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TEA WITH HERBAL ADDITIONS: THEIR ANTIOXIDANT ACTIVITY AND ITS DEPENDENCE ON HIGH PRESSURE PRE-TREATMENT BEFORE EXTRACTION

Ekaterina Vladimirovna Pastushkova[1*], Sergey Leonidovich Tikhonov[1], Olga Viktorovna Chugunova[1], Gennady Borisovich Pischikov[1]

[1]*Ural State University of Economics, 620219, Yekaterinburg, March 8 street, 62, Russian Federation*
**pastushkova.usue@list.ru*

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ABSTRACT

The antioxidant effects of developed tea drinks on the lipid peroxidation in rats' blood during swimming stress were investigated by malondialdehyde and conjugated dienes levels' measurement. Components of tea drinks from common Ural plants were selected based on their antioxidant action, contributing to prevention of oxidative stress, as well as high consumer properties. Developed tea drinks have high organoleptic characteristics, high content of flavonoids and high antioxidant activity. It was established that the use of developed tea drinks in the diet of rats can reduce the stress impact on the rat organism on biochemical and morphological levels. Processing of developed blends with high pressure (200 MPa, 60 s) significantly intensifies the subsequent yield of biologically active substances during extraction and the antioxidant activity of the extract. Good prospects of using common Ural plants for the development of tea drinks formulations with strong antioxidant effect are shown in our study.

1. Introduction

Tea and tea drinks are the most widely consumed beverages in the world and they are also an important agricultural product in many countries. A number of researchers have found that tea possesses a wide range of beneficial effects including depression of hypertension, reduction of cholesterol, anti-oxidation and anti-cancer activities (Kris-Etherton, Keen, 2002; Feng et al., 2016; Ganguly, 2017). These effects are commonly attributed to such chemical ingredients in tea leaves, as polyphenols, polysaccharides, alkaloids and amino acids, etc. (Nie & Xie, 2011; Wang et al., 2012).

Earlier it was generally believed that only green tea prepared by dehydration of *Camellia sinensis* leaves, which contain monomeric polyphenols, possesses antioxidant properties (Graham, 1992). Recent investigations, however, showed that black tea obtained by fermentation of tea leaves and containing only a small amount of catechins and theaflavins and thearubigins--whose biological activities are less studied, also has antioxidant activity (Frei & Higdon, 2003). As a result, black tea has been proved to protect against cancer, heart and other diseases (Alferink et al., 2017; Feng et al., 2016; Singh et al., 2017; Sur & Panda, 2017). There are some data about the similar effect of different herbal teas (Tan et al., 2016; Shannon et al., 2017). However, the biological activity of black and herbal tea as a source of antioxidants required further investigation.

Therefore, scientists have searched for potent antioxidants, especially among natural products. One such potentially health-promoting beverage is herbal tea drinks or tea with addition of natural plants. It was found that some herbal teas, which are a hot water infusions of dried or fresh plant parts: roots, leaves and fruits had excellent antioxidant capacities as compared with black tea (Dalar & Konczak, 2013; Deetae et al., 2012; Jin et al., 2016). Thus, it was found that is a kind of important resource worth of exploitation and utilization. Further studies showed that antioxidant properties and organoleptic parameters significantly varied among herbal teas and that total phenolic content in herbal teas was highly positively correlated with their antioxidant capacities (Qasim et al., 2017; Carabajal et al., 2017). So, these beverages could be important sources of antioxidant and antimutagenic compounds. The developed beverages from different herbs of different countries could be important dietary sources of antioxidant and antimutagenic compounds for prevention of chronic diseases (Carabajal et al., 2017; Kapepula et al., 2017).

On the other hand, it was found that most of herbal teas had lower antioxidant property values than green tea (Chan & Wong 2015; Pardau et al., 2017). It is known that extraction of bioactive compounds from plants strongly depends on the solvents used (Bhebhe et al., 2016) and extraction methods such as mechanical grinding, ultrasonic-assisted extraction (Wei & Yang 2015; Vinatoru et al., 2017), microwave-assisted extraction (Vinatoru et al., 2017), and also sample pretreatments using acid and alkali on the microstructure of plant sample (See et al., 2016). One of such promising extraction method which can intensify transition of biologically active substances in the extract from row material is the High Pressure Processing (HPP) (Balasubramaniam et al., 2015; Wang et al., 2016). Applying high-pressure processing can inactivate pathogenic and spoilage microorganisms, as well as modify structures with little or no effects on the nutritional and sensory quality of tea (Wang et al., 2016). Application of high-pressure reduces the thermal exposure of the food during processing; therefore protect a variety of bioactive compounds and can increase the extraction rate of these compounds from tea and herbs (Jun et al, 2011).

So, the aim of this study was to investigate antioxidant compounds extraction using conventional and HPP methods from different tea drinks made from Ural plants and the influence of these extracts on antioxidant activity in vivo.

2. Materials and methods

2.1. Plant materials

A number of plants were selected for study based on availability and popularity in the areas where research was being conducted (Yekaterinburg, Russia). This selection process was carried out on the basis of previous articles (Pastushkova et al., 2015) with the assumption that teas that are widely available and popular will represent an accurate cross-section of plants being consumed by the local population.

We used following dried plant raw materials for the study: stinging nettle (*Urtica dióica*), peppermint (*Mentha piperita L.*), Origanum ordinary (*Oríganum vulgáre*), Salvia officinalis (*Salvia officinalis*), Yarrow ordinary (*Achilléa millefólium*), Hypericum perforatum (*Hypéricum perforátum*), Thyme (*Thymus serpyllum L*), cowberry leaf (*Vitis idaeae* folia), black currant leaf (*Nigrum ribes* folia) and cherry leaf (*Cerasus* folia). Plant materials were harvested in 2015-2016 in ecologically clean areas: Nizhneserginsky and Shalinsky areas. Plant leaves from these locations of Ural region were washed and dried at ambient temperature for 7 days.

The end of drying process was determined by the following features: leaves, leaf stalks and grass stems should be without excessive fragility, the mass fraction of moisture should not exceed 14%. Next, the raw material was brought to a state that corresponds to the standard: parts that have lost their natural color, crushed parts, accidentally trapped impurities were removed

2.2. Extraction and pressure treatment

The technology used in our work for producing of tea drinks with addition of Ural plants consists in the joint processing of plant materials, which is based on black tea «Greenfield. Classic Breakfast» and plant components, which were placed in a blending drum and mixed at a rotation frequency of 4-5 rpm within 5-6 minutes and their packaging in hermetic vacuum package (Patent RU2462873C1, 2012). Obtained doses for one-time brewing and packaged compositions of tea drinks with the addition of Ural plant materials were treated with a high pressure of 100-200 MPa for 60-90 seconds. Tea drinks were placed in a high-pressure chamber in vacuum packaging and then the working chamber was filled with liquid (drinking water) to capacity and sealed. Then the required pressure was applied to high-pressure chamber.

2.3. Sample preparation

Each tea bag packaging of samples was brewed in a pot made of ceramic materials in 200 ml of mineral water with 85-95 ºC temperature for 5 minute without closed (Sharpe et al., 2016), and was used for experiments within 20 minutes after brewing.

2.4. Organoleptic measurements

During organoleptic characteristics of tea drinks such indicators as tea drinks taste, aroma, aftertaste and infusion were measured according to Russia standard GOST 32572-2013.

All experiments were conducted at one study site (Ural State University of Economics, Yekaterinburg) with seven judges. Ethical approval was obtained from the University ethical panel. Judges used three-time replicate sets of samples, with each set being completed within one session with rest periods between sessions. All judges were well-experienced. For all experiments, judges were asked to rate each tea drinks sample on a 0 to 5 scale.

2.5. Animals

Adult Wistar rats of either sex (8 weeks of age, weighing 190±10 g, mean±SEM) were used in experiments. Rats were housed under controlled environmental conditions (12-h light/dark cycle and temperature 20 °C) in cages. Food and water were available *ad libitum*. The project was reviewed and approved by the university animal care review committee. All procedures were in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and the Order of the Ministry of Health of the Russian Federation “On the approval of rules through laboratory practice" No. 267 on June 19, 2003”.

2.6 Experimental design. Swimming protocol

Rats were divided into three groups of 10 animals each as follows:

(a) The control group was treated intragastrically with 10 ml of physiological saline every day for 3 weeks (n = 10),

(b) The stress group was treated intragastrically with 10 ml of physiological saline every day for 3 weeks prior to stressing through the probe (n = 10), exercised for last 5 days and killed immediately after last exercise,

(c) The stress+tea group was treated intragastrically with 10 ml of black tea or tea drinks in a dose of 10 ml per animal every day for 4 weeks (n = 10), exercised for last 5 days and killed immediately after last exercise.

Stress was modeled by chronic forced swimming stress according to (Michailidis et al., 2007). The rats were subjected to swimming stress by keeping them in propylene tank of dimension (37X37X30 cm), filled with water to a height of 25cm. The duration of exercise was about 45 minutes a day for 5 days at a water temperature of 27–28°C.

2.7. Blood and tissue sampling

All animals were deeply anesthetized with chloral hydrate and euthanized by decapitation at the end of experiment. Samples of thymus and right adrenal gland were collected immediately after decapitation. Adrenal glands were also dissected out at this time.

Blood samples from the control and experimental rats were collected into heparinized tubes after decapitation (Nayanatara et al., 2005).

2.8. Lipid peroxidation measurements

Lipid peroxidation was assayed by spectrophotometric measurement of conjugated dienes at 234 nm (Recknagel and Glende, 1984) and by malondialdehyde (MDA) measurement as malondialdehyde-thiobarbituric acid adducts (Londero & Greco, 1996). MDA in serum was separated by connecting with thiobarbituric acid and serum proteins were precipitated by TCA centrifugation. Then, TBA complex was measured at a wavelength of 534 nanometers.

2.9. Antioxidant activity measurements

For preliminary measurement of potential antioxidant activity (potential AOA) of herbal extracts a potentiometric method was used. The value of antioxidant activity was measured as the redox potential difference of the $K_3[Fe(CN_6)]/K_4[Fe(CN_6)]$ mediator system using an antioxidant activity measuring device (IVA Co. Ltd., Yekaterinburg, Russia) (Brainina et al., 2007).

2.10. Flavonoids measurements

The total flavonoid content in the tea was determined by a method described by Matyuschenko & Stepanova (2003). The flavonoid content was calculated from a calibration curve using rutin as reference standard. Flavonoid content was expressed as g of ruthin equivalents/100g of tea leaves (g RE/100g). Measurements were conducted in triplicates.

2.11. Statistical analysis

Data are reported as mean values with standard deviation. Statistica 8.0 software was used for data analysis. *P* values less than 0.05 were considered statistically significant.

3. Results and discussions

3.1. Development of tea drinks based on common Ural plants

Based on the literature data about the pharmacological properties of Ural herbs and preliminary results of organoleptic compatibility studies (Pastushkova et al., 2015), we selected the most promising plants for use in herbal teas. Due to industrial production requirements, among the most significant selection criteria we used plant availability and best organoleptic characteristics of the plant (color and transparency of infusion, aroma and the influence on herbal tea taste).

Among selected herbs, the content of flavonoids and ascorbic acid, as well as their potential antioxidant activity (potential AOA) according to Brainina et al., (2007) were studied (Table 1). Black tea («Greenfield. Classic Breakfast»), which usually used as a main part of tea drinks, was used as a control.

Table 1. The content of biologically active substances in dry plant materials of common Ural plants (mean±SD**)**

Plant raw material	**Ascorbic acid, (mg/100 g)**	**Total flavonoids, (g RU/100 g)**	**Potential AOA, (equiv/l)**
Urtica dioica	3,40±0,04	0,20±0,03	8,53±0,41
Méntha piperíta	12,13±0,37	0,31±0,03	4,87±0,15
Oríganum vulgáre	5,65±0,04	0,41±0,02	4,91±0,10
Salvia officinalis	5,34±0,01	0,12±0,01	6,73±0,35
Achillea millefolium	1,70±0,02	0,34±0,03	2,67±0,15
Hypericum perforatum	6,58±0,03	0,16±0,02	3,84±0,15
Thymus vulgaris	1,89±0,04	0,29±0,01	5,38±0,20
Vaccinium vitis-idaea (folia)	1,41±0,03	0,11±0,02	2,47±0,15
Ríbes nígrum (folia)	3,20±0,01	0,50±0,03	2,16±0,10
Prunus cerasus (folia)	2,10±0,05	0,10±0,03	2,13±0,10
Camellia sinensis (black tea)	1.11+0.03	0.11+0.01	4.90+0.14

According to the data obtained, we can assert that at all plants studied ascorbic acid content was higher than in black tea (*Camellia sinensis).* Especially high it was in *Méntha piperíta*, Hypericum perforatum, *Oríganum vulgáre*, *Salvia officinalis* and *Urtica dioica*. Total flavonoids content was highest in *Ríbes nígrum* (folia), *Oríganum vulgáre, Achillea millefolium*, *Méntha piperíta* and *Thymus vulgaris* and in all of them it was higher, then in *Camellia sinensis*. In *Urtica dioica, Salvia officinalis* and *Thymus vulgaris* potential antioxidant activity higher, than in black tea (*Camellia sinensis)* was detected.

Therefore, we can suppose, that addition of this medicinal raw material can significantly increase the potential antioxidant activity of tea drinks due to the high content of antioxidants of different nature. Thereby, based on these data and on the organoleptic compatibility of tea drinks components, a number of herbal tea compositions were developed (Table 2).

Table 2. The composition of tea drinks with addition of plant materials

No.	**Composition**	**Content of components,%**
No. 1	*Prunus cerasus* (folia); *Ríbes nígrum* (folia); *Camellia sinensis* (black tea)	2,8 : 10,6 : 86,6
No. 2	*Urtica dioica*; *Hypericum perforatum*; *Salvia officinalis*; *Camellia sinensis* (black tea)	2,8 : 1,2 : 0,5 : 95,5
No. 3	*Urtica dioica*; *Hypericum perforatum*; *Vaccinium vitis-idaea* (folia); *Camellia sinensis* (black tea)	4,8 : 5,2 : 2,5 : 87,5
No. 4	*Oríganum vulgáre*; *Achillea millefolium*; *Thymus vulgaris*; *Camellia sinensis* (black tea)	2,5 : 10,5 : 1,6 : 85,4
No. 5	*Urtica dioica*; *Méntha piperíta*; *Ríbes nígrum* (folia); *Camellia sinensis* (black tea)	5,9 : 5,3 : 5,7 : 83,1
No. 6	*Oríganum vulgáre*; *Achillea millefolium*; *Salvia officinalis*; *Vaccinium vitis-idaea* (folia); *Camellia sinensis* (black tea)	4,8 : 13,9 : 2,8 : 2,9 : 75,6

These tea drinks has increased ascorbic acid content (up to 75% to black tea at No 5) and increased total flavonoids content (up to 40% to black tea at No 6). So, we developed a number of tea drinks from common Ural plants. The high content of natural antioxidants in herbs used allows us to achieve an increase of antioxidant activity using minimum dosage of added plant materials: the proportion of added in various combinations to black tea plant materials is not more than 15% by dry weight. It allows us to save consumer and organoleptic properties of tea drinks developed.

3.2. Influence of tea drinks on lipid peroxidation during swimming stress in rats

It is well known that green tea consumption had no effect on basic physiological parameters of rats (Alessio et al., 2002). It also had no effect on the basic rate of lipid peroxidation in different organs in non-stressed rats (Alessio et al., 2002). On the other hand, it has antioxidant activity *in vitro* and *in vivo* during swimming stress (Alessio et al., 2002) and this effect also known to other herbal drinks (Kothiyal & Ratan, 2011). So, we study the influence of consumption of 6 developed tea drinks on rate of lipid peroxidation in rats during stress.

The study of influence of developed tea drinks consumption on rate of lipid peroxidation in rats' blood showed that in animals consuming water and subjected to swimming stress ("stress" group) level of MDA content in blood significantly increased after acute exercise as compared to control (Figure 1A), wherein MDA levels in animals consuming black tea before stress did not significantly differ from those in stressed variant. These results differed from those obtained with animals that consumed tea drinks № 1- №6 (1 - 6 groups) in which swimming stress did not caused such significantly change in levels of MDA content from non-stressed variant (Fig. 1A), sowing protection against oxidative stress.

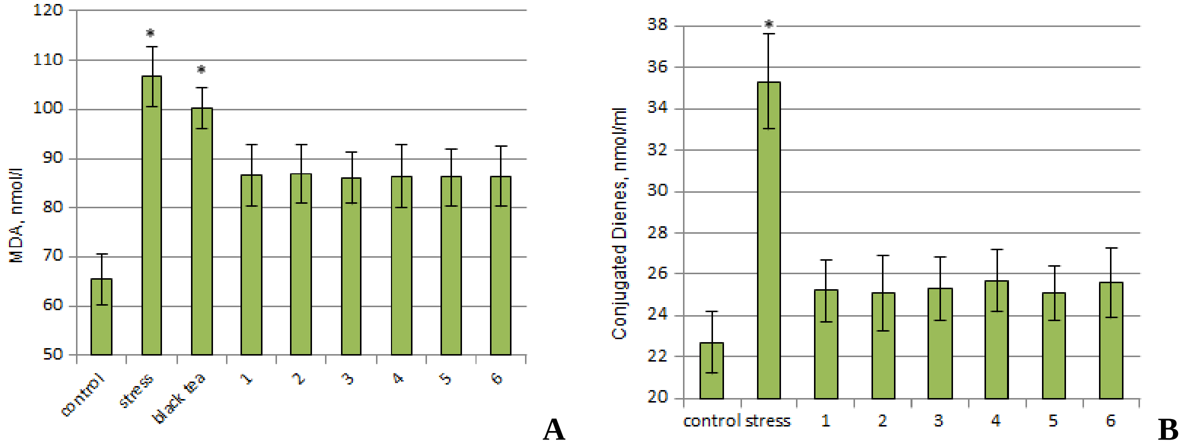

Figure 1. The influence of different tea compositions on MDA (A) and conjugated dienes content (B) in rats' blood during swimming stress.
* P < 0,05 compared to control non-stressed group

It is interesting to note that there was not any statistically significant difference between the influences of all tea drinks developed on the level of MDA content in blood during swimming stress (Figure 1A).

Conjugated dienes content in rat's blood showed even brighter picture (Figure 1B). If difference in MDA levels between "control" non-stressed group and groups which consumed tea drinks № 1- №6 during stress was statistically significant, the difference in conjugated dienes content between these groups was not statistically significant at all.

So, based on these data and the data about the organoleptic quality of 6 tea drinks developed, tea drink № 4 which has the best organoleptic indicators was chosen for the further experiments.

Evaluating the data obtained in general, we can say that the data about the influence of tea drinks consumption on rate of lipid peroxidation in rats' blood are in accordance with the data of about the influence of green tea on decrease of malondialdehyde level after green tea intake (Yokozawa et al., 1999, 2002) and the data of about the antioxidant effect of different herbal teas (Tan et al., 2016; Shannon et al., 2017).

3.3. Influence of tea drinks on morphological changes in rats during swimming stress

It is known that swimming stress cause changes in the biochemistry and even structure of many internal organs in rats (Avital et al., 2001), as particular in thymus mass (Živkovića et al., 2005) and in adrenal glands (Nayanatara et al., 2005). On the other hand, it is known that tea did not influence on structure and weight of internal organs of intact Ross broiler (Sarker et al., 2010). Therefore we studied the influence of tea drink № 4 consuming on stress-induced changes in these rats' organs. As a result of swimming stress, a significant decrease in the thymus mass in "stress" group was noted as compared to control (Figure 2A), in "stress+tea" group significantly less decrease of thymus mass was detected which showed good stress-protective properties of the tea drink tested. In addition, in animals of "stress" group the weight of the right adrenal gland significantly increased (Figure 2B). This hypertrophy of the adrenal cortex was observed due to the expansion of the beam zone as compared with control group. In the group of animals, which were given a tea drink ("stress+tea" group), the pathological changes in the adrenal glands after stress were less pronounced. In addition, it is noted that stress caused multiple hemorrhages on the gastric mucosa of the stressed group of animals, which were not given a tea drink ("stress group") which were not detected in "stress+tea" group.

The data about protective action of herbal tea drink studied on thymus mass and adrenal gland showed that herbal tea drink consumption can show not only biochemical (Tan et al., 2016; Shannon et al., 2017) and physiological (Lindenmuth & Lindenmuth 2000) impact, but morphological impact on organism too.

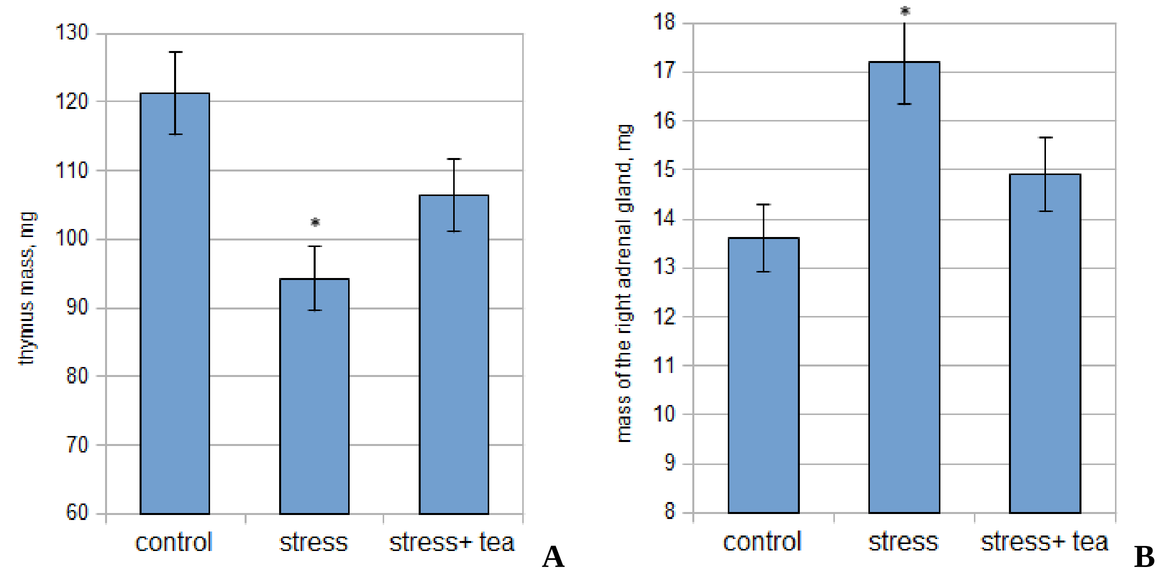

Figure 2. The influence of tea drink № 4 on mass of the right adrenal gland (A) and thymus mass (B) content in rats during swimming stress.
* P < 0,05 compared to control non-stressed group

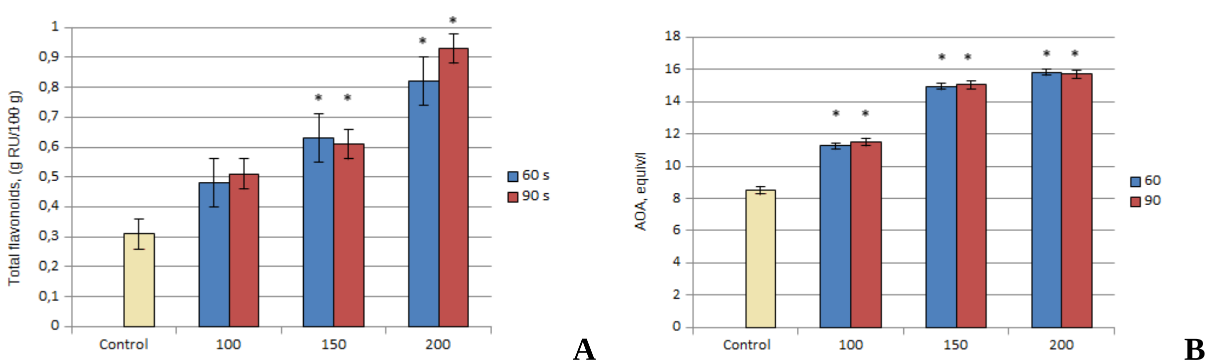

Figure 3. The influence of pressure (100, 150, 200 MPa) and treatment duration (60 and 90 secunds) on total flavonoids content (A) and potential AOA (B) in extracts of tea drink No. 4
* P < 0,05 compared to non-treated control Similar changes these processing caused in AOA. The highest AOA (15.82 mol eq. / l) was observed when a plant raw material was processed at pressure 200 MPa for 60 s. Further increase of treatment time did not cause any AOA increase in extract.

3.4. Influence of High Pressure Processing on the antioxidant features of tea drink

The next part of our research was to study the influence of High Pressure Processing of dried raw plant material on the antioxidant features of tea drink № 4 - total flavonoids content and antioxidant activity.

From the data of Figure 3 one can see, that the processing of tea drinks with high pressure significantly increase extraction of biologically active substances. In the samples of developed tea drinks, the content of Total flavonoids under pressure treatment 100 MPa for 60 s was about 40% higher, than in control, under pressure treatment 150 MPa for 60 s was about 100% higher, than in control and under pressure treatment 200 MPa for 60 s was about 130% higher, than in control sample (Figure 3A). The increase in the time of high pressure impact on plant materials does not lead to a significant increase in the intensity of extraction of Total flavonoids to infusion. On average, the difference in the amount of Total flavonoids, extracted into the drink during 60 or 90 seconds, was not statistically significant.

Therefore, one can say that the treatment of plant materials with a pressure of 200 MPa for 1 minute allows maximize the yield of biologically active substances and antioxidant activity in comparison with traditional technology.

The study of the organoleptic factors of tea drink with the addition of plant materials № 4 showed that, irrespective of the technological regimes, they correspond to the quality indicators. Taste and aroma were pronounced, well-coordinated, with pleasant, harmonious aftertaste and infusion was transparent.

The data about the influence of High Pressure Processing on the antioxidant features of tea drink showed, that this treatment can not only inactivate pathogenic and spoilage microorganisms or can intensify extraction of biologically active substances from fresh material (Balasubramaniam et al., 2015; Wang et al., 2016) bat can be used as pre-treatment of dried plant material with the aim of intensifying of biologically active substances extraction from it.

4.Conclusions

So, formulations and processing technology of tea drinks using some common Ural plants dried material are developed. Developed tea drinks have high organoleptic characteristics, high content of flavonoids and high antioxidant activity. It was established that the use of developed tea drinks in the diet of rats can reduce the stress impact on the rat organism on biochemical and morphological levels. Processing of developed blends with high pressure (200 MPa, 60 s) significantly intensifies the subsequent yield of biologically active substances during extraction and the antioxidant activity of the extract.

The study of the organoleptic characteristics of tea drinks with the addition of plant materials showed that, irrespective of the technological regimes, they correspond to the regulated indicators. Taste and aroma were pronounced, harmonious, aftertaste was pleasant, harmonious and infusion was transparent.

Therefore, good prospects of using common Ural plants for the development of tea drinks formulations with strong antioxidant effect are shown in our study. Moreover, because of the fact, that some of the common Ural plants studied are common for whole Europe, these formulations can be interesting not only in the investigated region.

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