GRAPE POMACE EXTRACT AND PEAR IN SNACK PRODUCTION TECHNOLOGY

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Abstract: The article is devoted to the study of the influence of process parameters for the production of pear snacks with the addition of grape extract secondary raw materials on the chemical composition and antioxidant activity of the resulting product. This paper describes the selection of the most optimum conditions for production of high quality, competitive product with the highest antioxidants activity. The object of the study were temperature, time of soaking pear slices in the extract, drying process. Analysis on the content of total amount of phenolic compounds the content of Gallic acid, flavonoids and tannins content of catechin, anthocyanin content cyanidin-3-glycoside, antiradical capacity using the free radical DPPH method (2,2-diphenyl-1-picrylhydrazyl), antioxidative activity by the method of ABTS (2,2'-settlement Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), which restores the force by the method of FRAP (ferric reducing antioxidant power), antioxidant activity in linoleic acid system. The obtained results are vivid enough and allow us to conclude that the best way to obtain high-quality resulting product out of pear fruit crop is freeze-drying, and the shelf life of an opened package in no longer than 38 hours. This study was financially supported by the Ministry of Education and Science of the Russian Federation within the basic part of the government task number 2014/199 FSBEI HPE Samara State Technical University by the project «Creating Scientific Methodology of Developing Formulation and Technologies of Food Products Fighting Oxidant Stress in Human's Body» code 974.

Keywords: Pears, snack, antioxidant activity, extract, grape, freeze drying

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INTRODUCTION

Pear is one of the major crops and is highly valued for its taste and technological qualities of the fruit. [1] Pear fruits are characterized by medical and nutritional value. They contain sugar, acids, tannins, mineral nutrients, vitamins, pectin substances. Especially pear fruits are valuable for containing such an important substance for the human organism as chlorogenic acid, and arbutin. There is more chlorogenic acid in pear fruit than in apples, and its amount depending on the class is of 30–70 mg%. It has healing properties for liver diseases [2].

Shelf life of pears is small, so raw fruit processing is becoming an important area of food industry. One of the main stages which determines the quality of the manufactured product is the selection of technological modes. The mode is called optimal when the product is produced with the best rates and efficiency of the process is the highest.

The aim of this work is the development of pear snack production technology with addition of an extract [3] of the secondary raw grape materials. In addition, we studied the effect of technological parameters on physical and chemical [4] and antioxidant rates [5] of the finished product. To develop the technology of obtaining a functional food Foods and Raw Materials, 2017, vol. 5, no. 1, pp. 63–72.

item - pear snacks – some research was conducted on the effect of pear slices thickness, a mass fraction of extract for soaking pear slices, temperature of soaking pear slices in the extract, drying technology, technological modes of drying on the organoleptic, physical and chemical, structural and mechanical rates of finished products.

OBJECTS AND METHODS OF STUDY

Chemicals and reagents. Folin-Ciocalteu reagent in sodium carbonate, gallic acid, catechin, ABTS (2,2'azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) were purchased from Fluka (Germany). DPPH (2,2diphenyl-1-picrylhydrazyl), Tween 40, hydrogen peroxide, sodium nitrite, aluminum chloride, thiobarbituric acid, trichloroacetic acid were purchased from Sigma-Aldrich Chem. mp. (USA).

Fruit collection. The grapes and pears collected on the territory of the Russian Federation at the enterprise wineries in the city of Samara in 2014.

Determination of total phenols. Total phenolic content of methanolic fruit extracts was assessed using a modified version of the Folin–Ciocalteu assay [6]. Gallic acid was used as a standard and the aqueous gallic acid solution (200 mg l 1) was diluted with distilled water to give appropriate concentrations for a

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standard curve. For the analysis, 100 ll of methanolic fruit extract or gallic acid standard, 100 ll of methanol, 100 ll of Folin–Ciocalteu reagent and 700 ll of Na_2CO_3 were added into 1.5 ml micro-centrifuge tube. The samples were vortexed immediately and the tubes were incubated in the dark for 20 min at room temperature. After incubation all samples were centrifuged at 13,000 rpm for 3 min. The absorbance of the supernatant was then measured at 735 nm in 1 ml plastic cuvettes using evolution 200 Series spectrophotometer. The results were expressed in mg gallic acid equivalent/100 g dry weight.

Determination of total flavonoids. The flavonoid content of the methanolic extracts was measured using a assay [7]. A known volume (0.5 ml) of the extract or standard solution of quercetin was added to a 10 ml volumetric flask. Distilled water was added to make a volume of 5 ml. At zero time, 0.3 ml of 5% w/v NaNO₂ was added to the flask. After 5 min, 0.6 ml of 10% w/v AlCl₃ was added and after 6 min, 2 ml of 1 M NaOH was added to the mixture followed by the addition of 2.1 ml distilled water. Absorbance was read at 350 nm against the blank (water) and flavonoid content was expressed as mg quercetin equivalents in 100 g of fresh material.

Determination of the anthocyanin profile. The concept of determining the amount of anthocyanin present in a material by measuring the change in absorbance at 2 different pH values (3.4 and 2.0) [8]. Researchers have proposed using the pH values of 1.0 and 4.5 (2-5). Monomeric anthocyanins undergo a reversible structural transformation as a function of pH (colored oxonium form at pH 1.0 and colorless hemiketal form at pH 4.5. Thus, the difference in absorbance at the vismax (520 nm) of the pigment is proportional to the concentration of pigment. Degraded anthocyanins in the polymeric form are resistant to color change with change in pH. Therefore, polymerized anthocyanin pigments are not measured by this method because they absorb both at pH 4.5 and 1.0.

Determination of condensed tannins. In presence of concentrated H_2SO_4 , condensed tanins were transformed by the reaction with vanillin to anthocyanidols [9]. 50 l of the methanolic seed extract appropriately dilute was mixed with 3 ml of 4% methanol vanillin solution and 1.5 ml of H_2SO_4 . After 15 min, the absorbance was measured at 500 nm. Condensed tannin contents of seeds (three replicates per treatment) were expressed as mg catechin equivalents (CE) per gram of dry weight through the calibration curve with catechin. The calibration curve range was 50–600 mg ml⁻¹.

DPPH radical scavenging activity. The scavenging activity of samples was measured in accordance with the method [10]. The method was based on the reduction of methanolic DPPH in the presence of a hydrogen-donating antioxidant. DPPH solution was an intense violet colour and showed an absorption band at 515 nm. Adsorption and colour lowered when DPPH was reduced by an antioxidant compound. The remaining DPPH corresponded inversely to the radical-scavenging activity of the

antioxidant. DPPH (2 mg) was dissolved in 54 ml of MeOH. Aliquots of investigated extract (50, 100, 200, 300, 500 and 1000 lg) were dissolved in 2 ml of MeOH. Then 1.0 ml of each solution was added to 2.0 ml of DPPH solution at room temperature. The absorbance at 515 nm was measured against a blank (2 ml MeOH in 2.0 ml of DPPH solution) using evolution 200 Series spectrophotometer. The results were expressed as percent-age of reduction of the initial DPPH adsorption by test samples:

% of reduction of the initial DPPH adsorption = ADPPH(t)_Asample (t)/ADPPH (t) _ 100,

ADPPH(t) is absorbance of DPPH at time t and Asample (t) is absorbance of sample at t the same time.

FRAP assay. The FRAP assay was carried [11]. The FRAP solution was freshly prepared on the day of use, by mixing acetate buffer (pH 3.6), ferric chloride solution (20 mM) and TPTZ solution (10 mM TPTZ in 40 mM HCl) in a proportion of 10 : 1 : 1, respectively. Following this the FRAP solution was heated, while protected from light, until it had reached a temperature of 37 LC. Appropriate dilutions of methanolic fruit extracts were prepared. One hundred microlitres of the diluted sample extract (or for blank 100 ll methanol and for Trolox standard curves 100 ll Trolox of appropriate concentration) and 900 ll of FRAP solution were added into a micro-centrifuge tubes. The tubes were vortexed and left at 37 LC for exactly 40 min, and the absorbance was measured at 593 nm. The Trolox standard curves were used to calculate the antioxidant activity of the samples in relation to Trolox and were expressed as mg Trolox equivalent/100 g dry weight sample (mg TE 100 g 1 DW).

ABTS free radical decolorization assay. The total antioxidant capacity assay conducted using evolution 200 Series spectrophotometer. The procedure was based on a method [12] with some modification. ABTS + was generated by reacting ABTS (7.4 mM) with potassium persulphate (2.6 mM). The solution was diluted to obtain an absorbance of 1.4 units at 414 nm (molar extinction coefficient $E = 3.6_{-104}$ mol_1 1 cm_1, Forni, Morla-Arellano, Packer, & Willison 1986) with 50 mM glycine-HCl buffer (pH 4.5) before use. Three millilitres of the solution were added to 20-80 ml of AA, trolox, hydroquinone, pyrogallol and fruit extracts separately. The changes in absorbance at 414 nm were recorded at 1, 3, 6, 10, 20, 30, 40, 60 and 90 min after mixing and until the absorbance reached a plateau. The antioxidant capacities, obtained by comparing the absorbance change at 414 nm in a test reaction mixture containing extract of fruit with that containing AA, were expressed as mg of AA equivalents per 100 g of homogenate (AEAC).

Determination of Antioxidant Activity in a Linoleic Acid System. The total antioxidant activity of FEHP was carried out by use of a linoleic acid system [13]. The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 as emulsifier, and 50 mL of phosphate buffer (0.2 M, pH 7.0), and then the mixture was homogenized. A 0.5 mL ethanol solution of different concentration of FEHP (50-500 ig/mL) was mixed with linoleic acid emulsion (2.5 mL, 0.2 M, pH 7.0) and phosphate buffer (2 mL, 0.2 M, pH 7.0). The reaction mixture was incubated at 37°C in the dark to accelerate the peroxidation process. The levels of peroxidation were determined according to the thiocyanate method by sequentially adding ethanol (5 mL, 75%), ammonium thiocyanate (0.1 mL, 30%), sample solution (0.1 mL), and ferrous chloride (0.1 mL, 20 mM in 3.5% HCl). After the mixture was left for 3 min, the peroxide value was determined by reading the absorbance at 500 nm on а spectrophotometer.

RESULTS AND DISCUSSION

Based on the example of soaking pear slices in grape extract the dependence of physical and chemical and antioxidant properties of snacks on mass fraction of the added extract was revealed. Four samples of the product with a mass fraction of the extract of 20, 40, 60 and 80% of the feedstock mass were prepared.

Comparative analysis has shown (Table 1) that with increasing concentrations of grapes pomace extract,

chemical composition rates increase. Thus, comparing the concentration of 20 and 80% we can observe that there is a 4 times increase in flavonoid (110 and 437 mg of catechin / 100 grams of feedstock, respectively), and the anthocyanins and tannins increase almost by 10 times.

Studying the rates in Fig. 1, we can also see an increase in the restoring force rates and the antioxidant capacity of pear snack with the addition of grapes pomace extract compared with the feedstock. Thus comparing the feedstock with pear snack soaked in 80% extract the restoring force rates and antioxidant capacity are increased by 6 times.

After analyzing the data of Fig. 2, in terms of antiradical activity rate E_{C50} (extract concentration required to bind 50% of free radicals DPPH) the feedstock does not show any anti-radical activity. The greatest antiradical activity was shown by pear slices soaked in 80% extract ($E_{C50} = 94$ mg / ml).

Thus, it can be concluded that soaking pear slices in the extract of different concentrations leads to an increase of the chemical composition and antioxidant properties as compared with the feedstock.

Extract %	Total content of phenols, mg of gallic acid/ 100 g of material	Total content of flavonoids, mg of catechine/ 100 g of material	Total content of anthocyanins, mg of cyanidin-3- glycoside/ 100 g of material	Total content of tannins, mg of catechine/ 100 g of material
0	118	47	Absent	0.12
20	110	31	3.18	0.12
40	189	136	4.23	0.16
60	224	189	25.24	0.88
80	437	314	31.6	1.02



Fig. 1. Study of restoring force and antioxidant activity.



0 10 20 30 40 50 60 70 80 90 100110120130140150160170

Fig. 2. Study of antioxidant activity.

The analysis of the data in the Table 2 allows to conclude that with the increasing temperature of pear samples soaked in 80% extract, chemical composition rates increase.

As seen in Fig. 3, the highest concentration of E_{C50} , and consequently, the lowest activity against trapping radicals found in pears soaked in extracts at 5°C. Pears soaked in room temperature extract and 35°C extract have lower values of the active concentration, hence higher antiradical activity. By the ability to capture radicals ABTS, all the temperatures of soaking pear slices in grape pomace extract are about the same level.

In the analysis of the Fig. 4 we can notice that pear samples soaked in the extract at 35°C have the greatest restoring force, the pear samples soaked in the extract

at room temperature differ slightly. The ability of pears to inhibit the oxidation of linoleic acid in model system, is characterized as the antioxidant activity. The highest antioxidant activity is shown in pear samples soaked in room temperature extract. In case of heating the extract pears showed the least antioxidant activity compared to other monitored temperatures.

The room temperature of 20–22°C was chosen from all the monitored temperatures, because in terms of chemical composition and antioxidant properties it is slightly different from the temperature of 35°C. In addition, the use of room temperature will allow to reduce extra energy usage.

There is some specific interest in measuring the mass fraction of soluble solids in the processed pear slices, both before and after heat treatment (Fig. 5).

Parametres	Total content of phenols, mg of gallic acid/ 100 g of material	Total content of flavonoids, mg of catechine/ 100 g of material	Total content of anthocyanins, mg of cyanidin-3- glycoside/ 100 g of material	Total content of tannins, mg of catechine/ 100 g of material
5°C	191	186	57.77	1.22
20°C	425	302	61.29	4.06
35°C	432	312	62.79	4.54

Table 2. The Change of chemical composition of pear snacks



 \blacksquare E_{c50} mg/cm³ \blacksquare ABTS, mmol trolox/g of material

Fig. 3. Study of antioxidant properties.



Fig. 4. Study of the restoring force and antioxidant activity.



Fig. 5. Mass fraction of soluble solids.

Fig. 5 shows the average data on the change of the mass fraction of soluble solids in fresh pears and pears soaked in the extract at different temperatures. Sliced pear fruits were kept in grapes pomace extract for one hour at 5°C, 25°C and 35°C. As a result of the work the direct dependence of the loss of soluble solids from the extract temperature was observed. During the data processing the loss of the mass fraction of soluble solids in the leaching and the removal of air contained in the intercellular tissue spaces of pear slices was revealed. On average, after treatment, the samples lost by 1 to 2% solids.

For obtaining the final product - pear snacks – three kinds of drying were researched: convective at 70°C, IR drying at 70°C, freeze-drying.

Table 3 shows that the highest content of phenolic compounds (458 mg of gallic acid / 100 g of feedstock) and flavonoids (317 mg of catechin / 100 g of feedstock) is had by pear snacks dried in freeze-drying. This type of drying preserves the greatest amount of anthocyanins (63.8 / 100 grams of feedstock) and tannins (2.52 mg of catechin / 100 grams of feedstock). It should also be noted that the heat treatment of pear

snack is inferior to freeze drying on the total content of phenolic compounds, flavonoids, anthocyanins and tannins.

According to the Fig. 6 data it can be concluded that the highest antiradical activity, characterized by the lowest concentration of the extract (EC50) necessary for the binding of free radicals 50% 2,2-diphenyl-1-picrylhydrazyl (DPPH), is shown in freeze-dried pear snacks.

The highest antioxidant activity (15.1%) and the restoring force (15.1 mmol Fe2 + / 1 kg IP) belongs to pear snacks which are also freeze-dried (Fig. 7). Low antioxidant activity (10.2%) and the restoring force (7.38 mmol Fe₂₊/ 1 kg IP) are observed in snacks dried using IR method.

Practically all kinds of heat treatment decrease the antioxidant capacity of snacks. This is explained by the following: not only the phenolic substances, flavonoids, but also vitamins C, A, E, the enzyme system of the cell possess the antioxidant activity in plant cells as well. When heated these antioxidants are apparently destroyed and there is antioxidant activity inherent in the phenolic antioxidant complex left.

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Drying type	Total content of phenols, mg of gallic acid/ 100 g of material	Total content of flavonoids, mg of catechine/ 100 g of material	Total content of anthocyanins, mg of cyanidin-3- glycoside/ 100 g of material	Total content of tannins, mg of catechine/ 100 g of material
IR	211	251	Absent	2.40
Convective	314	285	24.57	1.54
Freeze	458	317	63.80	2.52

Table 3. Study of chemical composition of final product dried in different ways



Fig. 6. Study of antioxidant activity of the final product.



Fig. 7. Study o restoring force and antioxidant activity of the finished product.

During the convective snack drying method initially drying process is sufficiently effective. However, as the product of dehydration and the consequent decrease in its heat- and mass-providing characteristics, an increasing share of thermal energy does not flow inside the dried products [14]. The energy output of the process is grows, drying time increases, there are local overheating of the product, which is reflected in the quality of the finished snack.

In terms of convective heating the product is heated up primarily due to heat-and-moisture providing which is determined by thermal diffusion of moisture. Because of the small thermal conductivity capacity at 70°C drying flows for long enough, even with a small sample thickness, moisture evaporation is slow, which is to be explained by increased content of strongly bound moisture as well.

Using a vacuum freeze drying allows to reduce the process duration in 2–3 times, to improve structural and rheological characteristics as well as to achieve a water activity rate that ensures microbiological stability of the product, organoleptic characteristics typical of dry products.

In this connection, the possibility of using freeze drying, resulting in the process which is the inverse of condensation has been studied. Thus the fluid is displaced by a completely different method, which contributes to a higher quality and, of course, useful products. First, the product undergoes a deep freeze, and then the moisture is "dried" out of it under the pressure.

The research resulted in development of the technology for snack production including the following operations: acceptance of raw materials, cleaning, inspection, seed chamber removal, cutting, soaking, freeze-drying, re-inspection, pre-packing, packing (Fig. 8).

These pear snacks dried with three different methods were analyzed on organoleptic and biochemical parameters. Fig. 9 shows comparative data of physical-chemical rates of fruit snacks prepared by freeze, convective and IR drying methods.

The content of moisture mass fraction in fruit snacks, prepared by convective drying method is $4.7 \pm 0.1\%$, at snacks dried by IR-method it is $4.6 \pm 0.1\%$, while for chips prepared by freeze method I is about 4.8 ± 0.1 %. The average content of titratable acids in fruit snacks, prepared by convective drying and IR methods is $2.3 \pm 0.1\%$, for the snacks prepared by freeze method is $2.4 \pm 0.1\%$. The content of reducing substances in the fruit snack prepared by convection, freeze and IR drying methods range from 27.3 to 27.9%.

So the pear snacks tasting with the addition of grape pomace extract obtained by convective, infrared, and freeze-drying, received the following tasting evaluation (Fig. 10).



Reducing substances content, %

Fig. 9. Comparative analysis of physical-chemical rates of fruit snacks prepared by convective, freeze and IR drying methods.



Fig. 10. Tasting evaluation of pear samples with the addition of grape pomace extract dried by different drying methods.

Analyzing the tasting evaluation data corresponding to the high quality of the final product, it must be concluded that the freeze drying method id the most advantageous one in the production of pear snacks with the addition of grape pomace extract, a freeze drying method.

Microbiological spoilage of food is determined by huge variety of bacteria, molds and yeasts. Safety of the studied pear snacks was evaluated by 3 factors (Table 4):

(1) The number of mesophilic aerobic and facultative anaerobic microorganisms QMAFAnM - a criterion which at 30°C for 48–72 hours allows to reveal all groups of microorganisms growing in certain environments. The identification of QMAFAnM was carried out according to All Union State Standard 10444.15-94 (23).

(2) The number of coliform bacteria (All Union State Standard 31747-2012) (34 ells).

(3) Mold and yeast were determined according to All Union State Standard P 10444.12 (36 ells).

Tests were conducted in an accredited testing laboratory of Hygiene and Epidemiology Centre in the Samara region.

Based on the data available, it can be concluded that the microbiological rates of pear snacks with high antioxidant properties prepared by freeze-drying, fully meet the requirements of TR CU 021/2011 "On food safety".

The shelf life of food - is one of the most important indicators, which depends on many factors: the quality of the raw material used, sanitary conditions of production, technology, equipment, storage and packaging conditions.

During the storage, studies of physical and chemical rates and antioxidant properties of natural

pear snacks and snacks with grape pomace extract added were carried out. Experimental snack samples were packaged in foil and paper bags, laminated with heat-sealable materials approved for use by the institutions of Russian Federal Service for Consumer Rights Protection and Human Welfare, and providing the quality of product safety during storage and transportation. Storaged packaged snacks were evaluated in terms of shelf life, physical, chemical, organoleptic rates and content of the antioxidant activity with a period of 3 months. Recommended storage time during freeze-drying method is 12 months with a relative humidity of 60% and a temperature of 23° C.

The results of physical and chemical rates are showed in Table 5.

Based on data in Table 5 we can conclude that by the study of physical and chemical parameters of snacks with higher antioxidant properties during 12 months' storage, the content of moisture mass fraction, acidity and reducing substances vary within established norms.

Studies of the content of antioxidant activity in pear snacks with enhanced antioxidant properties showed that during the storage rates have not changed unlike those of natural snacks (Table 6).

Study of Table 6 allows to conclude, that the grape pomace extract has a prolonging effect on the antioxidant properties of the final product during the storage.

The freshly collected samples were also analyzed for the duration of storage on the content of moisture mass fraction in accordance with All Union State Standard 5900-73 "Food concentrates. Methods for determination of moisture " (24) with a time period of 3 hours.

Table 4	4. N	Aicrobiologica	1 rates of	snacks	with	antioxidant	hyperactivity

Rates, measurement unit	easurement unit Allowable levels Actual Value		Normative Documents on Testing Methods
Total microbial number, KOE/g	Not more than 1×103	Less than 1.0×101	All Union State Standard 10444.15-94
Coliform, g	Is not allowed in 1.0	Not found в 1.0	All Union State Standard 31747-2012
Mold, KOE/g	Not more than 50	Less than 10	All Union State Standard 10444.12
Yeast, KOE/g	Not more than 50	Less than 10	All Union State Standard 10444.12

Table 5. Physical and chemical rates of pear snacks during the storage

Datas	Object			
Kales	Fresh snacks	After 12 months' storage		
Moisture Mass fraction, %	4.8 ± 0.3	4.9 ± 0.3		
Titratable acidity, % calculated as Apple acidity	2.4 ± 0.1	2.3 ± 0.1		
Content of soluble solids, %	27.9 ± 0.4	27.5 ± 0.4		

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Products of long-term storage	Total content of phenols, mg of gallic acid/ 100 g of material	Total content of flavonoids, mg of catechine/ 100 g of material	E _{c50} mg/cm ³	FRAP value mmol Fe2+/ 1 kg of raw material	Antioxidant activity, Inhibiting %
		Pear snacks	I		
3 months' storage	116.3	46.3	169.3	1.73	4.6
6 months' storage	112.7	43.4	43.4 174.8		4.1
9 months' storage	109.3	40.1	40.1 190.4		4.1
12 months' storage	87.6	39.7	198.7	1.14	3.8
	Pear snacks w	ith higher antioxidant p	oroperties	·	
3 months' storage	457.3	317.1	26.0	14.4	14.9
6 months' storage 457.1		316.8	26.2	14.3	14.8
9 months' storage 456.7		316.6	26.2	13.1	14.1
12 months' storage	456.4	315.8	26.6	13.2	13.4

Table 6. S	tudy of	chemical c	composition	and antioxic	lant activit	y of	pear snack	s during	the storage
								· · · · · · · · · · · · · · · · · · ·	



Fig. 11. Change of moisture mass fraction in final product without package.

It was found that in case of an opened package during the time crunch indicator decreases (Fig. 11), thus it is not recommended to store the opened package for more than 38 hours. The critical moisture content is 8%, as at higher humidity of the product development of microorganisms occurs.

The obtained results are quite illustrative and allow us to conclude that freeze-drying is the best way to produce a high quality final product of fruit crops of pears.

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