UTILIZAÇÃO DO EXTRATO DE ASTAXANTINA NA TECNOLOGIA DA PRODUÇÃO DE SALSICHAS COZIDAS COM BAIXO CONTEÚDO DE NITRITO DE SÓDIO

ASTAXANTHIN EXTRACT UTILIZATION IN THE TECHNOLOGY OF PRODUCING COOKED SAUSAGES WITH A LOW CONTENT OF SODIUM NITRITE

ИСПОЛЬЗОВАНИЕ ЭКСТРАКТА АСТАКСАНТИНА В ТЕХНОЛОГИИ ПОЛУЧЕНИЯ ВАРЕННЫХ КОЛБАС С ПОНИЖЕННЫМ СОДЕРЖАНИЕМ НИТРИТА НАТРИЯ

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RESUMO

A orientação prioritária no desenvolvimento da tecnologia de produtos cárneos é o desenvolvimento de receitas para salsichas cozidas com baixo teor residual de nitrito de sódio. A busca por substâncias de origem natural capazes de influenciar a formação da cor das salsichas cozidas e realizar propriedades antioxidantes é uma tarefa urgente atualmente. Na prática apresentada, foi utilizado um extrato de astaxantina da produção industrial (China). A atividade antioxidante do extrato de astaxantina foi traçada pelo método DPPH (2, 2-difenil-1-picrilhidrazila) a 517 nm com o espectrofotômetro Shimadzu UV-1800. O teor de mioglobina foi determinado a partir da densidade óptica do extrato de pigmento de salsicha cozida, obtido após extração com uma solução aquosa de acetona, no comprimento de onda de 540 nm. A astaxantina em concentrações de 0,08 e 0,1% afetou positivamente o processo de formação de cor e a preservação de gorduras na fórmula de carne de salsichas cozidas. Foi determinado que, antes da administração à fórmula de carne, o extrato de astaxantina deveria ser dissolvido em óleo vegetal e deixado por 3 horas à temperatura ambiente. Assim, seu espaçamento uniforme sobre o recheio (recheio) é alcançado sem a formação de “manchas vermelhas”. A astaxantina é bastante estável em fórmulas de carne e dá aos produtos de carne cozida uma cor rosa familiar. É determinado que o extrato de astaxantina é recomendado para uso na tecnologia de salsichas cozidas em concentrações de 0,08-0,1% para a massa do recheio de carne.

Palavras-chave: astaxantina, carotenoides, salsichas cozidas, nitrito de sódio.

ABSTRACT

The priority direction for the development of meat products technology is the development of recipes for cooked sausages with low residual sodium nitrite content. The search for substances of natural origin capable of influencing the formation of the cooked sausages color and exercising antioxidant properties is an urgent task today. In the submitted practice, an extract of astaxanthin of industrial production (China) was used. The antioxidant activity of the astaxanthin extract was traced by the DPPH method (2, 2-diphenyl-1-picrylhydrazyl) at 517 nm with the Shimadzu UV-1800 spectrophotometer. The content of myoglobin was determined from the optical density of the cooked sausage pigment extract, obtained after extraction with an aqueous solution of acetone, at a wavelength of 540 nm. Astaxanthin in concentrations of 0.08 and 0.1% positively affected the process of color formation and the preservation of fats in the meat formula of cooked sausages. It was determined that, before administration to the meat formula, the extract of astaxanthin should be dissolved in vegetable oil and left for 3 hours at room temperature. Thus, its uniform spacing over the stuffing (forcemeat) is achieved without the formation of “red spots”. Astaxanthin is quite stable in meat formulas and gives the cooked meat products a familiar pink color. It is determined that the extract of astaxanthin is recommended for use in the technology of cooked sausages in concentrations of 0.08-0.1% to the mass of the forcemeat.

Keywords: astaxanthin, carotenoid, cooked sausages, sodium nitrite.

АННОТАЦИЯ

Приоритетным направлением развития технологии мясных продуктов является разработка рецептур вареных колбас с пониженным остаточным содержанием нитрита натрия. Поиск веществ природного происхождения. Способных влиять на формирование цвета вареных колбасных изделий и проявлять антиоксидантные свойства, является актуальной задачей. В работе был использован экстракт астаксантина промышленного производства (Китай). Антиоксидантную активность экстракта астаксантина определяли методом cDPPH (2,2-дифенил-1-пикрилгидразин) при 517 нм на спектрофотометре Shimadzu UV-1800. Содержание миоглобина определяли по оптической плотности экстракта пигментов вареной колбасы, полученного после экстракции водным раствором ацетона, при длине волны 540 нм. Астаксантин в концентрации 0,08 и 0,1 % положительно влияет на процесс цветообразования и сохранность жиров в пищевой системе вареных колбас. Установлено, что перед введением в пищевую систему экстракт астаксантина следует растворять в растительном масле и оставлять на 3 часа при комнатной температуре. Так достигается его равномерное распределение по фаршу без образования «красных точек». Астаксантин достаточно устойчив в мясных пищевых системах и придает мясным вареным изделиям привычный для потребителя розовый цвет. Установлено, что экстракт астаксантина рекомендуется использовать в технологии вареных колбас в концентрациях 0,08-0,1% к массе фарша.

Ключевые слова: астаксантин, каротиноидный пигмент, вареные колбасы, нитрит натрия.

INTRODUCTION

An increasingly important trend in the technology of meat products is the development of the technology of meat products with the lower residual content of nitrite (Antipova *et al.*, 2013). The danger of nitrite entering the human body is associated with its nephrotoxic effect, with the emergence of methemoglobinemia and the formation of carcinogenic nitrite compounds (Antipova *et al.*, 2013). For human safety, it is very important to ensure a minimum of residual nitrite in the product, since it has a biological effect on the human body.

Nitrite of sodium (or potassium nitrite) plays an important role in the coloration of meat products. It gives the prepared, processed meat products a color that is close to the color of natural meat, which determines their consumer features. Along with the simultaneous participation in the color formation reaction, sodium nitrite performs a number of technological functions: it participates in the process of formation of taste and aromatic characteristics of salted raw materials, has a pronounced inhibitory effect on some microorganisms, and exercises the antioxidant action against lipids (Antipova *et*

al., 2013).

It is known that astaxanthin is one of the most powerful antioxidants found in nature (Jyotika *et al.*, 2012; Youstry, 2000). The sources of this dark red carotenoid pigment are green algae (*Haematococcus pluvialis*), red yeast fungi (*Phaffia rhodozyma*), plants, as well as fish and seafood (salmon, shrimp, and lobster) (Ambati, 2014). It positively affects human health, helps with the treatment and prevention of oncology, diabetes, diabetic nephropathy, metabolic syndrome, gastrointestinal tract diseases, liver, eyes, chronic fatigue syndrome, male infertility, renal insufficiency, etc. (Yamashita, 2013; Karppi *et al.*, 2007). The astaxanthin extract also positively affects the meat formula, being integrated into; it ensures the preservation of fats due to its high antioxidant activity (Martínez-Delgado *et al.*, 2016).

The purpose of this work is to investigate the opportunity of utilization of astaxanthin of natural origin in the technology of cooked sausages with a low content of sodium nitrite.

MATERIALS AND METHODS

An extract of astaxanthin of industrial production (China) was used. The extract was produced industrially on January 2, 2017, batch no. 17010272, and batch weight - 500 kg, expiration date - before January 2, 2019. The extract was stored in a vacuum package. The extract safety parameters are shown in Table 1.

Antioxidant activity of the astaxanthin extract was determined by the DPPH method (2, 2-diphenyl-1-picrylhydrazyl) (Khasanov *et al.*, 2004). The optical density of the solution was traced at 517 nm with the Shimadzu UV-1800 spectrophotometer. To determine anti-radical activity, a calibration curve was constructed that reflects the % - antioxidant activity, depending on the concentration of ascorbic acid.

Myoglobin content was determined from the optical density of the extract pigments of cooked sausage, obtained after extraction with an aqueous acetone solution, at a wavelength of 540 nm (GOST 25011-81). Solutions with known concentrations have a certain optical density. A calibration curve of the concentration versus optical density was plotted by Sigma myoglobin. The optical density for the calibration and for the solutions studied was determined with the Shimadzu UV-1800 spectrophotometer.

According to standard GOST practices, the following parameters were determined: protein, fat, salt, residual nitrite, humidity, acid, peroxide value, and also microbiological indexes. Methodic data are given in Table 2.

RESULTS AND DISCUSSION

The magnitude of antiradical activity was attributed to the amount of mg of ascorbic acid (according to the results of the calibration curve plotted against the %-antiradical activity of ascorbic acid concentration). Various solvents were used in the experiment: hexane, ethanol, distilled water, benzene, isopropanol, and deodorized vegetable soya oil. Antioxidant activity data are presented in Table 3.

Of the data presented, it is evident that the extract of astaxanthin shows maximum antioxidant activity when dissolved in fats, namely in vegetable oil, and is partially active when dissolved in hexane, benzene, isopropanol. It does not dissolve in distilled water and ethanol and does not exhibit anti-radical properties. The

extract of astaxanthin was utilized in the technology of "Asta" cooked sausages, reducing the content of salting mixture with sodium nitrite by 2 times. "Doktorskaya" sausage cooked according to the GOST R 52196-2003 standard was used as a control sample (Boiled sausage products...). The extract was used in four concentrations: 0.04%, 0.06%, 0.08%, and 0.1% to the mass of minced meat. The meat formula of sausages is presented in Table 4.

Primary raw meat materials were crushed to a size of pieces of 16-25 mm and salted down for 10 hours with a mixture of curing salt and edible salt at a temperature of 2 ± 2 °C. Minced meat for cooked sausages was prepared in a cutter in two stages.

At the first stage of cutting, raw low-fat meat materials, Optispice GOST "Doktorskaya" functional mixture, water and/or ice and premixed egg powder are added to the cutter. The extract of astaxanthin had to be dissolved in vegetable oil at room temperature 3 hours prior to administration into the meat formula. In the second stage, semi-fat pork was added; in order to achieve uniform spacing, the extract was added along with the fatty meat material. Lexolan polyamide barrier casing was used during the sausage forming process. Samples were cooked at 80 °C to the internal (measured in the midst of baloney) temperature of 72 °C, with the ageing of 5 minutes. The technological scheme for the production of cooked sausages with astaxanthin is shown in Figure 1.

In processed sausage products, the color characteristics were carried out in accordance with GOST R 52196-2011 "Cooked sausage products" ((Antipova *et al.*, 2014). The results are shown in Table 5.

It should be noted that at the end of the expiration date, in the samples of "Asta" 0.04% and "Asta" 0.06%, gray spots appear on cut-off sites, which indicates a loss of color. Also, the intensity of the color of cooked sausages was assessed by the content of myoglobin (GOST 25011-81). Data on the myoglobin content in the samples within the 35 days of storage are shown in Table 6.

The parameter closest in value to the control sample was a sample containing an extract of astaxanthin of 0.1%. The cooked sausage "Asta" with the content of the extract of astaxanthin of 0.08% also had a stable and natural color. The physicochemical parameters of

the cooked sausages quality are presented in Table 7.

Sodium nitrite in sausage products technology acts not only as a color former; it is also used as a preservative. It is known that cooked sausages have a lot of fat (up to 20%) in composition. To determine the quality and preservation of fat during storage, peroxide (GOST 9793-74) and acid (GOST 54346-2011) values of "Asta" cooked sausages and a control sample were determined by standard methods, that show the changes of the fat quality in sausages during storage - namely whether oxidation and rancidification of fat are occurring. Data on the peroxide and acid values are presented in Table 8.

From this table, it can be seen that samples with an astaxanthin extract content of 0.08% and 0.1% are more stable during storage. There is a direct correlation between the content of astaxanthin and the oxidation of lipids.

Microbiological safety indicators of cooked sausages are standardized by the Customs Union Technical Regulation 021/2011 "On the safety of food products" and the Customs Union Technical Regulation 034/2013 "On the safety of meat and meat products". Microbiological parameters of "Asta" series cooked sausages during storage are given in Table 9.

Sulphite-reducing Clostridia, S.aureus, Pathogenic and/or Salmonella, and L.monocetogenes were not detected in cooked sausages of the "Asta" series during storage.

CONCLUSIONS:

According to researches outcome, it is possible to draw a conclusion to recommend the utilization of astaxanthin extract in the technology of cooked sausages in concentrations of 0, 08% and 0, 1% to the weight of forcemeat.

Astaxanthin positively affects the preservation of fats in the meat formula of cooked sausages and does not impart an extraneous taste to sausage products that acquire a beautiful pink color. And it is quite stable in the samples of "Asta" 0.08% and "Asta" 0.1%.

The extract of astaxanthin should be dissolved in vegetable oil and left for 3 hours at room temperature prior to administration to the meat formula. This is the best way to achieve uniform spacing over the stuffing (forcemeat)

without the formation of "red spots". It is also worth noting that immediately after cooking, the products have a bright pink color, which becomes slightly less intense after cooling.

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Table 1. Astaxanthin extract safety parameters

Parameters	Results
Extractants utilized in extract production	Water and ethanol 45 - 55 mg/100 ml
Dry extract substancemoisture	3.07%
Solvent-Extractant ratio for extract production	10:1
Arsenic and heavy metals	Zero
E.coli	Zero
S.aureus in 1.0 g	Zero
- QMAFAnM, CFU/G	1×10^3
- Yeast and Mold, CFU/G /g	1×10^2

Table 2. Determination methodic for physicochemical and microbiological parameters of "Asta" cooked sausages

Parameter	State Standard
Protein content	GOST 25011-81
Fat content	GOST 23042-2015
Table salt content	GOST R 51480-99
Sodium nitrite content	GOST 29299-92
Acid value	GOST R 55480-2013
Peroxide value	GOST R 54346-2011
Moisture content	GOST 9793-74
QMAFAnM	GOST 10444.15-94
Escherichia coli group bacteria – CGB	GOST 31747-2012
Sulphite-reducing <i>Clostridia</i>	GOST29185-2014
<i>S.aureus</i>	GOST 31746-2012
<i>Salmonella</i>	GOST 31659-2012
<i>L.monocetogenes</i>	GOST 32031-2012

Table 3. Study of antioxidant activity of astaxanthin by the DPPH method

Solvent	Hexane	Benzene	Isopropanol	Water	Vegetable oil	Ethanol
Antioxidant (antiradical) activity of astaxanthin per 1 g of substance / mg	13.63±2.04	14.65±1.7	18.13 ±1.02	-	66.5±7.1	-
EC,50% , mg	0.191 ±0.001	0.179 ±0.015	0.171±0,02	-	0.042±0.002	-

Table 4. The meat formula of cooked sausages per 100 kg of primary raw materials, kg

Primary raw material	“Doktorskaya” GOST	“Asta” 0.04%, kg	“Asta” 0.06 %, kg	“Asta” 0.08%, kg	“Asta” 0.1%, kg
Beef, Grade A	25	25	25	25	25
Pork, Grade A	25	25	25	25	25
Pork, Semi-Fat	45	45	45	45	45
Egg powder	0.8	0.8	0.8	0.8	0.8
Egg powder/Water	2.1	2.1	2.1	2.1	2.1
Protelak	2	2	2	2	2
Mfunctional mix					
Salt	0.91	1.4	1.4	1.4	1.4
Sugar	0.2	0.2	0.2	0.2	0.2
Curing salt	1.19	0.595	0.595	0.595	0.595
Optispice					
functional mix, “Doktorskaya” GOST	1.4	1.4	1.4	1.4	1.4
Water/ice	30	29.8	29.7	29.65	29.57
Astaxanthin extract	-	0.053	0.080	0.107	0.133
Vegetable oil	-	0.15	0.2	0.250	0.300

Table 5. Color characteristics of cut-off samples of the “Asta” and “Doktorskaya” GOST cooked sausages

Storage time	Control sample (“Doktorskaya” GOST)	“Asta” 0.04%	“Asta” 0.06%	“Asta” 0.08%	“Asta” 0.1%
1 day	Pink	Light-pink	Light-pink	Pink	Rich-pink
10 days	Pink	Pale-pink	Light-pink	Pink	Rich-pink
20 days	Pink	Gray-pink	Light-pink	Pink	Rich-pink
30 days	Pink	Gray	Pale-pink	Pink	Pink
35 days	Pink	Gray	Gray-pink	Light-pink	Pink

Table 6. Myoglobin content in samples at different storage times (mg/1 g of sample)

Storage time (days)	Control sample (“Doktorskaya” GOST)	“Asta” 0.04%	“Asta” 0.06%	“Asta” 0.08%	“Asta” 0.1%
1	4.26±0.12	3.03±0.08	3.49±0.06	3.7±0.06	4.22±0.06
10	4.37±0.15	3.09±0.08	3.63±0.09	3.91±0.09	4.31±0.09
20	4.49±0.19	3.14±0.09	3.78±0.13	4.12±0.13	4.42±0.13
30	4.56±0.21	3.25±0.09	3.84±0.17	4.25±0.17	4.53±0.17
35	4.62±0.3	3.31 ±0.1	3.92 ±0.2	4.36 ±0.2	4.59 ±0.2

Table 7. Physico-chemical parameters of cooked sausage “Asta” and a control sample of “Doktorskaya”

Parameters	Control sample (“Doktorskaya”)	“Asta” 0.04%	“Asta” 0.06%	“Asta” 0.08%	“Asta” 0.1%
Protein content, %	11	10.9	10.9	10.9	11
Fat content, %	17.5	17.6	17.6	17.7	17.8
Table salt content, %	2.1	2.0	2.0	2.0	2.0
Moisture, %	68.1	67.8	67.7	68.2	68.3
Residual sodium nitrite content, %	0.0031±0.0002	0.0015±0.0002	0.0015±0.0002	0.0015±0.0002	0.015±0.0002

Table 8. Acid and peroxide values of sausage products during storage

Parameters	Standard by legal requirements	Storage time (days)	Control sample (“Doktorskaya”)	“Asta” 0.04%	“Asta” 0.06%	“Asta” 0.08%	“Asta” 0.1%
Acid value, mg, CFU/G /g, not more than	4.0	1	1.6	1.9±0.2	2.2±0.2	2.0±0.2	1.7±0.2
		15	1.9	2.4±0.2	2.2±0.2	2.0±0.2	1.9±0.2
		30	2.1	3.5±0.1	2.8±0.3	2.3±0.3	2.2±0.2
		35	2.2	3.8±0.1	3.0±0.2	2.4±0.2	2.3±0.2
Peroxide value, mole of active O ₂ / kg, not more than	10.0	1	2.5	4.8 ±0.1	3.3±0.1	2.5±0.2	2.3±0.2
		15	5.5	8.3±0.2	7.2±0.2	5.3±0.2	4.7±0.2
		30	7.0	11.9 ±0.2	10.6±0.2	7.7±0.2	6.7±0.3
		35	7.2	12.5±0.2	11.2±0.2	8.1±0.2	6.8±0.2

Table 9. Microbiological parameters of the “Asta” series cooked sausages during storage

Parameters	MPC, standard	Storage time (days)	“Asta” 0.04%	“Asta” 0.06%	“Asta” 0.08%	“Asta” 0.1%
QMAFAnM, CFU/G /g	Not more than 1×10^3	1	3×10^1	2×10^1	1.9×10^1	1×10^1
		15	1×10^2	1×10^2	5×10^1	3×10^1
		30	8×10^2	8×10^2	5×10^2	2×10^2
		35	4.8×10^3	3×10^3	8×10^2	3×10^2
Escherichia coli group bacteria – CGB in 1.0 g	Not allowed	1	N/d	N/d	N/d	N/d
		15	N/d	N/d	N/d	N/d
		30	N/d	N/d	N/d	N/d
		35	N/d	N/d	N/d	N/d
Sulphite- reducing <i>Clostridia</i> in 0.1 g	Not allowed	1	N/d	N/d	N/d	N/d
		15	N/d	N/d	N/d	N/d
		30	N/d	N/d	N/d	N/d
		35	N/d	N/d	N/d	N/d
<i>S.aureus</i> in 1.0 g	Not allowed	1	N/d	N/d	N/d	N/d
		15	N/d	N/d	N/d	N/d
		30	N/d	N/d	N/d	N/d
		35	N/d	N/d	N/d	N/d
Pathogenic, incl. <i>Salmonella</i> in 25 g	Not allowed	1	N/d	N/d	N/d	N/d
		15	N/d	N/d	N/d	N/d
		30	N/d	N/d	N/d	N/d
		35	N/d	N/d	N/d	N/d
- <i>L.monocetog</i> <i>enes</i> in 25 g	Not allowed	1	N/d	N/d	N/d	N/d
		15	N/d	N/d	N/d	N/d
		30	N/d	N/d	N/d	N/d
		35	N/d	N/d	N/d	N/d

* n/d – not detected

Figure 1. Technological scheme for the production of cooked sausages with astaxanthin