher. The differences among batches were greater on days 2 and 4. With regard to the evolution during storage time,control values were more stablethan for the other batches, no finding SD ($P>0.05$) for a* and b*; and pâtés with 5% and 15% did not show SD ($P>0.05$) for L* values. It is also important to highlight that samples with date had more similar evolution, except for L*, where the 5% followed the control tendency. On this basis it may be inferred that any of this three concentrations of date darkened the product, even when a higher moisture is related to higher L* values, same as occurred when added to bologna sausages [Sánchez-Zapata et. al. 2011]. Visually, also was appreciated a more greyish hue according to the date concentration, what was more accentuated a few minutes after slicing the pâtés. Therefore, in contact with the oxygen, pâtés with a high date paste concentration were darker, although a brown-grey colour is preferred for cooked products [Cornforth 1994]. Tarladgis [1962] asserted that the compound responsible of the brownish grey colour of cooked meats is a ferric-porphyrin coordination complex of the denatured globin molecule; and also modifications of this structure,the degradation of heme molecule and release of iron, might affect the colour displayed by the pâtés. However, as the results showed below no big differences were found for heme iron and metmyoglobin content, and samples with dates underwent a lesser oxidation. Therefore, the darkening in the pâtés may be due to polyphenol oxidase activity present in dates that still remain active after being scalded, since after slicing, with the contact with oxygen, a colour change was observed in samples with 10 and 15% of paste.

Moreover, except for day 0, the red coordinate was reduced more than 2 units; therefore, dates decreased the redness of the pâtés, independently of the concentration. The loss of redness may be due also to the formation of the MMb as a consequence of myoglobin oxidation. And comparing with other pork liver pâtés, the average value of a* (≈2 units) was lower than for Estévez and Cava [2004], close to 8 units; but the other two coordinates, L* and b*, presented very similar values. The less red product could be due to the higher content of dewlap. The yellow coordinatedecreased on day 4 ($P<0.05$) according to the date concentration, giving a less yellowish product.

NaCl content was significantly different ($P<0.05$) for each batch, being reduced from 0.92 g/100g in the control to 0.80 g/100 g with 15% of paste. Therefore, the addition of date decreased the salt content; approximately each 5% reduced in 0.03% the salt content. The less proportion of the other ingredients when the date paste was added made decrease the salt content.

Pigment and lipid oxidation

Metmyoglobin (MMb)

MMb content also showed differences ($P<0.05$) during storage time and due to the addition of dates, with a tendency to increase through the time and with the addition of date. No SD ($P>0.05$) were found among batches on day 2 and 4, with values close to 40% of MMb. Only SD ($P<0.05$) were shown on day 0; the control and 10% pâté presented more similar and lower values, while the 5% and 15% pâtés presented nearly the same values and tendency. Therefore, MMb was increased by the addition of dates on day 0, favouring the myoglobin oxidation, what also could explain the variations of the redness. However, on day 4 all they showed the same value (≈42%); thereby MMb was stabilized by the date paste.

Heme and Non Heme Iron

Heme iron content in $mg \cdot kg^{-1}$ showed SD ($P<0.05$) due to date content,being 0.15–0.45 $mg \cdot kg^{-1}$ lower for each 5% of date added; but not ($P>0.05$) due to storage time in samples with date. Therefore, the addition of the paste made the heme iron more stable, but lower, during storage time. Total iron decreased due to the substitution of liver by date, among 50-70 $mg \cdot kg^{-1}$ for each 5% added, but not SD ($P>0.05$) were found among batches. Thus, the differences in heme iron content would be due to the date addition. Only a 15% date reduced significantly its content, besides the less liver content, interactions of iron with the antioxidant compounds of dates might be the responsible.

Therefore, it may be concluded that only the addition of high amounts of date produced SD in the heme iron content, but not in the total iron respect to the control, while a 5 or 10% of date did not change significantly. For the non heme iron content (mg·kg-1) no SD ($P>0.05$) were found for storage time, but among the batches appeared some differences ($P<0.05$), being lower for the 15% pâté.

Regarding to the percentage of heme and non heme iron, to be able of a comparison according to the total iron content, no SD ($P>0.05$) were found neither for the storage time nor date content. Therefore, the proportion of heme and non heme iron regarding to the total iron no were really affected by the addition of dates. For the non heme iron, conversely as occurred for the heme iron, increased in the control during storage time, and was constant for the rest of the samples, although no SD ($P>0.05$) were detected. Thereby, the increase of MMb would be related with the non heme iron formation, in fact, oxidation of

the porphyrin ring and denaturation of myoglobin, giving MMb, seem to be related with the heme iron release in meat, what is normal during refrigerated storage [Lee., Hendricks et al., 1998].

Lipid oxidation

The amount of MA showed SD ($P<0.05$) during storage time and between batches. On day 0 no SD ($P>0.05$) were found among the batches, but on day 2 and 4 the control showed the highest values (≈1.85 mg MA·kg^{-1}) ($P<0.05$), while samples with 10 and 15% of paste maintained the values of day 0 (≈0.48 mg MA·kg^{-1}), and the 5% pâté had intermediate values. Therefore, lipid oxidation was significantly reduced in samples with date paste. This could be due to the presence of antioxidant compounds carried by the dates, but also for the lower amount non heme iron, since iron shows enhanced ability for promoting oxidation processes when is released from the heme molecule [Kanner and Doll 1991]. These higher amounts of non heme iron in the control may also have increased oxidative susceptibility [Estévez and Cava 2004], since when iron is released from myoglobin, haemoglobin, etc., it is become available to low molecular weight compounds such as amino acids, nucleotides and phosphates, with which they seem to form chelates [Decker and Crum 1993], and these chelates might be responsible for the lipid oxidation catalysis [Halliwell and Gutteridge 1990]. Also, the chelate activity of dates could help to prevent the oxidation. Oxidation is also promoted by sodium chloride, and pâtés with dates showed lower amounts of salt, what also could help to reduce oxidation. Furthermore, aqueous solutions of certain sugars may act as free-radical scavengers in some emulsions [Coupland and McClements 1996]. [Sims et al. 1979] suggested that sugar may decrease the concentration of oxygen in the aqueous phase, decreasing the diffusion coefficient of oxygen, since the aqueous phase increase its viscosity.

Conclusions

According to this study, addition of date paste reduced the lipid oxidation. No important differences were found among the heme pigments, the dates stabilized them during refrigerated storage of liver pâtés. Therefore, pâtés with dates are moresuited to refrigerated storage. Colour changesseemed not to be linked to lipid oxidation, but they were the unique drawback, the darker colour due to a high content of dates.Therefore, using date by-products in cooked meat products offers processors the opportunity to improve their nutritional and health qualities at low-cost. Moreover, adding ingredients considered beneficial for health could aid to the reduction of components considered harmful, such as those from the lipid oxidation. Also, they maintained the product stability. Further research to evaluate the most suitable concentration to do not affect the colour would be necessary.

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HYDROCOLLOIDS AS STABILIZERS OF LOW FAT EMULSION

Introduction

Increasing morbidity rate on civilization disease and growing awareness of consumers, are causes of changing habits of nutrition a lot of people. The result of this change is very interesting for food producers, who are focused on low-fat food. Lipids in food are general source of caloric value, but also are responsible for texture and taste of products. That is the reason of looking for substitutes of lipids, which will have influence on the product texture but not have caloric value.

Hydrocolloids have high-molecular-weight and are hydrophilic biopolymers. Hydrocolloids may be used in food production as a dietary fiber [Phillips 2005, Dłużewska 2007, Świderski 2009]. It means that are indigestible in human alimentary canal. Hydrocolloids have also many functional properties such as: thickening, gelling and stabilizing emulsions and that is the reason of their widely used in a variety of industrial sectors, including food industry [Świderski 1989, Rutkowski 1997, Schramm 1998, Gustaw 2003].

Emulsions are heterogeneous dispersions, which are made by mixing two or more immiscible phases. Boundary increase between phases is a necessary condition, to create stable emulsion. Increases of boundary require a huge amount of energy. In practice energy is generated by agitation or blending. The maximum of boundary increase is when interfacial tensions are higher or equal to shearing force [Stauffer 2001, Pijanowski 2004, Makarewicz 2008].

Factors, which have fundamental influence on stability of emulsion: interfacial tension, interfacial viscosity and intermolecular attractions. Stability of emulsion is determined by: density of continuous phase, scale dispersion and concentration of dispersed phase. Stable dispersion is characterized by resistance on: flocculation, creaming, coalescence, emulsion inversion and sedimentation [Komsta 2008, Lorenzo 2008].

The objective of this study was to evaluate the efficacy of selected hydrocolloids (locust bean gum – LBG, guar gum – G, konjac gum – K, tara gum – T and xanthan – X) for stabilization of low-fat emulsions (5.00% w/w).

Materials and Methods

Hydrocolloids, which were used in experiment are: locust bean gum, guar gum, konjac gum, tara gum and xanthan (Kerry Polska Sp. z o.o.). Rapeseed oil produced by Zakłady Tłuszczowe Kruszwica S.A was used.

Preparation of emulsions

Hydrocolloids were dissolved in water by mechanical stirrer CAT R-100C at 450 rpm. Next step was addition of oil. The solution were homogenized using homogenizer BUCHI B-400 at 9000 rpm for 15 s. Hydrocolloids were used in two different concentrations (1 – lower, 2 – higher): locust bean gum (LBG, L) konjac gum (K), tara gum (T) – 0.50, 1%, guar gum (G) – 0.75, 1.50%, and xanthan (X) – 0.15, 0.30%. Emulsion without hydrocolloids as reference sample was prepared (E0). Emulsions were produced according to variations presented in Table 1.

Table 1

Variants of hydrocolloids mixture

No	CODE		HYDROCOLLOIDS	
1	L-K-G	LBG	KONJAC GUM	GUAR GUM
2	L-G-X	LBG	GUAR GUM	XANTHAN GUM
3	L-T-G	LBG	TARA GUM	GUAR GUM
4	L-K-X	LBG	KONJAC GUM	XANTHAN GUM
5	L-T-K	LBG	TARA GUM	KONJAC GUM
6	L-T-X	LBG	TARA GUM	XANTHAN GUM
7	K-G-X	KONJAC GUM	GUAR GUM	XANTHAN GUM
8	T-K-G	TARA GUM	KONJAC GUM	GUAR GUM
9	T-K-X	TARA GUM	KONJAC GUM	XANTHAN GUM
10	T-G-X	TARA GUM	GUAR GUM	XANTHAN GUM

Storage Stability

Emulsions were stored at room temperature and Recorded last day when emulsion was still not stratified. Test lasted for 30 days.

Emulsifying Activity

Emulsifying activity was determined by the method of Yasurnatsu [Yasurnatsu 1972]. Emulsions were centrifuged at 5500 rpm for 10 minutes. Emulsifying activity was calculated as 100 x (height of emulsified layer) / (total height of mixture in tube).

Emulsion Stability

Emulsion stability was also determined by the method of Yasurnatsu [Yasurnatsu 1972]. For emulsion stability the emulsion was heated for 30 minutes at 80°C, cooled with tap water for 5min and centrifuged at 5500 rpm for 10 minutes. Emulsion stability was calculated as 100 x (height of remaining emulsified layer) / (total height of mixture in tube).

Viscosity

Viscosity determination was performed at rotation speed 60 rpm and temperature 25^{o}C. The rheological characteristic was conducted by rotational rheometer with stress-controlled system (Rheotest RN 4.1).

Statistical analysis

All experiments were replicated 3 times. Store stability results were analysed using analysis of variance (ANOVA). Emulsifying activity and emulsion stability effects were determined by Duncan's multiple-range test. Regression analysis of survival was used to assess change viscosity emulsions. Significance of difference was defined at $P<0.05$ (Tab. 2).

Table 2

Storage stability of emulsions [days]

EMULSION CONTENT	HYDROCOLLOIDS CONCENTRATION				EMULSION CONTENT	HYDROCOLLOIDS CONCENTRATION			
	0					1-1	2-1	1-2	2-2
E0	0				L-K	7.00	6.00	6.00	7.50
					L-G	30.0	30.0	30.0	30.0
EMULSION CONTENT	HYDROCOLLOIDS CONCENTRATION				L-X	30.0	30.0	30.0	30.0
	1	2			L-T	11.0	11.5	11.5	12.0
X	19.0	30.0			G-K	6.00	6.50	6.50	6.50
T	12.5	11.5			G-X	30.0	30.0	30.0	30.0
L	5.50	7.00			G-T	7.00	7.00	7.00	7.50
G	12.5	12.5			K-X	30.0	30.0	30.0	30.0
K	6.00	6.00			K-T	7.00	7.50	22.5	23.0
					X-T	17.0	17.5	17.5	17.5

EMULSION CONTENT	HYDROCOLLOIDS CONCENTRATION							
	1-1-1	1-1-2	1-2-1	1-2-2	2-1-1	2-1-2	2-2-1	2-2-2
L-K-G	5.00	6.00	6.00	6.00	6.50	13.5	7.50	14.0
L-G-X	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
L-T-G	3.00	6.00	6.00	6.00	6.00	6.50	6.50	7.00
L-K-X	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0

Table 2. continuous

L-T-K	3.00	3.00	3.00	3.00	3.00	6.50	4.00	7.00
L-T-X	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
K-G-X	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
T-K-G	14.0	15.0	14.0	15.0	14.5	30.0	14.5	30.0
T-K-X	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
T-G-X	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0

E0 – emulsion without hydrocolloids, X – xanthan gum, T – tara gum, L – locust bean gum, G – guar gum, K – konjac gum, 0 – without hydrocolloid, 1 – lower hydrocolloids concentration, 2 – higher hydrocolloids concentration

Results and discussion

Storage Stability

Significant influence of konjak gum on storage stability was not found. Tara gum and xanthan gum affected inequality on this parameter in all emulsions (Tab. 3 and 4).

Table 3

Main effects for storage stability

FACTORS	VARIANTS OF EMULSIONS				
	L(1)T(2)K(3)	L(1)T(2)G(3)	L(1)K(2)G(3)	T(1)K(2)G(3)	L(1)T(2)X(3)
R^2	0.61	0.69	0.79	0.72	0.92
R^2 corrected	0.41	0.52	0.68	0.56	0.88
Average/constant	8.04*	11.2*	10.5*	11.9*	20.9*
(1) [%] (L)	-3.56	4.44	6.89*	11.33*	7.44*
(1) [%] (Q)	-2.89	1.67	1.22	-1.33	3.61*
(2) [%] (L)	5.33*	-9.11*	-10.3	5.22	1.22
(2) [%] (Q)	-0.89	-5.00	-5.28	2.50	0.61
(3) [%] (L)	-1.67	2.89	8.11*	3.44	17.9*
(3) [%] (Q)	-1.72	1.00	2.39	-1.83	7.61*
1L regard to 2L	-7.17*	-7.33	-5.50	8.00*	0.83
1L regard to 3L	-3.83	1.67	4.33	-0.50	2.83*
2L regard to 3L	-1.50	-12.5	-8.67*	2.00	-5.83

X – xanthan gum, T – tara gum, L – locust bean gum, G – guar gum, K – konjac gum, R^2 – coefficient of determination, *significance for $\alpha \leq 0.05$

The best storage stability was obtained after addition of xanthan gum. Storage stability was grown when xanthan gum was added in lower concentration (0.15%). Increasing addition of this hydrocolloid was unjustified, because not improve storage stability emulsion. In emulsion, without xanthan gum, impossible is state what and how influence on storage stability, because its change essentially with modify component emulsion (Tab. 2).

Table 4

Main effects for store stability

FACTORS	VARIANTS OF EMULSIONS				
	L(1)K(2)X(3)	L(1)G(2)X(3)	T(1)K(2)X(3)	T(1)G(2)X(3)	K(1)G(2)X(3)
R^2	0.98	0.85	0.91	0.89	0.97
R^2 corrected	0.97	0.77	0.87	0.83	0.94
Average/constant	21.4*	25.4*	21.4*	20.6*	21.8*
(1) [%] (L)	2.22*	6.11*	3.78*	-1.11	0.67
(1) [%] (Q)	1.00	2.94	-1.55	-0.55	0.33
(2) [%] (L)	2.00	7.89*	8.11	6.89*	2.67
(2) [%] (Q)	1.00	3.94*	3.94	3.44	1.33
(3) [%] (L)	24.3*	12.6*	16.5*	18.9*	23.3*
(3) [%] (Q)	10.8*	4.94*	6.94*	8.11*	10.3*
1L regard to 2L	-3.00*	-0.17	2.83	-0.83	-4.00
1L regard to 3L	-1.33	-7.17*	-9.67	-2.33	1.00
2L regard to 3L	-1.00	-9.83*	1.83*	3.67	-2.00

X – xanthan gum, T – tara gum, L – locust bean gum, G – guar gum, K – konjac gum, R^2 – coefficient of determination, ∗significance for $\alpha \leq 0.05$

Dickinson et al. [Dickinson 2003] estimated impact hydrocolloids on rheological properties yoghurt. They also showed that xanthan gum (in mixture or singly) improve storage stability yoghurts but effect other hydrocolloids (locust bean gum, guar gum) depend on concentration this polysaccharide and interaction between other stabilizers. Paraskevopoulou et al. [Paraskevopoulou 2003] researched influence of polysaccharide addition on stability of a cheese whey kefir-milk mixture. They demonstrated that emulsion with 0.20%, 0.50% and 1.00% concetration xanthan gum not separated during 30 days test. However dispersion with 0.20%, 0.50% addition guar gum separated in 1 and 8 day test, but emulsion with 1.00% guar gum was stability during whole test. Koczo et al. [Koczo 1998] researched process flocculation of food dispersions stabilized by hydrocolloids. They showed that xanthan gum is better stabilizer than guar gum, even when is add in lower concentration. Emulsion with 0.10% xanthan gum as stability after 60 days store but with 0.15% guar gum was stability in 30.0%.

Table 5

Emulsifying activity emulsions [%]

EMULSION CONTENT	HYDROCOLLOIDS CONCENTRATION		EMULSION CONTENT	HYDROCOLLOIDS CONCENTRATION			
	0			1-1	2-1	1-2	2-2
E0	0.00		L-K	100	100	100	100
			L-G	100	100	100	100
EMULSION CONTENT	HYDROCOLLOIDS CONCENTRATION		L-X	100	100	100	100
	1	2	L-T	100	100	100	100
X	100	100	G-K	100	100	100	100
T	92.0	96.1	G-X	100	100	100	100
L	93.8	97.8	G-T	100	100	100	100
G	95.9	100	K-X	100	100	100	100
K	90.4	100	K-T	100	100	100	100
			X-T	100	100	100	100

EMULSION CONTENT	HYDROCOLLOIDS CONCENTRATION							
	1-1-1	1-1-2	1-2-1	1-2-2	2-1-1	2-1-2	2-2-1	2-2-2
L-G-K	100	100	100	100	100	100	100	100
L-G-X	100	100	100	100	100	100	100	100
L-G-T	100	100	100	100	100	100	100	100
L-K-X	100	100	100	100	100	100	100	100
L-K-T	100	100	100	100	100	100	100	100
L-X-T	100	100	100	100	100	100	100	100
G-K-X	100	100	100	100	100	100	100	100
G-K-T	100	100	100	100	100	100	100	100
G-X-T	100	100	100	100	100	100	100	100
K-X-T	100	100	100	100	100	100	100	100

E0 – emulsion without hydrocolloids, X – xanthan gum, T – tara gum, L – locust bean gum, G – guar gum, K – konjac gum, 0 – without hydrocolloid, 1 – lower hydrocolloids concentration, 2 – higher hydrocolloids concentration

Sahin and Ozdemir [Sahin 2007], screened impact hydrocolloids on ketchups stability, demonstrated that xanthan gum better improve ketchup stability than guar gum or locust bean

gum. Addition xanthan gum on level 1.00% cause separate emulsion in 2.00% after 30 days store, while in case of guar gum in 13.0% or locust bean gum in 18.0%.

Emulsifying Activity and Emulsion Stability

In work stated that emulsifying activity and emulsion stability are complete when emulsion contain 2 or 3 hydrocolloids or singly xanthan gum, guar gum, konjak gum already on level appropriately 0.15, 1.50, 1.00% (Tab. 5 and 6). Sahin and Ozdemir [Sahin 2007] showed that addition xanthan gum much more increase emulsifying activity than locust bean gum or guar gum.

Table 6

Emulsions stability [%]

EMULSION CONTENT	HYDROCOLLOIDS CONCENTRATION		EMULSION CONTENT	HYDROCOLLOIDS CONCENTRATION			
	0			1-1	2-1	1-2	2-2
E0	0,00		L-K	100	100	100	100
			L-G	100	100	100	100
EMULSION CONTENT	HYDROCOLLOIDS CONCENTRATION		L-X	100	100	100	100
	1	2	L-T	100	100	100	100
X	100	100	G-K	100	100	100	100
T	94.0	97.7	G-X	100	100	100	100
L	98.8	98.0	G-T	100	100	100	100
G	96.0	100	K-X	100	100	100	100
K	96.1	100	K-T	100	100	100	100
			X-T	100	100	100	100

EMULSION CONTENT	HYDROCOLLOIDS CONCENTRATION							
	1-1-1	1-1-2	1-2-1	1-2-2	2-1-1	2-1-2	2-2-1	2-2-2
L-G-K	100	100	100	100	100	100	100	100
L-G-X	100	100	100	100	100	100	100	100
L-G-T	100	100	100	100	100	100	100	100
L-K-X	100	100	100	100	100	100	100	100
L-K-T	100	100	100	100	100	100	100	100
L-X-T	100	100	100	100	100	100	100	100
G-K-X	100	100	100	100	100	100	100	100

Table 6. continuous

G-K-T	100	100	100	100	100	100	100	100
G-X-T	100	100	100	100	100	100	100	100
K-X-T	100	100	100	100	100	100	100	100

E0 – emulsion without hydrocolloids, X – xanthan gum, T – tara gum, L – locust bean gum, G – guar gum, K – konjac gum, 0 – without hydrocolloid, 1 – lower hydrocolloids concentration, 2 – higher hydrocolloids concentration

Sciarini et al. [Sciarini 2009] demonstrated that 0.10% concentration xanthan gum case 100% emulsifying activity and emulsion stability. Assay was pursuing after centrifuge at 800xg for 10 minutes. Sciarini et al. showed also that emulsifying activity was higher than emulsion stability. However in this work emulsifying activity was lower than emulsion stability.

Table 7

Emulsions viscosity [Pas]

EMULSION CONTENT	HYDROCOLLOIDS CONCENTRATION				EMULSION CONTENT	HYDROCOLLOIDS CONCENTRATION			
	0					1-1	2-1	1-2	2-2
E0	0.08				L-K	8.16	25.8	19.7	25.9
					L-G	10.6	22.8	63.0	29.7
EMULSION CONTENT	HYDROCOLLOIDS CONCENTRATION				L-X	62.2	70.1	55.1	77.6
	1	2			L-T	5.28	19.7	16.7	50.8
X	0.74	1.85			G-K	15.9	50.7	40.2	68.8
T	0.38	5.81			G-X	27.1	58.7	35.6	83.4
L	0.42	3.18			G-T	15.9	50.4	40.2	68.8
G	3.18	31.5			K-X	48.0	61.2	64.8	70.0
K	1.50	12.1			K-T	7.75	37.2	27.9	79.6
					X-T	37.7	57.8	70.1	77.6
EMULSION CONTENT	HYDROCOLLOIDS CONCENTRATION								
	1-1-1	1-1-2	1-2-1	1-2-2	2-1-1	2-1-2	2-2-1	2-2-2	
L-G-K	59.5	63.6	147	162	112	113	190	165	
L-G-X	70.2	111	127	116	100	136	152	202	
L-G-T	40.5	75.6	127	109	85.6	138	152	249	
L-K-X	42.5	63.7	56.4	44.3	121	93.6	48.8	88.3	

Table 7. continuous

L-K-T	29.9	89.5	82.9	141	84.5	105	120	209
L-X-T	72.9	155	63.2	148	73.5	122	148	185
G-K-X	114	39.0	140	282	111	32.1	21.2	241
G-K-T	57.7	123	80.8	160	122	133	75.3	179
G-X-T	82.1	134	202	116	88.2	107	141	215
K-X-T	75.7	144	272	34.5	93.0	178	72.3	214

E0 – emulsion without hydrocolloids, X – xanthan gum, T – tara gum, L – locust bean gum, G – guar gum, K – konjac gum, 0 – without hydrocolloid, 1 – lower hydrocolloids concentration, 2 – higher hydrocolloids concentration

Viscosity

In case emulsion with singly addition polysaccharide the highest viscosity (31.5 Pas) have emulsion with 1.50% addition guar gum and the lowest viscosity (0.38 Pas) with 0.50% tara gum. Doubling concentration hydrocolloid case increase viscosity: the highest, namely quintuple (0.38 Pas → 5.81 Pas) when stabilizer was tara gum, the lowest, double (0.74 Pas → 1.85 Pas) when added was xanthan gum (Tab. 7). Statistical analysis (Tab. 8 and 9) showed that guar gum and xanthan gum at most influence on viscosity emulsion, while locust bean gum the least. Xanthan gum and guar gum case increase viscosity, but locust bean gum decrease viscosity. Koksoy and Kilic [Koksoy 2004], researched possibility stabilizes yoghurt by hydrocolloids, showed that guar gum in higher grade influence on viscosity than locust bean gum. Namely addition guar gum on level 0.25% case increase viscosity from 15.0 mPas to 125 mPas (gain eight-time), while added was locust bean gum viscosity increase to 74.0 mPas (gain five-time). Khouryieh et al. [Khouryieh 2006] demonstrated that addition xanthan gum case highest viscosity (7.00 mPas) than mixture xanthan gum-guar gum which has viscosity 6.50 mPas, 6.00 mPas and 5.00 mPas appropriately for ratio: 3:2, 2:3, 1:4. Measurement was realized at shear rate 20 s^{-1} and temperature 25°C.

Table 8

Results of regression analysis for mixture viscosity

FACTORS	VARIANTS OF MIXTURE				
	L(1)T(2)K(3)	L(1)T(2)G(3)	L(1)K(2)G(3)	T(1)K(2)G(3)	L(1)T(2)X(3)
R	0.89	0.90	0.92	0.89	0.88
R^2	0.78	0.81	0.84	0.79	0.77
R^2 corrected	0.76	0.79	0.82	0.76	0.74
b (1)	0.41	0.48	0.37	0.46	0.45
b (2)	0.56	0.38	0.38	0.51	0.45
b (3)	0.56	0.66	0.75	0.57	0.59

X – xanthan gum, T – tara gum, L – locust bean gum, G – guar gum, K – konjac gum, R - coefficient of correlation, R^2 – coefficient of determination, β – coefficient of regression, * significance for $\alpha \leq 0.05$

Yamazaki et al. [2009] also showed that xanthan gum the most influence on viscosity, less guar gum and less locust bean gum. The higher concentration of hydrocolloid the smaller viscosity changes were observed. When concentration hydrocolloids were 0.25%, viscosity was on level: 400 mPas – xanthan gum, 60.0 mPas – guar gum, 50.0 mPas – locust bean gum. But when polysaccharides was addend on level 0.50%, viscosity amounted to: 500 mPas – xanthan gum, 300 mPas – guar gum, 100 mPas – locust bean gum. Mandala el at. [2004] were studied the influence of xanthan and locust bean gum on the rheology and structure of a white model-sauce. They showed that xanthan gum and locust bean gum similarly influence on viscosity.

Table 9

Results of regression analysis for mixture viscosity

FACTORS	VARIANTS OF MIXTURE				
	L(1)K(2)X(3)	L(1)G(2)X(3)	T(1)K(2)X(3)	T(1)G(2)X(3)	K(1)G(2)X(3)
R	0.78	0.82	0.72	0.87	0.97
R^2	0.61	0.68	0.52	0.75	0.48
R^2 corrected	0.56	0.64	0.46	0.72	0.42
b (1)	0.39	0.39	0.39	0.47	0.47
b (2)	0.23	0.52	0.38	0.47	0.29
b (3)	0.63	0.49	0.47	0.56	0.42

X – xanthan gum, T – tara gum, L – locust bean gum, G – guar gum, K – konjac gum, R – coefficient of correlation, R^2 – coefficient of determination, β – coefficient of regression, * significance for $\alpha \leq 0.05$

Conclusions

Xanthan gum presented the highest influence on storage stability of low-fat emulsion.

Addition of guar gum, locust bean gum and xanthan gum caused increases of storage stability of emulsion.

Influence of tara gum and konjak gum on storage stability depending on interaction of other stabilizers of emulsion was noted.

Xanthan gum and guar gum had the biggest influence on viscosity changes.

100% of emulsifying activity and emulsion stability were obtained for emulsions containing 2 or 3 hydrocolloids or xanthan gum, guar gum, konjak gum on levels 0.15%, 1.50%, 1.00% respectively.

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14

PROPERTIES OF EXTRUDATES PRODUCED FROM POTATO WASTE PULP

Introduction

Quick economy, social and cultural change, as well as technological advancement, caused modification of life style of modern society, unfortunately, often unfavorable for health. However consumer's health awareness is growing, regular interest and pursuit of healthy condition with desire to slow aging process is not uncommon. Proper nutrition significantly determines good health, which causes enlarging demand for functional food and development of new food market.

Functional food are mostly enriched products, because specific healthy characteristic is gained by addition of bioactive compounds, which stimulate desired course of metabolism. Food enrichment is one of the methods, which eliminates or reduces deficiency of nutrients in diet of whole populations or specific groups. Among bioactive compounds of food with identified, health beneficial properties, very important role is played by dietary fiber.

Dietary fiber is a group of compounds resistant on enzymatic hydrolysis in human gastrointestinal. It does not provide energy nor nutrients to organism, but due to specific properties (antioxidant activity, influence on mineral transmutation, metabolism of fats, proteins, cholesterol, stimulation of intestinal motility and normalization of its bacterial flora [Howarth et al. 2001, Roehring 1990] as well as antitumor activity [Mariadason et al. 2000], it ensures keeping organism in health and prevents civilization diseases. Despite proven beneficial effect of fiber on human body, intake of this component in highly developed countries is still unsatisfactory. In order to increase its consumption, food producers employ addition of fiber to products like: yogurt, dark bread, breakfast mix of muesli type, drinks. Fiber added to food not only increases nutritional value, but also positively affects its functional and sensorial properties.

Depending from formation, origin, structure, as well as content of individual fractions (soluble and insoluble) fiber exhibits diverse functional properties, stabilizing products during storage and texture forming [Nassar 2008, Raghavendra et al. 2006]. To most important functional properties of dietary fiber include moisturizing properties such as swelling, solubility, ability to absorb water and exchange cations, absorption of fat (cholesterol), bile acids and viscosity [Guillon et al. 2000]. Dietary fiber is used not only because of its functional and pro-health effect, but also due to its technological properties. It decreases fat content in

the final product and allows to maintain proper texture of product, in which it replaces fat, increases effectivity of production during heat treatment, improves stability of finished product, as well as consistency of bread and its greasiness, it also decrease size of ice crystals in products intended for deep freezing, simplifies emulsion creation and counteracts lumping [Stamatialis et al. 2000].

Currently sources of new sources of fiber are searched. Growing interest is directed on utilization of food industry wastes. The main reason of seeking new directions of those wastes procession is minimalization of their influence on the environment and utilization of valuable compounds, remaining in side products of food industry. One of examples of those side products, being also a source of dietary fiber, is potato pulp, which consist about 50% of dietary fiber [Meyer 1998].

In Europe, potatoes are the basic raw material for the obtainment of starch. The process generates vast amounts of potato pulp, one of the main side-products of the agro-food industry. It is estimated that starch plants in Europe produce approximately $1 \cdot 10^6$ tons of potato pulp [Meyer 2009] consisting mainly of juice water (over 90%). Potato pulp contains all compounds of potato, but during starch obtainment process most of the starch and soluble non-starch substances are being removed.

Pulp, in the past entirely collected by farmers, is now a serious problem for starch industry because, due to decreasing number of livestock, particularly cattle, its utilization for feeding purposes is lowering. Little interest of breeders is also caused by its low nutrition value. Potato pulp contains large amounts of fiber substances, while small amount of protein and fat, so, as a feed, it has to be enriched in protein and minerals. Potato pulp can also be used as a compost for plants growing, where it can be a source of major nutrients. However, utilization of pulp for fertilization requires previous soil pH adjustment and enrichment with mineral fertilizers crucial for plant cultivation. There are also reports about experimental use of dried pulp as a fully biodegradable material for glue, and in combination with starch as small food packages production [Meyer et al. 1997]. Concentrated potato pulp in the form of granules can also be used as material for the production ecological solid fuel or morphological component in fuels generated from wastes, as it is characterized by high energetic value [Obidziński 2009, 2010]. Attempts were also made to use potato pulp as a source of pectins [Turquois et al. 1999]. All these directions are, however, only partially successful, and large quantities are still deposited in landfills, which contributes to environmental degradation.

The solution of this problem may be greater utilization of potato pulp for production of fiber rich preparations. Potato fiber mainly consists of cellulose, hemicellulose, pectin and lignin [Meyer 1998]. It is characterized by low allergenicity (as it is gluten-free product), the stability in acidic pH, as well as high and low temperature – sterilization, and freezing. The most important features of potato fiber is capability of binding water and water-fat slurries. Addition of potato fiber may facilitate production of low caloric food, as well as food products with reduced fat, cholesterol or sugar. Mainly it is used in the meat industry due to the reduction of production losses, during boiling, smoking and cooking. It also positively affects the texture and structural stability of meat products, and reduces the leak in the package during prolonged storage. It is also favorable that products, like sausages, both whole and sliced are characterized by lesser shrinkage during cooking or frying. Conducted research, on the effects of potato fiber utilization as a substitute of fat in pâté, lead to conclusion that potato pulp preparation have highly positive effect on reducing fat content in the baked meat products. Liver pâtés were also characterized by lower caloric and properly unaltered organoleptic characteristics [Kaack

et al. 2006]. Experiments, in which part of the fat was replaced by potato fiber in canned meat products, allowed to obtain finely shredded, nutritionally beneficial, with structure and quality similar to control sample. This resulted in a significant increase of water and sodium chloride content, limited the thermal leakage and what is important did not significantly influenced the rheology of canned product (plasticity, elasticity and smoothness). Also other applications, than the meat industry, noted a beneficial effect of potato fiber produced from dried potato pulp. Studies on the effects of dietary fiber preparation on the quality of wheat bread have shown that it can be used to modify dough. Even a small addition improves the technological indices of wheat flour, as well as color and texture of bread, while it does not change most of the physicochemical characteristics. Therefore the fiber preparation from potato pulp can be a good raw material for the enrichment of bread [Kaack et al. 2006].

Although very applicable, potato pulp is still an enormous problem. One of the possible directions of waste potato pulp management can be extrusion process. Due to the pulp properties and chemical composition, it can be an excellent method of obtainment fiber rich preparations for food enrichment. Extrusion enables production of components easily adapting to food, without necessity to use foreign substances. During the process gelatinization occurs, as well as increase of starch to digestion susceptibility, increase of soluble substances content, protein digestibility of legume and oilseed plants, bio-availability of sulfur amino acids and fiber assimilation.

Extrusion process can produce broad range of products, due to possibility to extrudate diversified raw materials [Curvelo et al. 2001, Drożdż et al. 2004]. Process is used for obtainment of snacks, child and infant nutritions, modification of starch, proteins from both plants and animals, enrichment of fodder material and for production of fodder and pet food. Factors determining extrusion process and properties of obtained product are both proper choice of raw material, as well as conditions of process. Effects of heat, pressure and shear forces determine number of reactions, and transformations, that induce in processed material physical, chemical, and therefore quality change. All these modifications result with favorable influence on final product, improving its nutritional value, color flavor and structure, and as a result product with far different traits than raw material used in process [Wolf 2010].

Change in overall fiber content, as its fraction – soluble and insoluble fiber during extrusion were object of research by many authors [Gualberto et al. 1997, Stojceska et al. 2009]. Conducted researches proved, that during extrusion change of fiber and its fractions occur, which are mostly dependant on the plant type, that was used as fiber source and conditions of extrusion process. However changes taking place during extrusion of potato fiber have not been throughout investigated.

The purpose of research is invention of new products, rich in dietary fiber, based on potato pulp, obtained in effect of its extrusion, as well as determination of properties of obtained products.

Material and methods

Research based on potato pulp produced by Przedsiębiorstwo Przemysłu Ziemniaczanego S.A. in Niechlowo in 2009.

Potapo pulp was frozen and stored at -18°C for 7 days. After this time, within 24 hours, pulp was thawed and dried in fountain dryer at 110°C, with drying speed of 8 $m \cdot s^{-1}$. Potapo pulp was then milled in a Brabender's rotary-type laboratory mill B\4-MO-01 880 806 to granulation <1mm and its properties were determined.

Extrusion process

Potato pulp was moistured with distilled water to moisture content of 20, 24, 28%, posteriorly sieved, enclosed in air-tight bag and conditioned for 24 hours.

Extrusion was conducted in one-screw Brabender laboratory extruder of 20 DN type. Parameters of process are given below.

Screw revolutions	180 rev · min^{-1}
Feeder revolutions	30 rev · min^{-1}
Screw load	5.5 – 6 A
Nozzle diameter	3 mm
Screw compression	2:1

Table 1

Temperature distribution in extruder

Extrusion variant	Temperature in feeding section [°C]	Temperature in knead section [°C]	Temperature in final heating section [°C]
I	60	70	90
II	90	100	120
III	130	150	180

Analysis methods

Mechanical properties

Analysis were conducted on uncrushed extrudates.

Analysis was conducted with testing machine INSTRON 5544 with Bend Fixture. Load of head was 2kN, and speed of head travel 4.16 $mm \cdot s^{-1}$. The results were elaborated in computer program TableCurve 2D v5.01, which allowed graphical presentation of obtained data. Dependency of force affecting extrudate from head displacement showed on diagrams allowed to determine following mechanical properties:

Force max [N] – force causing intersection of extrudate.

Elongation [mm] – displacement of head from zero position, which causes maximal stress effecting with intersection of extrudate.

Work [J] – needed for complete intersection of extrudate. To determine its value the integral of area under the graph was calculated.

Water absorbtion and solubility

The obtained extrudates were crushed in blade laboratory mill of WŻ-1 type. From crushed extrudates 500 g of 1% water solutions were prepared, with correction for dry mass content. The samples were placed in water bath with shaking for 30 min, in temperature of 80°C, and afterwards cooled. Water evaporated during heating was supplemented with disstiled water to 500 g. The 50 g portions of solution was poured to 6 weighted and tared centrifuge vials and centrifuged in Biofuge 28 RS for 30 min with speed of 14500 rev min^{-1}. Supernatant was separated and its dry mass was determined by drying for 12 hours at 60°C and then for 3 hours at 105°C. The remaining sediment in centrifuge vial were weighed.

Solubility and water absorption was calculated from formulas:

$$R = \frac{A}{B} \cdot 100 ,$$

where:
R – solubility [%],
A – dry mass of supernatant [%],
B – dry mass of paste [%].

$$W = \frac{a - x \cdot \left(\frac{100 - R}{100} \right)}{x \cdot (100 - R)} \cdot 100 ,$$

where:
W – water absorption [g of water g of substance],
a – mean mass of sediment in centrifuge vial [g],
x – dry mass of solution in centrifuge vial [g].

Degree of saccharificataion

38 g of 0.72% suspension of crushed extrudates was prepared in 100 ml conical flask. The flask was held in boiling temperature for 5 min and after cooling it was supplemented with distilled water to initial mass. Subsequently 38 ml of acetate buffer was added. The flask was placed in water bath with shaking, in temperature of 37°C, and 4 ml glucoamylase enzyme solution was added. The ratio of enzyme to acetate buffer was 1:4. After 0.5, 1, 2, 3, 4, 5, 6 h 5 ml of hydrolyzate was collected to centrifuge vials and centrifuged for 5 min with 2500 rev min^{-1} in centrifuge of MPW 312 type. Afterwards 10µl supernatantu was collected and transferred to microcuvettes containing 1 ml of glucose agent from BIOSYSTEM company. After stirring and incubation for 20 min in temperature of 20°C, measurance of absorption was conducted with CECIL CE 2010 colorimeter, with wavelength set to λ=500 nm. Reference sample was reagent with acetate buffer. The amount of glucose was read from calibration curve.

Preparation of calibration curve.

Stock solution of glucose, containing 10 g of glucose per 1l, was prepared in volumetric flask. Calibration solutions were prepared as follows: to volumetric flasks of 100 ml volume 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50ml of stock solution was transferred, subsequently 50 ml of acetate buffer was added and filled with distilled water. After thorough mixing 10µl of solutions were collected and transferred to microcuvettesconatining 1ml of glucose concentration measurement agent.

After incubation (20 min, 20°C) measurement of absorbance was conducted with colorimeter CECIL CE 2010 at λ=500nm. Reference sample was reagent with acetate buffer. Measured values were placed on dependency diagram of absorbance from glucose concentration.

The degree of saccharification was calculated according to formula:

$$SC = \frac{x \cdot 100}{0.396},$$

where:

SC – degree of starch saccharification [%],

x – amount of glucose read from calibration curve [mg],

0.396 – maximum amount of glucose obtained from 0.36% potato starch gel [mg].

Obtained results were calculated on amount of starch contained in dry mass of potato pulp.

Method of results elaboration

Obtained results were subject of statistical analysis, based on two factor analysis of variance, with significance level α=0.05 in Statistica 9.0 program. The homogenic groups were calculated with Duncan's test.

Results and discussion

Potato pulp of determined moisture (20, 24, 28%) was subject to extrusion process in diverse temperatures. Obtained preparations were characterized by different color and degree of expansion, as well as mechanical and functional properties.

Figure 1 show 9 kinds of preparations obtained from diverse variants of temperature, and different moisture of potato pulp 20, 24, 28%. In the lowest temperature of extrusion (variant I) the significant influence of raw material moisture on degree of expansion and color of extrudates was observed. In this variant, with increasing moisture of potato pulp, used for extrusion, degree of extrudates expansion decreased, and the color changed from light brown to almost black. In II variant of extrusion similar influence of pulp moisture on color of extrudates was noted. The higher moisture of potato pulp the darker color of extrudates. Products obtained according to III variant of extrusion was characterized by light yellow color which was significantly lighter comparing to extrudates obtained in lower extrusion temperatures, and their degree of expansion was minimal.

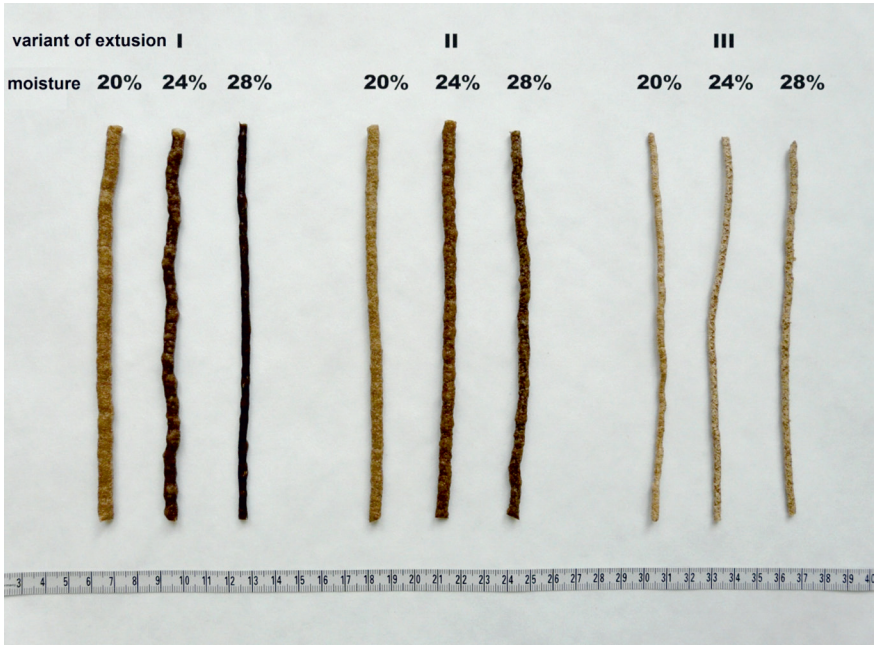

Fig. 1. Appearance of preparations obtained from diverse variants of temperature, and different moisture of potato pulp

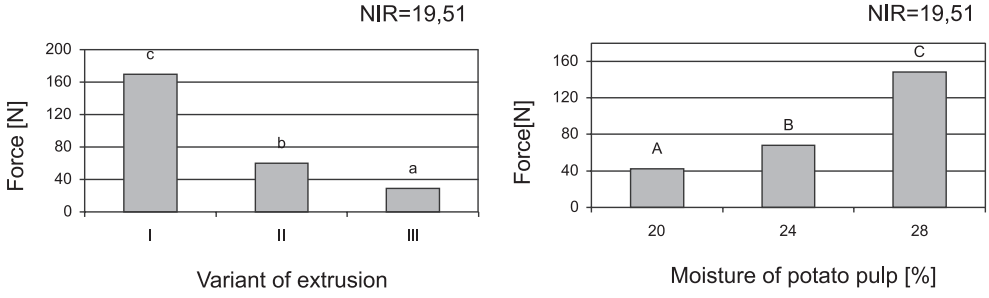

Fig. 2. Influence of extrusion temperature and moisture of potato pulp on maximum force needed for intersection of extrudates

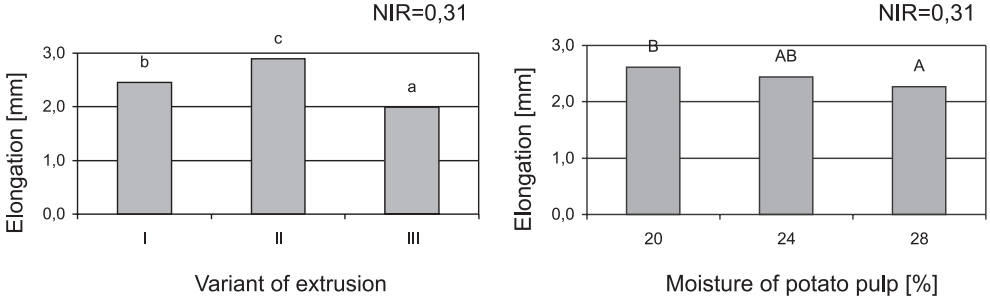

Fig. 3. Influence of extrusion temperature and moisture of potato pulp on elongation causing maximal stress needed for intersection of extrudates

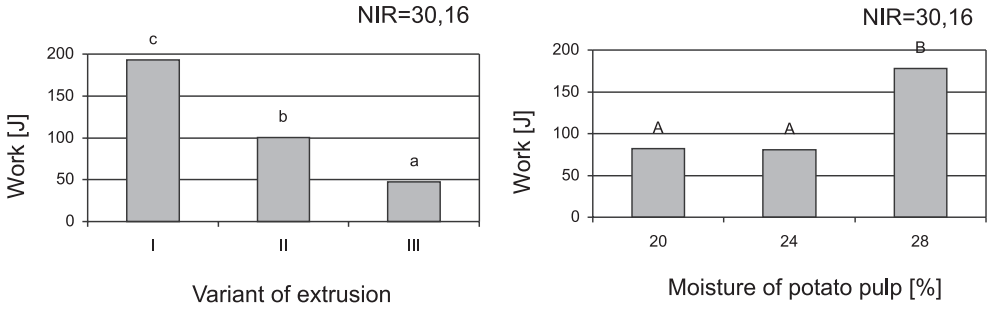

Fig. 4. Influence of extrusion temperature and moisture of potato pulp on work needed for intersection of extrudates

Dried potato pulp contains about 30% of starch and about 50% fiber substances [Meyer, 1998]. Change of potato pulp properties during extrusion are mostly caused by alternations of those two ingredients.

Extrudates produced from potato pulp were characterized by low expansion level, which is caused by high content of fiber. During extrusion, water is retained in the spaces beetwen fibers, therefore high moisture of raw material causes decrease in extrudates degree of expansion, increases irregularity of shape and roughness of surface [Camire et al. 1990, Stojeceska et al. 2009].

Obtained extrudates were subject of mechanical properties analysis on testing machine Instron. Ultimate strength effecting on extrudate during its intersection, elongation at maximal force effecting the extrudate, and work during intersection of single extrudates. These traits are interdependant, therefore should be interpreted together.

Conducted research showed, that maximum force causing intersection of extrudate was decreasing with increasing temperature of extrusion (Fig. 2). Force needed for intersection of extrudates produced in lowest temperature of extrusion (variant I) was far greater than maximum force required for intersection of extrudates obtained in remaining variants of extrusion. Increase of potato pulp moisture caused increase of maximum force, effecting the extrudate. The highest values of force needed for extrudate intersection were observed for products obtained from potato pulp of 28% moisture. Elongation, which is displacement of head, which causes maximal force is a parameter describing flexibility of obtained products. Extrudates produced according to II variant of temperature were characterized by greatest elongation values (Fig. 3) therefore they were most flexible. Decrease of moisture was effecting in increased elongation which caused intersection of extrudate. Increase of extrusion temperature was causing decreased values of work needed for extrudate intersection (Fig. 4). Increase of potato pulp moisture to 28% effected in almost twice higher work required for intersection of extrudates produced from potato pulp of 20 and 24% moisture.

Determination of ultimate strength and work performed during extrudates intersection showed, that preparations produced in lowest temperature of extrusion (variant I), with highest moisture of raw material, are the least susceptible on shearing, therefore they have the toughest structure. Increase of extrusion temperature caused weakening of obtained product structure. The least resistant on shearing were extrudates produced in III variant of extrusion. Internal structure of extrudate cell walls determines its mechanical properties. Lowered moisture of raw material favors formation of porous structure, increase of air cells number,

and thus creation of more expanded product. Since air cells of larger dimensions have thinner walls, extrudates durability on bending is inversely proportional to materials level of expansion [Mendonca et al. 2000, Yanniotis et al. 2007]. In variant III of extrusion, despite marginal expansion of products, influence of decreased moisture on toughness of extrudate was observed. Crunchiness and hardness of starch extrudates is dependant from degree gelatinization of starch in material [Gomez 1984]. Better gelatinization of starch in extruded raw materials, as well as low temperature of extrusion process causes greater expansion of final product, hence lower bending resistance of extrudates. Mechanical properties of obtained extrudates are also affected by high content of fiber in potato pulp. Research about extrusion of raw materials characterized by high content of fiber showed, that it may negatively effect texture properties of obtained products. High amounts of fiber cause formation of dense, hard texture and decrease of extrudates expansion degree. [Camirre et al. 1990, Altan et al. 2008, Stojceska et al. 2010]. Similarly Mendonca [2000], Yanniotis [2007], Ainsworth [2007] in their research claim, that increased amount of fiber in extruded products lead to higher hardness of obtained extrudates, effecting the thickness of air cells formed during extrusion process.

In crushed extrudates selected functional properties were analyzed. Conducted research showed, that solubility of potato pulp preparations, determined in temperature of 80°C, grew with increase of extrusion process temperature (Fig. 5). While opposite influence was observed for moisture of potato pulp on solubility of extrudates. With increasing moisture of raw material solubility of extrudates decreased.

Extrusion causes significant increase of starch products solubility. Increase of soluble substances content is caused by gelatinization and degradation of starch, to carbohydrates with shorter glucose chains, occurring under conditions of extrusion process. Lowered starch moisture and increased processing temperature effects in heightened solubility of obtained extrudates. It was concluded, that value of solubility for different starch products is raising with alternations of temperature and is inversely effected by moisture of raw material. Increase of extrudates solubility is also influenced by high content of dietary fiber in potato pulp. The extrusion process is causing increase of fiber soluble fraction content, in comparison to unprocessed material [Larrea et al. 2005, Wolf 2010]. It can be explained by break of covalent and non-covalent bonds in carbohydrates of insoluble fraction during extrusion, which effects in formation of smaller and more soluble fragments of those carbohydrates. Insoluble fraction of fiber decreases in favor of soluble fraction, with increase of extrusion temperature and decrease of water content in raw material [Larrea et al. 2005].

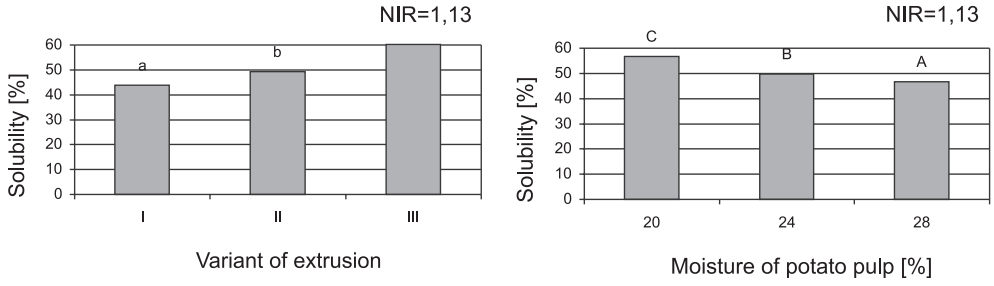

Fig. 5. Influence of extrusion temperature and moisture of potato pulp on solubility of obtained extrudates

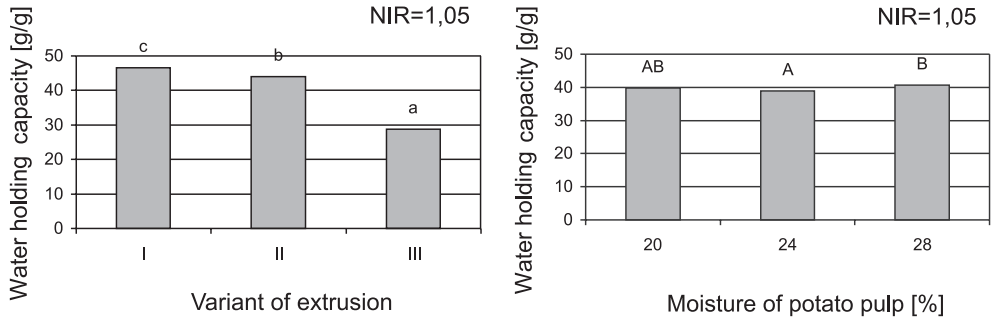

Fig. 6. Influence of extrusion temperature and moisture of potato pulp on water absorption of obtained extrudates

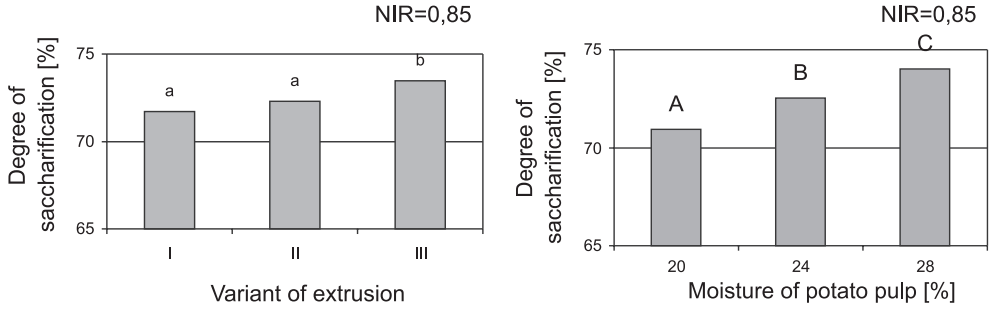

Fig. 7. Influence of extrusion temperature and moisture of potato pulp on degree of saccharification of obtained extrudates

Temperature of extrusion had significant influence on water absorption of obtained products. The higher temperatures of extrusion the lower water abosrption of obtained extrudates. Significant decrease of water absorption was observed for extrudates produced according to variant III of extrusion (Fig. 6). Similar influence of temperature on water absorption was observed by other authors [Guha et al. 1997, Ding et al. 2006]. Decrease of extrudates water absorption with increase of processing temperature was caused by high content of fiber in potato pulp. Altan et al proved in their research, that water absorption of extrudates rich in fiber decreases with increase of extrusion temperature, as well as with increasing content of fiber fraction [Altan et al. 2008]. With increasing temperature of extrusion, water absorption of extrudates based on apple fiber decreases, with simultaneous increase of solubility [Stojceska et al., 2010].

Water absorption of extrudates depending on moisture of potato pulp changed in limited degree, taking values from 38,92 $g \cdot g^{-1}$ to 40,60 $g \cdot g^{-1}$, however the changes were statistically significant. Extrudates obtained from potato pulp of greatest moisture were characterized by highest water absorption. Stojceska et al. [2009] researching influence of raw material moisture on water absorption of obtained extrudates also proved, that increasing amounts of water in starting material effects with enlargening water absorption of obtained products. Similar tendency are also confirmed in research by other authors [Mezreb et al. 2003].

Both heightened temperature of extrusion (Fig. 7) and increased moisture of starting material caused greater degree of saccharification of obtained preparations. As mentioned above during extrusion gelatinization and degradation of starch occurs. Degree of those

changes is dependant from kind of raw material used, its water content and work parameters of extruder, mainly the temperature. Comparing to starting material, extrudate contains higher amount of easy digestible low molecular carbohydrates formed during hydrolisation of starch [Krzyżaniak et al. 2005]. The case different from mentioned tendency may probably be explained by structure of extrudate produced in conditions of extrusion variant I from potato pulp of 28% moisture. Those extrudates were characterized by the hardest structure. Degree of saccharification was mostly affected by moisture of starting material. Despite lower solubility, preparations obtained from potato pulp with higher water content were more susceptible on conversion by amylolitical enzymes, when compared to potato pulp with lower water content. Similar results were obtained by Govindasamy [1997], who extruded sago starch of diverse moisture.

Conclusions

Intensive influence of temperature, pressure and mechanical shearing in conditions of continuous displacement and stirring, that effects on raw material during extrusion, causes number of changes, which transforms physico-mechanical properties. This contributes to formation of entirely new product. Conducted research showed, that selection of suitable extrusion temperature and moisture of raw material, allows to obtain potato pulp preparation with altered functional properties, such as solubility, water absorption and susceptibility on glucoamylase. Creation of this extruded preparation increases advantages of waste material, which potato pulp is, which leads to increased and broadened its potential utilization, as functional additive in food industry.

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15

CHARACTERISTICS OF MECHANICALLY DEBONED TURKEY MEAT AS A RAW MATERIAL FOR SURIMI LIKE ISOLATE PRODUCTION

Mechanically deboned meat (MDM/MRM) is a term used to describe the product recovered from animal bones or poultry carcasses by the application of pressure or shear forces. This process permits the recovery of most of the residual meat still remaining on bones after collecting the most valuable parts of the carcasses i.e. breast fillets, poultry legs, loins, etc. The resultant MDM has the structure like a highly comminuted meat. MDM is utilized in the production of a wide range of meat products, either as a binding agent or as an inexpensive source of meat [Day and Brown 2001, Püssa et al. 2009].

An increase of poultry production and growth in mechanically deboned poultry meat in the early 1970's resulted in focusing research efforts on improving the quality of products and thereby on increasing of MDM utilization in final products directed for human consumption [Betti and Fletcher 2005].

Mechanically separated chicken meat (MSCM) has a high content of haeme pigments, connective tissue, calcium and fat [Froning 1981, Yang and Froning 1992]. MDM has a dark color, undesired textural properties and is susceptible to lipid oxidation. [Hernandez et al. 1986, Smyth and O'Neill 1997, Calhoun et al. 1999].

The reason for fast progress in the oxidation processes can be due to the extensive stress and aeration during the machine-deboning process, and the compositional nature of the product (bone marrow, haeme and lipids). During frozen storage of mechanically deboned turkey meat, an intensive autooxidation occurs resulting in decreased functionality of the meat [Dawson and Gartner 1983, Mielnik et al. 2002]. Chemical composition of MDM can vary in very broad range. It contains from 11% to 18% protein, up to 30% fat and up to 76.5% moisture. The composition of mechanically deboned poultry meat (MDPM) is as follows: muscle tissue 39–57%, adipose tissue 36-53%, cartilage 1–11%, bone tissue 1–4%. [Fonkwe and Singh 1996]

Such big differences in the chemical composition are caused by the type of raw material used in the process and the type of separating machine. It led to lowering of the functional properties of MRM and undesirable red colour [Stangierski and Kijowski 2000]. The main problem for products formulated with MDPM is the rapid onset of oxidative rancidity, which results in off-flavours and off-odours consequences [Lee et al. 1975, MacNeil et al. 1973]. Secondary products of fatty acid oxidation, such as aldehydes, ketones, hydrocarbons, etc., are mainly responsible for rancid flavours and sensory deterioration of the products [Mielnik et al. 2002]. It should be stressed that microbiological quality of MRM is low, with possible

occurrence of pathogens, when compared to that of hand deboned meats. Numerous microbial infections, because of multiplied contact between people and machinery in food processing, are commonly known in MRM production. Depending on raw materials, the presence of bacteria in final product can range from 10^4 to 10^9 / gram of the product [Lee et al. 1984].

One of the negative effects, that is observed as the result of the recovery process, is comminuted structure of MRM characterized by a high level of connective tissue and bone particles apart from fat, which contribute to its low technological value. Therefore, MRM can be used in meat processing only in limited quantities regulated by national and international standards [Field 1983]. More liberal standards are applied to MDPM. The only requirement to be met to limit excessive usage of MRM is related to chemical composition and the content of some heavy metals. The technological characteristics of MRM become diminished during frozen storage, which does not eliminate the oxidation processes. After heat treatment the meat is characterised by developing strong warmed over flavour (WOF). Therefore, it is not recommended to store meat in frozen state longer than 2 or 3 months, if there is such a necessity at all [Trziszka et al. 1994].

To improve the quality of MDM an attempts were made to extract the functional proteins and remove excessive fat and haeme pigments by centrifugation of salt-soluble and extractable proteins from MDPM by using relatively mild ionic strength aqueous solutions at pH 7 and recovering the proteins by adjusting the pH to 4.5. The procedures are similar to those applied for production of surimi from fish wastes [Betti and Fletcher 2005]. Many studies have been conducted to elucidate optimal processing conditions to produce surimi-like protein isolates from mechanically deboned meat and poultry [Hernandez et al. 1986; Wimmer et al. 1993, Kijowski and Richardson 1996a b, Stangierski and Kijowski 2000]. Similar technologies to extract the pigments and fat from MDPM with sodium bicarbonate have been investigated [Hernandez et al. 1986, Dawson et al. 1988, Yang and Froning, 1992]. Although the process significantly reduced haeme and fat contents and resulted in a lighter-colored product, the final product yield was low and was still susceptible to oxidative rancidity [Dawson et al. 1989 1990]. It caused that fat reduction in MRM was of great importance as it improved the quality of this raw material. Laboratory experiments showed 95% reduction in fat content (Smith and O'Nill 1997) and it reached even 99% in pilot plants (Knight et al. 1991). Recently, a new method has been developed to separate proteins from low value muscle tissues [Betti and Fletcher 2005].

The process of "washing" myofibrillar proteins from fish with the resultant surimi product – raw material for highly profitable production of crab analogues – has been a stimulus for seeking similar procedures to be applied in poultry and red meats industry, using mechanically recovered meat [Kopeć et al. 1994].

The production of crab analogues requires surimi of white colour, and therefore at the first stage of research studies the efforts have been made to remove as much pigments as possible from MRM, by using carbonate, phosphate, acetate buffers, NaCl solutions or water. The biggest reduction in redness, by 74–82%, was achieved in buffered systems [Dawson et al. 1989, Kopeć et al. 1992, Yang and Froning 1992]. However, at least four stage extraction was necessary to reached the same level of decolourisation with the eluent excess of 2:1, which means about 8 dm^3 water (solutions) per 1 kg MRM [Kopeć et al. 1994].

The use of surimi-like materials as a substitute ingredient has been reported in numerous previous publications. Perlo et al. [2006] reported that washed chicken meat may be used as a substitute ingredient in chicken nuggets for up to 40% of the meat in the formulation. Ano-

ther study showed that spent hen surimi can replace around 40–60% of the meat in sausage formulations [Jin et al. 2009].

Although surimi-like material can be used in large proportions in product formulations, some research has shown that optimum results can be reached with lower proportions of surimi. Desmond and Kenny [1998] found that the optimum formulation of the frankfurter type sausage contained 7–10% beef heart surimi. Similary, McCormick et al. [1993] described good results, when 5% of restructured roast meat formula was replaced with mutton surimi. Jin et al. [2009] reported optimal results for imitation crab sticks using 5.5–11% spent hen surimi as a substitute ingredient [Kurina Ramadhan 2010].

The most simple and quick way of utilization in food industry is to substitute poultry breasts by the protein isolates. In the first phase of myofibrillar protein preparation from MDPM, implementation into food processing, it was found that the isolate can replace up to 20% of poultry breasts in such products as ham, loin, without deteriorating the quality and decreased productivity [Kopeć 1994].

The aim of the study was to verify the functional properties of different type of poultry meat raw materials, including various kinds of MDM, to be predicted as a raw material for further meat products processing and also for an isolation of myofibrillar proteins.

Material and method

The experimental material was turkey meat removed from hens carcasses (crossbreed Big6). Turkey hens were slaughtered in age of 15 weeks in "Bomadek" meat company (Trzebiechów, Poland). After post-mortem treatments (8 hours after slaughter) carcasses were subjected to mechanical cut up. Following types of meat were collected and used in the study: fillet from breast muscle (white meat) and meat from wing with or without skin (control). Subsequently bone elements (frame and cut off trunk, wing bones, leg bones) were separated from meat and deboned by Beehive RSTD-06 separator (Beehive, Utah, USA). As a result of deboning five kinds of MDPM were obtained: SMDM from sternum, FMDM from frames, WMDM from wing bones, LMDM from leg bones and mixed MMDM (bone elements proportionally obtained at dissection of carcasses).

The material for study was obtained from three different farms. The following analyses were performed:- basic chemical composition: dry matter PN-ISO 1442:2000, the nitrogen by Kjeldahl-method (984.13) using Kjeltec 2300 Foss Tecator apparatus (Sweden), crude protein by multiplying of N-content by 6.25, crude fat PN-ISO 1444:2000. Hyroxyprolina content according to PN-ISO 3496:2000.pH value was monitored by combined electrode with pHmeter at room temperature. Colour of studied kind of meats was measured using a Minolta Chromametr CR200, and expressed as a colour indicates L*, a* and b*. An average value for all determinants was obtained and calculated from three consecutive measurements.

Water binding capacity WBC of studied meats was performed according to Wierbicki method. Studied meats were homogenized with redistilled water or NaCl solution (1/w : 2/v) 4 minutes. Then homogenates were centrifuged at 3,500 turns per min for 10 minutes. The supernatant was poured into cylinder and the volume was measured.

For all other determinations experimental material was homogenized with redistilled water 1:4 (w/v). For pH analyses homogenates after 60 minutes was filtered through Whatmann filter paper. In obtaining filtrates acidity was monitored by combined electrode with pH-meter.

Surimi yield was calculated from protein content and volume of myofibrillar fraction collected after centrifugation of the homogenates.

The analyses were done in 6 replicates and differences were statistically verified with StaSoft Statistica Softwere 10.0.

Results and discussion

Type of raw materials significantly influenced the content of dry matter, protein, fat and slightly collagen. The lowest amount of dry matter was noticed in breast and wings muscle (26%), whereas the highest value was observed in mechanically deboned meat from wings, thighs and mix type of meats (Tab. 1). In mechanically deboned meat from trunk and sternum dry matter content amounted between 34.6–34.8%. Protein content in analyzed raw material varied from 13.5% for mechanically deboned meat mixed type to 23.6% for turkey hens breast muscles. Whereas, the collagen content amounted about 1% for MMDM and about 0.5% for breast muscle tissues.

Mayer et al. [2007] found also large variations in chemical composition of MRM derived from different bone components.

Table 1

Chemical composition of studied kinds of meat

Raw material	Parameters			
	Dry matter [%]	Total protein [%]	Fat [%]	Collagen [%]
MMDM	39.44 ^{C}	13.53 ^{A}	20.33 $^{C.D}$	1.17 ^{B}
FMDM	34.80 ^{B}	17.05 ^{C}	17.42 $^{B.C}$	1.34 ^{B}
SMDM	34.61 ^{B}	17.34 ^{C}	16.01 ^{B}	1.28 ^{B}
LMDM	39.91 ^{C}	15.38 ^{B}	23.19$^{D.E}$	1.16 ^{B}
WMDM	41.16 ^{C}	14.50 $^{A.B}$	24.77 ^{E}	1.25 ^{B}
Meat from wing with skin	26.42 ^{A}	23.21 ^{D}	3.73	1.58 ^{B}
Meat from wing without skin	27.77 ^{A}	22.77 ^{D}	3.53 ^{A}	1.23 ^{B}
Breast muscle	25.91 ^{A}	23.64 ^{D}	3.84 ^{A}	0.52 ^{A}

A,B,C,D – the same letter in indices of means in the column indicates no statistically difference ($p \leq 0.05$)

The highest content of total fat was observed in MDM from wings (24.8%) and MDM from leg bones (23.2%). The lower fat content was noticed in contamuscles. Total fat amounted 3.8 % in breast muscle and 3.7 in muscles cut off from wings. Pussa et al. [2009] reported that MDM derived from chicken, turkey and pork was characterized by fat content equaled 14.7, 20.3 and 25% respectively. Whilst, HDM contained 1.4, 2.1 and 2.0% fat for chicken, turkey and porcine raw materials, respectively.

A brighter red colour in mechanically deboned meat, compared to the colour of hand-boned meat, was due to the addition of haeme pigments from red bone marrow and to the elimination of connective tissue, which was devoid of pigments [Field 1981]. In Table 2 the results of colour trial analyses of different MDM types are presented. It was observed that meat from

wings and MDM from trunk were characterized by the highest L* value. Slightly lower L* value (but statistically significant) was observed for MDM mix type and MDM from sternum and breast muscles. MDM from thighs and MDM from wings were characterized by the lowest L* value. High value of a* parameter showed high amount of haeme compounds in mechanically deboned meat. The a* value was the highest for all studied mechanically deboned meat (17–18), while breasts and wings muscles were characterized by the low a* value (7–9). The b* value indicated contribution of yellow color. MDM from legs, sternum, wings bones and mix type were characterized by the highest value of b* parameter. The lowest contribution of yellow color was observed in breasts and wings muscles (control sample).

Table 2

Color characteristics depending on the kind of turkey meat

Raw material	**Colour**		
	L*	**a***	**b***
MMDM	50.28 ^{B}	17.07 ^{B}	10.99 $^{C.D}$
FMDM	52.75 $^{B.C}$	16.85 ^{B}	10.31 ^{C}
SMDM	51.67 ^{B}	17.18 ^{B}	10.92 $^{C.D}$
LMDM	45.29 ^{A}	17.96 ^{B}	12.03 ^{D}
WMDM	45.34 ^{A}	16.98 ^{B}	11.37 $^{C.D}$
Meat from wing with skin	55.42 ^{C}	8.94 ^{A}	8.42 ^{B}
Meat from wing without skin	52.54 $^{B.C}$	8.33 ^{A}	6.67 ^{A}
Breast muscle	51.80 ^{B}	6.96 ^{A}	7.78 $^{A.B}$

Supernatants obtained after centrifugation of homogenates of whole, control muscles were subjected to pH measurements, which revealed that the highest acidity was observed in not deboned sources (up to 6.8) (Tab. 3). The acidity of extracts from mechanically deboned meat amounted from 5.75 (MDM from sternum and thighs) to 6.10 (MDM mix type).

Mechanically deboned mix type meat was characterized by the highest pH value (6.28). The lowest pH value was observed in mechanically deboned meat from sternum (5.74). Breast and wings muscles were characterized by pH value about 6.2.

Table 3

Acidity and pH value of studied kinds of meat

Raw material	Parameters	
	pH	Acidity of the supernatants
MMDM	6.28^{B}	6.11 ^{A}
FMDM	6.04$^{A.B}$	5.83^{A}
SMDM	5.74^{A}	5.74 ^{A}
LMDM	5.80$^{A.B}$	5.74 ^{A}
WMDM	6.11$^{A.B}$	6.00 ^{A}
Meat from wing	6.22$^{A.B}$	6.85 ^{B}
Breast muscles	6.23$^{A.B}$	6.78 ^{B}

The highest water binding capacity was observed in control samples (breasts and wings muscles) (Tabl. 4). In case of deboned raw materials high value of WBC was noticed for MDM from legs and wings bones, but no significant differences among mechanically deboned meats were observed. The similar results were previously published by Kopeć et al. (1994) and Nurkheriyati et al. [2011].

Table 4

Water binding capacity of the studied kinds of meat

Raw material	Parameters	
	Water binding in NaCl solution (%)	Water binding in H_2O extracts (%)
MMDA	83.7 ^{A}	39.0 ^{A}
FMDM	83.6 ^{A}	41.3 ^{A}
SMDM	82.7 ^{A}	40.0 ^{A}
LMDM	85.8 ^{A}	43.3 ^{A}
WMDM	84.7 ^{A}	41.7 ^{A}
Meat from wing	91.2 ^{B}	49.3 ^{B}
Breast muscle	92.2 ^{B}	48.1 ^{B}

Extractability of myofibrillar proteins from various type of meat and mechanically recovered meat sources are presented in table 5. The highest amount of myofibrillar proteins (surimi) (19–23%) was isolated from breast and wings muscles. Whilst the lowest yield of recovered proteins was stated for LMDM and WMDM (5–8%).

Table 5

Extractability of myofibrillar protei from different kind of turkey meat

Raw material	Myofibrillar isolate surimi like (% of protein in relation to tantal protein)
MDA	10.35^{A}
FMDM	17.00^{B}
TMDM	19.38^{B}
LMDM	5.46^{A}
WMDM	8.30^{A}
Meat from wing without skin	19.18$^{B.C}$
Meat from wing with skin	23.10^{C}
Breast muscle	21.32^{C}

Kopeć et al. [1994] found about 25% of extractable myofibrillar proteins as a surimi like fraction, when MDM from sternum or spent duck meat were processed.

In own studies for high quality MDM from sternum 20% of total protein was obtained as myofibrillar isolate. For low quality MDM prepared from leg or wing bones the yield of surimi fraction was low (5–8%) and for MDM prepared from all bone raw metional 10% yield of myofibillar protein isolate was found.

Conclusion

The potential technological suitability of mechanically deboned turkey meat for further product processing assessed by functional properties and myofubrillar protein extractability depends on the bone raw materials used for separation process.

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16

CHICKEN BONES AS AN ALTERNATIVE RAW MATERIAL IN ACIDIC-ENZYMATIC PROCESS OF COLLAGEN EXTRACTION

Introductions

Meat industry by-products contain high amount of organic substances (51–81%), which are risky for an environmental and they have to be continuously neutralized. Non-utilization or under utilization of by-products not only lead to loss of potential revenues but also added [Jayathilakan et al. 2011] and increasing cost of disposal of these products. Solid by-products are utilized as an animal fodder, fertilizers, fuels additives, as well as they can be good raw material for collagen productions [Szabat 1989, Sobczak, Błyszczek 2009].

Eleven percentage of pork carcasses, 15% of beef carcasses and 16% of lamb carcasses are bones. These values are higher if they include the meat clinging to the bone. The marrow inside some of the bones can also be used as food. The marrow may be 4.0–6.0% of the carcass weight. For centuries, bones have been used to make soup and gelatin [Liu et al. 2001]

Bones are utilized most often for production of gelatin, which is extracted from ossein. To obtained ossein bones are prepared by cooking them at 80-95°C to remove the adhering meat, gristle and fat, They are then washed several times to get the bones clean. Next, the bones are washed in about 6% hydrochloric acid to remove the minerals. In general, the final ossein is about 1.0–2.0% of the total raw bone weight [Liu et al. 2001].

In the food industry collagen is mainly used for gelatin and protein's casings production. Gelatin is extracted from animal tissues rich in collagen type I (skin, bones) and type II (cartilages). In Europe gelatin is produced from pigs skin (49%), cattle skin (16%) and animal's bones (35%). Gelatin is utilized as an additive in dairy, meat, confectionary, fish, bakery and pastry products and also in beverages. In canned meat products gelatin bounds and holds the meat juices as well as emulsifies fat, in cooked ham it holds water and improves color stability, whereas in brawns and gelatin forms the matrix gel in witch solids particle are suspended. Moreover, collagen can be used as a gelatin coating to prevent drying and oxidation of the meat products. Gelatin is produced by the thermohydrolysis of collagen in two types: A by an acidic process with superior quality, and B after an alkine treatment. Below 40°C gelatin binds water and swells, while at higher temperatures is melts and gelatin gels are thermoreversible [Tederko1995, Makała, Tederko 1999, Smolińska, Kopeć 2009].

Most commercial gelatins are made from the hide of porcines and bovines and to a lesser extent from their bones. Poultry and fish by-products are seldom used as a source of ge-

latin. Traditional sources of gelatin posecertain problems. For example, Jewish and Muslim communities do not accept pork gelatin [Badii and Nazlin 2006], and beef gelatin is acceptable only if it has been processed according to their religious requirements, which will vary. On the other hand the major defect of fish gelatin is its fishy odor [Cho et al. 2004b]. These considerations have encouraged production of gelatin from poultry waste derived by mechanical deboning operations as a replacement for mammalian gelatins [Rafieian et al. 2011].

Collagen can be isolated from various tissues by use of organic (i.e. acetic, citric, lactic) or non-organic acids (i.e. hydrochloric), as well as by application of enzymes [Skierka, Sadowska 2007].

Acid extraction with pepsin hydrolysis was a common method for collagen extraction in recent time. Specifically, acetic acid has frequently been used as a solvent for collagen extraction and a number of collagens were extracted from marine animals by acetic acid [Nagai et. al. 2004, Sadowska et. al. 2003]. Liu et. al., [2001] had discussed the four acid extractions of collagen from broiler chicken feet [Cheng and Hsu 2009].

The process of acid extraction is carried out in cold conditions. In the first stage the material is washed with physiological salt solution to remove soluble proteins and polysaccharides. Following, the diluted low ionic strength acids (i.e. 0.5 M acetic acid, hydrochloric acid with pH 2.0–3.0) are applied in order to break the aldimine intermolecular bounds, which leads to swelling of the fibrous structure of collagen. Such acids do not act on more stable ketoimine bounds, thus collagen from bones, cartilages and old animals, containing high amount of this type of bonds, is almost insoluble. Only about 2% of collagen fibers are dissolved by diluted acids and salts, and they can be restored by pH or temperature adjustment. The remaining 98% of collagen is insoluble, and it can be isolated by strong alkyls or enzymes without the helical structure distortion [Freiss 1998].

Enzymatic hydrolysis is usually carried out with non-specific enzymes like pepsin, trypsin, pancreatin, ficin, bromelanin and papain [Skierka, Sadowska 2007]. Enzymes are obtained from animal's maw, lizosomes, various tissues, plants and microorganisms [Temiz et. al. 2008]. Collagenase is not utilized due to total and irreversible degradation of collagen fibers [Quinn et. al. 1990]. Non-specific enzymes do not decompose native collagen, but they act on the non-helical fragments at the edge of the molecule. By cutting out the telopeptides enzymes remove intermolecular bonds without the triple helical structure destruction [Hickman et al. 2000, Hang et al. 2007, Nalinanon et al. 2008].

The objective of the study was to compare collagen extraction in hydrochloric acid solution from pig and chicken bones and the method of acidic-enzmatic extraction using citric acid and pepsin.

Materials and methods

Materials used in the study were poultry and pigs bones. Bones were cut off from animals carcasses and cleaned by removing adjacent muscle and fat tissues and skin. Then, the materials were frozen at -18°C, mechanically comminuted and thermally treated in water conditions (pH 5.0) at 75°C for 40 minutes.

Acidic and enzymatic methods were applied for collagen isolation from raw pigs skin and bones, as well as pigs and poultry bones after thermal treatment. Acidic hydrolysis was performed in citric and hydrochloric acids at pH 2.0, whilst an enzymatic hydrolysis by

proteolytic enzyme pepsin (1 mg/g material) in citric and hydrochloric acids (pH 2.0). Hydrolysis of the thermally treated material was carried out in 6% hydrochloric acid. Collagen extraction was carried out by continuous shaking for seven days at room temperature (around 18°C). pH of the solution was controlled every two hours in first and second day, then every 12 hours up to the end of the process. The ratio between bones and the reaction solution was as 1:4 (w:w). After a certain time of the reaction samples were divided into solutions containing collagen and part consisted of undigested bones and skin. Samples were then analyzed for dry matter, total protein, ash, fat and hydroxyproline content. Collagen content was calculated on the basis of the mentioned above chemical composition results. All analyzes were duplicate in three consecutive experimental series. Collected data were statistically analyzed by Statistica 9.0. using ANOVA one-way analysis of variance Duncan test at $p>0.05$.

Results and discussion

The results collected in the study showed that protein content in raw materials changed significantly when the thermal treatment was applied, either for pigs or poultry bones (Tab. 1). Total protein content in dry matter increased after thermal operation for poultry bones from 42 to almost 55%. Whilst, the opposite results were obtained for pigs bones, when decrease in protein content was stated.

Table 1

Protein content in dry matter of an experimental materials

Protein content in dry matter [%]	Raw materials	Thermally treated materials
Pigs bones	28.7^{c}	38.5^{b}
Poultry bones	41.9^{b}	54.6^{a}

Statistical analysis of the collected results revealed significant effects of hydrolysis conditions applied in raw pigs bones on dry matter, collagen and ash content (Tab. 2). No influence of the working conditions on total protein and fat content in the hydrolyzed pig boness was noticed. The highest collagen content was analyzed in pigs bones after hydrolysis in hydrochloric acid (pH 2.0) – 16.8%. When citric acid was used to hydrolyze raw pigs bones around 16% of collagen was obtained. Enzymatic hydrolysis in citric acid was found the most effective, only 15% of collagen was analyzed in pigs bones post-extraction remaining.

Table 2

Collagen content and recovery after hydrolysis of pigs bones

parameter	Time of the hydrolysis [days]	Type of the hydrolysis				
		6% HCl	Acidic hydrolysis		Enzymatic hydrolysis	
			HCl pH 2.0	Citric acid pH 2.0	HCl + pepsin pH 2.0	Citric acid + pepsin pH 2.0
Hydroxyproline content [%]	3	0.065^{a}	0.005^{d}	0.012^{d}	0.046^{b}	0.040^{b}
	7	0.070^{a}	0.013^{d}	0.026^{c}	0.051^{b}	0.043^{b}

Table 2. continuous

collagen [%]	3	0.49^{a}	0.04^{d}	0.09^{d}	0.35^{b}	0.30^{b}
	7	0.53^{a}	0.10^{d}	0.20^{c}	0.39^{b}	0.33^{b}
Post-extraction solutions purity [%]	3	60.33^{a}	29.38^{de}	45.00^{bc}	40.76^{cd}	38.47^{cd}
	7	53.81^{ab}	45.55^{bc}	25.89^{e}	41.63^{cd}	36.67^{cde}
Collagen recovery [%]	3	9.65^{e}	1.04^{i}	4.07^{g}	9.22^{f}	12.62^{b}
	7	10.44^{d}	2.88^{h}	9.04^{f}	11.20^{c}	13.86^{a}

Application of hydrolysis had also a significant impact on collagen level in poultry bones (Tab. 3). The lowest collagen content was analyzed in poultry bones when enzymatic process either in hydrochloric or citric acids were performed (around 13.5%). Similarly to pigs bones, acidic hydrolysis of poultry bones in hydrochloric acid (pH 2.0) was characterized by the highest collagen loss (fibrous protein level in post-reaction mass 16%).

Table 3

Collagen content and recovery after hydrolysis of poultry bones

parameter \	Time of the hydrolysis [days]	Type of the hydrolysis				
		6% HCl	Acidic hydrolysis		Enzymatic hydrolysis	
			HCl pH 2.0	Citric acid pH 2.0	HCl + pepsin pH 2.0	Citric acid + pepsin pH 2.0
Hydroxyproline content [%]	3	0.071^{a}	0.003^{d}	0.007^{d}	0.032^{b}	0.030^{bc}
	7	0.076^{a}	0.004^{c}	0.014^{c}	0.070^{a}	0.045^{b}
collagen [%]	3	0.52^{a}	0.02^{d}	0.05^{d}	0.23^{b}	0.22^{bc}
	7	0.55^{a}	0.03^{c}	0.10^{c}	0.51^{a}	0.33^{b}
Post-extraction solutions purity [%]	3	43.51^{a}	6.33^{d}	15.02^{cd}	22.70^{bc}	23.06^{bc}
	7	42.84^{a}	9.38^{d}	31.24^{ab}	36.96^{a}	31.83ab
Collagen recovery [%]	3	13.20^{c}	0.56^{h}	2.56^{g}	6.94^{e}	11.28^{d}
	7	13.96^{b}	0.95^{h}	5.12^{f}	17.06^{a}	16.91^{a}

Collagen content and recovery in thermally treated pigs bones were significantly dependent on used hydrolysis method. Moreover, only in the case of citric acid (pH 2.0) significant differences in collagen concentration was observed. Processing of pigs bones in citric acid resulted in 0.09% and 0.2% of collagen content and 4% and 9% of protein recovery after 3 and 7 days of extraction, respectively (Tab. 4).

The highest recovery of collagen (14%) from bones was stated for post-hydrolysis solutions, when enzymatic process in hydrochloric acid was carried out. Slightly lower level

of collagen recovery (11%) was noticed for pepsin action in hydrochloric acid at pH 2.0. Hydrolysis of bones in weak acids i.e. citric and hydrochloric (pH 2.0) resulted in 9% and 2.9% of collagen recovery. Thus, it can be stated that pepsin used for hydrolyzing bones in hydrochloric acid at pH 2.0 was almost four times more effective than process carried out without the enzyme. When citric acid (pH 2.0) was applied for bones hydrolysis for 7 days around 9% of collagen was obtained, whereas pepsin addition to the reaction mixture resulted in 14% of collagen recovery.

Table 4

Protein content of an experimental materials after hydrolysis

protein content [%]	Type of the hydrolysis					
	control	6% HCl	Acidic hydrolysis		Enzymatic hydrolysis	
			HCl pH 2.0	Citric acid pH 2.0	HCl + pepsin pH 2.0	Citric acid + pepsin pH 2.0
Solid remainings of pigs bones after hydrolysis	17.8^{a}	16.9^{a}	16.1^{a}	16.6^{a}	17.0^{a}	15.1^{a}
Solid remaining of poultry bones after hydrolysis	17.4^{a}	16.5^{ab}	17.3^{a}	16.6^{ab}	16.8^{a}	15.8^{b}
Post-extraction solutions of pigs bones		0.99^{a}	0.22^{d}	0.78^{c}	0.93^{b}	0.90^{b}
Post-extraction solutions of poultry bones		12.0^{c}	6.79^{d}	17.3^{b}	25.5^{a}	25.3^{a}

All post-hydrolysis solutions were characterized by lower than 50% collagen content in the total protein, that caused low purity of analyzed solutions. Additionally, prolongation of the time of hydrolysis resulted in further lowering of collagen concentration and the purity of solutions, what was due to undergo hydrolysis of non-collagenous proteins.

Processing of thermally treated poultry bones effected in different results in relation to pigs bones. The most effective collagen recovery (17%) was found in solutions from enzymatic hydrolysis either in hydrochloric or citric acids, whereas the lowest yield of protein was stated for hydrochloric acid (pH 2.0). In all analyzed solutions an increase of collagen concentration was measured. An important disadvantage of the hydrolysis of thermally treated poultry bones was relatively low purity of obtained collagen solutions (less than 50%). Samples collected after three and seven days of extraction in hydrochloric acid (pH 2.0) were characterized by 6.3% and 9.4% collagen content in total protein, respectively. However, prolongation of the time of hydrolysis tend to an increase in collagen content and the purity of obtaining solutions.

Application of 6% hydrochloric acid resulted in high effectiveness of the total protein as well as collagen dissolution. Pigs bones demineralization by hydrochloric acid (pH 2.0) effected in 3% collagen content, whilst only 1% of the protein was recovered from poultry bones at the same conditions. When higher concentration of hydrochloric acid (6%) was

used for collagen extraction around 14% of the protein was recovered from poultry bones and 10% from pigs bones. It was also revealed that citric acid acted more effectively than hydrochloric acid (pH 2.0) but less effectively than concentrated hydrochloric acid. Respectively, 9% and 5% of collagen was recovered from pigs and poultry bones during hydrolysis by citric acid.

Skierka and Sadowska [2007] reported that acetic acid was the most effective in collagen isolation from Baltic cod skin, whilst the least recovery of the protein (18%) was obtained when hydrochloric acid was applied.

In our study collagen recovery was on the level of 0.23% when raw pigs bones where used as an experimental material and 0.9% and 2.9% when thermally treated pigs and poultry bones were processed. When citric acid was the working medium of the hydrolysis of previously heated pigs and poultry bones 9% and 5% of the fibrous proteins were recovered.

Skierka and Sadowska [2007] showed that maximum 60% of collagen was isolated by citric acid, whereas almost 90% when acetic and lactic acids were applied. According to the literature data solubility of collage in acidic conditions is not complete due to the fact that used acids did not fully release collagen molecules and intermolecular bonds were still found. Moreover, very low pH, for example for 0.15 M HCl solutions (pH 0.87), effected in lowering the ability to bind water by collagen particules. In self experiment pH of the applied solutions did not exceed 2.0 what resulted in low yield of the collagen extraction.

Results from Cheng et al. [2009] showed that acetic acid (FA) and lactic acid were the effective solvents for collagen extraction from silky fowl feet. Extraction yield of collagen amounted 7% (FA) and 8,34 % (FL).

Higher amount of collagen obtained during enzymatic hydrolysis in relation to acidic process showed that pepsin cut the netting bonds in telopeptides without disruption of the triple helical structure of the molecule Kopeć et. al. [2006] revealed the same relations for mechanically deboned poultry meat (MDOM) showing that citric acid was able to release 36% collagen from tendinous fraction of MDOM, whilst pepsin added to the reaction medium effected in 84% of the protein recovery. Nagai et. al. [2008] reported that only 1% collagen was recovered from Marine mammals when 0.5 M acetic acid was applied, whilst pepsin addition increased the recovery of the fibrous proteins up to 28%.

Skierka and Sadowska [2007] showed that apart from the increased effectiveness of the process enzymatic hydrolysis in lactic acid for 24 hours resulted in total collagen dissolution. In our study prolongation of the time of hydrolysis had a positive impact on the final solutions. The highest collagen recovery (90%) was analyzed for enzymatic hydrolysis of pigs skin in hydrochloric acid. Cheng et. al. [2009] reported that the effectiveness of an enzymatic hydrolysis of skin from poultry paws in hydrochloric acid was the same level as in acetic acid and collage recovery was around 51%. Result from Lin and Liu [2006] showed that pepsin digestion at a high temperature (12–18°C) and long time (48-72h) resulting in higher yield of telopeptide-poor collagen from bird feet (TPCBF) than at lower temperature (4°C) and shorter time.

Conclusion

Poultry bones are, as good raw material for collagen extraction, as pig bones when strong acidic or enzymatic extraction with pepsin is applied for the process.

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