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Scientific Research on the Production of plant-based Meat Analogs using the Wet Extrusion Process

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It is more and more necessary to select and provide model systems that fit to personalised nutrition, avoiding usage of main allergens. It can be achieved by combining raw materials of various origins, extracted using sustainable technologies, but at the same time maintaining the qualitative and quantitative characteristics of the product acceptable to specific user groups. Our research is distinguished by the processing of vegetable press-cakes and their combination with protein matrices, as well as the determination of the properties of these matrices. New methods of raw material processing can increase the bioavailability of essential nutrients in the raw material and in the matrix of final products and can promote positive changes in the microbiota. The consumption of fibrous materials is constantly growing, new sources of fibres are being sought, fibre-enriched food matrices are being developed, and consumer acceptability is being assessed.
The aim of this study is to analyse the possibilities of applying innovative wet extrusion technology to produce raw meat analogs by composing products with optimal composition and sensory properties, testing a new, non-thermal high-pressure processing preservation method. When developing models for the processing of matrices of plant and animal raw materials using high-pressure technology, for varying parameters of pressure, time, packaging and duration, it was established that the high-pressure exposure model must be selected taking into account the composition and properties of the product, the purposeful application of other microorganism inhibiting factors to achieve synergistic action, because exposure to high pressure alone does not ensure the destruction of bacteria in a model system. To create matrixes of raw materials of balanced composition of vegetable raw material, berry press-cakes and meat analogues of legume flour, five variations of balanced composition and nutritional value were created based on beetroot, pumpkin, carrot, black currant, Jerusalem artichoke and legume matrix.

1. Introduction

The purpose of this study is to analyse the possibilities of applying innovative wet extrusion technology to produce meat analogues. Vegetable and berry press-cakes were used as a source of soluble and insoluble fibre and other biologically active substances, combining them with protein matrices, composing products with optimal composition and sensory properties, testing the high-pressure preservation method. It is a preservation method in which harmful microorganisms are inactivated by pressure at a low temperature (<45 °C), so that the effect on taste, texture, appearance, or nutritional value is minimal (Hite, 1899).
This technology is considered one of the best innovations in food processing in 50 years (Dunne, 2005). High pressure treatment is effective in inactivating *E. coli, Salmonella spp.* and *Vibrio spp.*, yeasts, moulds and extending shelf life (Hayman et al., 2004; Ahn et al., 2007).
Gram-positive bacteria are more resistant than gram-negative, larger and more complex organisms are easier to inactivate (Hite, 1899). *Clostridium* or *Bacillus* spores can also be inactivated by pressure, and sterility is achieved with lower thermal effects (Margosch et.al., Matser et.al., 2006; Black et.al., 2007), only a longer duration of higher-pressure regime is required (Delacour et.al., Reddy et.al., 2006; Setlow, 2008).

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To eliminate spores at high pressure, the process can be divided into two stages, as in the case of tyndallisation, the first effect at lower pressure promotes the germination of spores, then the germinated spores are inactivated (Reddy et.al., 2006). Microorganisms with stiffer membranes are more sensitive to pressure (Ritz et.al., 2002). Under pressure the cell area and volume increase due to denaturation of membrane proteins or changes in lipids (Yaldagard et al., 2008; Moussaet al., 2006; Heremans 2005), which likely results in ion leakage from the cell (Tholozan et.al., 2000). The mechanism of inactivation of yeast and microscopic fungi by high pressure is analogous (Smeller 2002; Black et.al., 2007). Yeast mitochondria may be one of the elements damaged by pressure (Perrier-Cornet et al., 1999). The high structural diversity of viruses is also reflected by the unequal resistance to pressure (Brul et al., 2000).
High pressure processing has many advantages, including preservation of vitamins and flavor compounds and low energy requirements (Smel 1998). There is an opportunity to change the functional and sensory properties of various food components with little change in the feeling of freshness (Yaldagard et al., 2008; San Martin et al., 2002). Thus, a positive effect of high pressure on the functional and sensory properties of model systems based on vegetable pomace and plant proteins is expected.

2. Research objects and methods

2.1 Microbiological research methods

The total number of microorganisms was determined by the method of seeding in Petri plates, using the media for determining the total number of microorganisms (Plate Count Agar, LAB M). After the medium has solidified, the plates are inverted and stored for 72 h ± 3 h at 30 ℃.
Determination of individual bacteria as the number of coliform bacteria at 37 °C, CFU/g; *Salmonella spp*. detection 25 g, total number of mesophilic lactic acid bacteria, CFU/g, number of presumptive bifidobacteria, CFU/g, total number of sulfite-reducing bacteria (*Clostridia perfringens*), CFU/g, yeast count, CFU/g, number of molds, CFU/ g, detection of monocytogenes listeria (*Listeria monocytogenes*) at 37 °C, 25 g, performed using standard test methods.

2.2 Chemical research methods

Chemical parameters were determinated according to standard ISO methods. Total fat content (LST ISO 1443:2000;). Moisture content, % (LST ISO 1442:2000). Protein content, % (Calculated by multiplying the nitrogen content by a factor of 6.25 (Regulation (EU) No. 1169/2011 of the European Parliament and Council, Annex I, p. 10). Total ash content, % (LST ISO 936:2000). Fiber content, % (AOAC 985.29, 1990). Total carbohydrate content, %, (Carbohydrate (excluding fibrous substances) content, %, Calculated from the difference according to "Food composition", 2002, Vilnius). Energy value of 100 g, kcal; Energy value of 100 g, kJ (Calculation according to Regulation (EU) No. 1169/2011, Annex XIV).

2.3 Preparation of model systems

The composition of dry blackcurrant, Jerusalem artichoke, beetroot, pumpkin, carrot and leguminous plant flour mixed with water, and final bater was extruded. Extrusion: samples were extruded using a co-rotating twin-screw extruder ZE25Rx40D-UTXmi (KraussMaffei Berstorff GmbH, Germany) composed of eleven segmented barrels. The screws had a diameter of 26.6 mm, a length to diameter ratio (L/D ratio) of 40:1, and 100 rpm was set as the screw speed. The barrel diameter was 26.9 mm and die head with three circular holes had a diameter of 3.5 mm. Temperature profiles throughout the eleven-barrel segments were applied as follows (in °C): i) 18, 65, 85, 95, 100, 105, 110, 110, 110, 110 and product exit temperature (recorded).The extruded mass is placed in a vacuum package, 300 g each, frozen, and stored at -18 °C until the test. Parallel samples of 300 g each. processed in Hyperbaric 3000x3 min. and Hyperbaric 6000x3 min. mode, stored at +4 °C.

3. Results and discussion

The basis of model matrices is beetroot, pumpkin, carrot, blackcurrant, Jerusalem artichoke flour. Analysing their characteristics, we can see that blackcurrant flour has the most protein at 13.4±0.17% (Table 1), followed by beetroot at 10.6±1.1%, while pumpkin, carrot and Jerusalem artichoke flour has less protein. Blackcurrant flour also contains the most fat, 7.3±0.5%, the fat content of other raw materials does not exceed 2%, the most carbohydrates are in beetroot and pumpkin, fibers were not determined in all samples, but 48.3±3 were found in blackcurrant flour, 7%.
Next, these raw materials were mixed with pea flour and the mixture with water was processed by the wet extrusion method. The energy value of the model systems, expressed as the energy value of 100 g, kcal, reaches 179±6.57, thus a similar energy value of all compositions was achieved, and it was also possible to balance the moisture content, which fluctuates around 55.84±1.58%.

The amount of protein is an important indicator when composing the raw material of meat analogues, it ranges around 30.9±1.35%. The results of the assessment of fats (3,8±0,52), sugars and fibre (3,14±0,31) are presented in table 1.

One of the goals of creating raw materials for meat analogues is to increase fibre in food to normalize the growth of beneficial microbiota in the gastrointestinal tract. Their amount in the compositions has been increased, the amount of fibre fluctuates around 3.14±0.31%.

Next, these raw materials were mixed with pea flour and the mixture with water was processed by the wet extrusion method. The energy value of the model systems, expressed as the energy value of 100 g, kcal, reaches 179±6.57 (Table 2), thus a similar energy value of all compositions was achieved, and it was also possible to balance the moisture content, which fluctuates around 55.84±1.58%. The amount of protein is an important indicator when composing the raw material of meat analogues, it ranges around 30.9±1.35%. The results of the assessment of fats, sugars and fiber are presented.

Table 1: Quantitative and qualitative indicators of dry plant raw material for the production of model systems

No.	*Indicator*	*Beet flour*	*Pumpkin flour*	*Carrot flour*	*Blackcurrant flour*	*Jerusalem artichoke flour*
1	*Moisture, %*	*10,9±0,3*	*13,1±0,7*	*9,5±0,7*	*8,4±0,3*	*6,4±0,5*
2	*Proteins*	*10,6±1,1*	*7,1±0,5*	*6,4±0,3*	*13,4±0.17*	*6,5±0,21*
3	*Fats, %*	*2,0±0,15*	*1,7±0,13*	*1,9±0,15*	*7,3±0,5*	*1,2±0,0*
4	*Ashes, %*	*7,5±0,5*	*5,7±0,5*	*4,0±0,3*	*2,6±0,21*	*3,9±0,3*
5	*Sucrose, %*	*45,6±1,7*	*21,2±2,1*	*21,7±0,5*	*1,0±0,0*	*28,3±1,7*
6	*Glucose, %*	*5,9±0,9*	*11,8±0,5*	*8,0±0,5*	*5,6±0,5*	*0,4±0,0*
7	*Fructose, %*	*5,1±0,1*	*19,3±0,5*	*11,2±0,2*	*7,9±0,3*	*2,6±0,0*
8	*Fibers, %*	*-*	*-*	*-*	*48,3±3,7*	*-*

Salmonella spp. and *Listeria monocytogenes* were not detected in the raw material (Table 3) either before processing or after exposure to high pressure. The total number of microorganisms is small, $(7.3\pm1.7)\times10^3$ CFU/g, after exposure to pressure of 3000 and 6000 atmospheres, it decreases to single cells. Analogous results with mold and fungi, a small amount is found in the initial composition, $(7.4\pm 2.3)\times10^2$ CFU/g, when exposed to a pressure of 3000 atmospheres, it is reduced to single cells, when exposed to a pressure of 6000 atmospheres, there are no living microscopic fungi, and no spores of mesophilic aerobic microorganisms are found. Coliform bacteria are not found either in the initial formulation or after treatment. Yeast in the initial matrix found $(4.4\pm0.5)\times10^2$ CFU/g, the yeast does not survive the pressure.

Table 2: Nutritional, compositional, quantitative, and qualitative indicators of model system of raw materials for meat analogues of plant origin

Indicator	*The result*
Moisture content, %	*55,84±0,58*
Protein content, %	*30,9±1,35*
Fat content, %,	*3,8±0,52*
Total sugar content, %	*3,25±0,43*
Fiber content, %	*3,14±0,31*
Total carbohydrate content, %	*6,26±1,57*
Energy value of 100 g, kcal	*179±6,57*
Energy value of 100 g, kJ	*753±12,51*

Evaluating the possibilities of high-pressure treatment to reduce microbial contamination of meat analogues hermetically packed exposed to pressures of 5000 and 6000 atmospheres were studied.
Investigations of raw pressure-treated raw meat analogues show high bacterial contamination, after examining 5 products of various composition, it was found that the total number of microorganisms reaches as high as $(6.7\pm1.3)\times10^7$, the number of coliform bacteria in some samples reaches $(5.9\pm1.7)\times 10^5$, only one found $(1.8\pm0.3)\times10^2$ CFU/g. The number of yeasts ranges from $(1.6\pm0.3)\times10^4$ to $(4.6\pm0.9)\times10^5$ CFU/g, the number of molds is also quite high, reaching $(6.2\pm1.3)\times10^4$ CFU/g .

Table 3: Quantitative and qualitative indicators of dry plant raw material for the production of model systems

Indicator	*Microbiological indicators, CFU/g*		
	Control, without pressure treatment	*Treatment 3000 atm.*	*Treatment 6000 atm.*
Total bacteria count, CFU/g	*$(7,3\pm1,7)\times10^3$*	*Yes, but $< 4,0\times10^1$*	*Yes, but $< 4,0\times10^1$*
Coliforms, CFU/g, 37 °C, CFU/g	*$< 1,0\times10^1$*	*$< 1,0\times10^1$*	*$< 1,0\times10^1$*
Salmonella spp. detection, 25 g	*Not detected*	*Not detected*	*Not detected*
Listeria monocytogenes detection, 25 g	*Not detected*	*Not detected*	*Not detected*
Molds, CFU/g	*$(7,4\pm2,3)\times10^2$*	*Yes, but $< 4,0\times10^1$*	*$< 1,0\times10^1$*
Yeast, CFU/g	*$(4,4\pm0,5)\times10^2$*	*$< 1,0\times10^1$*	*$< 1,0\times10^1$*
Mesophylic aerobic microorganism spore count CFU/g	*$< 1,0\times10^1$*	*$< 1,0\times10^1$*	*$< 1,0\times10^1$*

Table 4: Studies on the effect of high-pressure processing on the microbial contamination of raw meat analogues

Product Raw meat analogues	Processing pressure, atm.	Total bacteria count, CFU/g	Coliforms, CFU/g	Yeast, CFU/g	Molds, CFU/g
Meat analogue with beetroots	Control	$(9,3\pm2,1)\times10^6$	$(1,8\pm0,3)\times10^2$	$(4,6\pm0,9)\times10^5$	$<1,0\times10^1$
Meat analogue with pumpkins	Control, no treatment	$(6,7\pm1,3)\times10^7$	$(2,3\pm0,7)\times10^5$	$(3,9\pm0,7)\times10^5$	$(9,0\pm1,5)\times10^1$
Meat analogue with carots	Control, no treatment	$(4,1\pm1,3)\times10^7$	$(5,9\pm1,7)\times10^5$	$(1,6\pm0,3)\times10^4$	$(6,2\pm1,3)\times10^4$
Meat analogue with artichoke	Control, no treatment	$(3,9\pm0,7)\times10^7$	$(4,3\pm0,9)\times10^5$	$(5,5\pm1,5)\times10^4$	$(3,8\pm0,7)\times10^4$
Meat analogue with beetroots	5000	$(5,8\pm1,5)\times10^2$	$<1,0\times10^1$	$<1,0\times10^1$	$<1,0\times10^1$
Meat analogue with beetroots	6000	$(4,4\pm0,7)\times10^2$	$<1,0\times10^1$	$<1,0\times10^1$	$<1,0\times10^1$
Meat analogue with pumpkins	5000	$(3,6\pm0,5)\times10^4$	$<1,0\times10^1$	$<1,0\times10^1$	$<1,0\times10^1$
Meat analogue with pumpkins	6000	$(1,9\pm0,3)\times10^4$	$<1,0\times10^1$	$<1,0\times10^1$	$<1,0\times10^1$
Meat analogue with carrots 3	5000	$(1,5\pm0,3)\times10^5$	$<1,0\times10^1$	$<1,0\times10^1$	$<1,0\times10^1$
Meat analogue with carots 3	6000	$(2,3\pm0,5)\times10^5$	$<1,0\times10^1$	$<1,0\times10^1$	$<1,0\times10^1$
Meat analogue with artichoke	5000	$(1,4\pm0,3)\times10^4$	$<1,0\times10^1$	$<1,0\times10^1$	$<1,0\times10^1$
Meat analogue with artichoke	6000	$(2,9\pm0,5)\times10^4$	$<1,0\times10^1$	$<1,0\times10^1$	$<1,0\times10^1$

For sufficiently high initial bacterial contamination, a 5-minute high pressure treatment of 5,000 or 6,000 atmospheres has been shown to be effective (Table 4). Treating the first raw meat analogue to 5,000 or 6,000 atmospheres reduced the bacteria count by 5,172 and 6,818 times, respectively, the second raw meat analogue by 83 and 157 times, with the smallest effect on the third raw meat analogue, only about 20 times.

Such differences in effectiveness can be explained by the different composition of the product and, accordingly, the different species composition of microorganisms.
A high effectiveness of the effect on coliform bacteria was established - treatment at a pressure of 5000 or 6000 atmospheres destroyed them. A similar effect was also found for yeasts and molds. Yeast and fungi are more sensitive to high pressure than bacteria. The mechanism of inactivation of yeast by high pressure is close to the mechanism of inactivation of bacteria, because high pressure affects the permeability of cell membranes and cell structures, denatures protein molecules (Black et al., 2007, Perrier-Cornet et al., 1999).
Thus, yeast, microscopic fungi and coliform bacteria can be eliminated from the raw meat analogue matrix using both 5,000 and 6,000 atmosphere pressures, although resistant species or forms of microorganisms cannot be destroyed, as shown by the results of studies on the total number of microorganisms.

4. Conclusions

1. When creating models for the processing of matrices of plant origin using high-pressure technology, it is established that the high-pressure exposure model must be selected taking into account the composition and properties of the product, the purposeful application of other microorganism-inhibiting factors in order to achieve synergistic action for the changing parameters of pressure, time, packaging and duration , because exposure to high pressure alone does not ensure the destruction of bacteria in a model system.
2. To create matrixes of raw materials of balanced composition of vegetable raw material, berry press-cakes and leguminous plant flour analogues, four compositions of balanced composition and nutritional value were created based on beetroot, pumpkin, carrot, Jerusalem artichoke and leguminous meal.
3. Wet extrusion processing models were created, with changes in pressure, humidity and temperature parameters, simulating the compositions of raw material matrices according to microbiological, chemical and sensory indicators.

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